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

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global cerebral blood flow (gCBF) was quantified by ultrasound and cardiac output via photoplethysmography. At FRC, stroke volume (SV) and cardiac output (Q̇) did not change from baseline (P>0.05). In contrast, during the TLC trial SV and Q̇ were decreased until 80% and 40% of apnoea, respectively (P<0.05). During the TLC trial, gCBF was significantly lower at 20%, but subsequently increased so that cerebral oxygen delivery was comparable to the FRC trial. IJVP was significantly higher throughout the TLC trial in comparison to FRC. MAP rose progressively in both trials but to a greater extent at TLC resulting in a comparable cerebral perfusion pressure between trials by apnoea end. In summary, although lung volume has a profound effect on Q̇ during prolonged breath-holding, these changes do not translate to the cerebrovasculature due to the greater sensitivity of CBF to arterial blood gases and MAP; regulatory mechanisms that facilitate the maintenance of cerebral oxygen delivery.

**New Findings:** To determine whether the reduction in cardiac output observed during extreme voluntary apnoea, secondary to high lung volume, results in a reduction in cerebral blood flow, perfusion pressure and oxygen delivery in a group of elite free divers. High lung volumes reduce cardiac output and ventricular filling during extreme apnoea, but changes in cerebral blood flow are only observed transiently during the early stages of apnoea. This reveals that whilst cardiac output is important in regulating cerebral hemodynamics, the role of mean arterial pressure in restoring cerebral perfusion pressure is of greater significance to the regulation of cerebral blood flow.

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

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Abstract

We investigated the role of lung volume-induced changes in cardiac output (Q) on cerebrovascular regulation during prolonged apnoea. Fifteen elite apnoea divers (1F; 185 ± 7 cm, 82 ± 12 kg, 29 ± 7 years) 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 (IJVP) and arterial blood gases were measured via cannulation, global cerebral blood flow (gCBF) was quantified by ultrasound and cardiac output via photoplethysmography. At FRC, stroke volume (SV) and cardiac output (Q) did not change from baseline (P>0.05). In contrast, during the TLC trial SV and Q were decreased until 80% and 40% of apnoea, respectively (P<0.05). During the TLC trial, gCBF was significantly lower at 20%, but subsequently increased so that cerebral oxygen delivery was comparable to the FRC trial. IJVP was significantly higher throughout the TLC trial in comparison to FRC. MAP rose progressively in both trials but to a greater extent at TLC resulting in a comparable cerebral perfusion pressure between trials by apnoea end. In summary, although lung volume has a profound effect on Q during prolonged breath-holding, these changes do not translate to the cerebrovasculature due to the greater sensitivity of CBF to arterial blood gases and MAP; regulatory mechanisms that facilitate the maintenance of cerebral oxygen delivery.
What is the central question of this study?

To determine whether the reduction in cardiac output observed during extreme voluntary apnoea, secondary to high lung volume, results 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 only observed transiently during the early stages of apnoea. This reveals that whilst cardiac output is important in regulating cerebral hemodynamics, the role of mean arterial pressure in restoring cerebral perfusion pressure is of greater significance to the regulation of cerebral blood flow.
Introduction

Prolonged breath-hold diving (‘apnoea diving’) has been performed for over 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, hypoxemia, 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 due 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 hypoxemia is most severe (Willie et al., 2015). These findings suggest that cerebral blood flow (CBF) and oxygen delivery (CDO2) may be attenuated by cardiac restriction secondary to high lung volumes. However, findings from Willie et al. (2015) demonstrate the maintenance of CDO2 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 (PaO2) and carbon dioxide (PaCO2), mean arterial pressure (MAP) was approximately 50% lower than during apnoea, highlighting the importance of cerebral perfusion pressure. Therefore, cardiovascular interaction independent of changes in blood gases appears to influence CDO2 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 to 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: total lung capacity (TLC) and functional residual capacity (FRC). Apnoea at TLC is characterised by a marked reduction in stroke volume (Breskovic et al., 2011; Willie et al., 2015); in contrast, during apnoea at FRC 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 under severe hypoxia and hypercapnia. We hypothesised 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.
Material and 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 seconds (range 296-560 seconds). All experimental procedures and protocols were approved by the Clinical Research Ethics Board of the University of British Columbia (H14-00922) and the ethical committee of the University of Split, School of Medicine (2181-198-03-04-14-0039), and conformed to the standards set by the Declaration of Helsinki.

Experimental Design

All participants attended the laboratory at the University of Split having abstained from vigorous exercise and alcohol for 48 hours and caffeine for 12 hours. 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 prior to any preparatory breath-hold manoeuvres in the supine position. Preparation for the breath-holds was standardised and consisted of one breath-hold at FRC until 6 IBMs followed by a two-minute 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 minutes 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 current study was collected concurrently with two other experiments performed in this novel population of elite divers (Bain et al., 2015; Hoiland et al., 2016). 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 does addresses a unique a priori research question, albeit in the same population.
Measurements

Cardiovascular, Cerebrovascular and Arterial Blood Gas Measurements

Cardiovascular: All cardiovascular variables were continuously acquired (1kHz) throughout the breath-holds (Powerlab, ADInstruments, Colorado Springs, CO) and recorded using dedicated software (Labchart V7, ADInstruments, Colorado Springs, CO). Beat-to-beat intra-arterial and jugular venous pressures were recorded continuously following catheterization of the radial artery and internal jugular vein performed under ultrasound guidance (8 MHz probe, Nanomaxx, Sonosite, Washington, 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 (Edwards Lifesciences VAMP). A jugular bulb catheter (Edwards PediaSat Oximetry Catheter, Edwards, Irvine, CA, USA) was placed in the right internal jugular vein and directed cephalad under 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, Finapress Medical Systems, Amsterdam, 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 (SpO$_2$%; Poet II, Criticare, USA) and transcutaneous PCO$_2$ (SenTec AG, Therwill, Switzerland) were also measured continuously throughout the apnoea.

Cerebrovascular: Cerebral blood velocity was measured in the right middle cerebral artery (MCAv) and the left posterior cerebral artery (PCAv) using a 2MHz pulsed-wave transcranial Doppler (TCD; Spencer Technologies, Seattle, WA) attached to a bi-lateral head frame (model M600 bilateral head frame, Spencer Technologies Seattle, WA). Both the MCA and PCA were insonated through the trans-temporal window as previously described (Bain et al., 2015). Standardised 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 10MHz multi-frequency linear array vascular ultrasound (Terason T3200, Teratech, Burlington, MA). The right ICA was insonated approximately 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 standardisation of location between measures within subject. In all trials the steering angle was fixed to 60 degrees 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, Michigan, US) and stored for offline analysis. Arterial
diameter and velocity were measured simultaneously using customised 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). Volumetric blood flow for both the ICA and VA was only possible in nine
participants (60%) and the data reported for these variables reflect this.

Beat-to-beat mean intra-arterial (MAP) and internal jugular venous pressures (IJVP) were
recorded continuously. Blood samples (2 mL) were obtained at baseline, the onset of
involuntary breathing movements (IBM) and apnoea end and analysed within 2 minutes using
an arterial blood gas analyser (ABL-90 CO-Ox, Radiometer, Copenhagen, Denmark) for arterial
PO$_2$, PCO$_2$ and O$_2$ saturation (SaO$_2$%).

**Involuntary Breathing Movements (IBM):** IBMs were identified from a chest plethysmography
belt placed around the chest and integrated into Labchart.

**Calculations:** 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 MCAv (ICA) or PCAv (VA) of the next stage, as previously described in detail
(Hoiiland et al., 2016) and utilised 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 due to measurement technique.

Blood flow (Q) within the ICA and VA were calculated as $Q = \pi r^2 v$, where $Q$ 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 $\text{gCBF (ml.min}^{-1}) = (Q_{\text{ICA}} \times 2) + (Q_{\text{VA}} \times 2)$, where $Q_{\text{ICA}}$ = blood
flow from the right ICA and $Q_{\text{VA}}$ = 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 $\text{CaO}_2$ (ml dl$^{-1}$) = $[\text{Hb}] \times 1.36 \times \text{SaO}_2$ (%)/100 + 0.003 x PaO$_2$,
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 $\text{CDO}_2 = \text{gCBF} \times \text{CaO}_2$. Cerebral
perfusion pressure (CPP) was calculated as $\text{CPP} = \text{MAP} - \text{IJVP}$, where IJVP is internal jugular
venous pressure 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 (LV) and right ventricular (RV) end-diastolic volume (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, due to motion artefact associated with IBMs, it was not possible to image following 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**

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 two-tailed paired t-tests. To analyse the effects of breath-holding at different lung volumes, apnoea duration was normalised to 100% and comparisons were made between TLC and FRC (condition) trials at 20% increments (time) vs. baseline using a 2 x 6 repeated measures analysis of variance (ANOVA). 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, paired-sample t-tests were employed 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. In each case, the participants (n=9) were included as random dichotomous factors. The analysis was done in SPSS version 22.0 (SPSS: an IBM Company, IBM: Armonk, NY) which arbitrarily produced a regression coefficient of 0 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 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.
Results

Apnoea characteristics

The longest apnoea time recorded in this study was 441 seconds and the group average was 304 ± 71 seconds. Maximal apnoea time was 38% longer at TLC vs. FRC (304 ± 71 vs. 188 ± 44 seconds, P<0.001) and IBM onset occurred earlier during the FRC trial (113 ± 36 vs. 150 ± 44 sec, P<0.001). As expected, lung volumes were significantly greater during the TLC trial (8.28 ± 1.06 vs. 4.16 ± 0.55 L, P<0.001). The SpO₂ decreased in both trials but there was no interaction effect (P>0.05; Figure 1). At apnoea end, PaO₂ was lower during the FRC trial (P<0.01; Table 1) but SaO₂ and CO₂ were comparable (Table 1). PaCO₂ and transcutaneous CO₂ were higher at the end of the TLC trial (both P<0.001) compared to FRC.

Cardiovascular responses to apnoea at TLC and FRC

Photoplethysmography: At FRC, stroke volume, heart rate and cardiac output did not change from baseline at any stage throughout the apnoea (P>0.05; Figure 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 vs. 199 ± 32), stroke volume (120 ± 21 vs. 115 ± 19) or cardiac output (7.95 ± 1.99 vs. 7.28 ± 1.79) during the FRC trial (all P>0.05), and a bi-ventricular reduction in EDV (200 ± 33 vs. 146 ± 32), stroke volume (120 ± 21 vs. 71 ± 12) and cardiac output (7.95 ± 1.99 vs. 5.59 ± 0.81) was present during TLC (all P<0.001).

A significant rise in MAP occurred in both trials, but to a greater extent at apnoea end during the TLC trial. The lower cardiac output and higher MAP during the TLC trial led to a significantly higher SVR throughout the apnoea (P<0.001). In addition, internal jugular venous pressure was also significantly higher during the TLC trial throughout the apnoea (Figure 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; Figure 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 QVA because QICA 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 approximately 10 mmHg higher during the
TLC trial, cerebral perfusion pressure was reduced (P<0.05, Figure 3). However, the disproportionate increase in MAP during the TLC trial meant 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 (CVR).

Results from the linear mixed modelling revealed that there was no significant relationship between changes in cardiac output and gCBF during apnoea the FRC apnoea (Figure 4A). 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, there was a significant correlation between changes in CPP and gCBF in both trials (Figure 4B) and substantial effect sizes were observed of 0.710 during TLC and 0.399 during FRC. Therefore, CPP explained 71% and 40% of the variance in gCBF during the TLC and FRC trial, respectively, with cardiac output only explaining 14% and 9%, respectively.
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 novel findings: (1) following an early reduction during breath-holding at TLC, gCBF is restored prior to a change in cardiac output, most likely owing to lower cerebral vascular resistance and (2) breath-holding at high lung volumes leads to a greater increase in IJVP and a decrease in cerebral perfusion pressure that is only restored 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 due to 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., 2016). 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 differences 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 SpO₂, transcutaneous CO₂ and MAP were not different during the early stages of apnoea, it is likely the restriction placed upon cardiac filling at high lung volumes translates to 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 following IBM onset

Lung compression whilst breath-holding at a depth of 10m has been shown to decrease stroke volume and cardiac output further than at the surface of the water (Marabotti et al., 2009a;
Marabotti et al., 2009b). However, each of these previous studies performed echocardiographic measurements within the first 90 seconds of apnoea. We demonstrate that despite restricted filling during the early stages of apnoea at TLC, cardiac output recovered to be comparable with 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 likely mediated via greater sympathetic activation in the TLC trial, as indicated by the consistently higher SVR throughout the TLC apnoea in the current study, and previous reports of substantially elevated muscle sympathetic nerve activity (Breskovic et al., 2011). In addition, cardiac output may also partially recover due to 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 due to the continued extraction of O₂ from the lung but the limitation of CO₂ 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 partially remove the constraint placed upon cardiac filling. Our MRI data prior to 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 (pre IBM onset; Table 2).

While 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 not been able to quantify flow nor the effect of CPP. In the current 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 PaCO₂ (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 processes 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 mmHg elevation in MAP (reviewed in: (Numan et al., 2014; Willie et al., 2014). Previously, MAP has been shown to be significantly higher during maximal apnoea compared to a simulated apnoea with the same blood gas profile achieved during normal breathing using end-tidal forcing to clamp end-tidal PO₂ and PCO₂ (Willie et al., 2015). In the current study, MAP was not different between trials up to 80% apnoea duration, but IJVP was on average 10 mmHg higher during 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) may 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 current study, heart rate decreases towards the end of the TLC trial (vs. 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 to 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 due to high lung volumes has been extensively discussed in the literature (Lindholm & Nyren, 2005; Marabotti et al., 2009b; Dzamonja et al., 2010; Batinic et al., 2011; Dujic & Breskovic, 2012; Kyhl et al., 2015), but the consequences for cerebral venous outflow are seldom mentioned. We speculate that direct compression of the intra-thoracic vessels (e.g. vena cava) due to high lung volumes, in combination with swings in intrathoracic pressure from IBMs (Cross et al., 2013), likely increased 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**

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 randomise the order. However, because there were no differences in any of the cardiovascular, hemodynamic and blood gas variables preceding the apnoea trials (e.g. transcutaneous CO₂ 35.1 ± 5.5. vs. 35.2 ± 6.2 mmHg), we are confident the standardised 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' following IBM onset affected our ability to obtain MRI and duplex ultrasound images. Following IBM onset, MRI imaging is not possible and we therefore only report measurements obtained via photoplethysmography. In the cerebral vessels, duplex ultrasound images of the extra-cranial vessels were supported by transcranial Doppler of the intra-cranial vessels, as this latter methodology is less susceptible to bodily movement. Lastly, data reported are expressed relative to apnoea duration due to the large difference in apnoea time between the TLC and FRC trials. The authors believe this to be the most useful comparison, as utilised 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.

**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, likely owing to the greater influence of cerebral perfusion pressure 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.
Competing Interests

The authors declare that there is no conflict of interest.

Author Contributions

M.S contributed to the study design, data collection and analysis, data 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 manuscript; O.F.B, I.D, D.B.M, D.M.M and D.M contributed to the data collection and critical review of manuscript; R.S and POD contributed to the study design, data interpretation, and critical review of manuscript; Z.D and P. N. A. contributed to the study design, data collection, data interpretation, and critical review of manuscript. P.N.A confirms that the study objectives and procedures are honestly disclosed. All experiments were performed at the University of Split, Croatia.

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Acknowledgements

We would like to acknowledge the apnoea divers from the Croatia National Apnoea team for their participation.


**Figure Legends**

**Figure 1- Peripheral oxygen saturation (SpO₂) and transcutaneous CO₂ during prolonged apnoea at TLC and FRC.** SpO₂ 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 hash (#) and red yen (¥) denote significant differences from baseline in TLC trial and FRC trial, respectively.

**Figure 2- Hemodynamic 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 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.

**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 due to the increase in mean arterial pressure (presented in Figure 2). Cerebral vascular resistance was higher during the FRC trial. Blue and red dotted lines denote IBM onset during the TLC and FRC trials, respectively. Asterisks (*) denote differences between TLC and FRC trials. Blue hash (#) and red yen (¥) denote significant differences from baseline in TLC trial and FRC trial, respectively.

**Figure 4- Relationship between changes in global cerebral blood flow (gCBF) with cardiac output (panel A) and cerebral perfusion pressure (CPP; panel 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.
Table 1- Arterial blood gases and cerebral blood flow at IBM onset and apnoea end.

<table>
<thead>
<tr>
<th></th>
<th>IBM Onset</th>
<th></th>
<th>Apnoea End</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>TLC</td>
<td>FRC</td>
<td>p value</td>
<td>TLC</td>
</tr>
<tr>
<td><strong>PaO₂ (mm Hg)</strong></td>
<td>71.1 ± 18.1</td>
<td>46.4 ± 14.2</td>
<td>&lt;0.001</td>
<td>35.1 ± 11.5</td>
</tr>
<tr>
<td><strong>PaCO₂ (mm Hg)</strong></td>
<td>42.5 ± 4.5</td>
<td>43.1 ± 4.7</td>
<td>NS</td>
<td>53.5 ± 4.7</td>
</tr>
<tr>
<td><strong>SaO₂ (%)</strong></td>
<td>93.7 ± 4.6</td>
<td>81.4 ± 11.8</td>
<td>&lt;0.001</td>
<td>63.9 ± 16.2</td>
</tr>
<tr>
<td><strong>CaO₂ (ml dl⁻¹)</strong></td>
<td>188 ± 18</td>
<td>16.6 ± 2.6</td>
<td>&lt;0.001</td>
<td>13.2 ± 3.1</td>
</tr>
<tr>
<td><strong>QICA (l min⁻¹)</strong></td>
<td>286.9 ± 75.8</td>
<td>285 ± 73</td>
<td>NS</td>
<td>427 ± 95</td>
</tr>
<tr>
<td><strong>QVA (l min⁻¹)</strong></td>
<td>119.5 ± 52.7</td>
<td>130 ± 63</td>
<td>NS</td>
<td>167 ± 81</td>
</tr>
<tr>
<td><strong>gCBF (l min⁻¹)</strong></td>
<td>812.8 ± 210.9</td>
<td>829 ± 247</td>
<td>NS</td>
<td>1094 ± 409</td>
</tr>
<tr>
<td><strong>CDO₂ (ml dl⁻¹ min⁻¹)</strong></td>
<td>146 ± 33</td>
<td>128 ± 34</td>
<td>NS</td>
<td>134 ± 60</td>
</tr>
</tbody>
</table>

PaO₂, arterial partial pressure of oxygen; PaCO₂, arterial partial pressure of carbon dioxide; SaO₂, arterial oxygen saturation; CaO₂, arterial oxygen content; QICA, internal carotid artery blood flow; QVA, vertebral artery blood flow; gCBF, global cerebral blood flow; CDO₂, cerebral oxygen delivery.

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

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>TLC Trial</th>
<th>FRC Trial</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Early EGP</td>
<td>Late EGP</td>
</tr>
<tr>
<td><strong>Left Ventricle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End-Diastolic Volume (ml)</td>
<td>200 ± 33</td>
<td>113 ± 20#</td>
<td>146 ± 24#</td>
</tr>
<tr>
<td>End-Systolic Volume (ml)</td>
<td>80 ± 17</td>
<td>51 ± 10#</td>
<td>75 ± 15</td>
</tr>
<tr>
<td>Stroke Volume (ml)</td>
<td>120 ± 21</td>
<td>62 ± 13#</td>
<td>71 ± 12#</td>
</tr>
<tr>
<td>Ejection Fraction (%)</td>
<td>60 ± 4</td>
<td>55 ± 5#</td>
<td>48 ± 4#</td>
</tr>
<tr>
<td>Cardiac Output (L min⁻¹)</td>
<td>7.95 ± 1.99</td>
<td>5.25 ± 1.11#</td>
<td>5.59 ± 0.81#</td>
</tr>
<tr>
<td><strong>Right Ventricle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End-Diastolic Volume (ml)</td>
<td>195 ± 29</td>
<td>112 ± 15#</td>
<td>140 ± 16#</td>
</tr>
<tr>
<td>End-Systolic Volume (ml)</td>
<td>77 ± 14</td>
<td>50 ± 7#</td>
<td>71 ± 6</td>
</tr>
<tr>
<td>Stroke Volume (ml)</td>
<td>118 ± 20</td>
<td>62 ± 12#</td>
<td>70 ± 12#</td>
</tr>
<tr>
<td>Ejection Fraction (%)</td>
<td>61 ± 4</td>
<td>55 ± 5#</td>
<td>49 ± 4#</td>
</tr>
<tr>
<td>Cardiac Output (L min⁻¹)</td>
<td>7.82 ± 1.9</td>
<td>5.21 ± 0.97#</td>
<td>5.53 ± 0.76#</td>
</tr>
</tbody>
</table>

Significant main effects for Condition and Time and a significant interaction effect Condition*TTime were observed for all right and left ventricular parameters listed in this table (P<0.001).
SpO₂ (%) vs Breath Hold Time (%)

- TLC
- FRC

Transcutaneous CO₂ (mm Hg) vs Breath Hold Time (%)

Condition P<0.05 & Time & Interaction P<0.001

Time P<0.001 & Interaction= P>0.05