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ORIGINAL ARTICLE

# Modeling the diving bradycardia: Toward an "oxygen-conserving breaking point"?

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**Abstract**
*Purpose* Although it has been demonstrated that the exponential decay model fits the heart rate (HR) kinetics in short static breath holding (BH), this model might be inaccurate when BH is maintained for several minutes. The aim of this study was to build a new meaningful model to quantify HR kinetics during prolonged static BH.
*Methods* Nonlinear regression analysis was used to build a model able to quantify the beat-to-beat HR reduction kinetics observed in prolonged static BH performed both in air and in immersed condition by 11 trained breath-hold divers. Dynamic changes in cardiac autonomic regulation through heart rate variability indices [root mean square of successive difference of R–R intervals (RMSSD); short-term fractal scaling exponent: (DFAα1)] and peripheral oxygen saturation ($SpO_2$) were also analyzed to strengthen the model.
*Results* The tri-phasic model showed a sharp exponential drop in HR immediately followed by a slight linear rise up until a breaking point preceding a linear drop in HR. The breaking points had similar level of $SpO_2$ whether in air or in immersed condition (95.1 ± 2.1 vs. 95.2 ± 3.0 %, respectively; $P = 0.49$), and the subsequent linear drop in HR was concomitant with a shift in cardiac autonomic regulation in air (RMSSD: +109.0 ± 47.8 %; $P < 0.001$; DFAα1: −18.0 ± 17.4 %; $P < 0.05$) and in immersion (RMSSD: +112.6 ± 55.8 %; $P < 0.001$; DFAα1: −26.0 ± 12 %; $P < 0.001$).
*Conclusion* In addition to accurately fitting the HR kinetics, the most striking finding is an "oxygen-conserving breaking point" highlighted by the model, which might be interpreted as unique adaptive feature against hypoxic damages in the human diving bradycardia.

**Keywords** Diving bradycardia · Cardiac autonomic regulation · Regression-based model

**Abbreviations**

| | |
|---|---|
| A | Theoretical amplitude of the exponential decay |
| $A_{\%HR}$ | Actual amplitude of the exponential decay |
| AIC | Akaike's information criterion |
| B | Slope of the linear increase in heart rate |
| BH | Breath holding |
| BHDs | Breath-hold divers |
| C | Slope of the linear decrease in heart rate |
| $\chi^2_{red}$ | Reduced Chi-squared |
| DFAα1 | Short-term fractal scaling exponent |
| ECG | Electrocardiogram |
| HR | Heart rate |
| %HR ($t$) | Relative heart rate change at any given time t |
| $HR_{max}$ | Heart rate peak |
| $\tau$ | Time constant of the exponential decay |
| HRV | Heart rate variability |
| $O_{2bp}$ | "Oxygen-conserving breaking point" |

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| | |
|---|---|
| $R^2$ | Coefficient of determination |
| $R^2_{adj}$ | Adjusted coefficient of determination |
| RMSE | Root mean squared errors |
| RMSSD | Root mean square of successive difference of R–R intervals |
| $SpO_2$ | Peripheral oxygen saturation |
| $SS_R$ | Sum of squared residuals |
| $T_{min}$ | Time at which the minimum value of the exponential decay occurs |

## Introduction

Human physiological response to static breath holding (BH) is called the diving response, and its main effects are bradycardia, decreased cardiac output, peripheral vasoconstriction and increased arterial blood pressure (Lindholm and Lundgren 2009; Heusser et al. 2009; Marabotti et al. 2008). Indeed, its primary goal is to save oxygen stores (i.e., the oxygen-conserving effect) toward hypoxia-sensitive tissues such as brain and heart (Joulia et al. 2009; Lindholm and Lundgren 2009). In healthy humans, these protective mechanisms against hypoxic damages are initially triggered by BH per se (Schuitema and Holm 1988; Perini et al. 2008) and strengthened by face immersion (Andersson et al. 2000) as well as whole-body immersion (Costalat et al. 2013; Marabotti et al. 2013).

It has recently been demonstrated that heart rate (HR) kinetics in response to short static BH (<60 s) displayed a diving bradycardia that decreased exponentially with and without face immersion (Caspers et al. 2011). The diving response is considered so far as one of the most powerful autonomic reflexes in humans (Schaller 2004; Cornelius et al. 2010); consequently, authors of this meta-analysis claimed that BH-induced bradycardia might be considered as a clinical tool to assess the integrity of cardiac autonomic pathways. However, their proposed exponential model (i.e., a mono-phasic model) could be not sufficient to accurately quantify the kinetics of the diving bradycardia when static BH is maintained for several minutes. Indeed, in both trained and elite breath-hold divers (BHDs), visual inspection of the diving bradycardia kinetics has shown to further decrease in the latter hypoxic stage of prolonged BH, likely due to the activation of both baroreflex and chemoreflex (Perini et al. 2008; Lemaître et al. 2008). To our knowledge, no study to date has used nonlinear regression analysis to describe the time course of HR reduction during prolonged BH.

Therefore, the purpose of this investigation was to quantify, using a nonlinear regression model, the beat-to-beat time course of heart rate (HR) reduction during maximal static BH in trained BHDs. Dynamic changes in cardiac autonomic regulation were also investigated through linear and nonlinear analysis of heart rate variability (HRV) analysis as well as peripheral oxygen saturation ($SpO_2$) to strengthen the physiological meaningfulness of the model. Maximal static BH was performed both in laboratory (i.e., dry-body BH) and in real environment condition (i.e., immersed-body BH at water surface) to raise the validity and the credibility of the model. We hypothesized that a tri-phasic model would be more accurate to quantify the bradycardic response and would be more meaningful from a physiological standpoint than the mono-phasic model when BH is prolonged for several minutes.

## Materials and methods

### Subjects

Regression-based modeling of HR kinetics and HRV analysis were performed on 11 healthy active BHDs (10 males and 1 female; age, 36.0 ± 9.6 years; height, 175.7 ± 4.2 cm; body mass, 71.2 ± 8.9 kg). The presence of a single female was fortuitous, and a recent article concluded that males and females have similar diving responses (Tocco et al. 2012). Their personal static BH performance was 316 ± 40 s. 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

Each subject performed two maximal voluntary static BH, as during their training, in two experimental conditions. The first BH was carried out in air and the second was performed during submersion at water surface (head-in). Dry-body BH and immersed-body BH were conducted in the same session with at least 4 h between each one and were preceded by a 15-min rest period. During rest periods, BHDs were asked to remain quiet. Before entering the water, the subjects put on wet suits, diving masks and snorkels. Before starting immersed-body BH, all subjects assumed the prone position at the surface of the water and breathed through the snorkel with their heads submersed in water. BHDs were also instructed to keep their chest relaxed and to perform BH without prior hyperventilation. Ambient air and pool water temperature were, respectively, 26 and 27 °C.

### R–R recording and peripheral oxygen saturation measurements

$SpO_2$ was assessed by fingertip pulse oximetry (PalmSat 2500, Nonin Medical, Inc., USA). Beat-to-beat R–R

intervals were continuously recorded by means of a two-lead ECG system included in the PhysioFlow PF-05 device (Manatec Biomedical, France) whose sampling rate recording is 250 Hz (i.e., a temporal resolution of 4 ms). This sampling rate recording has already been used to record the diving bradycardia during both static and dynamic BH (Kiviniemi et al. 2012). A MATLAB-based package provided by the PhysioFlow's designers was applied to extract ECG recording of each subject. Then, R-wave peaks from ECG signal were detected by a digital filtering method implemented in Kubios HRV 2.1 software (Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland), based on Pan–Tompkins algorithm (Pan and Tompkins 1985).

Careful attention has been paid to the detection of occasional physiological artifacts such as ectopic beats (i.e., premature beats) or missed beats detections since it has been showed that abnormal beats crucially distort subsequent short-term HRV analysis (Peltola 2012). Briefly, the spurious R–R intervals were identified by using an artifact detection algorithm based on the intra-individual calculation of an artifact threshold criterion (Berntson et al. 1990), and they were then replaced using cubic spline interpolation. Both artifact detections and their consequent replacements were performed using a recent data processing software [ArTiiFACT version 2.06 (Kaufmann et al. 2011)].

## Diving bradycardia modeling

All datasets were time-aligned in each condition using cubic spline interpolation/extrapolation since BH times were slightly different between BHDs. To highlight the comparison of both conditions, we chose to normalize HR using the percentage change from the maximum (peak) value for each BH as follows:

$$\text{relative HR change } (\%) = \frac{\text{absolute HR value}}{\text{absolute HR peak value}} \cdot 100$$

HR datasets were analyzed using nonlinear curve fitting toolbox implemented in OriginPro software (OriginLab, Northampton, MA). Parameters were calculated by using least square nonlinear regression in which the convergence criterion was satisfied by minimizing the sum of squared errors (Levenberg–Marquardt algorithm). The number of maximum iterations allowed was 400, and the iteration process continued until successive repetitions reduced the sum of squared residuals by $<10^{-9}$. Based on the observed mean HR kinetic, we hypothesized that a piecewise regression analysis would describe HR behaviour during prolonged BH. Therefore, the parameter estimates in both conditions were determined as a function of time ($t$) by using a piecewise function defined as follows:

When $t < O_{2bp}$ (first sub-function):

$$\%\text{HR}(t) = \text{HR}_{\max} - A\left[1 - e^{\left(\frac{-t}{\tau}\right)}\right] + B \cdot t$$

when $t > O_{2bp}$ (second sub-function):

$$\%\text{HR}(t) = \text{HR}_{\max} - A \cdot \left[1 - e^{\left(\frac{-O_{2bp}}{\tau}\right)}\right] + B \cdot O_{2bp} - C \cdot (t - O_{2bp})$$

where %HR ($t$) is the relative HR change at any given time $t$; $HR_{max}$ is the peak HR and equals 100 (%) because HR was previously normalized using the percentage change from the peak value; $\tau$ is the time constant of the exponential decay (i.e., the time needed to reach 63 % of the lowest HR during the normoxic phase of BH); $A$ is the theoretical amplitude of the exponential decay; $B$ is the slope (i.e., sensitivity) of the linear increase in %HR following the exponential drop; $O_{2bp}$ ("oxygen-conserving breaking point") is the time delay at which HR kinetics changes its behaviour; $C$ is the slope (sensitivity) of the linear decrease in %HR following $O_{2bp}$.

Setting the derivative of the first sub-function equal to zero and finding the solution give the time ($T_{min}$) at which the minimum value of the exponential decay occurs. Hence, substituting the algebraic expression for $T_{min}$ back into the sub-function gives the expression for the actual amplitude of the exponential decay, i.e., the magnitude of the bradycardia during the normoxic phase of BH ($A_{\%HR}$). Below are the expressions for these two additional derived parameters depending on the fitted parameters:

$$T_{\min} = \tau \cdot \ln\left(\frac{A}{B \cdot \tau}\right)$$

$$A_{\%\text{HR}} = A - B \cdot \tau \cdot \left[1 + \ln\left(\frac{A}{B \cdot \tau}\right)\right]$$

The model parameters as well as the derived parameters described above are presented in Fig. 1 (panel a).

## HRV analysis

During resting phases, HRV parameters were calculated over the most stable period of 5 min chosen from the 15-min recording time of each resting phase. Before HRV analysis, the non-stationary trends of the signal were removed using the smoothness prior approach (Tarvainen et al. 2002) and the smoothing parameter was set to $\lambda = 500$ which corresponds to a cutoff frequency of 0.035 Hz (i.e., below LF frequency band). A widely accepted time-domain parameter known as the root mean square of successive difference of R–R intervals (RMSSD) was calculated, and it is considered as a marker of parasympathetic activity (Heart

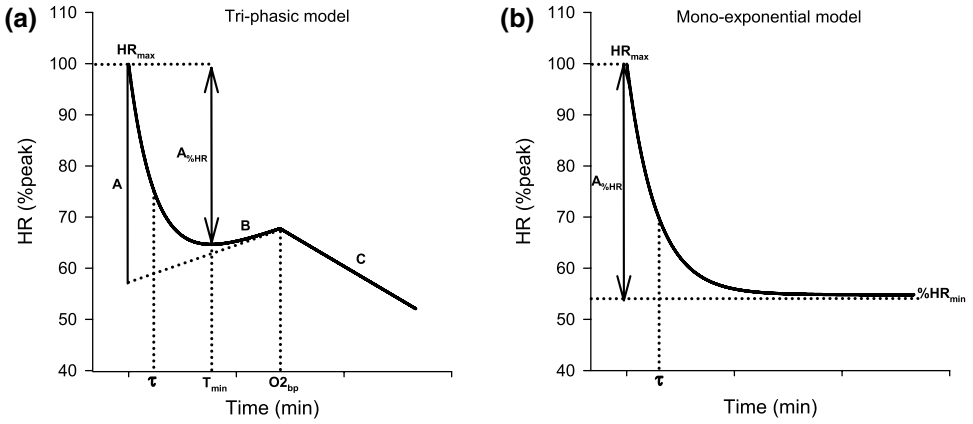


**Fig. 1** Description of model parameters regarding the tri-phasic model (**a**) and the mono-exponential model of Caspers et al. (**b**). $HR_{max}$, maximum heart rate expressed as percentage; $\tau$, time constant of the exponential decay; *A*, theoretical amplitude of the exponential decay; *B*, slope (sensitivity) of the increase in %HR following the exponential drop; $O_{2bp}$ ("oxygen-conserving breaking point"), time delay at which HR kinetic changes its behaviour; *C*, slope (sensitivity) of the decrease in %HR following $O_{2bp}$; $T_{min}$, time at which %HR reached its minimum value during exponential decay; $A_{\%HR}$, amplitude of the actual exponential decay; $\%HR_{min}$, minimum heart rate expressed as percentage of maximum heart rate

rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). In addition, the short-term fractal scaling exponent (DFA$\alpha$1) from nonlinear methods was calculated over the range of $4 \leq n \leq 16$ heart beats, and it quantifies the short-term fractal organization in human HRV (Peng et al. 1995). The output of DFA is an exponent ($\alpha$1), which is the slope obtained by linear regression of a log–log fluctuation plot against window size (Peng et al. 1995). It has been demonstrated that decreased $\alpha$1 is physiologically related to co-activation of peripheral sympathetic and central vagal outflows, whereas an increased $\alpha$1 corresponded to peripheral sympathetic activation associated with vagal withdrawal to the heart (Tulppo et al. 2005). All HRV indices were computed by means of Kubios HRV software version 2.1. During dynamic HR changes (i.e., while in BH), RMSSD was calculated for each of the 30 s while in BH. This time window of analysis has been previously validated to capture the instantaneous change in parasympathetic regulation when the usual conditions for signal stableness are affected (Goldberger et al. 2006; Smith et al. 2013). In addition, both RMSSD and DFA$\alpha$1 were calculated during normoxic phase of BH and hypoxic phase of BH, according to periods specified by the tri-phasic model (Figs. 2 and 3).

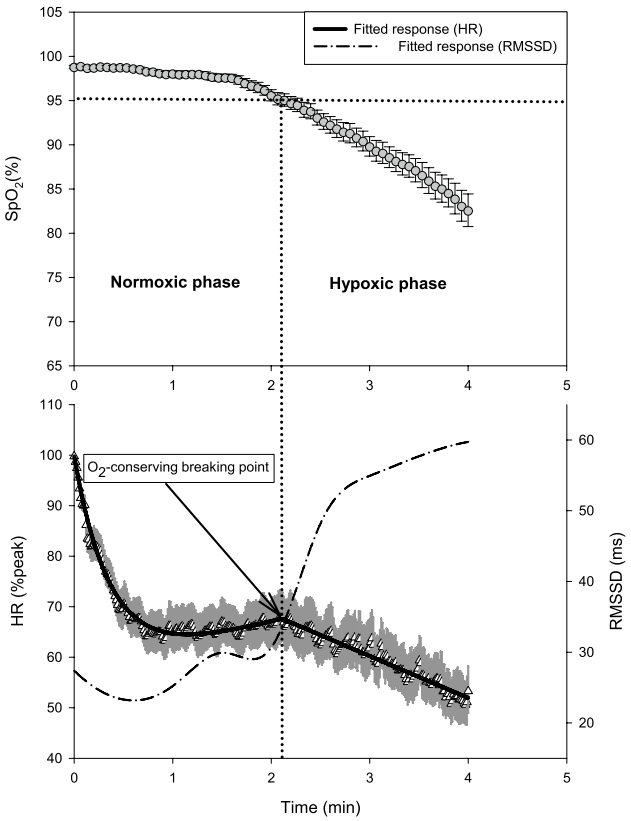


**Fig. 2** Mean (±SE) time courses of diving bradycardia (%HR), peripheral oxygen saturation ($SpO_2$) and *cubic spline* interpolation of the root mean square of successive difference of R–R intervals (RMSSD) during dry-body breath holding. Breath holding is separated into two phases with a *vertical dotted line* passing through the "oxygen-conserving breaking point"

## Goodness of fit and models comparison

The adjusted version for the proportion of the variation ($R^2_{adj}$), root mean squared errors (RMSE) and reduced

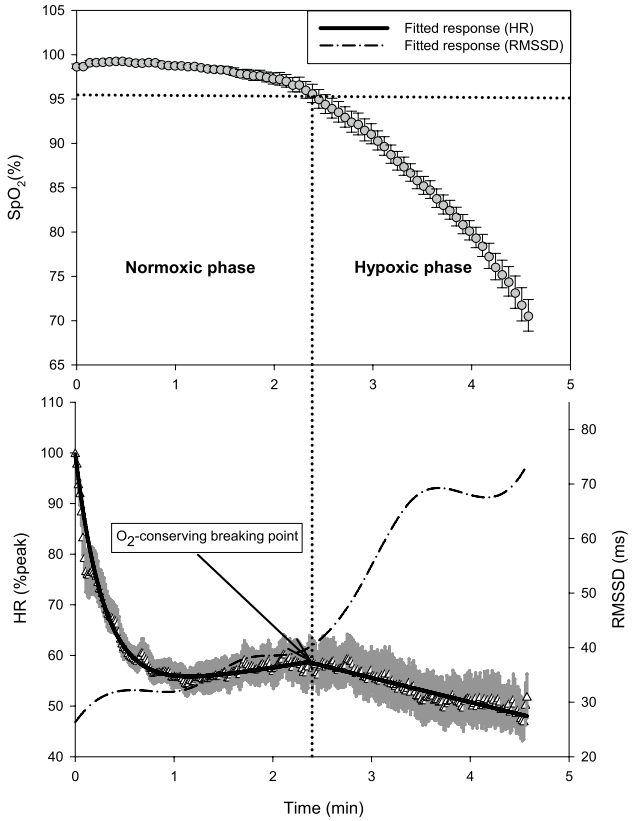


**Fig. 3** Mean (±SE) time courses of diving bradycardia (%HR), peripheral oxygen saturation ($SpO_2$) and *cubic spline* interpolation of the root mean square of successive difference of R–R intervals (RMSSD) during immersed-body breath holding. Breath holding is separated into two phases with a *vertical dotted line* passing through the "oxygen-conserving breaking point"

Chi-squared ($\chi^2_{red}$) were used to assess goodness of fit for each model (Motulsky and Christopoulos 2004). The most suitable model to describe the kinetics of the diving bradycardia would be the model with the highest $R^2_{adj}$ and both lowest $\chi^2_{red}$ and RMSE. Akaike's information criterion (AIC) based on information theory (Burnham and Anderson 2002) was reported to compare the tri-phasic model (Fig. 1, panel a) with the mono-exponential model of Caspers et al. (Fig. 1, panel b) during prolonged BH. This comparison was made to ensure that the proposed tri-phasic model is more accurate than the mono-phasic one of Caspers to describe HR kinetics when BH is maintained for several minutes. In addition, the extra sum of square *F* test (*F* test) was calculated on the basis of the tri-phasic model applied on dry-body BH datasets and immersed-body BH datasets to compare the overall kinetics between the two conditions.

### HRV 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 one-way repeated-measure ANOVA was used to compare HRV indices between the three phases (resting phase, normoxic phase of BH and hypoxic phase of BH) in both conditions. In addition, a two-way repeated-measure ANOVA was used to analyze the interactions of HRV indices between the two conditions (dry-body BH and immersed-body BH) across the three phases (rest phase, normoxic phase of BH and hypoxic phase of BH). If main effect was significant, multiple pairwise comparisons adjusted with Holm–Sidak correction were conducted. Statistical analysis was performed and graphs were plotted using OriginPro software version 9.0 and Sigmaplot software version 12.3 (SPSS, Chicago, USA), respectively. The results in tables and in the text are expressed as the mean value ± SD. A *P* value <0.05 was considered statistically significant for all analyses.

## Results

Dry-body BH and immersed-body BH lasted 240.5 ± 18.5 and 268.0 ± 40.2, respectively ($P < 0.05$).

### Goodness of fit and models comparison

All models successfully converged within a few iterations (<20) and reached the $\chi^2$ tolerance value of $10^{-9}$. The parameter estimates and their respective confidence intervals are reported in Table 1. The *F* test calculated on the residual sum of square from the tri-phasic model showed that HR kinetics was different between dry-body BH and immersed-body BH ($P < 0.05$; Table 1). In addition, AIC, $SS_R$, $\chi^2_{red}$ and RMSE from the tri-phasic model were lower than the ones from the mono-phasic model whether in dry-body BH or immersed-body BH (Table 2). Conversely, $R^2_{adj}$ from the tri-phasic model was higher than the mono-phasic model in both conditions (Table 2).

### HRV indices and $SpO_2$

Time courses of the diving bradycardia (%HR), peripheral oxygen saturation ($SpO_2$) and cubic spline interpolation of the root mean square of successive difference of R–R intervals (RMSSD) during dry-body and immersed-body BH are presented in Figs. 2 and 3. BH is separated into a normoxic and a hypoxic phase according to the time at which the "oxygen-conserving breaking point" occurred. At this breaking point, absolute HR values were lower in immersed condition than in air (58.7 ± 11.0 vs. 67.9 ± 14.8 bpm, respectively; $P < 0.001$), whereas $SpO_2$ percentages were similar for both dry-body and immersed-body BH (95.1 ± 2.1 vs.

**Table 1** Model parameters and their respective confidence limits for the tri-phasic model applied on datasets of prolonged breath holding in the two experimental conditions

| Parameters | Dry-body BH* | LCL–UCL (95 %) | Immersed-body BH* | LCL–UCL (95 %) |
|---|---|---|---|---|
| *Tri-phasic model* | | | | |
| $\tau$ (min) | 0.36 | 0.31–0.42 | 0.29 | 0.25–0.32 |
| $A$ (%) | 42.7 | 37.37–48.12 | 48.28 | 45.44–51.12 |
| $B$ (%.min$^{-1}$) | 5.00 | 1.80–8.19 | 3.08 | 1.39–4.78 |
| $O2_{bp}$ (min) | 2.10 | 1.77–2.21 | 2.36 | 2.10–2.61 |
| $C$ (%.min$^{-1}$) | 8.27 | 6.87–9.67 | 4.80 | 3.71–5.89 |
| $T_{min}$ (min) | 1.15 | 1.09–1.23 | 1.16 | 1.07–1.22 |
| $A_{\%HR}$ (%) | 35.17 | 30.60–40.94 | 43.82 | 40.22–46.92 |

*BH* breath holding, $\tau$ time constant of the exponential decay, $A$ theoretical amplitude of the exponential decay, $B$ slope (sensitivity) of the increase in %HR following the exponential drop, $O_{2bp}$ ("oxygen-conserving breaking point"), time delay at which HR kinetic changes its behaviour; $C$ slope (sensitivity) of the decrease in %HR following $O_{2bp}$; $T_{min}$, time at which %HR reached its minimum value during exponential decay, $A_{\%HR}$, amplitude of the actual exponential decay, *LCL* lower confidence limit, *UCL* upper confidence limit

* $P < 0.05$ between kinetics ($F$ test)

**Table 2** Statistical comparison of the goodness of fit between the mono-phasic model and the tri-phasic model

| Models | $SS_R$ | $R^2_{adj}$ | RMSE | $\chi^2_{red}$ | Iterations | AIC |
|---|---|---|---|---|---|---|
| *Dry-body BH* | | | | | | |
| Mono-phasic model[a] | 4984.8 | 0.75 | 4.10 | 16.78 | 10 | 851.1 |
| Tri-phasic model | 396.3 | 0.98 | 1.16 | 1.34 | 11 | 95.5 |
| *Immersed-body BH* | | | | | | |
| Mono-phasic model[a] | 2649.3 | 0.85 | 2.99 | 8.92 | 6 | 661.5 |
| Tri-phasic model | 713.0 | 0.96 | 1.55 | 2.41 | 19 | 271.8 |

$SS_R$ sum of squared residuals, $R^2_{adj}$ adjusted coefficient of variation, *RMSE* root mean squared errors, $\chi^2_{red}$ reduced Chi-squared, *AIC* Akaike's information criterion, *BH* breath holding

[a] Model proposed by Caspers et al.

**Table 3** Heart rate variability indices of the two breath-holding phases and their respective baseline values

| HRV indices | $Rest_{dry}$ | Dry-body BH | | $Rest_{H2O}$ | Immersed-body BH | |
|---|---|---|---|---|---|---|
| | | $NX_{dry}$ | $HX_{dry}$ | | $NX_{H2O}$ | $HX_{H2O}$ |
| RMSSD (ms) | 38.2 ± 13.3 | 27.5 ± 14.0* | 54.3 ± 23.0$^{\#}$ | 40.1 ± 22.2 | 32.9 ± 17.0 | 67 ± 27.9$^{\#}$ |
| DFAα1 | 1.35 ± 0.16 | 1.40 ± 0.20 | 1.16 ± 0.30$^{\#}$ | 1.46 ± 0.22 | 1.39 ± 0.22 | 1.02 ± 0.20$^{\#}$ |
| mHR (bpm) | 64 ± 7 | 70 ± 11 | 58 ± 13$^{\S}$ | 72 ± 10 | 65 ± 9 | 54 ± 12$^{\S}$ |

*BH* breath holding, *HRV* heart rate variability, *mHR* mean heart rate, $NX_{dry}$ normoxic phase in dry-body BH, $HX_{dry}$ hypoxic phase in dry-body BH, $NX_{H2O}$ normoxic phase in immersed-body BH, $HX_{H2O}$ hypoxic phase in immersed-body BH, *RMSSD* root mean square of successive difference of R–R intervals, *DFAα1* short-term fractal scaling exponent

* $P < 0.05$ versus respective baseline value

$^{\S}$ $P < 0.05$ versus respective value of normoxic phase

$^{\#}$ $P < 0.05$ versus both respective normoxic and baseline values

95.2 ± 3.0 %, respectively; $P = 0.49$; Figs. 2 and 3). The latency time to reach nadir $SpO_2$ after breathing was resumed was similar for dry-body BH and immersed-body BH (11.2 ± 6.6 vs. 16.0 ± 8.0 s, respectively; $P = 0.37$).

As expected, the normoxic phases of BH had higher level of $SpO_2$ than the hypoxic phases had both in air (97.9 ± 1.0 vs. 89.3 ± 3.6 %, respectively; $P < 0.001$) and in immersion (98.3 ± 1.0 vs. 85.0 ± 3.4 %, respectively; $P < 0.001$). HRV indices are presented in Table 3. There were significant effects of phase type on both RMSSD and DFA α1 whether in air or immersed condition (all $P < 0.001$). During normoxic phase of BH, RMSSD

reached lower values than its respective baseline value in air ($P < 0.05$), whereas no difference was found between these two phases in immersed condition ($P = 0.83$). The RMSSDs were higher in the hypoxic phase of BH compared with their respective baselines values (dry-body BH: $+39.5 \pm 31.4$ % $P < 0.001$; immersed-body BH: $+93.9 \pm 102$ %; $P < 0.001$) and their respective normoxic phase of BH (dry-body BH: $+109.0 \pm 47.8$ %; $P < 0.001$; immersed-body BH: $+112.6 \pm 55.8$ %; $P < 0.001$). As a result of increased RMSSDs, mean HR reached lower absolute values in the hypoxic phase of BH than in the normoxic phase whether in air ($P < 0.001$) or in immersion ($P < 0.001$) (Table 3). Nonlinear DFA$\alpha$1 indexes decreased during the hypoxic phase of BH when compared to their respective baseline values (dry-body BH: $-13.6 \pm 21.4$ %; $P < 0.001$; immersed-body BH: $-29.2 \pm 14.3$ %; $P < 0.001$) and their respective normoxic phase of BH (dry-body BH: $-18.0 \pm 17.4$ %; $P < 0.05$; immersed-body BH: $-26.0 \pm 12$ %; $P < 0.001$). Finally, two-way ANOVA did not reveal any significant interaction on HRV indices between conditions and phase types.

## Discussion

This study is the first to quantify the beat-to-beat heart rate kinetics in prolonged static apneas performed by trained breath-hold divers both in air and in immersed condition by means of a regression-based model. Similar nonlinear regression approaches have already been used to quantify the heart rate kinetics during other non-stationary physiological conditions like the reactivation of parasympathetic tone following exercise (Pierpont et al. 2013). In the early stage of BH, the present model has shown that HR decreased exponentially, which is in line with the model previously used to fit the bradycardia in response to short static BH (Caspers et al. 2011). Then, the model has shown a slight linear rise in HR carried on up until a breaking point we have chosen to name "oxygen-conserving breaking point" and preceding an additional drop in HR. This final linear drop in HR was paralleled by the visible decrease in $SpO_2$ (Figs. 2, 3). The tri-phasic model had similar patterns whether BH was performed in air or in immersed condition.

In addition to accurately fitting diving bradycardia, the regression-based model highlighted a breaking point in the HR kinetics at the onset of a second HR drop precisely occurring at equivalent level of $SpO_2$ (~95 %) between dry-body BH and immersed-body BH despite different BH durations (Figs. 2, 3). Consequently, this finding supports the occurrence of a physiological event, i.e., the "oxygen-conserving breaking point" since this breaking point is immediately followed by progressive decrease in myocardial work. The occurrence of this particular event is delayed in immersed-body BH due to the bradycardia in the beginning of BH being more pronounced in immersed-body BH than in dry-body BH. Indeed, the fitted model parameters indicate a reduced exponential time constant ($\tau$) and greater HR amplitude ($A_{\%HR}$) when BH was performed in immersed condition. Consequently, the lagging of the breaking point in immersed-body BH immediately preceding a second bradycardia is thereby supporting an amplified oxygen-sparing effect associated with face immersion (de Bruijn et al. 2009; Andersson and Evaggelidis 2009).

It should be noted that the level of $SpO_2$ at which the second bradycardia occurred corresponds to increase in sensory discharge frequency of the carotid sinus nerve where chemoreceptors are located (González et al. 1992). As a result, this breaking point allows the distinction between a normoxic phase of BH and a hypoxic phase of BH, terminology which has recently been adopted to split prolonged BH into two phases (Laurino et al. 2012). According to the mathematical model, the normoxic phase of BH includes two opposite patterns in HR kinetics. The first one showing a large bradycardia first in exponential decay then finishing with a slight linear rise; however, both patterns were not associated with any concomitant increase in RMSSD (from baseline values) whether in air or in immersed condition. Similar results have already been reported in elite BHDs (Kiviniemi et al. 2012; Lemaître et al. 2008) and were likely due to BH-induced respiratory arrest, since HRV is greatly dependent upon both respiratory frequency and tidal volume (Ritz 2009). The transition from normoxic to hypoxic phase is marked by an additional drop in HR concomitant with a shift in cardiac autonomic regulation as indicated by a substantial change in both linear and nonlinear HRV indexes. In the hypoxic phase, increased RMSSD is in accordance with previous investigations which showed fairly similar results during final stage of prolonged BH (Lemaître et al. 2008; Kiviniemi et al. 2012). It is now well established that voluntary breathing of a hypoxic mixture leads to both tachycardia (Naeije 2010) and hyperventilation (Teppema and Dahan 2010) mediated by concomitant vagal withdrawal and increase in sympathetic activity. In the absence of ventilation (i.e., no afferent influence from stretch receptors in the lungs), a reverse mechanism is observed and peripheral chemoreceptor stimulation induces an enhancement of vagal activity resulting in bradycardia (de Burgh et al. 1988; Chapleau and Sabharwal 2011). The final linear decrease in HR vagally mediated aimed to further reduce myocardial work, and it is likely to be a unique adaptive feature of human diving bradycardia against hypoxic environment. Although RMSSD reached similar values during hypoxic phases in both conditions, we observed a more pronounced decrease in HR during dry-body BH than immersed-body BH as indicated by a steeper

slope in the final linear drop in HR (C model parameter; Table 3). This finding was not unexpected since the relationship between HR and HRV has shown to be curvilinear (Goldberger et al. 2001), i.e., increase in parasympathetic activity will have a lesser impact upon low HR values than upon high HR values. This nonlinear relationship may explain the present dissociation between HR and HRV across the two conditions since HR reached lower absolute values in immersed condition than in air at the occurrence of the "oxygen-conserving breaking point."

The drop in DFAα1 in the hypoxic phase of BH showed that increase in parasympathetic activity to the sinoatrial node was accompanied by an increase in peripheral sympathetic activity, therefore leading to autonomic co-activation (Tulppo et al. 2005). Additional increase in peripheral sympathetic tone illustrated by decreased DFAα1 is consistent with studies which have reported a massive rise in both mean blood pressure and muscle sympathetic nerve activity only in the second half of prolonged BH (Heusser et al. 2009; Perini et al. 2010; Guaraldi et al. 2009). The onset of involuntary breathing movements, i.e., physiological breaking point, was not recorded in the present study. Consequently, it does not allow us to state whether or not there are similarities between onsets of the well-established physiological breaking point and the proposed "oxygen-conserving breaking point." Moreover, involuntary breathing movements result from stimulation of the respiratory drive due to carbon dioxide accumulation (Lin et al. 1974), thereby leading to an increase in venous return, and thus stroke volume, which maintain cerebral perfusion (Dujic et al. 2009). Therefore, progressive BH-induced hypercapnic challenge and subsequent increased stroke volume should also be considered as potential contributors to the reduced HR through a baroreflex mechanism in the latter stage of static BH, i.e., the third phase of the model. Accordingly, decrease in DFAα1, i.e., breakdown of fractal organization in HR, indirectly suggests that vagally mediated bradycardia following the "oxygen-conserving breaking point" might result from complex synergistic interactions of peripheral chemoreceptor activation and baroreceptor activation.

Using eyeballing and statistical goodness of fit parameters criteria showed that the tri-phasic model is accurate to fit the diving bradycardia when BH is maintained for several minutes. Statistical parameters such as $SS_R$, RMSE and $\chi^2_{red}$ as well as Akaike's information criterion (AIC) revealed that tri-phasic model is more accurate than the mono-exponential one (Motulsky and Ransnas 1987). In addition, our model converged within less than 20 iterations although the parameters were constrained within wide physiological ranges, thus enhancing the reliability of the model. Surprisingly, Caspers et al. showed in non-divers reduced exponential time constants ($\tau$) than the ones of the present study both in air (16.2 vs. 21.6 s, respectively) s and in immersion (10.4 vs. 17.4 s, respectively). It would have been expected that BHDs would have shown a faster decrease in HR than non-divers. However, it should be noted that τ of Caspers' study was calculated from BH performed at lower water temperature (range 6–25 °C) than the present investigation (27 °C); this might explain the discrepancies between τ values since the rate at which HR declines is known to be increased at low water temperature (Jay et al. 2007). Conversely, our results revealed substantially greater HR amplitudes ($A_{\%HR}$) than the ones reported by Caspers et al. both in air (35 vs. 17 %, respectively) and in immersion (44 vs. 30 %, respectively), which is in accordance with a recent work that demonstrated a more pronounced bradycardia in BHDs than in non-divers (Tocco et al. 2012). BHDs wore a mask during immersed-body BH which might have partly obscured the full expression of the diving response (de Bruijn et al. 2009). We took the precaution to use a specific diving mask, i.e., a little mask that did not cover the whole facial area, since the main facial cold afferent is through the ophthalmic branch of the trigeminal nerve [forehead and eye region (Schuitema and Holm 1988)]. Model-derived parameters such as the amplitude ($A_{\%HR}$) and the exponential time constant ($\tau$) correspond, respectively, to the magnitude of the bradycardia and the rate at which HR decreases during the normoxic phase of BH. As both these parameters indicated a more pronounced bradycardia in immersed-body BH than in dry-body BH, the diving mask worn by the subjects likely had little effect on the magnitude of the diving bradycardia. Given their ability to differentiate the two conditions ($A_{\%HR}$ and $\tau$), our results suggest that these parameters describing the early stage of the human diving bradycardia might be valuable clinical parameters to quantify efferent autonomic pathways liable to be impaired (Caspers et al. 2011). In addition, we suggest that this quantitative approach may have practical application in the field of training, such as monitoring the effects of BH training on the diving response in the short and/or in the long term. Finally, this model seems well-adapted to prolonged BH since it takes into account additional cardioprotective mechanisms to counteract hypoxic challenge as illustrated by the breaking point ($O_{2bp}$) and the subsequent linear decrease in HR (C parameter). However, statistical results from the meta-analysis of Caspers et al. (2011) using a mono-exponential decay have showed high coefficient of determination ($R^2$) for short BH with and without face immersion ($R^2 > 0.94$; $R^2 > 0.93$, respectively); thus, a single-phase model as the one proposed by Caspers et al. is sufficient to fit the time course of HR reduction just until the oxygen-conserving breaking point has not been reached (i.e., for short BH).

$SpO_2$ reached its nadir after breathing was resumed during both dry-body BH and immersed-body BH,

representing a mean latency time to detect arterial oxygen saturation (from our finger pulse oximeter) of 18 and 24 s, respectively. Fairly similar lag times in detecting central hypoxia by means of finger probe have been observed and were explained by the circulation time between the lungs and the radial artery, a phenomenon that would tend to be increased via BH-induced vasoconstriction (Lindholm et al. 2007; Andersson and Evaggelidis 2009). Accordingly, the level of $SpO_2$ measured at the finger throughout BH may not be considered as representative of central hypoxia where chemoreceptors are located. To estimate the contribution of this latency time on level of $SpO_2$ at which the breaking point occurred, we have reported a weighted $SpO_2$ for each condition taking into account the lag times above-mentioned. These levels of $SpO_2$ compared to the ones recorded at the finger during dry-body BH and immersed-body BH revealed mean (±SD) percentage errors of 0.7 % (0.4) and 1.9 % (1.0), respectively. Given the likely shorter lag times at the breaking point than the lag times reported at the end of BH (i.e., 18 and 24 s), it is reasonable to assume that the latency phenomenon, at least until the breaking point, had little effect over $SpO_2$ percentages measured at the finger; thus, they have been considered as good estimates of arterial oxygen saturation.

In conclusion, nonlinear regression analysis was used to quantify changes in heart rate kinetic during prolonged static breath holding. The tri-phasic pattern is both simple and well-adapted to fitting the diving bradycardia. Besides, it provides physiological meaningfulness as indicated by the "oxygen-conserving breaking point" found out in heart rate kinetics whose occurrence was concomitant with a shift toward a synergistic sympathetic and parasympathetic activation. Both modeling and heart rate variability approaches, as a whole, offer further insight into the adaptive changes in cardiac autonomic regulation in response to progressive hypercapnic hypoxia to which trained breath-hold divers are frequently exposed.

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**Conflict of interest** The authors declare that they have no conflict of interest.

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