

Dynamic Cerebral Autoregulation Is Acutely Impaired during Maximal Apnoea in Trained Divers

Troy J. Cross^{1,3*}, Justin J. Kavanagh¹, Toni Breskovic², Bruce D. Johnson³, Zeljko Dujic²

1 Griffith Health Institute and Heart Foundation Research Centre, Griffith University, Gold Coast Campus, Queensland, Australia, **2** Department of Physiology, University of Split School of Medicine, Split, Croatia, **3** Division of Cardiovascular Diseases, Mayo Clinic, Rochester, Minnesota, United States of America

Abstract

Aims: To examine whether dynamic cerebral autoregulation is acutely impaired during maximal voluntary apnoea in trained divers.

Methods: Mean arterial pressure (MAP), cerebral blood flow-velocity (CBFV) and end-tidal partial pressures of O₂ and CO₂ (PETO₂ and PETCO₂) were measured in eleven trained, male apnoea divers (28±2 yr; 182±2 cm, 76±7 kg) during maximal “dry” breath holding. Dynamic cerebral autoregulation was assessed by determining the strength of phase synchronisation between MAP and CBFV during maximal apnoea.

Results: The strength of phase synchronisation between MAP and CBFV increased from rest until the end of maximal voluntary apnoea ($P<0.05$), suggesting that dynamic cerebral autoregulation had weakened by the apnoea breakpoint. The magnitude of impairment in dynamic cerebral autoregulation was strongly, and positively related to the rise in PETCO₂ observed during maximal breath holding ($R^2=0.67$, $P<0.05$). Interestingly, the impairment in dynamic cerebral autoregulation was not related to the fall in PETO₂ induced by apnoea ($R^2=0.01$, $P=0.75$).

Conclusions: This study is the first to report that dynamic cerebral autoregulation is acutely impaired in trained divers performing maximal voluntary apnoea. Furthermore, our data suggest that the impaired autoregulatory response is related to the change in PETCO₂, but not PETO₂, during maximal apnoea in trained divers.

Citation: Cross TJ, Kavanagh JJ, Breskovic T, Johnson BD, Dujic Z (2014) Dynamic Cerebral Autoregulation Is Acutely Impaired during Maximal Apnoea in Trained Divers. PLoS ONE 9(2): e87598. doi:10.1371/journal.pone.0087598

Editor: Gennady Cymbalyuk, Georgia State University, United States of America

Received: October 23, 2013; **Accepted:** December 21, 2013; **Published:** February 3, 2014

Copyright: © 2014 Cross et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This study was supported by the Croatian Ministry of Science, Education and Sports Grant No. 216-2160133-0130 (procured by ZD). TJC was supported by an Australian Postgraduate Award, a Griffith University International Exchange Incentive Scheme Grant, and a grant from the Mayo Clinic. BDJ was supported by a grant to the Mayo Clinic from the Gonda Family. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: cross.troy@mayo.edu

Introduction

Cerebral autoregulation is a compensatory mechanism which acts to maintain cerebral blood flow at a near constant value, despite fluctuations in arterial blood pressure or intracranial pressure [1,2]. It can be argued that autoregulation of cerebral blood flow and, by extension, cerebral O₂ delivery is nowhere more important for human survival than during prolonged asphyxia. And perhaps an extreme model of asphyxia can be found in the breath-hold diver performing a maximal apnoea [3,4].

In trained breath-hold divers, arterial O₂ tension may approach <30 mmHg, and CO₂ tension may reach as high as 91 mmHg, by the breakpoint of maximal apnoea [5–9]. In spite of this extreme asphyxia, cerebral tissue oxygenation is well-maintained during maximal apnoea due to a compensatory cerebral hyperaemia [10]. The majority of the rise in cerebral blood flow during apnoea is achieved by vasodilation of cerebral resistance vessels, consequent to hypercapnia [11,12]. Maximal apnoea also evokes a powerful vasoconstriction of the peripheral tissues, leading to a pronounced arterial hypertension [10,13,14]. Indeed, approxi-

mately one-third of the cerebral blood flow response to brief apnoea (i.e., 20 s) is contributed by the increase in arterial blood pressure [11]. The observation that arterial hypertension partly explains the rise in cerebral blood flow suggests that cerebral autoregulation is functionally impaired during maximal voluntary apnoea. To date, no study has examined dynamic cerebral autoregulation (dCA) during prolonged breath holding.

The primary aim of this study was to quantify the effectiveness of dCA during maximal “dry” apnoea in trained breath-hold divers. Previous studies indicate that the relationship between arterial CO₂ on cerebrovascular pressure-reactivity is nonlinear, primarily affecting the phase more so than the amplitude dynamics of the cerebral autoregulatory response [15–18]. Moreover, it is well-established that arterial O₂ tension exerts a modulatory effect on dCA, wherein arterial hypoxaemia seemingly impairs the autoregulatory response [19–21]. Consequently, dCA was assessed using phase synchronisation analysis [22,23] on spontaneous, beat-to-beat values of mean arterial blood pressure (MAP) and middle cerebral artery blood flow-velocity (CBFV) during maximal apnoea. It was hypothesised that trained divers would display signs of impaired dCA by the breakpoint of apnoea, evidenced by

a significant increase in the strength of phase synchronisation between arterial pressure and cerebral blood flow-velocity. Moreover, it was expected that those divers with the highest levels of arterial hypercapnia would demonstrate the largest increases in phase synchronisation by the apnoea breakpoint.

Methods

Ethics Statement

The present study conformed to the principles outlined in the Declaration of Helsinki and was approved by the research ethics board at the School of Medicine, University of Split, Croatia.

Subjects and Experimental Design

Eleven trained, male apnoea divers (28 ± 2 yr; 182 ± 2 cm, 76 ± 7 kg) volunteered to participate in the present study, and provided written informed consent. Within the 6 months prior to experimental testing, the divers were engaged in apnoea training at least 3 times a week, were each session lasted approximately 60 to 90 min. The subjects underwent a pre-participatory health screening to ensure they were physically active non-smokers, with no history of cardiac, pulmonary or metabolic disease.

Subjects first performed a number of pulmonary function tests in the supine position, after which they were instructed to remain supine for 10 min before performing breath-hold manoeuvres (i.e., rest period). Breath-hold manoeuvres commenced after full inflation to total lung capacity (TLC) while wearing a nose-clip. The subjects were instructed to keep their glottis closed during each breath-hold. Subjects were discouraged from performing preparatory hyperventilation, and were not allowed to perform glossopharyngeal insufflations prior to the manoeuvre. The subjects completed two practice trials, followed by 3 experimental breath holds, separated by at least 10 min of supine rest. A repeat apnoea trial did not begin until haemodynamic variables had returned to baseline. Subjects were encouraged to completely relax their respiratory musculature during the early period of the breath-hold (i.e., easy-going phase) and, should the urge arise, to allow respiratory contractions to develop “naturally” toward the end of breath holding. The subjects were also instructed to avoid activation of their peripheral musculature. No further explanation of the apnoeic effort was provided.

Pulmonary Function Testing and End-tidal Gases

Before each experiment, the subjects performed a forced vital capacity (FVC) manoeuvre while in the supine posture (Quark PFT, Cosmed, Rome, Italy). All pulmonary function testing was performed and reported in accordance with the American Thoracic Society guidelines [24]. Expired gases were continuously sampled at the mouth using a mass spectrometer (AMIS 2000, Innovision A/S, Odense, Denmark) and, from these data, the end-tidal partial pressures of O_2 and CO_2 (PET_{O_2} and PET_{CO_2}) were computed.

Electrocardiogram, Arterial Oxygen Saturation, Blood Pressure and Cerebral Blood Flow-velocity

Arterial O_2 saturation of haemoglobin (SaO_2) was measured via finger pulse oximetry (Poet II, Criticare Systems, Waukesha, WI). Beat-by-beat arterial blood pressure was measured using a pneumatic cuff placed around the middle phalanx of the non-dominant hand, and was connected to a photoplethysmograph (Finometer, Finapres Medical Systems, Arnhem, Netherlands). The hand bearing the finger cuff was positioned at the level of the heart in order to negate hydrostatic pressure artifact. The hand was kept in this position for the duration of the experimental

protocol. Heart rate and rhythm were monitored continuously using a standard 3-lead electrocardiogram module that was interfaced with the Finometer unit. CBFV was obtained via transcranial Doppler ultrasound of the proximal middle cerebral artery, measured using a 2-MHz probe fixed at a constant angle over the right posterior temporal “window” (Transcranial Doppler, Multigon, Yonkers, NY, USA).

Data Collection and Signal Processing

All signals were sampled continuously at 1000 Hz (Powerlab 16 SP, ADInstruments Inc., Castle Hill, Australia) and stored on a personal computer for off-line analyses. Mean arterial pressure (MAP) and mean CBFV were computed as the arithmetic mean of the arterial pulse wave and transcranial Doppler flow-velocity signals over each cardiac beat interval, respectively [25]. Cerebrovascular resistance index (CVRi) was calculated as $MAP/CBFV$. The discontinuous, beat-by-beat time series were resampled to 2 Hz using cubic spline interpolation.

Phase Synchronisation and Dynamic Cerebral Autoregulation

Phase synchronisation analysis describes the strength of interaction between the intrinsic phases (Φ) of two weakly-coupled, nonstationary, nonlinear chaotic oscillators [26,27]. Two oscillators are said to be ‘synchronised’ or ‘phase-locked’ when the difference in their phases remain constant over a given length of time [22,23,28,29]. In the present study, the strength of phase synchronisation between MAP and CBFV was used to assess the effectiveness of dCA during maximal apnoea. That is, MAP and CBFV were considered to act as two autonomous oscillators, weakly-coupled in their phases through the action of the effector, dCA. According to this paradigm, dCA is intact when the oscillators CBFV and MAP are not synchronised in their phase dynamics [22,23]; i.e., the oscillator CBFV operates near-independently of MAP. Conversely, when the dynamics of MAP and CBFV are tuned such that their phases become synchronised, fluctuations in arterial pressure are “passively” transmitted through the cerebral vasculature unabated, portending autoregulatory failure.

The strength of phase coupling between MAP and CBFV, and thus the effectiveness of dCA, was quantified using the phase synchronisation index (PhSI). The method used to calculate PhSI was modified from that described by others [22,23,30]. In brief, the 2 Hz interpolated data for MAP and CBFV were band-pass filtered using a 5th order, zero-phase Butterworth filter, with a low and high cut-off frequency of 0.01 and 0.15 Hz, respectively. These frequency cut offs were chosen based on previous observations that dCA is most active within the low to very-low frequency region [i.e., 0.01–0.15 Hz; 1,2]. This filtering approach also had the effect of removing variability in each time-series due to respiration and the cardiac cycle. The intrinsic phases of the filtered MAP and CBFV time series, $\Phi^{MAP}(t)$ and $\Phi^{CBFV}(t)$, were extracted using the Hilbert transform [23,30]. The distribution of phase differences between MAP and CBFV was then determined as: $\Delta\Phi(t) = \Phi^{MAP}(t) - \Phi^{CBFV}(t)$. $\Delta\Phi(t)$ was wrapped to the interval -180° to 180° , such that a negative value suggests that changes in MAP ‘lead’ those observed in CBFV. PhSI is a quantity that represents the time-dependent stability of $\Delta\Phi(t)$, and was calculated as:

$$PhSI(t) = \sqrt{\langle \cos \Delta\Phi(t) \rangle^2 + \langle \sin \Delta\Phi(t) \rangle^2} \quad (1)$$

where the brackets $\langle \rangle$ denotes a 30-s moving average window. PhSI lies within the interval $0 \leq \text{PhSI} \leq 1$. A value of $\text{PhSI} = 1$ indicates perfect phase synchronisation, while $\text{PhSI} = 0$ suggests complete absence of phase synchronisation between signals MAP and CBFV. Therefore, in the context of the present study, a decrease in PhSI during apnoea was taken to represent an increased effectiveness of dCA, whereas an increase in PhSI was interpreted as an impairment of autoregulatory processes while breath holding. Phase synchronisation analysis was performed using MATLAB® (The MathWorks, Inc., Natick, USA).

Statistical Analyses

Data were averaged across repeated trials. The dependent variables were averaged into four time-epochs representing: the resting period, the first and last 30-s of maximal apnoea, and the final 30-s of the 2 min recovery period. PETO_2 and PETCO_2 could not be measured while subjects were breath holding. As such, PETO_2 and PETCO_2 data were organised into 4 bins representing: 1) the resting period; 2) the mean of values observed 30-s immediately prior to apnoea; 3) the minimum and maximum values of PETO_2 and PETCO_2 , respectively, within the first 3 breaths immediately following the apnoea breakpoint; and: 4) the final 30-s of the 2-min post-apnoea recovery period. One-way analyses of variance were used to evaluate the effect of these time epochs on cardiovascular and cerebrovascular variables, and on parameters derived the phase synchronisation analysis. Pair-wise comparisons were assessed using the *Bonferroni* post-hoc adjustment. Pearson's correlation coefficient was used to determine whether any change in dCA function, assessed via PhSI, could be related to the changes in either PETO_2 and PETCO_2 observed during maximal voluntary apnoea. Results are presented as mean \pm standard error of the mean (SEM) unless stated otherwise. All data were analysed using SPSS 20.0 (SPSS, Inc., Chicago, IL, USA). Statistical analyses were considered significant if $P < 0.05$.

Results

Pulmonary Function and Breath Holding Performance

All subjects presented with normal pulmonary function, where FVC, forced expiratory volume in 1 s (FEV_1) and FEV_1/FVC were 6.30 ± 0.41 L ($116 \pm 7\%$ predicted), 5.14 ± 0.24 L ($113 \pm 5\%$ predicted) and 0.82 ± 0.02 ($100 \pm 2\%$ predicted). The average duration of maximal voluntary apnoea was 199 ± 11 s (range 154 to 261 s). The coefficient of variation computed for the subject's repeated apnoeic efforts was $7 \pm 2\%$.

Expired Gases, Cardiovascular and Cerebrovascular Variables during Maximal Voluntary Apnoea

During the resting period before apnoea, PETO_2 and PETCO_2 were 121 ± 3 mmHg and 41 ± 1 mmHg, respectively. In the 30-s period before apnoea, PETO_2 increased to 131 ± 3 mmHg ($P < 0.05$) while PETCO_2 fell to 38 ± 1 mmHg ($P < 0.05$). Thus, despite verbal instruction, the subjects hyperventilated (albeit mildly) before performing maximal apnoea. Immediately post-apnoea, PETO_2 and PETCO_2 were 61 ± 4 mmHg and 56 ± 1 mmHg – both values were different from those observed at rest ($P < 0.05$). The average changes in PETO_2 and PETCO_2 from rest until the apnoea breakpoint were -57 ± 7 mmHg and $+14 \pm 1$ mmHg, respectively. By the end of the 2-min recovery period, PETO_2 and PETCO_2 remained different from rest (104 ± 5 mmHg and 46 ± 4 mmHg, respectively; both P -values were < 0.05).

Figure 1 displays the changes in MAP and CBFV during maximal apnoea for a representative trained diver. The time-

course changes in cardiovascular and cerebrovascular variables during maximal apnoea are reported in **Table 1**. CVRi increased from rest to the beginning of maximal apnoea ($P < 0.05$). Conversely, MAP and CBFV decreased over the same period of time ($P < 0.05$). Thereafter, MAP and CBFV progressively increased ($P < 0.05$), while CVRi and SaO_2 continuously decreased ($P < 0.05$), from the beginning until the end of apnoea. After 2-min of recovery, the above parameters returned to baseline. Apart from an initial rise at the beginning of apnoea ($P < 0.05$), HR was not different from rest during and after breath holding.

Dynamic Cerebral Autoregulation during Maximal Voluntary Apnoea

Figure 1 also displays the changes in $\Delta\Phi$ and PhSI during maximal apnoea for representative trained diver. **Figure 2** displays the group-averaged values for $\Delta\Phi$ and PhSI obtained before, during and after maximal voluntary apnoea. PhSI had increased to values higher than resting values by the breakpoint of maximal voluntary apnoea ($P < 0.05$). The mean change in PhSI from rest until the apnoea breakpoint (Δ) was 0.18 ± 0.03 . PhSI had returned to baseline by the end of the post-apnoea recovery

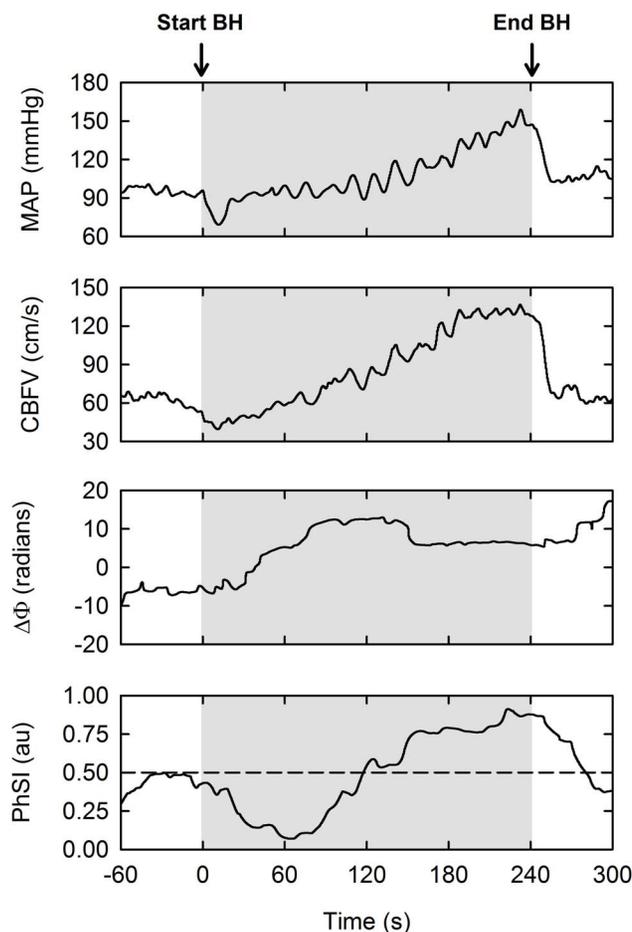


Figure 1. Individual data from a representative subject during maximal voluntary apnoea. BH: breath-hold; MAP: mean arterial pressure; CBFV: cerebral blood flow-velocity; $\Delta\Phi$: phase angle between the MAP and CBFV time series; PhSI: phase synchronisation index. N.B.: values for $\Delta\Phi$ and PhSI were obtained from the low-frequency region of the MAP–CBFV relationship (i.e., 0.01–0.15 Hz), where dynamic cerebral autoregulation is considered most active. doi:10.1371/journal.pone.0087598.g001

Table 1. Cardiovascular and cerebrovascular parameters before, during and after maximal voluntary apnoea.

	Rest	Maximal apnoea		$\Delta_{\text{End-Rest}}$	Post
		Start	End		
HR (beats \cdot min $^{-1}$)	72 \pm 2	94 \pm 8*	71 \pm 4†	-1 \pm 4	81 \pm 4
MAP (mmHg)	92 \pm 4	67 \pm 4*	123 \pm 4*†	31 \pm 3	96 \pm 4†
CBFV (cm \cdot s $^{-1}$)	51 \pm 3	35 \pm 2*	93 \pm 9*†	42 \pm 8	52 \pm 4†
CVRI (units)	2.1 \pm 0.2	2.3 \pm 0.1*	1.6 \pm 0.1*†	-0.6 \pm 0.2	2.2 \pm 0.2†
SaO $_2$ (%)	99 \pm 1	98 \pm 1	87 \pm 2*†	-12 \pm 2	99 \pm 1

Values represent means \pm SEM from 11 divers. HR: heart rate; MAP: mean arterial pressure; CBFV: middle cerebral artery blood flow-velocity; CVRI: cerebrovascular resistance index in units of mmHg \cdot cm $^{-1}$ \cdot s; SaO $_2$: arterial haemoglobin oxygenation.

* P <0.05 compared with resting values.

† P <0.05 compared with previous time-point.

doi:10.1371/journal.pone.0087598.t001

period (P <0.05 compared to end of apnoea). The phase angle between MAP and CBFV ($\Delta\Phi$) decreased below resting values during maximal apnoea (P <0.05), approaching values close to zero by the breakpoint.

The absolute rise in PETCO $_2$ from rest until the apnoea breakpoint was strongly, and positively correlated with the increase in PhSI over the same period of time ($R^2=0.67$, P <0.05, left-panel **Figure 3**). That is, the larger the rise in PETCO $_2$ and, by extension, arterial tension of CO $_2$, the greater the impairment in dCA during maximal voluntary apnoea.

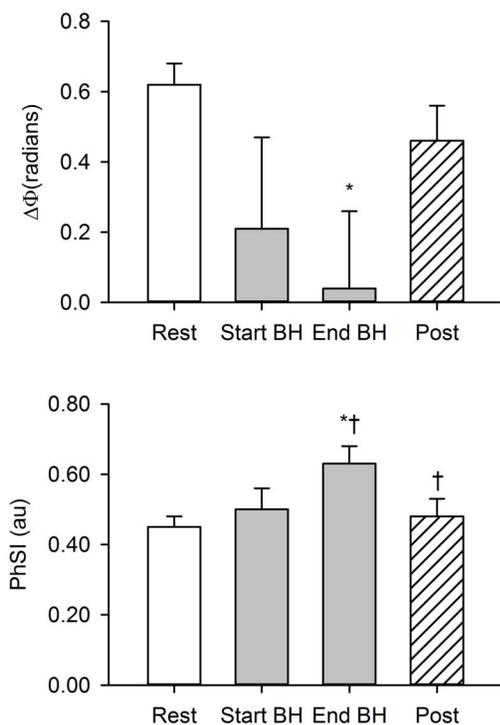


Figure 2. Group-averaged data for phase angle ($\Delta\Phi$) and phase synchronisation index (PhSI) during maximal voluntary apnoea. Bars are means \pm SEM from 11 divers. BH: breath-hold. * P <0.05 compared with resting values. † P <0.05 compared with previous time-point.

doi:10.1371/journal.pone.0087598.g002

Interestingly, the magnitude of rise in PETO $_2$ was not identified as significant correlate of the rise in PhSI throughout breath holding ($R^2=0.01$, $P=0.75$, right-panel **Figure 3**).

Discussion

The novel findings of the present study were that: (i) the effectiveness of dCA is reduced during maximal voluntary apnoea in trained divers; and that (ii) this reduction was strongly related to the rise in PETCO $_2$ during apnoea. Our data suggest that the ability of the cerebral vessels to autoregulate blood flow, and therefore to protect the brain against abrupt oscillations in driving pressure, is acutely impaired in divers performing maximal “dry” apnoea. These findings have important implications for the cerebrovascular health and safety of trained apnoea divers.

We report that dCA was acutely impaired during prolonged, voluntary apnoea. This inference is substantiated by two lines of evidence. First, PhSI had increased above resting values by the breakpoint of maximal apnoea. The rise in phase-coupling strength between MAP and CBFV indicates a progressive weakening of the autoregulatory response over time [22,23]. Second, not only had $\Delta\Phi$ decreased during maximal apnoea, but its value approached zero by the breakpoint. It has been argued that when $\Delta\Phi$ approximates zero, changes in MAP affect those in CBFV with no temporal delay, signifying autoregulatory failure [31,32]. The present finding that maximal voluntary apnoea weakens dCA is consistent with observations made by others. For example, Przybyłowski et al. [11] reported that approximately one-third of the cerebral blood flow response to brief apnoea (~20 s) is mediated by increases in arterial blood pressure, indicating a partial failure of cerebral autoregulatory mechanisms during voluntary breath holding. More recently, Dineen et al. [33] demonstrated that the effectiveness of dCA is transiently reduced during voluntary apnoeas lasting ~35 s. These findings, in conjunction with those of our own, supports the idea that voluntary breath holding acutely impairs dCA.

Many investigators have demonstrated that elevated arterial CO $_2$ acutely impairs dCA during spontaneous breathing [1,15–18,34] – the precise mechanism(s) by which hypercapnia weakens the autoregulatory response is unclear. Nevertheless, our findings are in agreement with the above reports, insofar as the trained divers who displayed the largest rise in PETCO $_2$ during maximal apnoea presented with the greatest impairment in dCA. It is well-established that isocapnic hypoxia impairs the cerebral autoregulatory response in awake, spontaneously breathing humans [19–21]. It is therefore surprising that we observed no direct relationship between the fall in PETO $_2$ and the rise in PhSI during maximal apnoea. This finding does not necessarily obviate the thesis that arterial hypoxaemia contributes to the weakened autoregulatory response observed during maximal apnoea. Rather, it is possible that arterial O $_2$ tension impacts on dCA in a manner not elucidated by the correlation analysis performed on our group of trained divers.

It must be appreciated that factors other than arterial blood gas tensions are known to modulate the effectiveness of dCA, these include: (i) sympathetic activity; (ii) metabolite production; and (iii) perivascular innervation [35–40]. Our data do not allow us to comment on the role that these factors played in disrupting autoregulatory processes during maximal apnoea. Further investigations are required to elucidate the precise mechanisms responsible for impairing dCA during prolonged, voluntary breath holding.

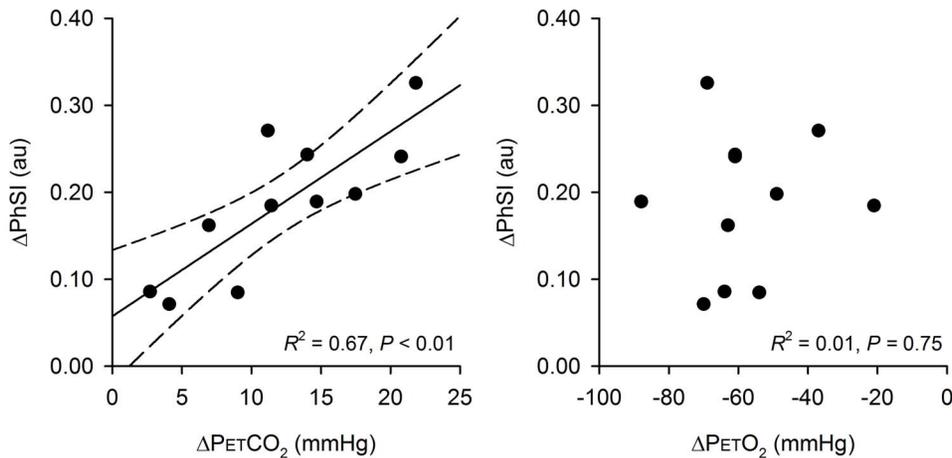


Figure 3. The influence of CO₂ and O₂ on the dynamic cerebral autoregulation during maximal voluntary apnoea. BH: breath-hold; PhSI: phase synchronisation index between the MAP and CBFV time series; PETCO₂: end-tidal partial pressure of CO₂; PETO₂: end-tidal partial pressure of O₂; Δ: absolute difference in magnitude between resting values and those observed at the breakpoint of apnoea. This figure demonstrates that those individuals who displayed the largest rise in PETCO₂ also presented with the greatest impairment in dynamic cerebral autoregulation during apnoea. The fall in PETO₂, and thus magnitude of arterial hypoxaemia, was not related to the impaired autoregulatory response observed during maximal apnoea in trained divers.

doi:10.1371/journal.pone.0087598.g003

Implications for the Cerebrovascular Health of Breath-hold Divers

The observation that PhSI had increased by the breakpoint of maximal apnoea begs the question: what is the impact of impaired autoregulation on cerebrovascular health during breath-hold diving? Certainly, there are numerous reports of serious brain damage and neurological impairment in habitual breath-hold divers [41–46]. However, the primary cause of these brain injuries is often attributed to cerebral arterial gas embolism, resulting from pulmonary barotrauma and/or shunting of venous gas emboli formed in peripheral tissues at depth. In a state of impaired dCA, the cerebral vessels behave more like a “pressure passive” system, wherein low to very-low frequency variations in arterial pressure (i.e., 0.01–0.15 Hz) are passively transmitted through the cerebrovasculature unabated, resulting in potentially severe fluctuations in cerebral blood flow and intracranial pressure. Following the above, one may speculate that arterial hypertension induced by apnoea [10,13,14], and concurrent impairment of cerebral autoregulation, may together promote the development of intracranial hypertension, and thus heighten the risk of cerebrovascular insult during breath-hold diving. Further research is warranted in order to determine whether impaired dCA during maximal breath holding leads to cerebrovascular insult in trained divers.

Methodological Considerations

We used transcranial Doppler ultrasound of the right middle cerebral artery (MCA) to estimate bulk cerebral blood flow during maximal breath holding [47,48]. This technique does not measure volumetric blood flow *per se*, rather it quantifies the velocity of blood passing through the insonated artery [49]. Changes in the radius of the insonated artery may therefore alter blood velocity, independent of volumetric flow through the MCA. Importantly, however, evidence suggests that MCA diameter is stable over a wide range of MAP and arterial blood gases [50,51]. With this point in mind, we are confident that our values of cerebral blood flow-velocity is a valid marker of bulk cerebral blood flow. It must be acknowledged that only trained breath-hold divers were recruited in this study, and that all apnoeas were performed

under “dry” laboratory conditions. Therefore, we caution that our findings may not apply to untrained/naïve participants, or to the conditions imposed on subjects when breath holding under water at depth. On this latter point, it was not feasible to use transcranial Doppler ultrasound to measure CBFV under water. Future studies may overcome this limitation via computational modelling of the pressure-flow characteristics of peripheral and cerebral vessels [52–54].

That the trained diver in **Figure 1** demonstrated a fall in PhSI within the first 60-s of apnoea suggests dCA transiently improved after the beginning of breath holding. This improved dCA may have been related to our observation that subjects, on average, were mildly hypocapnic immediately prior to breath holding. If this hypocapnia persisted throughout the first 60-s of apnoea, it is conceivable that the lowered arterial CO₂ tension mediated an improvement in dCA – at least for the individual displayed in **Figure 1**. However, it was not feasible to measure PETCO₂ during apnoeic efforts and, in turn, we cannot comment on whether arterial hypocapnia modulated dCA during the early phase of maximal apnoea. In relation to the above, it is of note that other investigators have reported that arterial hypercapnia does not develop to any great extent within the first 60 s of voluntary breath holding [55–57].

Conclusions

The present study clearly demonstrates that the phase dynamics of cerebral autoregulation are acutely impaired during maximal “dry” apnoea in trained divers. Moreover, we provide evidence that the degree of impairment in dCA is related to the magnitude of rise in PETCO₂ observed during apnoea. The potential for this impaired dCA to heighten the risk of cerebrovascular injury during breath-hold diving should be further explored.

Acknowledgments

The Authors thank each subject for their participation in the present study. The Authors are also grateful for the guidance and advice provided by Dr Surendran Sabapathy.

Author Contributions

Conceived and designed the experiments: TJC TB BDJ ZD. Performed the experiments: TJC TB BDJ ZD. Analyzed the data: TJC JJK. Contributed reagents/materials/analysis tools: ZD BDJ. Wrote the paper: TJC JJK.

References

- Aaslid R, Lindegaard KF, Sorteberg W, Normes H (1989) Cerebral autoregulation dynamics in humans. *Stroke* 20: 45–52.
- Zhang R, Zuckerman JH, Giller CA, Levine BD (1998) Transfer function analysis of dynamic cerebral autoregulation in humans. *American Journal of Physiology - Heart and Circulatory Physiology* 274: H233–H241.
- Dujić Z, Breskovic T, Bakovic D (2013) Breath-hold diving as a brain survival response. *Translational Neuroscience* 4: 302–313.
- Dujic Z, Breskovic T (2012) Impact of breath holding on cardiovascular respiratory and cerebrovascular health. *Sports Medicine* 42: 459–472.
- Ferris EB, Engel GL, Stevens CD, Webb J (1946) Voluntary Breathholding. Iii. The Relation of the Maximum Time of Breathholding to the Oxygen and Carbon Dioxide Tensions of Arterial Blood, with a Note on Its Clinical and Physiological Significance. *Journal of Clinical Investigation* 25: 734–743.
- Klocke FJ, Rahn H (1959) Breath holding after breathing of oxygen. *Journal of Applied Physiology* 14: 689–693.
- Schneider EC (1930) Observations on holding the breath. *American Journal of Physiology* 94: 464–470.
- Andersson JPA, Linér MH, Jönsson H (2009) Increased serum levels of the brain damage marker S100B after apnea in trained breath-hold divers: a study including respiratory and cardiovascular observations. *Journal of Applied Physiology* 107: 809–815.
- Overgaard K, Friis S, Pedersen R, Lykkeboe G (2006) Influence of lung volume, glossopharyngeal inhalation and P ET O₂ and P ET CO₂ on apnea performance in trained breath-hold divers. *European Journal of Applied Physiology* 97: 158–164.
- Palada I, Obad A, Bakovic D, Valic Z, Ivancev V, et al. (2007) Cerebral and peripheral hemodynamics and oxygenation during maximal dry breath-holds. *Respiratory Physiology & Neurobiology* 157: 374–381.
- Przybyłowski T, Bangash M-F, Reichmuth K, Morgan BJ, Skatrud JB, et al. (2003) Mechanisms of the cerebrovascular response to apnoea in humans. *The Journal of Physiology* 548: 323–332.
- Kjeld T, Pott FC, Secher NH (2009) Facial immersion in cold water enhances cerebral blood velocity during breath-hold exercise in humans. *Journal of Applied Physiology* 106: 1243–1248.
- Heusser K, Dzamonja G, Tank J, Palada I, Valic Z, et al. (2009) Cardiovascular Regulation During Apnea in Elite Divers. *Hypertension* 53: 719–724.
- Andersson JPA, Linér MH, Rünow E, Schagatay EKA (2002) Diving response and arterial oxygen saturation during apnea and exercise in breath-hold divers. *Journal of Applied Physiology* 93: 882–886.
- Mitsis GD, Poulin MJ, Robbins PA, Marmarelis VZ (2004) Nonlinear modeling of the dynamic effects of arterial pressure and CO₂ variations on cerebral blood flow in healthy humans. *IEEE Transactions on Biomedical Engineering* 51: 1932–1943.
- Panerai RB, Deverson ST, Mahony P, Hayes P, Evans DH (1999) Effect of CO₂ on dynamic cerebral autoregulation measurement. *Physiological Measurement* 20: 265.
- Birch AA, Dirnhuber MJ, Hartley-Davies R, Iannotti F, Neil-Dwyer G (1995) Assessment of Autoregulation by Means of Periodic Changes in Blood Pressure. *Stroke* 26: 834–837.
- Ursino M, Lodi CA (1998) Interaction among autoregulation, CO₂ reactivity, and intracranial pressure: a mathematical model. *American Journal of Physiology - Heart and Circulatory Physiology* 274: H1715–H1728.
- Mardimac A, Balaban DY, Machina MA, Battisti-Charbonney A, Han JS, et al. (2012) The interaction of carbon dioxide and hypoxia in the control of cerebral blood flow. *Pflügers Arch* 464: 345–351.
- Querido JS, Ainslie PN, Foster GE, Henderson WR, Halliwill JR, et al. (2013) Dynamic cerebral autoregulation during and following acute hypoxia: role of carbon dioxide. *Journal of Applied Physiology* 114: 1183–1190.
- Ogoh S, Nakahara H, Ainslie PN, Miyamoto T (2010) The effect of oxygen on dynamic cerebral autoregulation: critical role of hypocapnia. *Journal of Applied Physiology* 108: 538–543.
- Latka M, Turalska M, Glaubic-Latka M, Kolodziej W, Latka D, et al. (2005) Phase dynamics in cerebral autoregulation. *American Journal of Physiology - Heart and Circulatory Physiology* 289: H2272–2279.
- Ocon AJ, Kulesa J, Clarke D, Taneja I, Medow MS, et al. (2009) Increased phase synchronization and decreased cerebral autoregulation during fainting in the young. *American Journal of Physiology - Heart and Circulatory Physiology* 297: H2084–2095.
- Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, et al. (2005) Standardisation of spirometry. *European Respiratory Journal* 26: 319–338.
- O'Leary DD, Shoemaker JK, Edwards MR, Hughson RL (2004) Spontaneous beat-by-beat fluctuations of total peripheral and cerebrovascular resistance in response to tilt. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 287: R670–R679.
- Rosenblum MG, Kurths J, Pikovsky A, Schafer C, Tass P, et al. (1998) Synchronization in noisy systems and cardiorespiratory interaction. *IEEE Engineering in Medicine and Biology Magazine* 17: 46–53.
- Rosenblum MG, Cimponeriu L, Bezerianos A, Patzak A, Mrowka R (2002) Identification of coupling direction: application to cardiorespiratory interaction. *Physical review E, Statistical, nonlinear, and soft matter physics* 65: 041909.
- Le Van Quyen M, Foucher J, Lachaux J, Rodriguez E, Lutz A, et al. (2001) Comparison of Hilbert transform and wavelet methods for the analysis of neuronal synchrony. *Journal of neuroscience methods* 111: 83–98.
- Quiñero R, Kraskov A, Kreuz T, Grassberger P (2002) Performance of different synchronization measures in real data: a case study on electroencephalographic signals. *Physical review E, Statistical, nonlinear, and soft matter physics* 65: 041903.
- Ocon AJ, Medow MS, Taneja I, Stewart JM (2011) Respiration drives phase synchronization between blood pressure and RR interval following loss of cardiovagal baroreflex during vasovagal syncope. *American Journal of Physiology - Heart and Circulatory Physiology* 300: H527–540.
- Diehl RR, Linden D, Lücke D, Berlit P (1995) Phase Relationship Between Cerebral Blood Flow Velocity and Blood Pressure: A Clinical Test of Autoregulation. *Stroke* 26: 1801–1804.
- Reinhard M, Roth M, Müller T, Guschlbauer B, Timmer J, et al. (2004) Effect of Carotid Endarterectomy or Stenting on Impairment of Dynamic Cerebral Autoregulation. *Stroke* 35: 1381–1387.
- Dineen NE, Brodie FG, Robinson TG, Panerai RB (2010) Continuous estimates of dynamic cerebral autoregulation during transient hypocapnia and hypercapnia. *Journal of Applied Physiology* 108: 604–613.
- Maggio P, Salinet AS, Panerai RB, Robinson TG (2013) Does hypercapnia-induced impairment of cerebral autoregulation affect neurovascular coupling? A functional TCD study. *Journal of Applied Physiology* 115: 491–497.
- ter Laan M, van Dijk JMC, Elting JWJ, Staal MJ, Absalom AR (2013) Sympathetic regulation of cerebral blood flow in humans: a review. *British Journal of Anaesthesia* 111: 361–367.
- Zhang R, Zuckerman JH, Iwasaki K, Wilson TE, Crandall CG, et al. (2002) Autonomic Neural Control of Dynamic Cerebral Autoregulation in Humans. *Circulation* 106: 1814–1820.
- Mitsis GD, Zhang R, Levine BD, Tzanalaridou E, Katritsis DG, et al. (2009) Autonomic neural control of cerebral hemodynamics. *IEEE Engineering in Medicine and Biology Magazine* 28: 54–62.
- Hamel E (2006) Perivascular nerves and the regulation of cerebrovascular tone. *Journal of Applied Physiology* 100: 1059–1064.
- Peterson EC, Wang Z, Britz G (2011) Regulation of cerebral blood flow. *International Journal of Vascular Medicine* 2011: 823525.
- Toda N, Ayajiki K, Okamura T (2009) Cerebral Blood Flow Regulation by Nitric Oxide: Recent Advances. *Pharmacological Reviews* 61: 62–97.
- Tamaki H, Kohshi K, Sajima S, Takeyama J, Nakamura T, et al. (2010) Repetitive breath-hold diving causes serious brain injury. *Undersea & hyperbaric medicine* 37: 7–11.
- Melamed Y, Shupak A, Bitterman H (1992) Medical problems associated with underwater diving. *New England Journal of Medicine* 326: 30–35.
- Kohshi K, Katoh T, Abe H, Okudera T (2000) Neurological accidents caused by repetitive breath-hold dives: two case reports. *Journal of the Neurological Sciences* 178: 66–69.
- Kohshi K, Wong RM, Abe H, Katoh T, Okudera T, et al. (2005) Neurological manifestations in Japanese Ama divers. *Undersea & hyperbaric medicine* 32: 11–20.
- Gempp E, Blatteau JE (2006) Neurological disorders after repetitive breath-hold diving. *Aviation Space and Environmental Medicine* 77: 971–973.
- Ridgway L, McFarland K (2006) Apnea diving: long-term neurocognitive sequelae of repeated hypoxemia. *The Clinical neuropsychologist* 20: 160–176.
- Dahl A, Lindegaard KF, Russell D, Nyberg-Hansen R, Rootwelt K, et al. (1992) A comparison of transcranial Doppler and cerebral blood flow studies to assess cerebral vasoreactivity. *Stroke* 23: 15–19.
- Cross TJ, Kavanagh JJ, Breskovic T, Zubin Maslov P, Lojpur M, et al. (2013) The Effects of Involuntary Respiratory Contractions on Cerebral Blood Flow during Maximal Apnoea in Trained Divers. *PLoS One* 8: e66950.
- Kontos HA (1989) Validity of cerebral arterial blood flow calculations from velocity measurements. *Stroke* 20: 1–3.
- Poulin MJ, Robbins PA (1996) Indexes of Flow and Cross-sectional Area of the Middle Cerebral Artery Using Doppler Ultrasound During Hypoxia and Hypercapnia in Humans. *Stroke* 27: 2244–2250.
- Serrador JM, Picot PA, Rutt BK, Shoemaker JK, Bondar RL (2000) MRI Measures of Middle Cerebral Artery Diameter in Conscious Humans During Simulated Orthostasis. *Stroke* 31: 1672–1678.

52. Wong KKL, Mazumdar J, Pincombe B, Worthley SG, Sanders P, et al. (2006) Theoretical modeling of micro-scale biological phenomena in human coronary arteries. *Medical & biological engineering & computing* 44: 971–982.
53. Wong KKL, Tu J, Mazumdar J, Abbott D (2010) Modelling of blood flow resistance for an atherosclerotic artery with multiple stenoses and poststenotic dilatations. *ANZIAM Journal* 51: C66–C82.
54. Wong KKL, Tu J, Sun Z, Dissanayake D (2013) *Methods in Research and Development of Biomedical Devices*. Singapore: World Scientific Publishing Co.
55. Hong SK, Lin YC, Lally DA, Yim BJB, Kominami N, et al. (1971) Alveolar Gas Exchanges and Cardiovascular Functions during Breath Holding with Air. *Journal of Applied Physiology* 30: 540–&.
56. Ferretti G, Costa M, Ferrigno M, Grassi B, Marconi C, et al. (1991) Alveolar gas composition and exchange during deep breath-hold diving and dry breath holds in elite divers. *Journal of Applied Physiology* 70: 794–802.
57. Liner MH, Ferrigno M, Lundgren CE (1993) Alveolar gas exchange during simulated breath-hold diving to 20 m. *Undersea Hyperb Med* 20: 27–38.