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Cerebral oxidative metabolism is decreased with extreme apnoea in humans; impact of hypercapnia

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Key points

- The present study describes the cerebral oxidative and non-oxidative metabolism in man during a prolonged apnoea (ranging from 3 min 36 s to 7 min 26 s) that generates extremely low levels of blood oxygen and high levels of carbon dioxide.
- The cerebral oxidative metabolism, measured from the product of cerebral blood flow and the radial artery-jugular venous oxygen content difference, was reduced by ∼29% at the termination of apnoea, although there was no change in the non-oxidative metabolism.
- A subset study with mild and severe hypercapnic breathing at the same level of hypoxia suggests that hypercapnia can partly explain the cerebral metabolic reduction near the apnoea breakpoint.
- A hypercapnia-induced oxygen-conserving response may protect the brain against severe oxygen deprivation associated with prolonged apnoea.

Abstract Prolonged apnoea in humans is reflected in progressive hypoxaemia and hypercapnia. In the present study, we explore the cerebral metabolic responses under extreme hypoxia and hypercapnia associated with prolonged apnoea. We hypothesized that the cerebral metabolic rate for oxygen ($CMRO_2$) will be reduced near the termination of apnoea, attributed in part to the hypercapnia. Fourteen elite apnoea-divers performed a maximal apnoea (range 3 min 36 s to 7 min 26 s) under dry laboratory conditions. In a subset study with the same divers, the impact of hypercapnia on cerebral metabolism was determined using varying levels of hypercapnic breathing, against the background of similar hypoxia. In both studies, the $CMRO_2$ was calculated from the product of cerebral blood flow (ultrasound) and the radial artery-internal jugular venous oxygen content difference. Non-oxidative cerebral metabolism was calculated from the ratio of oxygen and carbohydrate (lactate and glucose) metabolism. The $CMRO_2$ was reduced by ∼29% ($P < 0.01$, Cohen's $d = 1.18$) near the termination of apnoea compared to baseline, although non-oxidative metabolism remained unaltered. In the subset study, in similar backgrounds of hypoxia (arterial O_2 tension: ∼38.4 mmHg), severe hypercapnia (arterial CO_2 tension: ∼58.7 mmHg), but not mild-hypercapnia (arterial CO_2 tension: ∼46.3 mmHg), depressed the $CMRO_2$ (∼17%, $P = 0.04$, Cohen's $d = 0.87$). Similarly to the apnoea, there was no change in the non-oxidative metabolism.

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These data indicate that hypercapnia can partly explain the reduction in $CMRO_2$ near the apnoea breakpoint. This hypercapnic-induced oxygen conservation may protect the brain against severe hypoxaemia associated with prolonged apnoea.

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Abbreviations CO, cardiac output; CDO_2, cerebral delivery of oxygen; $CMRO_2$, cerebral metabolic rate of oxygen; gCBF, global cerebral blood flow; Glu, glucose; Glu Ext, glucose extraction; HR, heart rate; ICA, internal carotid artery; Lac, lactate; Lac Ext, lactate extraction; MAP, mean arterial pressure; MCA, middle cerebral artery; MCAv, middle cerebral artery blood velocity; mild-HH, mild-hypercapnic hypoxia; OCI, oxidative carbohydrate index; OGI, oxidative glucose index; O_2 Ext, oxygen extraction fraction; S_{aO_2}, oxygen saturation; P_{CO_2}, partial pressure of carbon dioxide; P_{O_2}, partial pressure of oxygen; P_{ETCO_2}, partial pressure of end-tidal carbon dioxide; P_{ETO_2}, partial pressure of end-tidal oxygen; PCA, posterior cerebral artery; PCAv, posterior cerebral artery blood veleocity; QICA, blood flow of the right internal carotid artery; QVA, blood flow of the left vertebral artery; Severe-HH, severe-hypercapnic hypoxia; SV, stroke volume; v, venous; VA, vertebral artery.

Introduction

Although it accounts for only 2% of total body mass, the human brain utilizes a disproportionate 20% of the body's basal oxygen consumption. The relatively high cerebral metabolic rate of oxygen ($CMRO_2$) is required to support a high rate of ATP production and neuronal activity (Brown and Ransom, 2007). A constant oxygen supply is therefore obligatory for the maintenance of normal brain function. With respect to cerebral energy demands for oxygen, the current world record apnoea duration in humans of 11 min 35 s is truly remarkable.

In terms of oxygen conservation, decreasing the cerebral oxidative metabolism and increasing non-oxidative metabolism is of teleological benefit. Not surprisingly, a reduction in the $CMRO_2$ during deep hypothermia is readily utilized in medicine (e.g. during bypass surgery; Fukui and Takanashi, 2016) and can partly explain extreme survival following prolonged anoxic (>15 min) cold-water immersion (Young *et al.* 1980; Antretter *et al.* 1994). It appears, however, that moderate hypoxia alone does not provide the stimulus of oxygen conservation. For example, no appreciable changes in the $CMRO_2$ have been reported during isocapnic or poikilocapnic hypoxic breathing (P_{aO_2} of ∼40–55 mmHg) (Kety and Schmidt, 1948; Cohen *et al.* 1967; Bailey *et al.* 2009; Overgaard *et al.* 2012; Ainslie *et al.* 2014). Indeed, using recent magnetic imaging techniques, some studies report significant elevations in $CMRO_2$ by 5–10% with acute poikilocapnic hypoxia (Xu *et al.* 2012; Vestergaard *et al.* 2015). Recent and available data further indicate no increases in cerebral non-oxidative metabolism (estimated via the oxidative carbohydrate index; OCI) with isocapnic hypoxic breathing (Ainslie *et al.* 2014).

An increase in $CMRO_2$ with acute hypoxia shown by some studies (Vestergaard *et al.* 2015), but not all (Ainslie *et al.* 2014), may relate to the background P_{aCO_2} or pH. In one study (Ainslie *et al.* 2014), subjects were kept at eucapnia, whereas, in another study (Vestergaard *et al.* 2015), the hypoxia-induced increase in ventilation reduced P_{aCO_2} by an average of 10 mmHg (absolute 31 mmHg). A large body of both human and animal data indicates that changing P_{aCO_2} may impact $CMRO_2$, with hypocapnia increasing, and hypercapnia decreasing it; for a synopsis, see Yablonskiy (2011). Indeed, synaptic transmission (Dulla *et al.* 2005; Thesen *et al.* 2012) and phosphofructokinase activity (Folbergrova *et al.* 1975) are each dependent on pH. Because a prolonged breath-hold yields both extreme levels of hypoxia and hypercapnia (Willie *et al.* 2014; Bain *et al.* 2015*a*), it stands to reason that, in contrast to hypoxia alone, the $CMRO_2$ may be reduced during apnoea. Additionally, in contrast to hypoxic breathing (Ainslie *et al.* 2014), a greater increase in sympathetic nerve activity during apnoea, despite the same chemoreflex stimuli (Steinback *et al.* 2010*a*), may promote a shift towards non-oxidative metabolic pathways. For example, infusion of high dose adrenaline (0.08 μg kg^{-1} min^{-1} for 15 min) in humans significantly increases the percentage of non-oxidative cerebral metabolism (Seifert *et al.* 2009) and adrenaline appears to underlie the increased non-oxidative metabolism during exercise (Larsen *et al.* 2008). A shift towards non-oxidative cerebral metabolism, and a decrease in the $CMRO_2$, may in turn help explain how some elite breath-hold divers may hold their breath for over 10 min.

Despite obvious implications in neuropathology, describing the cerebral metabolic profile under severe apneic stress in humans has remained a methodological and ethical challenge. Elite breath-hold divers provide a unique population to test the limits of hypoxaemia tolerance beyond what is possible in otherwise healthy humans. As such, the primary purpose of the present study was to examine the oxidative and non-oxidative

cerebral metabolism during a prolonged dry-land apnoea in elite human breath-hold competitors. In a subset study, we gathered mechanistic information by quantifying the impact of hypercapnia/pH on cerebral metabolism against the background of hypoxia. We examined the hypotheses that (1) compared to a resting baseline, $CMRO_2$ will be reduced and indices of cerebral non-oxidative metabolism will be increased during a prolonged apnoea and (2) the reductions in $CMRO_2$ will partly be a function of a hypercapnic-mechanism.

Methods

Participants

Fourteen competitive and elite breath-hold divers (two females; age 29.5 ± 7.3 years; body mass index 23.5 ± 2.5 kg m^{-2}) were recruited from the Croatian national apnoea team, and provided their written informed consent for participation. All participants were actively competing, and had been practicing competitive breath hold diving for between 1.5 and 14.0 years (mean 5.2 ± 3.7 years). The majority of subjects were competitive in the discipline of dynamic apnoea (maximal swimming distance underwater). Some were also involved in various depth disciplines (with regular exposure to high pressure). Seven of the subjects were world-class free diving competitors, having been placed in the top ten within the last 3 years in international competition in at least one event. Three subjects had recently set new official world records. All subjects were normotensive and free from cardiovascular and respiratory disease. The ethical committees of the University of Split School of Medicine, the University of British Columbia and the University of South Wales approved the experimental procedures.

Experimental design

Experimentation was completed on a single day, following strict adherence to pre-testing protocol, including abstinence from vigorous exercise and alcohol at least 48 h, as well as from caffeine at least 12 h, before arriving at the laboratory. We did not obtain information on previous caffeine use. All testing was performed at the University of Split, School of Medicine, Department of Integrative Physiology. Upon arrival, a medical history and standard anthropometric and pulmonary functioning metrics were assessed. After, a 20-gauge arterial catheter (Arrow, Markham, Ontario, Canada) was placed in the right radial artery, and a central venous catheter (PediaSat Oximetry Catheter; Edwards Lifesciences, Irvine, CA, USA) was placed in the right internal jugular vein and directed cephalad to the jugular bulb. Using the identical technique and performed by the same anaesthesiologist, correct placement of the jugular bulb catheter has been confirmed by lateral skull X-ray (Ainslie *et al.* 2014). Arterial and jugular cannulations were completed under ultrasound guidance with local anaesthesia (1% lidocaine) and under sterile conditions. Following cannulation, the catheters were attached to an in-line waste-less sampling system and a pressure transducer located at the height of the right atrium (VAMP System; Edwards Lifesciences) and a TruWave transducer (Edwards Lifesciences). Subjects were then instrumented with the remaining measurements (see measurements).

Apnoea. Baseline measurements were acquired following a minimum of 30 min of supine rest, and prior to preparatory apnoeas. After baseline measurements were acquired, each participant completed two preparatory (non-experimental) apnoeas. The first preparatory apnoea was performed after a normal end-expiration until seven involuntary breathing movements were attained. Two minutes later, the second preparatory apnoea was performed at total lung capacity until ten involuntary breathing movements were attained. These preparatory apnoeas were performed to generate the longest possible time for the experimental maximal apnoea, chosen by the national Croatian apnoea team coach (Ivan Drvis) based on a best-suited standardized preparatory phase for all subjects. Following the preparatory phase, subjects rested for 6 min before commencing the maximal apnoea. Subjects were allowed to lung pack (glossopharyngeal insufflation) prior to the experimental apnoea, based on individual preference to attain the longest apnoea possible. Of note, the individual magnitude of glossopharyngeal insufflation will have profound effects on the initial haemodynamic changes at the apnoea onset (Batinic *et al.* 2011). The magnitude of glossopharyngeal insufflation was not measured. Data were collected throughout the maximal breath-hold until breathing was resumed. Arterial and jugular venous blood draws were attained prior to the preparatory apnoeas (baseline), every 30 s throughout the maximal apnoea, and immediately upon termination (100% of apnoea).

Subset study: end-tidal forcing. Following the maximal apnoea, participants' rested supine for a minimum of 30 min before starting the hypercapnic-hypoxic breathing subset trials. The mild-hypercapnic hypoxic (mild-HH) and severe-hypercapnic hypoxia (severe-HH) breathing was performed using a custom built end-tidal (P_{ET}) forcing system. Subjects were equipped with a mouthpiece and nose clip, and were instructed to breath normally. Gases were sampled at the mouthpiece and analysed by a calibrated gas analyser (ML206; ADInstruments, Colorado Springs, CO, USA). Respiratory flows were measured by pneumotachography (HR 800L; Hans Rudolph, Shawnee, KS, USA). Custom written software (Labview, Austin, TX, USA) determined

the breath-by-breath tidal volumes and end-tidal partial pressures of oxygen and carbon dioxide (P_{ETO_2} and P_{ETCO_2}). The end-tidal forcing system prospectively delivered inspired gases to clamp P_{ETO_2} and P_{ETCO_2} at desired input levels. Independently controlled solenoid valves delivered the desired volumes of O_2, CO_2, and N_2 as determined by an error reduction algorithm incorporating P_{ETO_2}, P_{ETCO_2} and inspiratory and expiratory tidal volume from the last breath. Levels of desired P_{ETO_2} for both mild-HH and severe-HH were individualized to the average P_{aO_2} measured during the last 90 s of the maximal breath-hold. Levels of P_{ETCO_2} during the mild-HH trial were individualized to the P_{aCO_2} achieved at ∼50% of the maximal apnoea. Levels of P_{ETCO_2} during the severe-HH trial were targeted for +10 mmHg from the mild-HH. Each condition (mild-HH and severe-HH) lasted 5 min, and the severe-HH trial was performed immediately following mild-HH, at the same time as continually maintaining hypoxia. Arterial and jugular venous blood draws were taken after 4 min 30 s in each stage.

Measurements

Blood gases, oximetry and metabolites. Measurements of arterial and jugular venous PO_2, PCO_2, O_2 saturation (S_{aO_2}), glucose (Glu) and lactate (La) were analysed immediately following each draw using a commercially available cassette based analyser (ABL90 Flex; Radiometer, Copenhagen, Denmark). Each measure required <2 ml of blood. Arterial (radial) and jugular venous draws were taken at the same time.

Catecholamines. Blood samples (7 ml) were collected into tubes containing K-EDTA and centrifuged at 600*g* for 10 min at 4°C. Plasma (2 ml sample volume) was transferred into cryovial tubes and immediately snap-frozen under liquid nitrogen (N_2, Cryopak CP100, Taylor-Wharton, Theodore, AL, USA) and then stored at –80°C prior to analysis. Analysis was performed within 1 month of receipt of the sample. All chemicals including reagents and standards were of the highest available purity from Sigma-Aldrich (Poole, UK). Plasma concentrations of adrenaline, and noradrenaline were measured by reverse-phase ion pair high performance liquid chromatography (Gilson ASTED.XL; Anachem Ltd, Luton, UK) with electrochemical detection (ESA Coulochem II; ESA Analytical, East Grinstead, UK) (Bouloux *et al.* 1985). The coefficient of variation was 5% for noradrenaline at 4.8 nmol l^{-1} and 8% for adrenaline at 1.0 nmol l^{-1}.

Cardiovascular. Heart rate (HR) was obtained from the R-R intervals measured from a three-lead ECG. Beat-to-beat arterial blood pressure was measured by finger photoplethysmography (Finometer PRO; Finapress Medical Systems, Amsterdam, Netherlands) normalized to manual cuff measurements of the brachial artery. Intra-radial arterial pressure and jugular venous pressure were also recorded; however, as a result of the frequency of blood sampling, measurements were available for ∼5- to 10-s bins around each blood draw. Online calculations of stroke volume were obtained using the three-element nonlinear arterial model of the arterial blood pressure waveform (Finometer). Cardiac output was then derived from the product of stroke volume and HR.

Cerebrovascular. The cerebral blood velocity of the right middle cerebral artery (MCAv) and left posterior cerebral artery (PCAv) was measured using a 2-MHz pulsed transcranial Doppler ultrasound system (Spencer Technologies, Seattle, WA, USA). A specialized headband fixation device (model M600 bilateral head frame; Spencer Technologies) was used to secure the probes in position. Signal quality was optimized using standardized search techniques that produce test–retest reliability of ∼3% and 2% for MCAv and PCAv, respectively. MCAv was insonated through the left temporal window, at a depth 1 cm distal to the MCA–anterior cerebral artery bifurcation. PCAv was insonated at the P1 segment through the right temporal window.

Volumetric blood flow of the right internal carotid artery (QICA) and left vertebral artery (QVA) was concomitantly measured using duplex vascular ultrasound (Terason 3000; Teratech, Burlington, MA, USA). The right ICA was insonated ∼2cm from the carotid bifurcation, whereas the left VA was insonated at the C5-C6 or C5-C4 space depending on individual anatomy. Care was taken to standardize the insonation location between measurements within subjects. The steering angle was fixed to 60° among all trials, and the sample volume was placed in the centre of the vessel adjusted to cover the entire vascular lumen. All files were collected in AVI format for offline analysis at 30 Hz using custom designed software (Woodman *et al.* 2001). Simultaneous measurements of luminal diameter and velocity over a minimum of 10 cardiac cycles were used to calculate flow.

Calculations

Under the assumption of symmetrical blood flow of contralateral ICA and VA arteries, global CBF (gCBF) was calculated as: gCBf (ml min^{-1}) = (QICA × 2) + (QVA × 2).

As a result of the onset of involuntary breathing movements during the latter ∼60% of the breath-hold (struggle phase), which encompasses large movements of the chest wall and movement of the sternocleidomastoid muscles, we were unable to attain reliable QICA and QVA values in all subjects for the entire breath-hold. However, we were able to reliably track ICA and VA diameter up to

the breath-hold termination. As such, where simultaneous measurements of ICA or VA velocity with diameter were missing, gCBF was derived from incorporating changes in MCAv (indicative of ICA velocity) and PCAv (indicative of VA velocity) with correction for diameter. That is, the percentage change in MCAv or PCAv was estimated to equate with ICA or VA velocity changes ($r^2 = 0.7$, where simultaneous measurements are available). The inclusion of changes in diameter measurements ensured that gCBF was not underestimated using the metrics of ΔMCAv and ΔPCAv only (Coverdale *et al.* 2014).

Contents of arterial (CaO_2) and venous (CvO_2) oxygen were calculated as:

$$CaO_2(\text{ml dl}^{-1}) = [\text{Hb}] \times 1.36 \times \frac{SaO_2(\%)}{100} + 0.003 \times P_{aO_2}$$

$$CvO_2(\text{ml dl}^{-1}) = [\text{Hb}] \times 1.36 \times \frac{SvO_2(\%)}{100} + 0.003 \times P_{VO_2}$$

where 1.36 is the affinity for oxygen to haemoglobin for a given arterial saturation and 0.003 is the percentage of oxygen dissolved in the blood. Values are expressed as ml of O_2 per 100 ml of blood (ml dl^{-1}).

Cerebral delivery of oxygen (CDO_2) was calculated as:

$$CDO_2(\text{ml min}^{-1}) = CaO_2 \times \frac{g\text{CBF}}{100}$$

The cerebral metabolic rate of oxygen ($CMRO_2$) was calculated as:

$$CMRO_2(\text{ml min}^{-1}) = (CaO_2 - CvO_2) \times \frac{g\text{CBF}}{100}$$

Cerebral oxygen, glucose and lactate extraction fraction were calculated from the arterial-venous content difference divided by the arterial value, and then multiplied by 100.

The OCI, which provides an estimation of oxidative *vs.* non-oxidative metabolism (Ainslie *et al.* 2014), was calculated from the equation below. The oxidative glucose index (OGI) was calculated the same way but with omission of the addition of the lactate arterial–venous difference. In short, a reduction of 6 reflects the presence of non-oxidative metabolism given that the oxidization of glucose by oxygen requires one oxygen molecule per six carbon atoms found in glucose, and carboxylation of lactate to pyruvate acid yields only one molecule of pyruvate, compared to the two derived from the breakdown of glucose. This was then converted to a percent OCI by dividing by 6 and then multiplying by 100.

$$\text{OCI}(\%) = \frac{CaO_2 - CVO_2}{\frac{(Glu_a - Glu_v) + 0.5(Lac_a - Lac_v)}{6}} \times 100$$

Statistical analysis

Data are the mean ± SD. Baseline measurements were acquired during quite rest prior to the preparatory apnoeas (~15 min before the maximal apnoea) and 1 min before the end-tidal forcing trials. Baseline measurements were averaged over 1 min around the baseline blood draw. Mean values for MAP (Finometer corrected with intra-arterial) HR and gCBF during the apnoea were averaged over 20 s around the blood draws.

Statistical analysis for both apnoea and the end-tidal forcing was performed using one-way repeated measures ANOVA. When a main effect was observed, pre-planned paired comparisons were performed to baseline only using two-tailed Student *t* tests. A Bonferroni adjustment was applied to correct for multiple comparisons (six stages of the breath hold and three stages of end-tidal forcing). When significant, Cohen's *d* was calculated for effect size of the primary outcome variable ($CMRO_2$).

Results

Apnoea

Maximal apnoea times ranged from 3 min 36 s to 7 min 26 s with an average of 5 min 14 s.

Blood gases, oximetry and metabolites. Arterial and jugular venous blood gas, oximetry, pH and metabolite data are provided in Table 1. To illustrate the near normalization of arterial with venous blood at the end of the apnoea, arterial and jugular venous PO_2 from baseline to 100% of the apnoea are shown in Fig. 1. As expected, there was a main effect for all blood gas variables (all $P < 0.05$). Significant *post hoc* comparisons to baseline are denoted in Table 1.

Haemodynamics. Heart rate (HR), mean arterial pressure (MAP), stroke volume (SV), cardiac output (CO) and global cerebral blood flow (gCBF) are provided in Table 2. There was a main effect of for all variables (all $P < 0.05$). Despite a reduction in CO from baseline, the MAP was elevated throughout the majority of the apnoea (all $P < 0.05$), indicating an elevated total peripheral resistance. The reductions in indices of SV from 20–80% of the apnoea (all $P < 0.05$) are attributable to the glossopharyngeal insufflation prior to the start of the apnoea. As expected, gCBF was reduced at the onset of the apnoea, and then was progressively elevated by ~70% at 100% of the apnoea. All

Table 1. Arterial (radial) and cerebral venous (internal jugular bulb) measurements of the P_{CO_2}, S_{aO_2}, haematocrit (Hct), haemoglobin (tHb), Glu, Lac and pH at baseline and during 20% increments of the maximal apnoea

	P_{CO_2} (mmHg)	S_{aO_2} (%)	Hct (%)	tHb (mmol L^{-1})	Glu (mmol L^{-1})	Lac (mmol L^{-1})	pH
Baseline							
Arterial	40.9 ± 3.3	97.8 ± 0.9	44.4 ± 3.1	14.5 ± 1.0	5.6 ± 0.8	0.8 ± 0.3	7.41 ± 0.02
Venous	51.8 ± 3.6	62.1 ± 5.1	44.1 ± 2.6	14.4 ± 0.9	4.9 ± 0.8	0.8 ± 0.3	7.36 ± 0.02
20%							
Arterial	32.4 ± 5.1*	99.0 ± 0.4*	44.3 ± 3.2	14.4 ± 1.0	5.3 ± 0.6	0.9 ± 0.2	7.48 ± 0.04*
Venous	47.6 ± 3.5*	50.5 ± 8.1*	45.3 ± 3.4	14.8 ± 1.1	4.6 ± 0.6	0.9 ± 0.2	7.40 ± 0.02*
40%							
Arterial	39.6 ± 4.3	96.9 ± 1.9	44.8 ± 4.2	14.6 ± 1.4	5.3 ± 0.6	0.9 ± 0.2	7.42 ± 0.02
Venous	50.0 ± 3.1	61.2 ± 6.5	45.5 ± 3.6	14.9 ± 1.2	4.8 ± 0.6	0.9 ± 0.3	7.38 ± 0.02
60%							
Arterial	46.1 ± 5.0*	89.2 ± 5.1*	45.4 ± 4.4	14.8 ± 1.5	5.3 ± 0.7	1.0 ± 0.3	7.38 ± 0.03*
Venous	52.5 ± 3.4	63.7 ± 6.0	45.7 ± 3.8	14.9 ± 1.2	4.9 ± 0.6	1.0 ± 0.3	7.36 ± 0.02
80%							
Arterial	49.7 ± 4.7*	80.6 ± 7.8*	45.5 ± 3.7*	14.8 ± 1.2*	5.5 ± 0.6	1.0 ± 0.3	7.36 ± 0.02*
Venous	54.9 ± 3.7	59.5 ± 6.0	45.7 ± 3.2*	14.9 ± 1.0*	5.1 ± 0.6	1.0 ± 0.3	7.34 ± 0.02*
100%							
Arterial	53.4 ± 4.8*	60.9 ± 14.0*	45.8 ± 3.4*	14.9 ± 1.1*	5.4 ± 0.7	1.1 ± 0.3*	7.34 ± 0.02*
Venous	57.8 ± 4.7*	45.6 ± 13.0*	46.0 ± 3.7*	15.0 ± 1.2*	5.1 ± 0.6	1.2 ± 0.4*	7.32 ± 0.02*

*Significant difference from baseline.

significant *post hoc* comparisons to baseline are denoted in Table 2.

Cerebral metabolic dynamics and oxygen delivery. Cerebral delivery of oxygen (CDO_2), oxygen extraction (O_2 Ext), lactate extraction (Lac Ext), glucose extraction (Glu Ext), OCI and $CMRO_2$ are provided in Table 3. Individual $CMRO_2$ at baseline and at 100% of the apnoea are shown in Fig. 2. There was a main effect of CDO_2 ($P < 0.01$), O_2 Ext ($P < 0.01$), Glu Ext ($P < 0.01$), OCI ($P = 0.02$) and $CMRO_2$ ($P < 0.01$). Compared to baseline, the $CMRO_2$ was reduced by ∼29% at 100% of the apnoea ($P < 0.01$, $d = 1.18$) but similar at all other time points (all $P < 0.05$). The O_2 Ext was increased at 20% of the apnoea, and decreased at the latter half of the apnoea (all $P < 0.05$). The CDO_2 fell below baseline values at 20% of the apnoea ($P < 0.01$), above baseline at 60% and 80% of the apnoea (both $P < 0.01$) and was similar to baseline at 100% of the apnoea. All significant *post hoc* comparisons to baseline are denoted in Table 3. Although there was a main effect of OCI, no *post hoc* comparisons were different. Values for OGI were identical to the OCI. There was no correlation between the absolute or change in P_{aCO_2} or P_{aO_2} with the change in $CMRO_2$ from baseline to 100% of the apnoea.

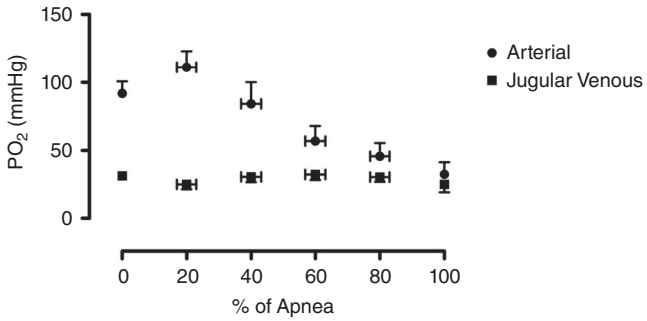

Figure 1. Absolute arterial and internal jugular venous artial pressure of oxygen
Mean ± SD of absolute arterial (P_{aO_2}) and internal jugular venous (P_{VO_2}) partial pressure of oxygen throughout the duration of the maximal apnoea.

Catecholamines. The radial arterial plasma adrenaline and noradrenaline increased by ∼380% (670 ± 380 to 2012 ± 1020 pmol l^{-1}) and ∼483% (1893 ± 673 to 8260 ± 4375 pmol l^{-1}) from baseline to 100% of the apnoea, respectively (both $P < 0.01$). Similarly, the jugular venous plasma adrenaline and noradrenaline increased by ∼258% (584 ± 258 to 1762 ± 801 pmol l^{-1}) and ∼385% (1860 ± 577 to 8559 ± 3707 pmol l^{-1}) (both $P < 0.01$). The arterial–venous adrenaline and noradrenaline differences were not different from baseline to 100% of the apnoea (both $P < 0.05$).

Subset study: variable hypercapnic breathing with similar hypoxia

Three participants were unable to complete the end-tidal forcing trial as a result of ventilatory volumes during the

Table 2. HR, SV, CO, MAP and gCBF at baseline and during 20% increments of the maximal apnoea

	HR (beats min^{-1})	SV (ml)	CO (mmol L^{-1})	MAP (mmHg)	gCBF (ml min^{-1})
Baseline	64 ± 11	108 ± 21	6.78 ± 1.65	88 ± 8	610 ± 153
20%	80 ± 17*	58 ± 16*	4.52 ± 1.47*	92 ± 10	469 ± 140*
40%	77 ± 16*	62 ± 13*	4.54 ± 1.03*	99 ± 11*	613 ± 196
60%	66 ± 16	81 ± 15*	5.11 ± 1.46*	115 ± 21*	828 ± 267*
80%	59 ± 14	95 ± 18	5.41 ± 1.52*	131 ± 21*	991 ± 325*
100%	56 ± 15	96 ± 33	5.17 ± 1.91*	139 ± 23*	1041 ± 318*

*Significant difference from baseline.

Table 3. CDO_2, O_2 Ext, Lac Ext, Glu Ext, OCI and $CMRO_2$ at baseline and during 20% increments of the maximal apnoea

	CDO_2 (ml min^{-1})	O_2 Ext (%)	Lac Ext (%)	Glu Ext (%)	OCI (%)	$CMRO_2$ (ml min^{-1})
Baseline	122 ± 30	37 ± 6	−2 ± 11	12 ± 3	83 ± 13	45 ± 11
20%	92 ± 24*	47 ± 10*	−3 ± 17	12 ± 6	127 ± 75	43 ± 10
40%	120 ± 34	36 ± 7	−3 ± 20	9 ± 6	125 ± 61	41 ± 10
60%	152 ± 48*	28 ± 7*	0 ± 15	7 ± 4*	139 ± 94	41 ± 12
80%	163 ± 50*	26 ± 4*	−3 ± 17	7 ± 2*	92 ± 37	41 ± 11
100%	128 ± 36	25 ± 9*	−2 ± 13	5 ± 3*	92 ± 57	32 ± 12*

*Significant difference from baseline.

severe-hypercapnic hypoxia (severe-HH) that exceeded the capacity of the end-tidal forcing system (i.e. >100 L min^{-1}). The sample size for the hypoxic breathing trial was in turn reduced from 14 to 11 subjects.

Blood gases, oximetry and metabolites. Arterial and jugular venous blood gas, oximetry, pH and metabolite data are provided in Table 4. By design, there was an increase in P_{aCO_2} and a reduction in P_{aO_2}, SaO_2 and pH in both mild-HH and severe-HH from baseline. There was no main effect of glucose or lactate (both $P < 0.05$).

Haemodynamics. Absolute HR, MAP, SV, CO and gCBF are provided in Table 5. Except for SV, all measurements in both conditions were elevated from baseline (all $P < 0.05$).

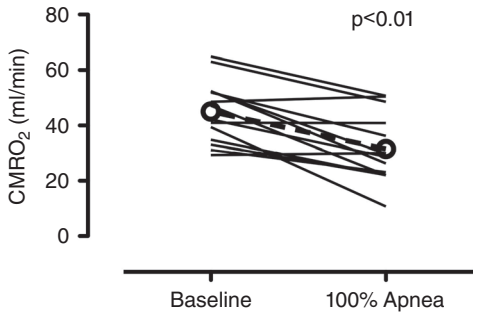

Figure 2. Individual data of the absolute $CMRO_2$ before the apnoea (baseline) and at apnoea termination (100% apnoea)
Dashed line with open circles denotes mean data. Compared to baseline, the $CMRO_2$ was reduced by ∼29% at 100% of the apnoea ($P < 0.01$, $d = 1.18$).

Cerebral metabolism and oxygen delivery. Absolute CDO_2, O_2 Ext, Glu Ext, Lac Ext, $CMRO_2$ and OCI are provided in Table 6. Individual $CMRO_2$ are shown in Fig. 3. There was a main effect of $CMRO_2$ ($P < 0.01$). Compared to baseline, *post hoc* comparisons revealed no change in $CMRO_2$ during mild-HH ($P > 0.05$), although there was an ∼17% reduction in the severe-HH condition ($P = 0.04$, $d = 0.87$). The OCI was unchanged from baseline in both conditions. Values for OGI were identical to the OCI. As expected in both mild-HH and severe-HH, the O_2 Ext was reduced from baseline, and the CDO_2 was elevated (all $P < 0.05$).

Discussion

The present study reports the first known measurements of cerebral metabolism in humans during a prolonged dry apnoea lasting on average greater than 5 min. A marked reduction in $CMRO_2$ was observed immediately prior to the termination of the apnoea (∼29%, $d = 1.18$) compared to baseline measurements. By contrast, there was no change at any time point in the indirect estimation of non-oxidative metabolism, despite an ∼380% increase in arterial adrenaline concentrations from baseline to the end of apnoea. With manipulation of end-tidal gases, mild hypercapnic hypoxia had little influence on $CMRO_2$; however, the same level of hypoxia but with severe hypercapnia reduced the $CMRO_2$ by ∼17% ($d = 0.87$). We

Table 4. Arterial (radial) and cerebral venous (internal jugular bulb) measurements of P_{O_2} and P_{CO_2}, S_{aO_2}, haematocrit (Hct), haemoglobin (tHb), Glu, Lac and pH at baseline and during similar hypoxic breathing with mild-HH and severe-HH

	P_{O_2} (mmHg)	P_{CO_2} (mmHg)	S_{aO_2} (%)	Hct (%)	tHb (mmol L^{-1})	Glu (mmol L^{-1})	Lac (mmol L^{-1})	pH
Baseline								
Arterial	91.6 ± 13.7	38.6 ± 5.1	98 ± 1	43.9 ± 3.7	14.3 ± 1.2	5.4 ± 0.5	0.9 ± 0.3	7.43 ± 0.05
Venous	29.5 ± 4.1	50.1 ± 4.2	60 ± 5	44.6 ± 4.1	14.6 ± 1.3	4.9 ± 0.6	1.0 ± 0.3	7.37 ± 0.04
Mild-HH								
Arterial	38.9 ± 7.8*	46.3 ± 3.2*	75 ± 8*	44.7 ± 3.8*	14.6 ± 1.2*	5.3 ± 0.5	0.9 ± 0.3	7.38 ± 0.03*
Venous	28.0 ± 3.4	52.6 ± 2.6*	54 ± 7*	44.6 ± 3.4	14.6 ± 1.1	5.0 ± 0.5	0.9 ± 0.3	7.35 ± 0.02*
Severe-HH								
Arterial	38.0 ± 8.9*	58.7 ± 4.3*	68 ± 12*	45.3 ± 3.4*	14.8 ± 1.1*	5.3 ± 0.4	0.8 ± 0.3	7.30 ± 0.02*
Venous	30.8 ± 5.5	62.3 ± 4.1*	55 ± 11*	45.2 ± 3.6	14.8 ± 1.2	5.2 ± 0.4	0.9 ± 0.3	7.29 ± 0.02*

*Significant difference from baseline.

Table 5. HR, SV, CO, MAP and gCBF during baseline and hypoxic breathing with mild-HH and severe-HH

	HR (mmHg)	SV (ml)	CO (L min^{-1})	MAP (mmHg)	gCBF (ml min^{-1})
Baseline	69 ± 18	102 ± 18	7 ± 1	89 ± 6	593 ± 151
Mild-HH	88 ± 14*	104 ± 17	9 ± 2*	102 ± 11*	983 ± 204*
Severe-HH	94 ± 16*	107 ± 15	10 ± 3*	113 ± 18*	1340 ± 343*

*Significant difference from baseline.

Table 6. QICA, QVA, flow velocity in the MCA and PCA, total CDO_2 and total $CMRO_2$ during baseline and hypoxic breathing with mild-HH and severe-HH

	CDO_2 (ml min^{-1})	O_2 Ext (%)	Lac Ext (%)	Glu Ext (%)	OCI (%)	$CMRO_2$ (ml min^{-1})
Baseline	117 ± 32	38 ± 7	–5 ± 6	10 ± 5	88 ± 16	44 ± 13
Mild-HH	149 ± 32*	28 ± 5*	2 ± 8	7 ± 3	80 ± 18	41 ± 11
Severe-HH	186 ± 53*	19 ± 4*	–5 ± 15	3 ± 3*	80 ± 26	34 ± 11*

*Significant difference from baseline.

interpret these findings to indicate that the increased levels of hypercapnia and reduced pH partly explain the reduction in $CMRO_2$ near the termination of apnoea.

Impact of hypercapnia on the $CMRO_2$

The notion of a reduced $CMRO_2$ resulting from hypercapnia is not novel but has remained debatable (Yablonskiy, 2011). With obvious implications in general medicine and anaesthesia (Waaben *et al.* 1989), the impact of P_{aCO_2} on $CMRO_2$ deserves attention. Unfortunately, the results obtained in human studies with similar MRI-based experimental designs and in the absence of anaesthesia have remained inconsistent. For example, Jain *et al.* (2011) report no change in $CMRO_2$ with an ∼8 mmHg increase in end-tidal CO_2; Chen *et al.* (2010) report an ∼7% reduction in $CMRO_2$ with an ∼9 mmHg increase in end-tidal CO_2; and Xu *et al.* (2011) report an ∼13% reduction in $CMRO_2$ with an ∼7 mmHg increase in end-tidal CO_2. Related to this, an increase in $CMRO_2$ has been shown during high altitude (Smith *et al.* 2013), which is suspected to be a consequence of the hyperventilatory-induced hypocapnia and therefore brain alkalosis. This finding is corroborated by the finding that an increase in $CMRO_2$ following 6 h of normobaric hypoxia is removed with acetazolamide (Wang *et al.* 2015).

Using arterial and cerebral venous sampling, the present study provides evidence for reductions in $CMRO_2$ with hypercapnia, at least in the presence of hypoxia. The pH-dependent activity of phosphofructokinase (the enzyme responsible for the phosphorylation of fructose 6-phosphate in glycolysis) provides mechanistic support for reductions in $CMRO_2$ with hypercapnia. Indeed, an accumulation of glucose 6-phosphate and fructose 6-phosphate is shown in rats exposed to acute hypercapnia, which cannot be explained by glycogen breakdown (Folbergrova *et al.* 1975). Additionally, hypercapnia has consistently been shown to depress cortical activity, which is suspected to result from acidotic-induced increases in extracellular adenosine (Dulla *et al.* 2005; Zappe *et al.*

2008; Thesen *et al.* 2012). Ultimately, the bulk of literature now collectively supports a P_{aCO_2} modulation of $CMRO_2$. The end-tidal forcing data in the present study may indicate that this modulation is dose-dependent, whereby a threshold change in P_{aCO_2} is required.

Non-oxidative metabolism and adrenaline

During apnoea, cessation of ventilation, chemosensory stimuli and baroreflex inhibition significantly increase muscle sympathetic nerve activity (burst frequency, magnitude and recruitment) (Heusser *et al.* 2010; Steinback *et al.* 2010*b*) and therefore catecholamine release. Ascribed to the known effect of catecholamines on muscle metabolism (Tank and Lee Wong, 2015), an increase in whole-body anaerobic metabolism is often held integral to the mammalian dive reflex (Blix and Folkow, 2011). Whether the cerebral tissue significantly partakes in the non-oxidative metabolism is unclear, although it is generally accepted that, similar to in the muscle, adrenaline (but not noradrenaline) increases the cerebral non-oxidative metabolism (Seifert *et al.* 2009). The ∼380% increase in arterial adrenaline from baseline to the termination of apnoea therefore offers potential for an increased cerebral non-oxidative metabolism (Seifert *et al.* 2009). However, there was no reduction in the indirect estimation of non-oxidative carbohydrate use (OCI) at any time point during the apnoea compared to baseline. Indeed, from 20% to 60% of the apnoea, non-oxidative metabolism trended above 100%. An oxidative carbohydrate index above 100% may indicate the presence of carbon oxidation from sources other than arterial glucose or lactate. Based on animal studies, these data may in turn indicate an allosteric glycogen mobilization at the astrocytes (Saez *et al.* 2014).

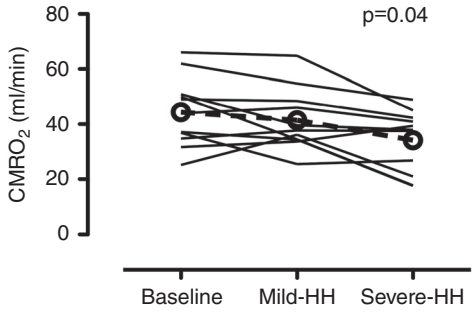

Figure 3. Individual data of the absolute $CMRO_2$ at baseline, during mild-HH and severe-HH
Hypoxia was on average a partial pressure of arterial oxygen of ∼38.5 mmHg for both mild-HH and severe-HH. Hypercapnia was on average set at an arterial partial pressure of carbon dioxide of ∼46.3 mmHg and ∼58.7 mmHg in the mild-HH and severe-HH conditions respectively. Dashed line with open circles denotes mean data. There was a ∼17% reduction in the severe-HH condition ($P = 0.04$, $d = 0.87$) compared to baseline.

Under the same metabolic paradigm by which adrenaline increases non-oxidative metabolism, adrenaline should also increase the oxidative metabolism (Tank and Lee Wong, 2015). For example, I.V. adrenaline infusion (∼ 8 μg ml^{-1} min^{-1}), with concurrent increases in MAP to ensure amine passage across the blood–brain barrier, significantly increases the $CMRO_2$ in humans (King *et al.* 1952). However, when the adrenaline concentrations result in glycogenolytic and glycolytic flux being increased and impacting the $CMRO_2$, circulating blood glucose would also be elevated (Seifert *et al.* 2009; Tank and Lee Wong, 2015). Therefore, the slightly reduced arterial glucose, reduced $CMRO_2$ and unchanged oxidative carbohydrate index collectively indicate that the ∼380% increase in adrenaline was insufficient to elicit noticeable metabolic changes. This is perhaps not surprising given that, when non-oxidative metabolism does increase, plasma adrenaline may be increased >10-fold (e.g. during exhaustive exercise) (Pott *et al.* 1996; Tank and Lee Wong, 2015). Of note, anaerobic-tolerant animals adapt to hypoxia by slowing the oxidative metabolism, rather than increasing the non-oxidative metabolism (Hochachka *et al.* 1996). Our findings reveal that a similar metabolic outcome is initiated in humans during prolonged apnoea.

Other potential modulating factors of the cerebral metabolic response

Although we emphasize the impact of P_{aCO_2} on $CMRO_2$, other factors, including oxygen-conserving reflexes attending the dive reflex, cannot be dismissed. Indeed, it is conceivable that the $CMRO_2$ may be reduced to a greater extent had the apnoea been performed in water, with activation of the trigeminal nerve and therefore a stronger oxygen-conserving/dive reflex (Schaller *et al.* 2009; Lemaitre *et al.* 2015). However, it appears that the activation of the trigeminal nerve has a greater responsibility for blood redistribution (from the periphery to the brain) and bradycardia, without directly influencing the $CMRO_2$ (Reis *et al.* 1997; Schaller *et al.* 2009; Lemaitre *et al.* 2015). Reductions in $CMRO_2$ associated with oxygen-conserving reflexes may relate more to mechanisms observed in rapid ischaemic preconditioning (Gidday, 2006; Schaller *et al.* 2009). In such a case, neural inhibitory factors (e.g. adenosine and ATP sensitive potassium channels) are probably responsible, as reported in the anoxic tolerant turtle (Perez-Pinzon *et al.* 1993). It is difficult to speculate on these potential oxygen-conserving mechanisms in the present study; however, given that the average level of acidosis was greater in the severe-hypercapnic hypoxia trial than it was at the termination of apnoea (arterial pH: 7.30 *vs.* 7.34, respectively), oxygen-conserving reflexes independent of acidosis probably also contributed to the reduction in

$CMRO_2$ during apnoea. It should also be emphasized that the metabolic changes associated with P_{aCO_2} described in the present study are against the background of hypoxia. Nevertheless, although acidosis decreased the oxygen saturation for the same P_{aO_2} during severe-hypercapnic hypoxia compared to mild-hypercapnic hypoxia (via the Bohr effect; Table 5), this probably did not influence the $CMRO_2$. Indeed, using the same technique, we have demonstrated that similar eucapnic oxygen saturations (∼70%) of 15 min in duration do not alter the $CMRO_2$ (Ainslie *et al.* 2014). If anything, the further oxygen desaturation would have increased the $CMRO_2$, rather than decrease it (Xu *et al.* 2012; Vestergaard *et al.* 2015). Finally, the gCBF was higher in the severe-HH condition compared to the mild-HH condition. Therefore, it may be suggested that reduced temperature (via increased tissue to blood heat transfer; Bain *et al.* 2015*b*) or a potential oxygen diffusion limitation can partly explain the reduction in $CMRO_2$. A diffusion limitation, however, is not probable given that cerebral O_2 extraction is well maintained even during exercise under both normoxic and hypoxic conditions (Smith *et al.* 2014). A diffusion limitation is also not probable based on the mathematical cerebral blood flow/metabolism relationship described by Gjedde (2005).

Surprisingly, at the termination of apnoea, neither the P_{aO_2}, nor the P_{aCO_2} was independently correlated with the reduction in $CMRO_2$. This may suggest individual variability in the $CMRO_2$ response to extreme blood gas changes, an interacting facet of hypoxia and hypercapnia, or the impact of other poorly defined brain oxygen-conserving reflexes in humans (Schaller *et al.* 2009).

Conclusions

In summary, the present study reports a clear reduction in $CMRO_2$ immediately prior to the termination of a prolonged apnoea, which is attributable in part to hypercapnia. By contrast, there appears to be no change in non-oxidative metabolism, despite a three- to four-fold increase in arterial adrenaline concentrations. To our knowledge, this is the first study to examine the cerebral metabolism in healthy humans during prolonged apnoea, yielding some of the most extreme levels of hypoxia and hypercapnia reported to date. It is suggested that hypercapnia promotes brain oxygen-conservation, and therefore provides a tenable protective mechanism against severe hypoxia relating to apnoea.

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Additional information

Competing interests

The authors declare that they have no competing interests.

Author contributions

ARB, PNA, ZD, ID and DBM conceived and designed the research. All authors performed the experiments. ARB analysed the data. All authors interpreted the results of the experiments. ARB prepared the artwork. ARB drafted the manuscript. All authors edited and revised the manuscript and approved the final version of the manuscript submitted for publication. All authors agree to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part 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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