

Research Paper

Influence of lung volume on the interaction between cardiac output and cerebrovascular regulation during extreme apnoea

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New Findings

• What is the central question of this study?

Does the reduction in cardiac output observed during extreme voluntary apnoea, secondary to high lung volume, result in a reduction in cerebral blood flow, perfusion pressure and oxygen delivery in a group of elite free divers?

• What is the main finding and its importance?

High lung volumes reduce cardiac output and ventricular filling during extreme apnoea, but changes in cerebral blood flow are observed only transiently during the early stages of apnoea. This reveals that whilst cardiac output is important in regulating cerebral haemodynamics, the role of mean arterial pressure in restoring cerebral perfusion pressure is of greater significance to the regulation of cerebral blood flow.

We investigated the role of lung volume-induced changes in cardiac output ($\dot{Q}$) on cerebrovascular regulation during prolonged apnoea. Fifteen elite apnoea divers (one female; 185 ± 7 cm, 82 ± 12 kg, 29 ± 7 years old) attended the laboratory on two separate occasions and completed maximal breath-holds at total lung capacity (TLC) and functional residual capacity (FRC) to elicit disparate cardiovascular responses. Mean arterial pressure (MAP), internal jugular venous pressure and arterial blood gases were measured via cannulation. Global cerebral blood flow was quantified by ultrasound and cardiac output was quantified by via photoplethysmography. At FRC, stroke volume and $\dot{Q}$ did not change from baseline ($P > 0.05$). In contrast, during the TLC trial stroke volume and $\dot{Q}$ were decreased until 80 and 40% of apnoea, respectively ($P < 0.05$). During the TLC trial, global cerebral blood flow was significantly lower at 20%, but subsequently increased so that cerebral oxygen delivery was comparable to that during the FRC trial. Internal jugular venous pressure was significantly higher throughout

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the TLC trial in comparison to FRC. The MAP increased progressively in both trials but to a greater extent at TLC, resulting in a comparable cerebral perfusion pressure between trials by the end of apnoea. In summary, although lung volume has a profound effect on $\dot{Q}$ during prolonged breath-holding, these changes do not translate to the cerebrovasculature owing to the greater sensitivity of cerebral blood flow to arterial blood gases and MAP; regulatory mechanisms that facilitate the maintenance of cerebral oxygen delivery.

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Introduction

Prolonged breath-hold diving ('apnoea diving') has been performed for >2000 years to harvest food and commodities from the ocean (Ferretti, 2001). The remarkable physiological feat of prolonged breath-holding has recently evolved from a primal hunter-gatherer technique to a competitive sport that exists in numerous forms (Dujic & Breskovic, 2012). The complex and integrative physiological stress has, however, remained unchanged. During prolonged apnoeas, trained divers experience severe sympathoexcitation, hypertension, hypoxaemia and hypercapnia (Breskovic *et al.* 2011; Willie *et al.* 2015); they also tolerate forceful diaphragmatic contractions termed 'involuntary breathing movements' (IBMs) for up to 50% of the apnoea duration (Dujic *et al.* 2009).

A prominent response to breath-hold diving is a marked reduction in cardiac output that is partly mediated by bradycardia resulting from the mammalian dive reflex. In addition to a reduced heart rate, breath-holding at total lung capacity (TLC) induces a bi-ventricular decrease in end-diastolic volume (EDV) and stroke volume (Batinic *et al.* 2011; Breskovic *et al.* 2011). This reduction in ventricular filling is attributable to the high intrathoracic pressure that results in compression of the intrathoracic vessels, and therefore, decreased venous return. Perhaps unsurprisingly, syncope is reported during the initial stages of prolonged apnoea when lung volumes are at their highest (Stevens *et al.* 1946; Dzamonja *et al.* 2010); there are also anecdotal reports of syncope at apnoea end, when hypoxaemia is most severe (Willie *et al.* 2015). These findings suggest that cerebral blood flow (CBF) and oxygen delivery (CDO_2) might be attenuated by cardiac restriction secondary to high lung volumes. However, findings from Willie *et al.* (2015) demonstrate the maintenance of CDO_2 at the end of a maximal apnoea at TLC. Cerebral oxygen delivery was, however, reduced below baseline during replication of the arterial blood gas profile of a maximal apnoea via rapid manipulations of end-tidal gases during spontaneous breathing (Willie *et al.* 2015). During this simulated apnoea, despite comparable arterial partial

pressure of oxygen (P_{aO_2}) and carbon dioxide (P_{aCO_2}), mean arterial pressure (MAP) was ~50% lower than during apnoea, highlighting the importance of cerebral perfusion pressure. Therefore, cardiovascular interaction independent of changes in blood gases appears to influence CDO_2 during prolonged apnoea.

A causal link between cardiac output and cerebral blood flow seems likely given that any alteration to cardiac output (acutely or chronically) can lead to a change in CBF that is independent of other CBF-regulating mechanisms (Levine *et al.* 1994; van Lieshout *et al.* 2001; Brown *et al.* 2003; Ogoh *et al.* 2005; Ogawa *et al.* 2007). In a recent review (Meng *et al.* 2015), the results of five studies were collated to demonstrate that, for each percentage change in cardiac output, a 0.35% change in CBF is observed. These changes were, however, observed during manipulations to central blood volume in the absence of changes in other regulatory mechanisms, such as arterial blood gases. How cardiac output influences CBF during rapid and concomitant changes in cerebral perfusion pressure and arterial blood gases is currently unknown.

The purpose of this study was to explore the role of lung volume-induced changes in cardiac output on cerebrovascular regulation during prolonged apnoea. We examined CBF, oxygen delivery and perfusion pressure during maximal apnoea at two lung volumes intended to elicit disparate cardiovascular responses, i.e. TLC and functional residual capacity (FRC). Apnoea at TLC is characterized by a marked reduction in stroke volume (Breskovic *et al.* 2011; Willie *et al.* 2015); in contrast, during apnoea at FRC the stroke volume and cardiac output are maintained. Thus, breath-holding at different lung volumes allows investigation of the unique interaction between cardiovascular control and cerebrovascular regulation during severe hypoxia and hypercapnia. We hypothesized that the lung volume-induced changes in cardiac output will be reflected in CBF during the early stages of apnoea, but arterial blood gases will have an overriding effect towards the end of apnoea, when hypoxia and hypercapnia are most severe.

Methods

Participants

Fifteen elite breath-hold divers (one female) from the national Croatian apnoea team volunteered to participate and provided written informed consent. Participants were free from cardiorespiratory disease, determined by a thorough screening process that included a medical history, pulmonary function testing and a standard anthropometry assessment. The participants had been competing for an average of 5.2 years (range 1.5–14 years) and had an average personal best static apnoea of 401 s (range 296–560 s). All experimental procedures and protocols were approved by the Clinical Research Ethics Board of the University of British Columbia (approval no. H14-00922) and the ethical committee of the University of Split, School of Medicine (approval no. 2181-198-03-04-14-0039), and conformed to the standards set by the *Declaration of Helsinki*, except for registration in a database.

Experimental design

All participants attended the laboratory at the University of Split having abstained from vigorous exercise and alcohol for 48 h and caffeine for 12 h. A subset of the participants ($n = 11$) completed a second visit to corroborate the cardiac measures obtained by photoplethysmography in the experimental visit and to quantify lung volume at FRC and TLC (see '*Confirmation of the cardiac response to breath-holding*' below).

During the experimental visit, baseline measures were collected before any preparatory breath-hold manoeuvres in the supine position. Preparation for the breath-holds was standardized and consisted of one breath-hold at FRC until six IBMs, followed by a 2 min rest, and a second breath-hold at TLC lasting until 10 IBMs. The divers then performed two maximal breath-holds at TLC and FRC separated by at least 30 min of recovery. The required lung volume was self-identified by the divers, as they regularly train and compete at TLC and FRC. Data for the present study were collected concurrently with two other experiments performed in this novel population of elite divers (Bain *et al.* 2015; Hoiland *et al.* 2017). The authors would like to acknowledge that there is minor overlap in some of the parameters reported (e.g. blood gases and CBF) during the TLC trial in visit 1 only, with otherwise no data duplication. Each study addresses a unique *a priori* research question, albeit in the same population.

Measurements

Cardiovascular, cerebrovascular and arterial blood gas measurements. All cardiovascular variables were continuously acquired (1 kHz) throughout the breath-holds

(Powerlab; ADInstruments, Colorado Springs, CO, USA) and recorded using dedicated software (Labchart V7; ADInstruments, Colorado Springs, CO, USA). Beat-to-beat intra-arterial and jugular venous pressures were recorded continuously after catheterization of the radial artery and internal jugular vein performed under ultrasound guidance (8 MHz probe, Nanomaxx; Sonosite, Bothell, WA, USA). A 20-gauge radial arterial catheter (Arrow, Markham, Ontario, Canada) was placed for blood gas sampling and beat-to-beat arterial blood pressure measurement, with the pressure transducer positioned at the level of the right atrium (VAMP; Edwards Lifesciences, Irvine, CA, USA). A jugular bulb catheter (Edwards PediaSat Oximetry Catheter; Edwards Lifesciences) was placed in the right internal jugular vein and directed cephalad in sterile conditions for the assessment of internal jugular venous pressure (IJVP), as previously described (Ainslie *et al.* 2014). Stroke volume was estimated using finger photoplethysmography (Finometer PRO; Finapres Medical Systems, Amsterdam, The Netherlands). Heart rate was recorded from the R–R intervals measured from a three-lead ECG, and multiplied by stroke volume to calculate cardiac output. Peripheral arterial oxygen saturation (S_{pO_2} , expressed as a percentage; Poet II; Criticare, Waukesha, WI, USA) and transcutaneous P_{CO_2} (SenTec AG; Therwill, Switzerland) were also measured continuously throughout the apnoea.

Cerebral blood velocity was measured in the right middle cerebral artery (MCAv) and the left posterior cerebral artery (PCAv) using a 2 MHz pulsed-wave transcranial Doppler (TCD; Spencer Technologies, Seattle, WA, USA) attached to a bilateral head frame (model M600 bilateral head frame; Spencer Technologies). Both the MCA and the PCA were insonated through the trans-temporal window as previously described (Bain *et al.* 2015). Standardized search techniques were applied that have been shown to produce test–retest reliability of 2–3% (Willie *et al.* 2011).

Assessment of volumetric blood flow was simultaneously measured in the right internal carotid artery (ICA) and left vertebral artery (VA) using a 10 MHz multifrequency linear array vascular ultrasound probe (Terason T3200; Teratech, Burlington, MA, USA). The right ICA was insonated ~2 cm from the carotid bifurcation, and the left VA was insonated at the C5–C6 or C5–C4 vertebral space. The precise location between subjects was varied to achieve the best possible signal, and specific care taken to ensure standardization of the location between measures within subject. In all trials, the steering angle was fixed to 60 deg, with the sample volume placed in the centre of the vascular lumen. Video files (AVI) were recorded at 30 Hz using a frame-grabber (Camtasia; TechSmith, Okemos, MI, USA) and stored for offline analysis. Arterial diameter and velocity were measured

simultaneously using customized edge-detection software for a minimum of 10 cardiac cycles (Woodman *et al.* 2001); this automated approach improves the accuracy and reliability of the ultrasound measures and reduces experimenter bias (Thomas *et al.* 2015). The inclusion of vessel diameter from two-dimensional ultrasound, instead of relying on blood velocity alone, provides a more accurate assessment of cerebral blood flow, because vessel diameter is likely to change during severe hypercapnic hypoxia (Hoiland & Ainslie, 2016), although this is currently under debate (Brothers & Zhang, 2016). Volumetric blood flow for both the ICA and the VA was possible in only nine participants, and the data reported for these variables reflect this.

Beat-to-beat mean intra-arterial pressure (MAP) and IJVP were recorded continuously. Blood samples (2 ml) were obtained at baseline, the onset of IBMs and apnoea end and analysed within 2 min using an arterial blood gas analyser (ABL-90 CO-Ox; Radiometer, Copenhagen, Denmark) for P_{aO_2} , P_{aCO_2} and S_{aO_2} .

Involuntary breathing movements were identified from a chest plethysmography belt placed around the chest and integrated into Labchart.

In some instances, during assessment of the ICA and VA the force and frequency of the IBMs did not allow reliable imaging of blood flow velocity. Therefore, volumetric flow was calculated from the velocity of the last successful velocity measurement multiplied by the percentage change in MCAv (ICA) or PCAv (VA) of the next stage, as previously described in detail (Hoiland *et al.* 2017) and used repeatedly within the literature (Fisher *et al.* 2013; Smith *et al.* 2014; Bain *et al.* 2015; Willie *et al.* 2015). This approach was kept consistent between trials for each individual to avoid within-subject trial differences attributable to measurement technique.

Blood flow ($\dot{Q}$) within the ICA and VA were calculated as $\dot{Q} = \pi r^2 v$, where $\dot{Q}$ is the flow, r is the radius of the vessel and v is the velocity of the blood moving through the vessel. Global cerebral blood flow was then calculated as gCBF (ml min^{-1}) = $(\dot{Q}_{ICA} \times 2) + (\dot{Q}_{VA} \times 2)$, where $\dot{Q}_{ICA}$ is the blood flow from the right ICA and $\dot{Q}_{VA}$ is the blood flow from the left VA, assuming blood flow is consistent between the contralateral ICA and VA as shown previously (Zarrinkoob *et al.* 2015). Arterial oxygen content was calculated as C_{aO_2} (ml dl^{-1}) = $[\text{Hb}] \times 1.36 \times S_{aO_2}$ (%) / 100 + $0.003 \times P_{aO_2}$, where $[\text{Hb}]$ is the concentration of haemoglobin in arterial blood, 1.36 is the affinity for O_2 to haemoglobin for a given oxygen saturation and 0.003 is the percentage of O_2 dissolved in the blood. Cerebral oxygen delivery (CDO_2) was calculated as $CDO_2 = \text{gCBF} \times C_{aO_2}$. Cerebral perfusion pressure (CPP) was calculated as $\text{CPP} = \text{MAP} - \text{IJVP}$, and cerebrovascular resistance (CVR) was calculated as $\text{CVR} = \text{CPP} / \text{gCBF}$.

Confirmation of the cardiac response to breath-holding.

Magnetic resonance imaging (MRI) was completed in a subset of the divers ($n = 11$) to corroborate the cardiac output response reported via plethysmography and to quantify the lung volumes self-selected at TLC and FRC by the divers. Left and right ventricular EDV, end-systolic volume (ESV), stroke volume and ejection fraction were assessed during both TLC and FRC apnoea. Lung volume was also quantified during TLC and FRC apnoea. Unfortunately, owing to motion artefact associated with IBMs, it was not possible to image after IBM onset; data are therefore reported during the early and late 'easy-going phase' defined as the period before the first IBM. The MRI measurements were obtained with a clinical 1.5 T MR scanner (Avanto; Siemens Medical Solutions AG, Erlangen, Germany) using a dedicated cardiac phased-array coil and performed as previously described by our group in this population (Batinic *et al.* 2011).

Statistical analyses

Data presented are means $\pm$ SD. Differences in apnoea duration, time to IBM onset and the cardiovascular, cerebrovascular and blood gas measurements at IBM onset and end breath-hold were compared using Student's two-tailed paired t tests. To analyse the effects of breath-holding at different lung volumes, apnoea duration was normalized to 100% and comparisons were made between TLC and FRC (condition) trials at 20% increments (time) *versus* baseline using a 2×6 repeated-measures ANOVA. Data were normally distributed, as confirmed via Shapiro–Wilk normality test. Where sphericity could not be assumed (Mauchly's) the Greenhouse–Geisser correction was applied. When a significant ($P < 0.05$) main or interaction effect was observed, Student's paired t tests were used *post hoc*, with the Bonferroni correction applied to account for multiple comparisons. The relationships between variables during the trials were analysed using mixed model linear regression for each of the pairs of variables of interest. Shapiro–Wilk tests revealed that the residual values were normally distributed, and there were no large positive correlations between independent variables and absolute residual values, meaning that the data satisfied the assumption of homoscedasticity. In each case, the participants ($n = 9$ because of missing volumetric CBF data) were included as random dichotomous factors. The analysis was done in SPSS version 22.0 (SPSS: an IBM Company, IBM, Armonk, NY, USA), which arbitrarily produced a regression coefficient of zero for the final participant, who was considered to be a redundant parameter. Therefore, the regression coefficients for the participants were adjusted so that their mean would be zero and that the regression line would pass through a

Table 1. Arterial blood gases and cerebral blood flow at the onset of involuntary breathing movements and the end of apnoea

Parameter	IBM onset			Apnoea end		
	TLC	FRC	<i>P</i> value	TLC	FRC	<i>P</i> value
Arterial blood gases						
P_{aO_2} (mmHg)	71.1 ± 18.1	46.4 ± 14.2	<0.001	35.1 ± 11.5	29.6 ± 8.1	<0.01
P_{aCO_2} (mmHg)	42.5 ± 4.5	43.1 ± 4.7	n.s.	53.5 ± 4.7	45.0 ± 7.5	<0.001
S_{aO_2} (%)	93.7 ± 4.6	81.4 ± 11.8	<0.001	63.9 ± 16.2	60.4 ± 13.3	n.s.
CaO_2 (ml dl ⁻¹)	18.8 ± 1.8	16.6 ± 2.6	<0.001	13.2 ± 3.1	12.3 ± 2.5	n.s.
Cerebral blood flow						
$\dot{Q}_{ICA}$ (ml min ⁻¹)	286.9 ± 75.8	285 ± 73	n.s.	427 ± 95	350 ± 93	n.s.
$\dot{Q}_{VA}$ (ml min ⁻¹)	119.5 ± 52.7	130 ± 63	n.s.	167 ± 81	160 ± 88	n.s.
gCBF (ml min ⁻¹)	812.8 ± 210.9	829 ± 247	n.s.	1094 ± 409	1019 ± 318	n.s.
CDO ₂ (ml dl ⁻¹ min ⁻¹)	146 ± 33	128 ± 34	n.s.	134 ± 60	116 ± 35	n.s.

Abbreviations: CaO_2 , arterial oxygen content; CDO₂, cerebral oxygen delivery; FRC, functional residual capacity; gCBF, global cerebral blood flow; IBM, involuntary breathing movement; P_{aCO_2} , arterial partial pressure of carbon dioxide; P_{aO_2} , arterial partial pressure of oxygen; $\dot{Q}_{ICA}$, internal carotid artery blood flow; $\dot{Q}_{VA}$, vertebral artery blood flow; S_{aO_2} , arterial oxygen saturation; and TLC, total lung capacity.

point formed by the mean of the two continuous variables being analysed. This adjustment in the regression coefficients for the participants was mirrored by an equivalent adjustment to the intercept for the regression equation. The mixed model linear regression resulted in the gradient of the regression line being closer to the mean gradient within participants rather than being dictated by inter-individual differences. As part of the analysis, effect sizes were also determined using partial η^2 values for each of the four mixed model regression analyses. This was done by running equivalent analysis of variance tests in SPSS, including the independent variable as a covariate and participant as a random effect.

Results

Apnoea characteristics

The longest apnoea time recorded in this study was 441 s, and the group average was 304 ± 71 s. Maximal apnoea time was 38% longer at TLC *versus* FRC (304 ± 71 *versus* 188 ± 44 s, $P < 0.001$), and IBM onset occurred earlier during the FRC trial (113 ± 36 *versus* 150 ± 44 s, $P < 0.001$). As expected, lung volumes were significantly greater during the TLC trial (8.28 ± 1.06 *versus* 4.16 ± 0.55 l, $P < 0.001$). The S_{pO_2} decreased in both trials, but there was no interaction effect ($P > 0.05$; Fig. 1). At apnoea end, P_{aO_2} was lower during the FRC trial ($P < 0.01$; Table 1), but S_{aO_2} and CDO₂ were comparable (Table 1). The P_{aCO_2} and transcutaneous CO₂ were higher

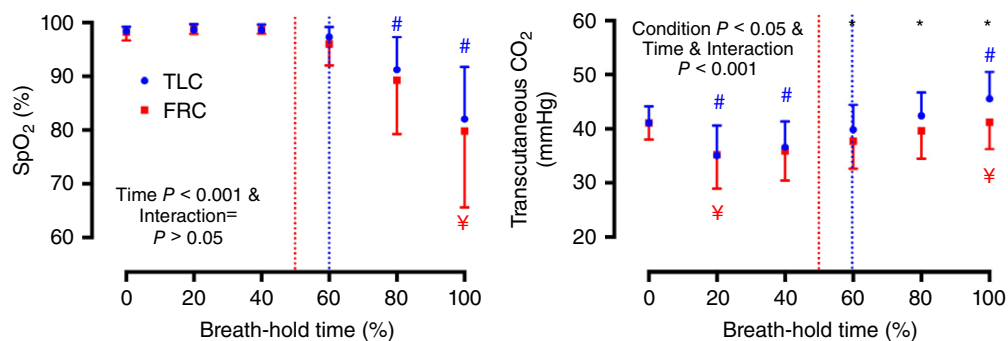


Figure 1. Peripheral oxygen saturation (S_{pO_2}) and transcutaneous CO₂ during prolonged apnoea at total lung capacity (TLC) and functional residual capacity (FRC)

The S_{pO_2} decreased with apnoea time ($P < 0.001$) but did not differ between trials. In contrast, transcutaneous CO₂ increased to a greater extent during the TLC trial. Asterisks (*) denote differences between TLC and FRC trials. Blue and red dotted lines denote IBM onset during the TLC and FRC trials, respectively. Blue hash (#) and red yen (¥) denote significant differences from baseline in TLC trial and FRC trial, respectively.

at the end of the TLC trial (both $P < 0.001$) compared with FRC.

Cardiovascular responses to apnoea at TLC and FRC

At FRC, stroke volume, heart rate and cardiac output did not change from baseline at any stage throughout the apnoea ($P > 0.05$; Fig. 2). In contrast, during the TLC trial, stroke volume was decreased until 80% apnoea duration ($P < 0.01$). The reduction in stroke volume was not accompanied by a significant change in heart rate at TLC, resulting in a marked reduction in cardiac output at 20 and 40% apnoea duration (both $P < 0.05$). In agreement

with measures derived from photoplethysmography, MRI revealed that there were no changes in left ventricular EDV (200 ± 33 ml versus 199 ± 32 ml), stroke volume (120 ± 21 ml versus 115 ± 19 ml) or cardiac output (7.95 ± 1.99 L min⁻¹ versus 7.28 ± 1.79 L min⁻¹) during the FRC trial (all $P > 0.05$), and a bi-ventricular reduction in EDV (200 ± 33 ml versus 146 ± 32 ml), stroke volume (120 ± 21 ml versus 71 ± 12 ml) and cardiac output (7.95 ± 1.99 L min⁻¹ versus 5.59 ± 0.81 L min⁻¹) was present during TLC (all $P < 0.001$).

A significant increase in MAP occurred in both trials, but to a greater extent at apnoea end during the TLC

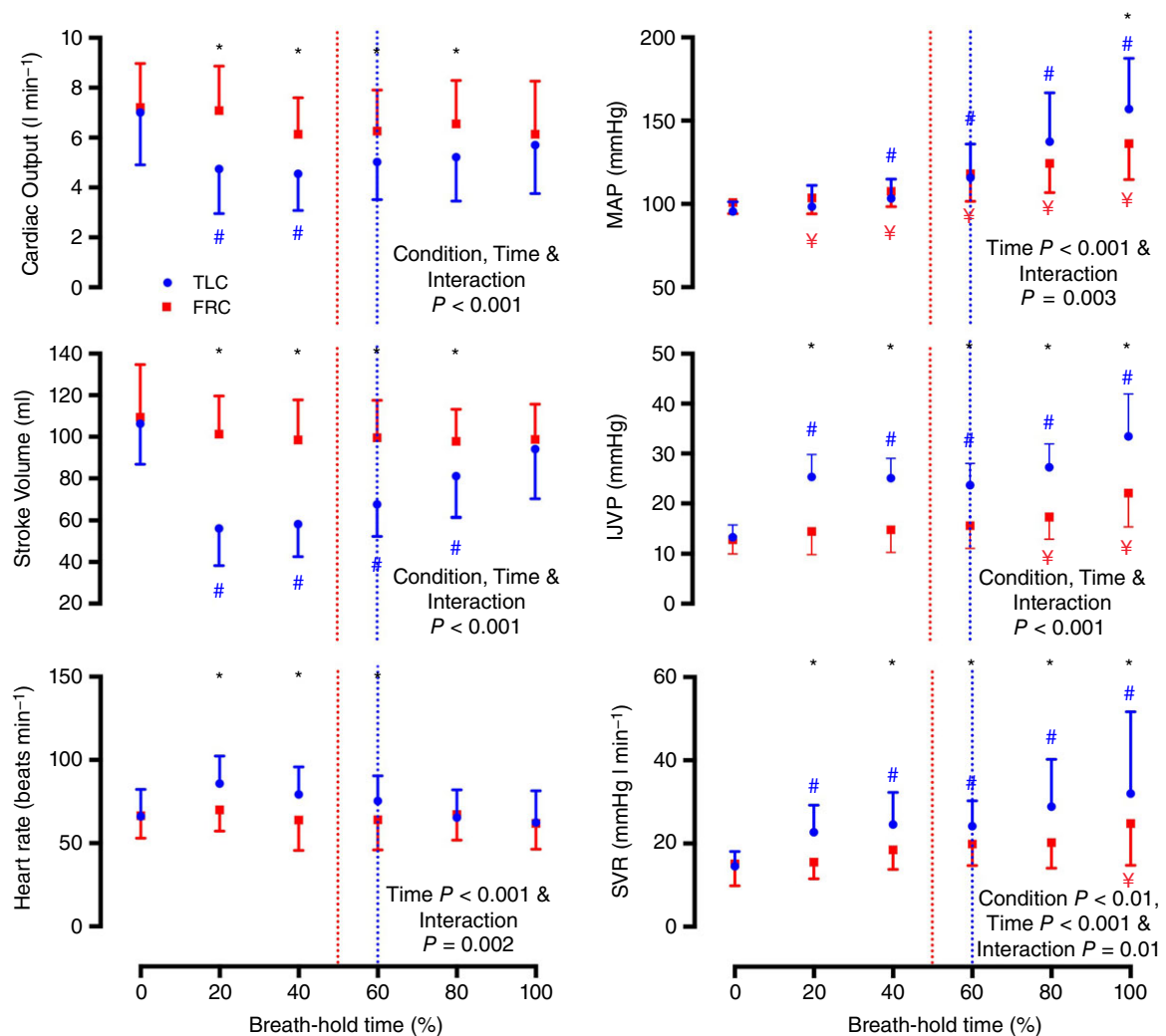


Figure 2. Haemodynamic responses to prolonged breath-holding at TLC and FRC

Stroke volume and cardiac output (via photoplethysmography) decreased significantly during the TLC trial (blue circles) but recovered by apnoea end. Mean arterial pressure (MAP) did not differ between trials until apnoea end despite a significantly higher internal jugular venous pressure (IJVP) throughout the TLC trial. The lower cardiac output but comparable MAP meant that systemic vascular resistance (SVR) was higher during the TLC trial. Blue and red dotted lines denote IBM onset during the TLC and FRC trials, respectively. Asterisks (*) denote significant differences between TLC and FRC trials. Blue hash (#) and red yen (¥) denote significant differences from baseline in TLC trial and FRC trial, respectively.

trial. The lower cardiac output and higher MAP during the TLC trial led to a significantly higher systemic vascular resistance throughout the apnoea ($P < 0.001$). In addition, internal jugular venous pressure was also significantly higher during the TLC trial throughout the apnoea (Fig. 2).

Cerebrovascular responses to apnoea at TLC and FRC

During the early stages of breath-holding there was a decrease in gCBF during the TLC trial ($P < 0.01$), with no change during FRC ($P > 0.05$; Fig. 3). During the latter stages of the breath-hold there was a marked increase in gCBF in response to breath-holding in both trials. The

early differences between the trials were exclusively driven by changes in $\dot{Q}_{VA}$, because $\dot{Q}_{ICA}$ was not different between trials at any stage throughout the breath-holds. As MAP was not different between trials during the early stages but IJVP was ~ 10 mmHg higher during the TLC trial, CPP was reduced ($P < 0.05$; Fig. 3). However, the disproportionate increase in MAP during the TLC trial meant that CPP was comparable between trials by apnoea end. During the FRC trial, CPP was higher but gCBF was comparable, resulting in a higher cerebral vascular resistance.

Results from the linear mixed modelling revealed that there was no significant relationship between changes in cardiac output and gCBF during the FRC apnoea (Fig. 4A).

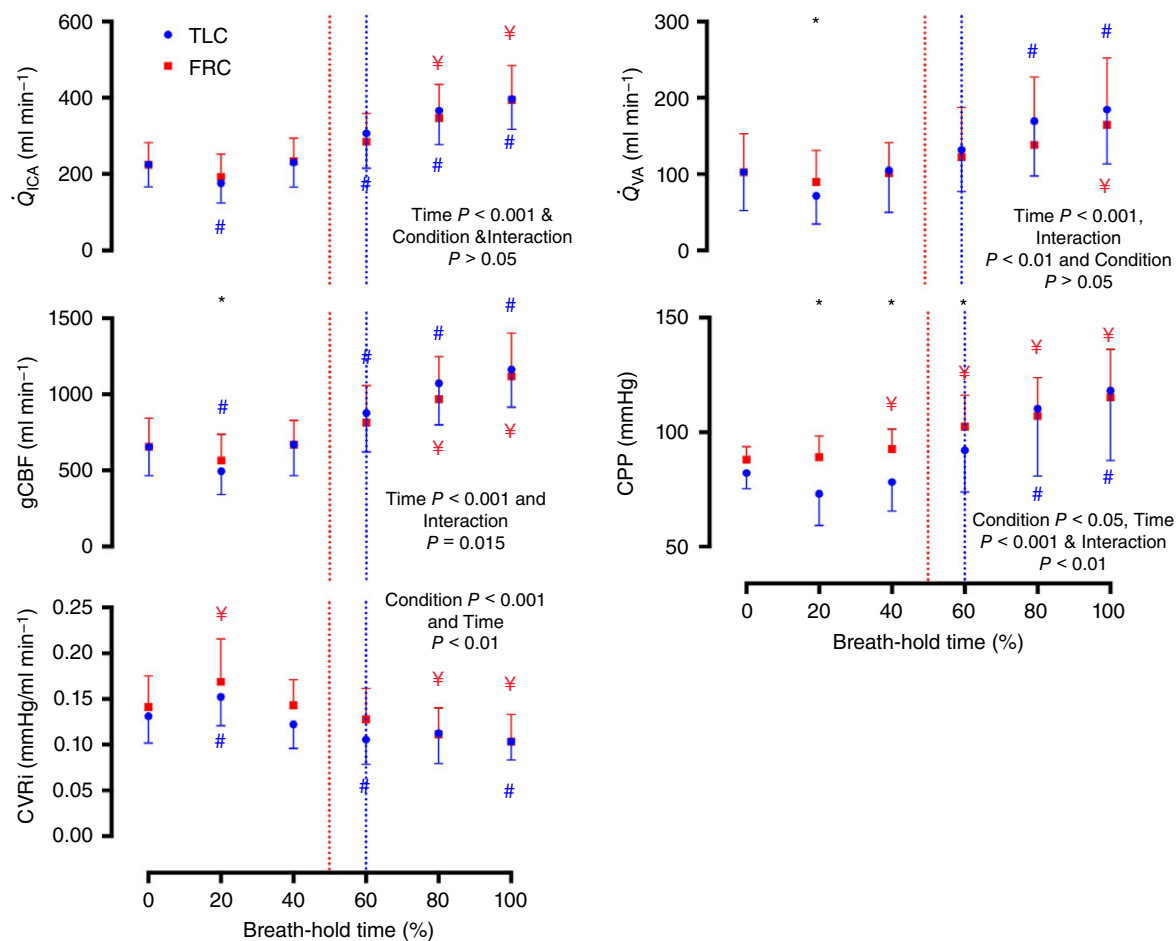


Figure 3. Cerebral blood flow, perfusion pressure and vascular resistance during prolonged breath-holding at TLC and FRC

Global cerebral blood flow (gCBF) decreased early in the TLC trial but recovered for the remainder of the apnoea and was driven by changes in the vertebral artery. In addition to the decrease in gCBF, cerebral perfusion pressure (CPP) was also decreased early in the apnoea, but was comparable to the FRC trial by apnoea end owing to the increase in mean arterial pressure (presented in Fig. 2). Cerebral vascular resistance was higher during the FRC trial. Abbreviations: CVRI, cerebrovascular resistance index; $\dot{Q}_{ICA}$, internal carotid artery blood flow; and $\dot{Q}_{VA}$, vertebral artery blood flow. Blue and red dotted lines denote IBM onset during the TLC and FRC trials, respectively. Asterisks (*) denote differences between TLC and FRC trials, respectively. Blue hash (#) and red yen (¥) denote significant differences from baseline in TLC trial and FRC trial, respectively.

There was a significant relationship between gCBF and cardiac output during the TLC, but the effect sizes were relatively small (0.088 during FRC and 0.136 during TLC). In contrast to cardiac output, there was a significant correlation between changes in CPP and gCBF in both trials (Fig. 4B), and substantial effect sizes were observed of 0.399 during FRC and 0.710 during TLC apnoea. Therefore, CPP explained 40 and 71% of the variance in gCBF during the FRC and TLC trials, respectively. In contrast, cardiac output explained only 9% during FRC and 14% during TLC breath-holding.

Discussion

This is the first study to assess the integrative role of lung volume on cardiac function and CBF during prolonged apnoea. There were two new findings, as follows: (i) after an early reduction during breath-holding at TLC, gCBF is restored before a change in cardiac output, most probably owing to lower cerebral vascular resistance; and (ii) breath-holding at high lung volumes leads to a greater increase in IJVP and a decrease in cerebral

perfusion pressure that is restored only during the latter stages of the apnoea via increased MAP. In agreement with our hypothesis, these findings demonstrate that lung volume has a profound effect on cardiac output during prolonged breath-holding, but these changes do not influence CBF because of the greater influence of other regulatory mechanisms.

Cardiac output and cerebral blood flow during early apnoea

A reduction in heart rate and cardiac output is a universal feature of the mammalian dive reflex that is particularly marked during facial immersion. The lower heart rate can result in a reduction in cardiac work and may help oxygen sparing, thus prolonging apnoea duration (Hoiland *et al.* 2017). Despite the positive effects of bradycardia, a balance must exist between oxygen sparing whilst also maintaining the perfusion of important vascular beds. Herein, we have shown that high lung volumes significantly restrict ventricular filling and stroke volume during breath-holding, with the most pronounced

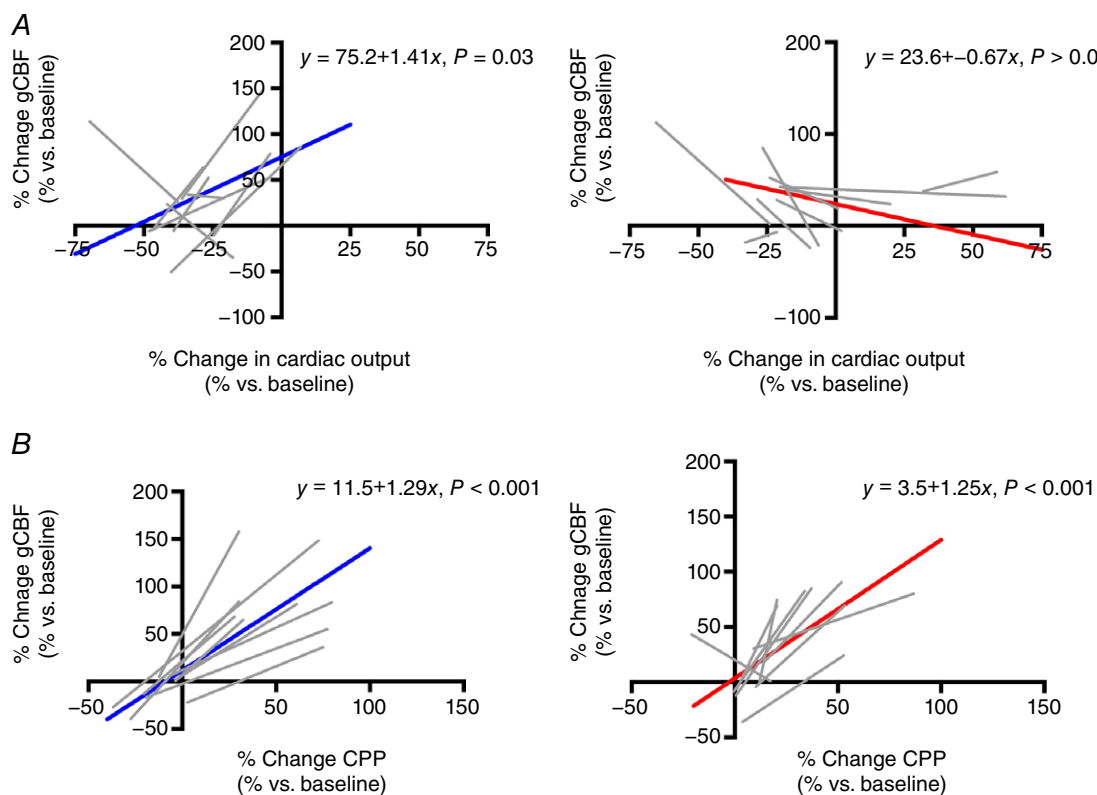


Figure 4. Relationship between changes in global cerebral blood flow (gCBF) with cardiac output (A) and cerebral perfusion pressure (CPP; B) during TLC and FRC apnoea

Grey lines represent each individual's response to apnoea, and blue (TLC) and red (FRC) lines represent the slope and intercept calculated from the linear mixed model accounting for participants as a random effect. Abbreviation: CPP, cerebral perfusion pressure.

Table 2. Left and right ventricular volumes and function during the early and late stages of the 'easy-going phase' (EGP)

Parameter	Baseline	TLC trial		FRC trial	
		Early EGP	Late EGP	Early EGP	Late EGP
Left ventricle					
End-diastolic volume (ml)	200 ± 33	113 ± 20*	146 ± 24*	193 ± 34	199 ± 32
End-systolic volume (ml)	80 ± 17	51 ± 10*	75 ± 15	78 ± 17	84 ± 17
Stroke volume (ml)	120 ± 21	62 ± 13*	71 ± 12*	115 ± 20	115 ± 19
Ejection fraction (%)	60 ± 4	55 ± 5*	48 ± 4*	60 ± 4	58 ± 4
Cardiac output (l min ⁻¹)	7.95 ± 1.99	5.25 ± 1.11*	5.59 ± 0.81*	7.54 ± 2	7.28 ± 1.79*
Right ventricle					
End-diastolic volume (ml)	195 ± 29	112 ± 15*	140 ± 16*	191 ± 29	199 ± 30
End-systolic volume (ml)	77 ± 14	50 ± 7*	71 ± 6	77 ± 11	84 ± 12
Stroke volume (ml)	118 ± 20	62 ± 12*	70 ± 12*	114 ± 19	115 ± 21
Ejection fraction (%)	61 ± 4	55 ± 5*	49 ± 4*	59 ± 3	58 ± 4
Cardiac output (l min ⁻¹)	7.82 ± 1.9	5.21 ± 0.97*	5.53 ± 0.76*	7.41 ± 1.95	7.33 ± 1.83

Abbreviations: EGP, easy-going phase; FRC, functional residual capacity; and TLC, total lung capacity. Significant main effects for condition and time and a significant interaction effect condition × time were observed for all right and left ventricular parameters listed in this table ($P < 0.001$). *indicate $P < 0.05$ vs. baseline.

differences being observed early in the apnoea. During the early stages of apnoea at TLC, the marked decrease in cardiac output was accompanied by a concomitant decrease in gCBF and CPP, a change that was not evident at FRC when cardiac output was maintained. As S_{pO_2} , transcutaneous CO_2 and MAP were not different during the early stages of apnoea, it is likely that the restriction placed upon cardiac filling at high lung volumes translates into an increased IJVP, reduced CPP, and ultimately, a decrease in gCBF. The early reduction in gCBF is in agreement with evidence of syncope during 'air packing' to achieve greater lung volumes; indeed, syncope is commonly observed in the early phase of maximal breath-holding in elite apnoea divers (Dzamonja *et al.* 2010). The competitive diver must therefore balance the positive performance effect of 'air packing' against the negative impact of a high lung volume on cardiac output, and subsequently, gCBF. Ultimately, once CBF is reduced by >50%, cerebral oxygen extraction becomes maximized and syncope readily occurs (Gjedde, 2005).

Cardiac output and cerebral blood flow after onset of IBM

Lung compression whilst breath-holding at a depth of 10 m has been shown to decrease stroke volume and cardiac output further than at the surface of the water (Marabotti *et al.* 2009a,b). However, each of these previous studies performed echocardiographic measurements within the first 90 s of apnoea. We demonstrate that despite restricted filling during the early stages of apnoea at TLC, cardiac output recovered to be comparable to FRC breath-holding by apnoea end. The recovery of stroke volume in combination with a modest increase in heart

rate restored cardiac output. The increase in heart rate at this stage was probably mediated via greater sympathetic activation in the TLC trial, as indicated by the consistently higher systemic vascular resistance throughout the TLC apnoea in the present study and by previous reports of substantially elevated muscle sympathetic nerve activity (Breskovic *et al.* 2011). In addition, cardiac output might also recover in part because of the moderate decrease (200–500 ml min⁻¹) in lung volume that has previously been observed over the course of the apnoea (Stevens *et al.* 1946). The reduction in lung volume is attributable to the continued extraction of O_2 from the lung but the limitation of CO_2 exchange as equilibrium between the lung and blood is reached. Absolute lung volume therefore decreases towards the end of maximal apnoea, and such a reduction may partly remove the constraint placed upon cardiac filling. Our MRI data before IBM onset in the present study support this theory. For example, there was a bi-ventricular increase in EDV from the early to late easy-going phase (before IBM onset; Table 2).

Although cardiac output remained lower in the TLC trial until the final stage of apnoea, gCBF was restored by 40% of the apnoea duration and continued to rise equally in both trials from this point onwards. Similar observations have been made previously, where cardiac output remains lower than baseline, but cerebral blood velocity is higher than baseline (Palada *et al.* 2008; Cross *et al.* 2013). Previous studies, however, have only reported cerebral velocity, and therefore, have not been able to quantify flow nor the effect of CPP. In the present study, despite the reduction in cardiac output and CPP early in the apnoea, the sensitivity of the cerebrovasculature to changes in blood gases appears to alter cerebrovascular resistance and facilitate the maintenance of CBF (Willie

et al. 2012). The uncoupling of cardiac output and CBF is further demonstrated by our linear mixed modelling that revealed cardiac output to explain 14 and 9% of the variance in gCBF during the TLC and FRC apnoea, respectively. A much greater influence of CPP was observed, which was responsible for 71 and 40% of the variance in gCBF during the TLC and FRC trial, respectively. Thus, although a recent review concluded that alteration of cardiac output, either acutely or chronically, leads to a change in CBF that is independent of other CBF-regulating parameters, including CPP and P_{aCO_2} (Meng *et al.* 2015), it seems that this relationship is dissociated during the unique situation of apnea. This difference may be driven by a protective mechanism during this extreme scenario, as maintaining cerebral oxygenation is paramount to survival.

Cerebral autoregulation during apnoea

Cerebral autoregulation is an umbrella term for a process that aims to protect CBF through a range of fluctuations in CPP, and can be examined by assessing the relationship between CPP and gCBF (Willie *et al.* 2014). Studies have demonstrated that CBF increases by 0.4–0.6% per millimetre of mercury elevation in MAP (reviewed by Numan *et al.* 2014; Willie *et al.* 2014). Previously, MAP has been shown to be significantly higher during maximal apnoea compared with a simulated apnoea with the same blood gas profile achieved during normal breathing using end-tidal forcing to clamp end-tidal P_{O_2} and P_{CO_2} (Willie *et al.* 2015). In the present study, MAP was not different between trials up to 80% apnoea duration, but IJVP was on average 10 mmHg higher during the TLC trial. This elevation in IJVP resulted in a lower CPP during the initial stages of the TLC trial. However, MAP increased disproportionately towards the end of the TLC trial such that CPP was comparable to the FRC trial. We speculate that the disproportionate increase in MAP during the TLC trial (and the apnoea trial in a previous study; Willie *et al.* 2015) might be a consequence of elevated intracranial pressure (evidenced by the IJVP pressure) via the Cushing reflex (Cushing, 1901). The Cushing reflex is known to drive sympathetic output (and therefore MAP) in response to increased intracranial pressure in order to maintain CPP. Another prominent feature of the Cushing reflex is bradycardia. In the present study, heart rate decreased towards the end of the TLC trial (*versus* 20–40% apnoea duration), further supporting our speculation. Indeed, breath-holding at TLC is known to induce a greater magnitude response in sympathetic activity compared with FRC apnoea (Breskovic *et al.* 2011) and normal breathing under the same chemoreflex stress (Steinback *et al.* 2010). Although we cannot establish causation, it is possible that the increase in MAP towards the end of apnoea was at least partly driven by the Cushing reflex.

The elevated IJVP is also noteworthy in its own right, as this may reflect mechanical restriction of cerebral venous outflow. The restriction on venous return to the heart attributable to high lung volumes has been extensively discussed in the literature (Lindholm & Nyrén, 2005; Marabotti *et al.* 2009b; Dzamonja *et al.* 2010; Batinic *et al.* 2011; Dujic & Breskovic, 2012; Kyhl *et al.* 2016), but the consequences for cerebral venous outflow are seldom mentioned. We speculate that direct compression of the intrathoracic vessels (e.g. vena cava) attributable to high lung volumes, in combination with swings in intrathoracic pressure from IBMs (Cross *et al.* 2013), are likely to increase back-pressure and vascular resistance, in turn reducing the passive pressure gradient for cerebral venous drainage. Therefore, intracranial pressure would increase, resulting in increased MAP via the Cushing reflex. In the context of extreme apnoea, the elevations in MAP are to counteract the increase in intracranial pressure in order to restore perfusion pressure to maintain favourable cerebral (and potentially myocardial) oxygen delivery.

Limitations and future directions

Although the measurement techniques and experimental design were carefully selected, there are a number of considerations that need to be taken into account. Maximal apnoea is a voluntary process that is subject to individual motivation. The very nature may therefore result in both intra- and inter-individual differences between trials. For consistency, all apnoea preparation was performed under the guidance of the divers' coach and kept constant between trials and individuals. Care was taken to ensure enough rest was given between breath-hold attempts, as it was not possible to randomize the order. However, because there were no differences in any of the cardiovascular, haemodynamic and blood gas variables preceding the apnoea trials (e.g. transcutaneous CO_2 35.1 ± 5.5 *versus* 35.2 ± 6.2 mmHg), we are confident that the standardized preparation by the apnoea coach and sufficient rest between efforts meant there was no order effect.

As indicated in our methodology, the movements associated with the 'struggle phase' after IBM onset affected our ability to obtain MRI and duplex ultrasound images. After IBM onset, MRI is not possible, and we therefore report only measurements obtained via photoplethysmography. In the cerebral vessels, duplex ultrasound images of the extracranial vessels were supported by transcranial Doppler of the intracranial vessels, as this latter methodology is less susceptible to bodily movement.

Data reported are expressed relative to apnoea duration because of the large difference in apnoea time between the TLC and FRC trials. The authors believe this to be the most useful comparison, as used previously in the diving

literature (Cross *et al.* 2013; Bain *et al.* 2015; Willie *et al.* 2015), but it must be considered when interpreting the results.

Finally, the changes in CBF and relative contributions of cardiac output and CPP may differ significantly if investigated in less severe conditions in a 'normal' population. The purpose of the present study was to investigate the regulation of CBF during extreme apnoea, but future work should assess whether mild changes in CBF-regulating mechanisms, such as arterial blood gases, would change the relative influence of cardiac output on CBF.

Conclusions

Together, our findings demonstrate that cerebral blood flow and oxygen delivery are maintained despite a reduction in cardiac output during breath-holding at TLC, probably owing to the greater influence of CPP and arterial blood gases on gCBF. In addition, given the large increase in IJVP observed during breath-holding at high lung volumes, an increase in MAP towards the end of a maximal breath-hold is essential to restore CPP and cerebral oxygen delivery.

References

- Ainslie PN, Shaw AD, Smith KJ, Willie CK, Ikeda K, Graham J & Macleod DB (2014). Stability of cerebral metabolism and substrate availability in humans during hypoxia and hyperoxia. *Clin Sci (Lond)* **126**, 661–670.
- Bain AR, Dujic Z, Hoiland RL, Barak OF, Madden D, Drvis I, Stembridge M, Macleod DB, MacLeod DM & Ainslie PN (2015). Peripheral chemoreflex inhibition with low-dose dopamine; new insight into mechanisms of extreme apnea. *Am J Physiol Regul Integr Comp Physiol* **309**, R1162–R1171.
- Batinic T, Utz W, Breskovic T, Jordan J, Schulz-Menger J, Jankovic S, Dujic Z & Tank J (2011). Cardiac magnetic resonance imaging during pulmonary hyperinflation in apnea divers. *Med Sci Sports Exerc* **43**, 2095–2101.
- Breskovic T, Steinback CD, Salmanpour A, Shoemaker JK & Dujic Z (2011). Recruitment pattern of sympathetic neurons during breath-holding at different lung volumes in apnea divers and controls. *Auton Neurosci* **164**, 74–81.
- Brothers RM & Zhang R (2016). CrossTalk opposing view: The middle cerebral artery diameter does not change during alterations in arterial blood gases and blood pressure. *J Physiol* **594**, 4077–4079.
- Brown CM, Dütsch M, Hecht MJ, Neundörfer B & Hilz MJ (2003). Assessment of cerebrovascular and cardiovascular responses to lower body negative pressure as a test of cerebral autoregulation. *J Neurol Sci* **208**, 71–78.
- Cross TJ, Kavanagh JJ, Breskovic T, Zubin Maslov P, Lojpur M, Johnson BD & Dujic Z (2013). The effects of involuntary respiratory contractions on cerebral blood flow during maximal apnoea in trained divers. *PLoS One* **8**, e66950.
- Cushing H (1901). Concerning a definite regulatory mechanism of the vaso-motor centre which controls blood pressure during cerebral compression. *Bull Johns Hopkins Hosp* **12**, 290–292.
- Dujic Z & Breskovic T (2012). Impact of breath holding on cardiovascular respiratory and cerebrovascular health. *Sports Med* **42**, 459–472.
- Dujic Z, Uglesic L, Breskovic T, Valic Z, Heusser K, Marinovic J, Ljubkovic M & Palada I (2009). Involuntary breathing movements improve cerebral oxygenation during apnea struggle phase in elite divers. *J Appl Physiol* **107**, 1840–1846.
- Dzamonja G, Tank J, Heusser K, Palada I, Valic Z, Bakovic D, Obad A, Ivancev V, Breskovic T, Diedrich A, Luft FC, Dujic Z & Jordan J (2010). Glossopharyngeal insufflation induces cardioinhibitory syncope in apnea divers. *Clin Auton Res* **20**, 381–384.
- Ferretti G (2001). Extreme human breath-hold diving. *Eur J Appl Physiol* **84**, 254–271.
- Fisher JP, Hartwich D, Seifert T, Olesen ND, McNulty CL, Nielsen HB, van Lieshout JJ & Secher NH (2013). Cerebral perfusion, oxygenation and metabolism during exercise in young and elderly individuals. *J Physiol* **591**, 1859–1870.
- Gjedde A (2005). The pathways of oxygen in brain. I. Delivery and metabolism of oxygen. *Adv Exp Med Biol* **566**, 269–275.
- Hoiland RL & Ainslie PN (2016). CrossTalk proposal: The middle cerebral artery diameter does change during alterations in arterial blood gases and blood pressure. *J Physiol* **594**, 4073–4075.
- Hoiland RL, Ainslie PN, Bain AR, Macleod DB, Stembridge M, Drvis I, Madden D, Barak OF, MacLeod DM & Dujic Z (2017). β_1 -Blockade increases maximal apnea duration in elite breath hold divers. *J Appl Physiol* **122**, 899–906.
- Kyhl K, Drvis I, Barak OF, Mijacika T, Engström T, Secher NH, Dujic Z, Buca A & Madsen PL (2016). Organ perfusion during voluntary pulmonary hyperinflation; a magnetic resonance imaging study. *Am J Physiol Heart Circ Physiol* **310**, H444–H451.
- Levine BD, Giller CA, Lane LD, Buckley JC & Blomqvist CG (1994). Cerebral versus systemic hemodynamics during graded orthostatic stress in humans. *Circulation* **90**, 298–306.
- Lindholm P & Nyrén S (2005). Studies on inspiratory and expiratory glossopharyngeal breathing in breath-hold divers employing magnetic resonance imaging and spirometry. *Eur J Appl Physiol* **94**, 646–651.
- Marabotti C, Scalzini A, Cialoni D, Passera M, L'Abbate A & Bedini R (2009a). Cardiac changes induced by immersion and breath-hold diving in humans. *J Appl Physiol* **106**, 293–297.
- Marabotti C, Scalzini A, Cialoni D, Passera M, Ripoli A, L'Abbate A & Bedini R (2009b). Effects of depth and chest volume on cardiac function during breath-hold diving. *Eur J Appl Physiol* **106**, 683–689.
- Meng L, Hou W, Chui J, Han R & Gelb AW (2015). Cardiac output and cerebral blood flow: the integrated regulation of brain perfusion in adult humans. *Anesthesiology* **123**, 1198–1208.
- Numan T, Bain AR, Hoiland RL, Smirl JD, Lewis NC & Ainslie PN (2014). Static autoregulation in humans: a review and reanalysis. *Med Eng Phys* **36**, 1487–1495.

- Ogawa Y, Iwasaki K, Aoki K, Shibata S, Kato J & Ogawa S (2007). Central hypervolemia with hemodilution impairs dynamic cerebral autoregulation. *Anesth Analg* **105**, 1389–1396, table of contents.
- Ogoh S, Brothers RM, Barnes Q, Eubank WL, Hawkins MN, Purkayastha S, O-Yurvati A & Raven PB (2005). The effect of changes in cardiac output on middle cerebral artery mean blood velocity at rest and during exercise. *J Physiol* **569**, 697–704.
- Palada I, Bakovic D, Valic Z, Obad A, Ivancev V, Eterovic D, Shoemaker JK & Dujic Z (2008). Restoration of hemodynamics in apnea struggle phase in association with involuntary breathing movements. *Respir Physiol Neurobiol* **161**, 174–181.
- Smith K, MacLeod D, Willie C, Lewis N, Hoiland R, Ikeda K, Tymko M, Donnelly J, Day T, MacLeod N, Lucas S & Ainslie P (2014). Influence of high altitude on cerebral blood flow and fuel utilization during exercise and recovery. *J Physiol* **592**, 5507–5527.
- Steinback CD, Breskovic T, Frances M, Dujic Z & Shoemaker JK (2010). Ventilatory restraint of sympathetic activity during chemoreflex stress. *Am J Physiol Regul Integr Comp Physiol* **299**, R1407–R1414.
- Stevens CD, Ferris EB, Webb JP, Engel GL & Logan M (1946). Voluntary breathholding. I. Pulmonary gas exchange during breathholding. *J Clin Invest* **25**, 723–728.
- Thomas KN, Lewis NC, Hill BG & Ainslie PN (2015). Technical recommendations for the use of carotid duplex ultrasound for the assessment of extracranial blood flow. *Am J Physiol Regul Integr Comp Physiol* **309**, R707–R720.
- van Lieshout JJ, Pott F, Madsen PL, van Goudoever J & Secher NH (2001). Muscle tensing during standing: effects on cerebral tissue oxygenation and cerebral artery blood velocity. *Stroke* **32**, 1546–1551.
- Willie CK, Ainslie PN, Drvis I, MacLeod DB, Bain AR, Madden D, Maslov PZ & Dujic Z (2015). Regulation of brain blood flow and oxygen delivery in elite breath-hold divers. *J Cereb Blood Flow Metab* **35**, 66–73.
- Willie CK, Colino FL, Bailey DM, Tzeng YC, Binsted G, Jones LW, Haykowsky MJ, Bellapart J, Ogoh S, Smith KJ, Smirl JD, Day TA, Lucas SJ, Eller LK & Ainslie PN (2011). Utility of transcranial Doppler ultrasound for the integrative assessment of cerebrovascular function. *J Neurosci Methods* **196**, 221–237.
- Willie CK, Macleod DB, Shaw AD, Smith KJ, Tzeng YC, Eves ND, Ikeda K, Graham J, Lewis NC, Day TA & Ainslie PN (2012). Regional brain blood flow in man during acute changes in arterial blood gases. *J Physiol* **590**, 3261–3275.
- Willie CK, Tzeng YC, Fisher JA & Ainslie PN (2014). Integrative regulation of human brain blood flow. *J Physiol* **592**, 841–859.
- Woodman RJ, Playford DA, Watts GF, Cheetham C, Reed C, Taylor RR, Puddey IB, Beilin LJ, Burke V, Mori TA & Green D (2001). Improved analysis of brachial artery ultrasound using a novel edge-detection software system. *J Appl Physiol* **91**, 929–937.
- Zarrinkoob L, Ambarki K, Wåhlin A, Birgander R, Eklund A & Malm J (2015). Blood flow distribution in cerebral arteries. *J Cereb Blood Flow Metab* **35**, 648–654.

Additional information

Competing interests

None declared.

Author contributions

All experiments were performed at the University of Split, Croatia. M.S. contributed to the study design, data collection, analysis and interpretation and drafting of the manuscript; R.L.H., A.R.B. and T.B. contributed to the data collection and analyses and critical review of the manuscript; O.F.B., I.D., D.B.M., D.M.M. and D.M. contributed to the data collection and critical review of the manuscript; R.S. and P.O'D. contributed to the study design, data interpretation and critical review of the manuscript; Z.D. and P.N.A. contributed to the study design, data collection, data interpretation and critical review of the manuscript. P.N.A. confirms that the study objectives and procedures are honestly disclosed. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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