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RESEARCH PAPER

Alterations in resting cerebrovascular regulation do not affect reactivity to hypoxia, hyperoxia or neurovascular coupling following a SCUBA dive

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Abstract

Reductions in vascular function during a SCUBA dive – due to hyperoxia-induced oxidative stress, arterial and venous gas emboli and altered endothelial integrity – may also extend to the cerebrovasculature following return to the surface. This study aimed to characterize cerebral blood flow (CBF) regulation following a single SCUBA dive to a depth of 18 m sea water with a 47 min bottom time. Prior to and following the dive, participants ($n = 11$) completed (1) resting CBF in the internal carotid (ICA) and vertebral (VA) arteries (duplex ultrasound) and intra-cranial blood velocity (v) of the middle and posterior cerebral arteries (MCAv and PCAv, respectively) (transcranial Doppler ultrasound); (2) cerebrovascular reactivity to acute poikilocapnic hypoxia (i.e. F_{IO_2}, 0.10) and hyperoxia (i.e. F_{IO_2}, 1.0); and (3) neurovascular coupling (NVC; regional CBF response to local increases in cerebral metabolism). Global CBF, cerebrovascular reactivity to hypoxia and hyperoxia, and NVC were unaltered following a SCUBA dive (all $P > 0.05$); however, there were subtle changes in other cerebrovascular metrics post-dive, including reductions in ICA ($-13 \pm 8\%$, $P = 0.003$) and VA ($-11 \pm 14\%$, $P = 0.021$) shear rate, lower ICAv ($-10 \pm 9\%$, $P = 0.008$) and VAv ($-9 \pm 14\%$, $P = 0.028$), increases in ICA diameter ($+4 \pm 5\%$, $P = 0.017$) and elevations in PCAv ($+10 \pm 19\%$, $P = 0.047$). Although we observed subtle alterations in CBF regulation at rest, these changes did not translate into any functional changes in cerebrovascular reactivity to hypoxia or hyperoxia, or NVC. Whether prolonged exposure to hyperoxia and hyperbaria during longer, deeper, colder and/or repetitive SCUBA dives would provoke changes to the cerebrovasculature requires further investigation.

KEYWORDS
cerebral blood flow, duplex ultrasound, SCUBA diving

1 | INTRODUCTION

Recreational participation in diving with a self-contained underwater breathing apparatus (SCUBA) presents ubiquitous physiological stressors such as hyperoxia, hyperbaria, exercise and water temperature. Cerebral blood flow (CBF) regulation is an integrative process principally mediated via alterations in cerebral metabolism, arterial blood gases and blood pressure (reviewed

in Willie, Tzeng, Fisher, & Ainslie, 2014). Throughout SCUBA diving, reductions in cardiac output, diffusing lung capacity and pulmonary gas exchange (Dujic, 2005), as well as hyperoxia-induced vasoconstriction (Wunderlich et al., 2017), likely contribute to attenuated systemic vascular blood flow. As a consequence, SCUBA-related reductions in peripheral vascular function (Lambrechts et al., 2013; Marinovic et al., 2011) – due to hyperoxia-induced oxidative stress (Obad et al., 2010; Wang et al., 2020), arterial and venous gas emboli (Barak et al., 2015), and altered endothelial integrity (Bilopavlovic et al., 2013) – have been reported during typical diving profiles (e.g. 18–30 m sea water with 30–47 min bottom time). Emerging data indicate that adverse changes in vascular function may also extend to cerebrovascular changes post-SCUBA dive (Barak et al., 2016, 2018).

Recent studies indicate intra-cranial cerebral blood velocity (CBV) is increased following 47 min exposure to hyperoxia during a SCUBA dive (Barak et al., 2016, 2018). This transient increase in CBV is perhaps attributable to free radical-mediated reductions in downstream resistance (i.e. vasodilatation of pial arteries) provoked via elevations in oxidative stress (Leffler et al., 1990; Rosenblum, 1983; Wei, Christman, Kontos, & Povlishock, 1985) as antioxidant treatment abolishes the temporary elevation in CBV following a SCUBA dive (Barak et al., 2018). Conversely, lower nitric oxide bioavailability following a SCUBA dive (Theunissen et al., 2013) may contribute to reductions in resting CBF regulation (Mashour & Boock, 1999) via cerebral vasoconstriction (Kety & Schmidt, 1948; Lambertsen et al., 1953; Omae et al., 1997; Visser, Van Hulst, Wieneke, & Van Huffelen, 1996; Watson, Beards, Altaf, Kassner, & Jackson, 2000). Additionally, previous animal experiments (Faraci & Breese, 1993; Meng, Tobin, & Busija, 1995; Yang & Iadecola, 1997) and recent evidence in humans (Hoiland et al., unpublished data) indicate neurovascular coupling (NVC; regional CBF response to local increases in cerebral metabolism) is regulated in part by nitric oxide-mediated signalling; therefore, conceivably higher oxidative stress following the SCUBA dive (Modun et al., 2012; Obad et al., 2010) may inactivate nitric oxide (Demchenko, Boso, Bennett, Whorton, & Piantadosi, 2000; Elayan, Axley, Prasad, Ahlers, & Auker, 2000; Zhang, Sam, Klitzman, & Piantadosi, 1995) and reduce NVC. As such, the balance between countervailing pial arteriole dilatation and reductions in nitric oxide bioavailability following exposure to hyperoxia experienced during a SCUBA dive will likely dictate resting CBF regulation and NVC.

Notably, two gaps are apparent with respect to CBF regulation following a single SCUBA dive: (1) validation of indirect surrogate measures of CBF (e.g. transcranial Doppler ultrasound derived CBV) to accurately assess cerebral vasomotor changes; and (2) characterization of the functional responsiveness of the cerebral vasculature following a SCUBA dive. Particularly, assessment of cerebrovascular function – inclusive of its vascular reactivity (Willie et al., 2012) and neurovascular coupling (Phillips, Chan, Zheng, Krassioukov, & Ainslie, 2016) – is needed to provide better insight into the influence of a SCUBA dive on cerebrovascular health. To address these gaps, we performed a comprehensive cerebrovascular assessment before and immediately following a single SCUBA dive (18 m sea water with 47 min bottom time) with the following measures: (1) resting CBF regulation of the internal carotid artery (ICA), vertebral artery (VA), middle and posterior cerebral arteries (MCAv, and PCAv, respectively); (2) cerebrovascular reactivity to acute poikilocapnic hypoxia (i.e. F_{IO_2}, 0.10) and hyperoxia (i.e. F_{IO_2}, 1.0) to index CBF regulation via arterial oxygen changes; and (3) NVC (regional CBF response to local increases in cerebral metabolism). We hypothesized the following: (1) resting global CBF will be unaffected following a single SCUBA dive; (2) the SCUBA dive will attenuate cerebrovascular reactivity to hypoxia post-dive; and (3) the NVC response will be reduced post-SCUBA dive.

New Findings

- **What is the central question of this study?**
  What are the characteristics of cerebral blood flow (CBF) regulation following a single SCUBA dive to a depth of 18 m sea water with a 47 min bottom time.
- **What is the main finding and its importance?**
  Acute alterations in CBF regulation at rest, including extra-cranial vasodilatation, reductions in shear patterns and elevations in intra-cranial blood velocity were observed at rest following a single SCUBA dive.
  These subtle changes in CBF regulation did not translate into any functional changes in cerebrovascular reactivity to hypoxia or hyperoxia, or neurovascular coupling following a single SCUBA dive.

2 | METHODS

2.1 | Ethical approval

Following verbal and written explanation of the study, written informed consent was acquired. This study was approved by the University of Split School of Medicine Institutional Ethics Committee (reg. no. 2181-198-03-04-14-0042) and all procedures were conducted in accordance with the *Declaration of Helsinki*, except registration in a database.

2.2 | Participants

Eleven healthy male divers (39 ± 9 years, 186 ± 6 cm, 88 ± 12 kg) participated in the study. Diving experience ranged from 4 to 24 years with 100 to 2900 dives. Participants had no history of cerebrovascular, cardiovascular or respiratory disease.

2.3 | Experimental protocol

The study was performed at a military base of the Croatian Navy Force. The divers were asked to abstain from vigorous physical activity and diving for 48 h before the study. All divers performed a single dive at a depth of 18 m sea water with a 47 min bottom time and 2 min ascent to surface (9 m sea water min^{-1}) without any decompression stops; compressed ambient air was used as the breathing gas (i.e. inspired P_{O_2} approx. 60 kPa). The divers performed swimming of moderate intensity throughout the dives. Sea water temperature at the bottom was approximately 17°C. Each diver was accompanied by a safety diver, and both of them were equipped with a diving computer (Uwatec Galileo sol, Johnson Outdoors, Inc., Racine, WI, USA). Participants completed a cerebrovascular assessment pre- and post-SCUBA dive as described below. Following the SCUBA dive, participants were re-instrumented immediately and resting measurements were performed starting at approximately 10 min from return to the surface. Experimental tests were performed supine in the following order prior to and following the SCUBA dive: (1) resting CBF; (2) neurovascular coupling (NVC); (3) cerebrovascular reactivity to hypoxia and hyperoxia.

2.4 | Cerebral blood flow

Right ICA ($n = 8$) and left VA ($n = 10$) were assessed with ipsilateral measures of middle (MCAv) and posterior (PCAv) cerebral artery blood velocities, respectively (with the exception of left ICA ($n = 2$) and right VA ($n = 1$) for adequate image quality). Transcranial Doppler (TCD) ultrasound (Spencer Technologies, Seattle, WA, USA) was used to assess cerebral blood velocity (CBV), as an index of CBF, in the right MCA and left PCA. The 2 MHz TCD probes were attached to a specialized headband (model M600 bilateral head frame, Spencer Technologies), and each vessel was insonated through the trans-temporal window, using previously described location and standardization techniques (Willie et al., 2011). The ICA and VA blood velocity and vessel diameter were measured using a 10 MHz multifrequency linear array Duplex ultrasound (Terason 3000; Teratech, Burlington, MA, USA) using previously described location and standardization techniques (Thomas, Lewis, Hill, & Ainslie, 2015). Pulse-wave mode was used to measure peak blood velocity, while arterial diameter was concurrently measured using B-mode imaging. The ICA blood velocity and vessel diameter were measured ≥1.5 cm from the carotid bifurcation to avoid any turbulent or retrograde flow patterns; and the VA blood velocity and vessel diameter were measured between C4 and C5 or C5 and C6. The vessel location was decided on an individual basis to allow for reliable image acquisition, with the same location repeated within participants and between trials. The insonation angle (60°) was unchanged throughout each test and, following acquisition of the first ultrasound image, there was no alteration of B-mode gain or dynamic range to avoid changes in arterial wall brightness/thickness. Our within-day coefficients of variation in the current study for ICA diameter, velocity and blood flow were 2.8%, 5.5% and 7.2%, respectively.

2.5 | Cerebrovascular O_2 reactivity

The ICA blood flow ($\dot{Q}_{ICA}$) response to hypoxia and hyperoxia was assessed pre- and post-SCUBA dive. Participants were instrumented with a two-way non-rebreathing valve (Hans Rudloph masks M and L, two-way valve; Hans Rudolph, Shawnee, KS, USA). A Douglas bag filled with either 10% O_2 or 100% O_2 was fitted with a three-way valve to allow the breathing circuit to alternate between ambient air and the contents of the Douglas bag. Following a 1 min resting baseline (BSL), participants breathed the appropriate O_2 mixture (i.e. 10% O_2 *vs.* 100% O_2) for 10 min. The order of these reactivity tests was counter-balanced between participants and approximately 5–10 min was allowed for washout between conditions.

2.6 | Neurovascular coupling

The NVC test evoked selective changes in PCAv in response to activation of the visual cortex, and the MCAv allowed for regional comparisons. Following 2 min of rest, five cycles of repeated, alternating, 40 s exposure to eyes-closed, and then 20 s eyes-open with reading was completed, according to standardized guidelines (Phillips et al., 2016). The researcher confirmed that the participant's eyes were closed and open during the respective trials. The PCAv and MCAv response to five cycles were exported on a breath-by-breath (respiratory) and beat-by-beat (cardiovascular and cerebrovascular) basis, cubic spline interpolated at 5 Hz, and used for data analysis using custom software developed in MATLAB (The MathWorks, Natick, MA, USA) (Phillips et al., 2016). The NVC test provides an index of metabolic and myogenic regulation that is normalized to any temporal changes in arterial blood gases (Phillips et al., 2016).

2.7 | Cardiorespiratory measures

Heart rate (HR) was continuously measured using a lead-II electrocardiogram (ECG; BioAmp ML132, ADInstruments, Colorado Springs, CO, USA). Beat-by-beat blood pressure was acquired using non-invasive finger photoplethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam, Netherlands) and was calibrated prior to data collection using the return-to-flow function. The Finometer blood pressure waveform was averaged to calculate MAP after calibrating values to the average of two manual brachial blood pressure measurements (Welch Allyn, Hillrom, Chicago, IL, USA; aneroid sphygmomanometer). Stroke volume (SV) was estimated from the blood pressure waveform (Beat Scope, Finapres Medical Systems). Cardiac output (CO) was calculated by multiplying HR and SV. Peripheral oxygen saturation (S_{pO_2}) was measured using pulse oximetry (VacuMed, Ventura, CA, USA). Lastly, breath-by-breath CO_2 and O_2 were sampled using a calibrated gas analyser (model ML206, ADInstruments), and the pressure of end-tidal CO_2 and O_2 (i.e. P_{ETCO_2} and P_{ETO_2}, respectively) was calculated in LabChart (ADInstruments) using peak detection analysis with correction for daily barometric pressure.

2.8 | Data analyses

All cardiorespiratory measures were sampled continuously at 1 kHz using an analog-to-digital converter (Powerlab, 16/30; ADInstruments) and data were interfaced with LabChart (Version 7.1) and analysed offline. A 60 s average of resting CBF values (e.g. $\dot{Q}_{ICA}$, $\dot{Q}_{VA}$, MCA*v*, PCA*v*), as well as 60 s before the onset of NVC and cerebrovascular reactivity were used as resting BSL values. The change in $\dot{Q}_{ICA}$ from BSL to the last 3 min of exposure was used to index cerebrovascular reactivity. Duplex ultrasound recordings were captured and saved for offline analysis using custom edge-detection and wall tracking software (BloodFlow Analysis, Chris Reed, Perth, AU; version 5.1). This analysis method utilizes integration of diameter and velocity traces to calculate mean beat-to-beat flow at 30 Hz independent of observer bias; it has been validated and is described in detail elsewhere (Woodman et al., 2001).

Blood flow was calculated as:

$$\dot{Q} = \text{peak envelope blood velocity}/2 \times \left[\pi(0.5 \times \text{diameter})^2\right] \times 60.$$

Global cerebral blood flow (CBF) was estimated as:

$$\text{Global } CBF = 2 \times \left(\dot{Q}_{ICA} + \dot{Q}_{VA}\right).$$

Mean shear rate was calculated as:

$$\text{Shear rate} = 4 \times \text{peak envelope blood velocity}/\text{arterial diameter}.$$

Cerebrovascular conductance (CVC) was calculated as: global CBF, $\dot{Q}_{ICA}$, $\dot{Q}_{VA}$, MCA*v* or PCA*v*/MAP.

2.9 | Statistical analyses

All cerebrovascular and haemodynamic variables at rest were compared using one-way, paired Student's *t* test (pre- *vs.* post-SCUBA dive). Absolute $\dot{Q}_{ICA}$, MCA*v* and PCA*v* were plotted against either S_{pO_2} (hypoxia) or P_{ETO_2} (hyperoxia) using linear regression to calculate individual cerebrovascular reactivity slope values. Additionally, a linear mixed model with fixed effects of time (pre- *vs.* post-SCUBA dive) and either S_{pO_2} (hypoxia) or P_{ETO_2} (hyperoxia) with P_{ETCO_2} as a covariate was used to calculate estimates of fixed effects for group cerebrovascular reactivity averages (Atkinson et al., 2011). A Bonferroni correction was applied for multiple comparisons when significant interactions were detected. All NVC variables (PCA*v*, MCA*v*, PCA_{CVC}, MCA_{CVC}, MAP, P_{ETCO_2}) were compared as absolute peak response, *change* in absolute peak response from BSL, time to peak response, average absolute response, average *change* in absolute response, relative peak response, and average relative peak response using one-way, paired Student's *t* test (pre- *vs.* post-SCUBA dive). Statistical analyses were performed using SPSS Statistics Version 22.0 (IBM Corp., Armonk, NY, USA) and statistical significance was set at $P < 0.05$.

3 | RESULTS

3.1 | Cerebrovascular and haemodynamic parameters

Overall, global CBF was unchanged pre- *vs.* post-SCUBA ($P = 0.087$; Table 1). Following the SCUBA dive, ICA diameter was increased by +4 ± 5% ($P = 0.017$; Table 1 and Figure 1b) whereas blood velocity was reduced by −10 ± 9% ($P = 0.008$; Table 1 and Figure 1c); as a result, $\dot{Q}_{ICA}$ was unchanged (−1 ± 17%, $P = 0.384$; Table 1). The VA blood velocity was also reduced by −9 ± 14% ($P = 0.028$; Table 1 and Figure 1c) following the SCUBA dive but diameter was not different pre- *vs.* post-SCUBA dive (+2 ± 8%, $P = 0.175$; Table 1 and Figure 1b); therefore, $\dot{Q}_{VA}$ was also unchanged (−5 ± 19%, $P = 0.148$; Table 1). Shear rates were reduced following the SCUBA dive for both ICA (−13 ± 8%, $P = 0.003$; Table 1 and Figure 1a) and VA (−11 ± 14%, $P = 0.021$; Table 1 and Figure 1a). As MAP was not different pre- and post-SCUBA dive ($P = 0.211$; Table 2), both ICA_{CVC} and VA_{CVC} were unaltered following SCUBA ($P = 0.373$ and $P = 0.167$, respectively; Table 1). Both PCA*v* (+10 ± 19%, $P = 0.047$; Table 1 and Figure 1d) and PCA_{CVC} (+8 ± 16%, $P = 0.049$; Table 1) were elevated following the dive, whereas MCA*v* and MCA_{CVC} tended to be higher, but were not significantly different ($P = 0.051$ and $P = 0.072$, respectively; Table 1 and Figure 1d). Both S_{pO_2} and P_{ETO_2} were not different pre- *vs.* post-SCUBA dive ($P = 0.432$ and $P = 0.252$, respectively; Table 2). Participants were relatively hypocapnic following the SCUBA dive as indicated by a −1.4 ± 2.4 mmHg reduction in P_{ETCO_2} ($P = 0.037$; Table 2). Lastly, HR (−9 ± 10%, $P = 0.008$; Table 2), SV (−10 ± 13%, $P = 0.017$; Table 2) and CO (−19 ± 12%, $P = 0.001$; Table 2) were reduced following the SCUBA dive.

3.2 | Cerebrovascular O_2 reactivity

Hypoxia increased $\dot{Q}_{ICA}$ both pre- and post-SCUBA dive (hypoxia effect: $P = 0.008$), and although $\dot{Q}_{ICA}$ was lower throughout this response following the SCUBA dive (dive effect: $P = 0.001$), the reactivity slopes (i.e. $\Delta\dot{Q}_{ICA}$ *vs.* ΔS_{pO_2}) were not different between trials (interaction effect: $P = 0.677$; Figure 2a). This $\dot{Q}_{ICA}$ response was evoked via slight vasodilatation (BSL: 5.22 ± 0.57 mm *vs.* Hypoxia: 5.33 ± 0.57 mm, hypoxia effect: $P = 0.037$) with no change in blood velocity (BSL: 39.68 ± 9.95 cm s^{-1} *vs.* Hypoxia: 41.63 ± 9.95 cm s^{-1}; hypoxia effect: $P = 0.083$) throughout the hypoxia trials. Further, the vasodilatation reactivity slope (i.e. ΔICA diameter *vs.* ΔS_{pO_2}) was not different between trials (interaction effect: $P = 0.542$). The ICA shear rate, ICA_{CVC}, MCA*v*, PCA*v*, MCA_{CVC} and PCA_{CVC} hypoxic reactivities did not change pre- or post-SCUBA dive (interaction effects: all $P > 0.05$, respectively). Including P_{ETCO_2} as a covariate did not significantly influence the $\dot{Q}_{ICA}$ ($P = 0.904$; Figure 2c) or ICA_{CVC} reactivity slopes ($P = 0.133$).

Hyperoxia reduced $\dot{Q}_{ICA}$ both pre- and post-SCUBA dive (hyperoxia effect: $P < 0.001$); however, there was no difference in this response between trials (dive effect: $P = 0.449$) or with respect to the reactivity

TABLE 1 Cerebrovascular parameters pre- and post-SCUBA dive at rest

	Pre-SCUBA	Post-SCUBA	Sample size	*P*
ICA				
Diameter (mm)	5.01 ± 0.48	5.22 ± 0.59*	9	**0.017**
Velocity (cm s^{-1})	43.95 ± 12.07	38.98 ± 8.12*	9	**0.008**
$\dot{Q}$ (ml min^{-1})	259 ± 70	255 ± 77	9	0.384
Shear rate (s^{-1})	356 ± 113	302 ± 74*	9	**0.003**
CVC (ml min^{-1} $mmHg^{-1}$)	2.88 ± 0.89	2.83 ± 0.89	9	0.373
VA				
Diameter (mm)	4.09 ± 0.53	4.19 ± 0.63	10	0.175
Velocity (cm s^{-1})	25.39 ± 7.36	22.53 ± 5.54*	10	**0.028**
$\dot{Q}$ (ml min^{-1})	103 ± 43	96 ± 38	10	0.148
Shear rate (s^{-1})	251 ± 77	218 ± 58*	10	**0.021**
CVC (ml min^{-1} $mmHg^{-1}$)	1.13 ± 0.53	1.07 ± 0.45	10	0.167
Global CBF (ml min^{-1})	721 ± 227	673 ± 185	8	0.087
MCAv (cm s^{-1})	59.99 ± 12.39	62.06 ± 12.66	11	0.051
MCA_{CVC} (cm s^{-1} $mmHg^{-1}$)	0.66 ± 0.15	0.69 ± 0.17	11	0.072
PCAv (cm s^{-1})	39.34 ± 10.70	43.42 ± 13.82*	10	**0.047**
PCA_{CVC} (cm s^{-1} $mmHg^{-1}$)	0.44 ± 0.13	0.49 ± 0.17*	10	**0.049**

Data are mean ± SD. Values shown in bold are considered statistically significant. CBF, cerebral blood flow; CVC, cerebrovascular conductance; ICA, internal carotid artery; MCAv, middle cerebral artery mean velocity; PCAv, posterior cerebral artery mean velocity; $\dot{Q}$, flow; VA, vertebral artery.

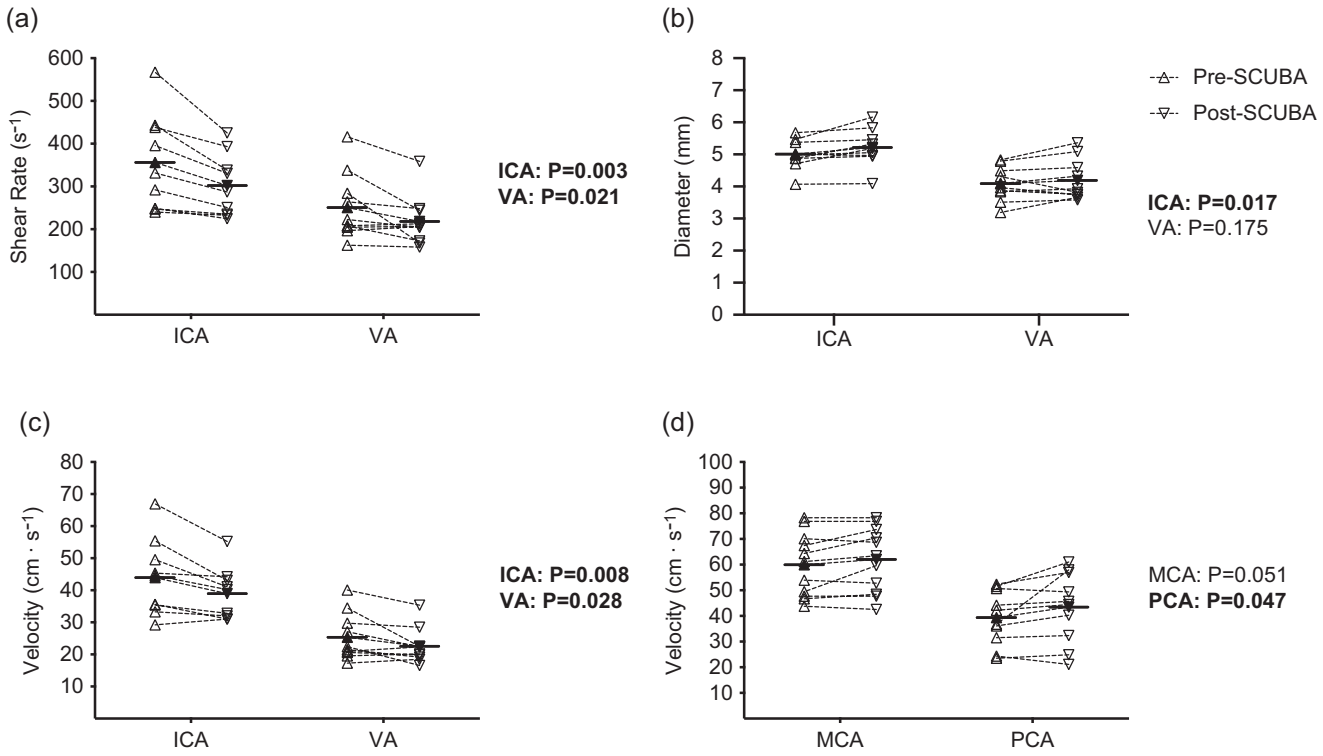

FIGURE 1 Acute alterations in cerebrovascular regulation at rest following a SCUBA dive. (a) Shear rate in the internal carotid artery (ICA) and vertebral artery (VA). (b) Diameter of the ICA and VA. (c) Blood velocity in the ICA and VA. (d) Blood velocity in the middle cerebral artery (MCA) and posterior cerebral artery (PCA). Following a SCUBA dive subtle reductions in ICA and VA shear patterns (a) and blood velocities (c) as well as elevations in arterial diameter (b) and intra-cranial blood velocities (d) were observed at rest. Data are individual responses with respective group average lines for ICA: $n = 9$; VA: $n = 10$; MCA: $n = 11$; PCA: $n = 10$

TABLE 2 Haemodynamic parameters pre- and post-SCUBA dive at rest

	Pre-SCUBA	Post-SCUBA	Sample size	*P*
S_{pO_2} (%)	98.9 ± 0.6	98.8 ± 0.8	11	0.432
P_{ETO_2} (mmHg)	102.9 ± 5.2	101.6 ± 4.5	11	0.252
P_{ETCO_2} (mmHg)	38.9 ± 2.3	37.4 ± 2.4*	11	**0.037**
MAP (mmHg)	90 ± 5	91 ± 6	11	0.211
SBP (mmHg)	120 ± 7	121 ± 10	11	0.315
DBP (mmHg)	75 ± 6	76 ± 5	11	0.238
HR (bpm)	63 ± 6	58 ± 8*	11	**0.008**
SV (ml)	123 ± 20	109 ± 15*	11	**0.017**
CO (l min^{-1})	7.8 ± 1.6	6.3 ± 1.1*	11	**0.001**

Data are mean ± SD. Values shown in bold are considered statistically significant. *$P < 0.05$ pre- *vs.* post-SCUBA. CO, cardiac output; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; P_{ETCO_2}, end-tidal P_{CO_2}; P_{ETO_2}, end-tidal P_{O_2}; SBP, systolic blood pressure; S_{pO_2}, peripheral oxygen saturation; SV, stroke volume.

slopes (interaction effect: $P = 0.405$; Figure 2b). This response was perhaps in part mediated by ICA vasoconstriction (BSL: 5.13 ± 0.54 mm *vs.* Hyperoxia: 4.97 ± 0.54 mm, hyperoxia effect: $P = 0.001$) as well as a reduction in blood velocity (BSL: 41.53 ± 8.36 cm s^{-1} *vs.* Hyperoxia: 38.77 ± 8.36 cm s^{-1}; hyperoxia effect: $P = 0.008$) throughout the hyperoxia trials. Further, the vasoconstriction reactivity slope (i.e. ΔICA diameter *vs.* ΔP_{ETO_2}) was not different between trials (interaction effect: $P = 0.219$). The ICA shear rate, ICA_{CVC}, MCAv, PCAv, MCA_{CVC} and PCA_{CVC} response to hyperoxia did not change pre- or post-SCUBA dive (interaction effects: all $P > 0.05$). The $\dot{Q}_{ICA}$ and ICA_{CVC} were reduced to the same extent pre- *vs.* post-SCUBA (hyperoxia effects: $P < 0.001$ and $P = 0.001$, respectively). However, covariate analysis for P_{ETCO_2} ($P = 0.012$ and $P = 0.023$, respectively) revealed no influence of hyperoxia on the reduction in CBF (i.e. the decrease in CBF was driven by hypocapnia); this again was not different pre- *vs.* post-SCUBA (interaction effects: $P = 0.320$ and $P = 0.519$, respectively; Figure 2d).

3.3 | Neurovascular coupling

The absolute peak response for PCAv and PCA_{CVC} tended to be higher following SCUBA dive (PCAv 44.71 ± 15.14 *vs.* 48.60 ± 16.71 cm s^{-1}; and PCA_{CVC} 0.48 ± 0.18 *vs.* 0.52 ± 0.19 cm s^{-1} $mmHg^{-1}$; both $P = 0.058$); however, this was likely due to higher resting PCAv following SCUBA (Table 1 and Figure 1d). Indeed, when expressed as an absolute peak *change* from BSL, there was no influence of SCUBA

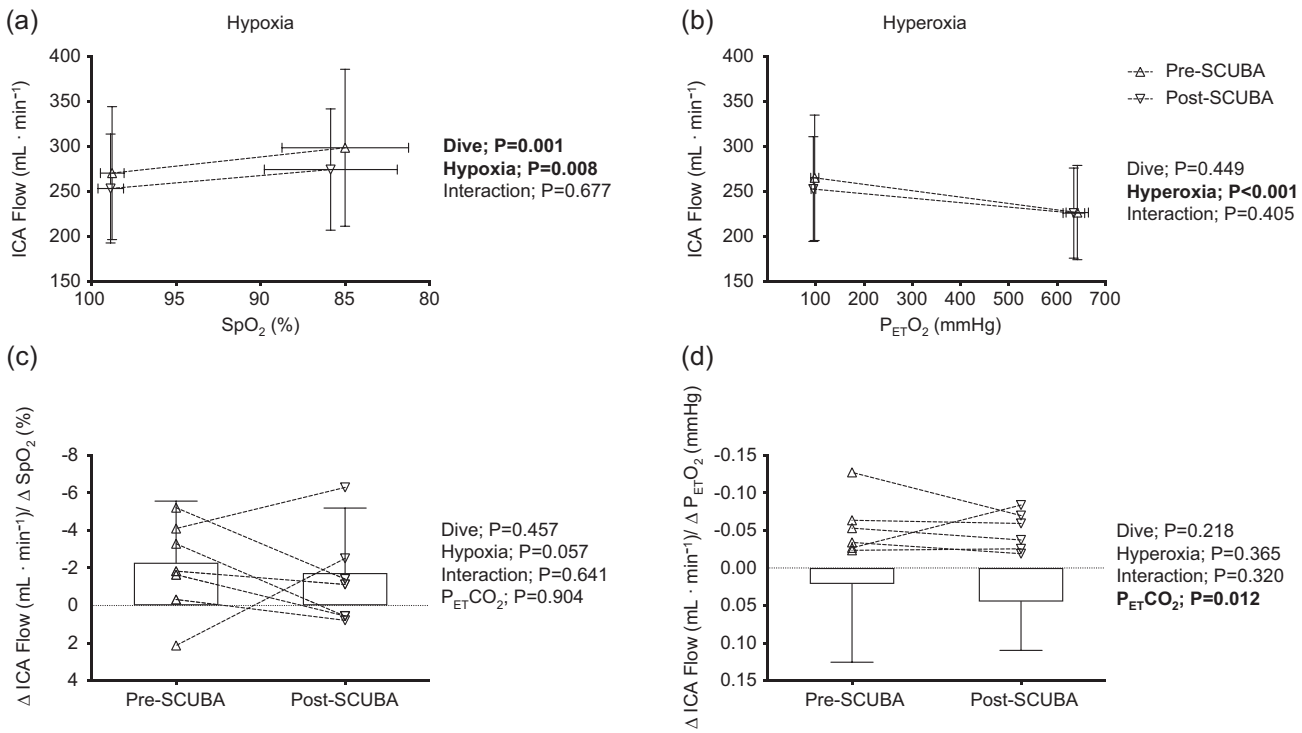

FIGURE 2 Cerebrovascular reactivity to hypoxia and hyperoxia pre- and post-SCUBA dive. (a) Internal carotid artery (ICA) blood flow *vs.* peripheral oxygen saturation (S_{pO_2}) during normoxia and within the last 3 min of exposure to 10% O_2 (hypoxia). (b) Internal carotid artery (ICA) blood flow *vs.* pressure of end-tidal oxygen (P_{ETO_2}) during normoxia and within the last 3 min of exposure to 100% O_2 (hyperoxia). (c) Individual hypoxic reactivity slopes (i.e. relative change in ICA flow/S_{pO_2}); there was no influence of end-tidal P_{CO_2} (P_{ETCO_2}) as a covariate (bar graph). (d) Individual hyperoxic reactivity slopes (i.e. relative change in ICA flow/P_{ETO_2}); covariate analysis for P_{ETCO_2} revealed no influence of hyperoxia on the reduction in $\dot{Q}_{ICA}$ presented in (b) (i.e. the decrease in $\dot{Q}_{ICA}$ was driven by hypocapnia within both trials). For (c,d), the bar graphs represent average slope values corrected for P_{ETCO_2} as a covariate. Data are means ± SD for hypoxia: $n = 7$; hyperoxia: $n = 6$

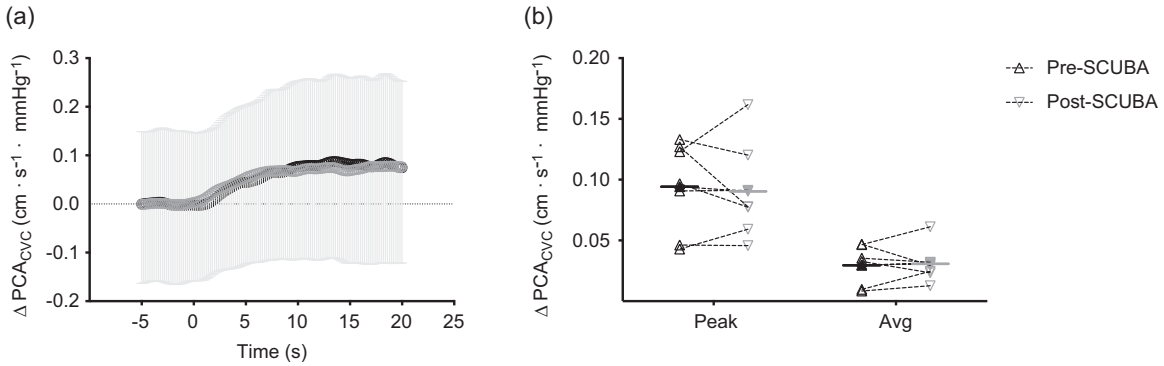

FIGURE 3 Neurovascular coupling response of the posterior cerebral artery cerebrovascular conductance (PCA_{CVC}) pre- and post-SCUBA dive. (a) Absolute change in PCA_{CVC}. (b) Individual data with respective group averages for the absolute change in peak and average responses of PCA_{CVC}. At time 0 s there is cerebral activation during 'eyes-open' following resting 'eyes-closed'. Absolute PCA_{CVC} was elevated following the SCUBA dive; however, there is no difference in this response when expressed as an absolute change score. Data are means ± SD for $n = 7$

dive on the absolute peak PCA*v* response (pre: +8.81 ± 3.46 cm s^{-1} *vs.* post: +8.41 ± 3.39 cm s^{-1}, $P = 0.350$). Additionally, the PCA*v* average absolute *change* from BSL was not different following the SCUBA dive (pre: +2.86 ± 1.61 cm s^{-1} *vs.* post: +2.91 ± 1.39 cm s^{-1}, $P = 0.457$), and this was consistent with the PCA_{CVC} results (Figure 3a,b). The PCA*v* relative peak response was also not different pre- *vs.* post-SCUBA dive (24.73 ± 6.53% *vs.* 21.53 ± 5.16%, respectively; $P = 0.219$), and this response was unaltered when expressed as PCA_{CVC}. The average relative response for PCA*v* and PCA_{CVC} was not affected by the SCUBA dive ($P = 0.451$ and $P = 0.488$, respectively). Time to peak response was lower (i.e. faster) post-SCUBA dive for PCA*v* (pre: 14.94 ± 3.44 s *vs.* post: 12.69 ± 4.15 s, $P = 0.034$).

4 | DISCUSSION

The main findings of this study indicate that following a 47 min SCUBA dive to 18 m sea water: (1) although global CBF was not affected, subtle reductions in ICA and VA shear patterns as well as elevations in intra-cranial blood velocities were observed at rest; (2) cerebrovascular reactivity to both hypoxia and hyperoxia was preserved; and (3) the NVC response was maintained. These findings indicate that a single 47 min SCUBA dive to 18 m sea water does not acutely affect functional cerebrovascular regulation as indexed by measures of vascular reactivity to hypoxia and hyperoxia as well as NVC.

4.1 | Cerebrovascular regulation at rest following a SCUBA dive

The approximately 10% increase in PCA*v* observed in the current study is consistent with the 8–10% elevation in PCA*v* reported in two separate studies by Barak et al. (2016, 2018) following a single SCUBA dive with the same dive profile; that is, 18 m sea water with a 47 min bottom time. Further, our data extend these findings and reveal subtle alterations in changes in extra-cranial blood flow patterns, including vasodilatation and reductions in shear rate. The increases in intra-cranial CBV are perhaps explained by hyperoxia-induced cerebral vasoconstriction due to (1) compensatory increases in respiration and resultant reductions in P_{CO_2} (Eldridge & Kiley, 1987); and/or (2) increased reactive oxygen species (ROS) (Modun et al., 2012; Obad et al., 2010) and related reductions in nitric oxide bioavailability (Theunissen et al., 2013) and cerebral metabolism (Mattos et al., 2019); and such changes are reflected in additional stresses of diving (e.g. high hydrostatic pressure, water temperature, exercise) independent of hyperoxia *per se* (Barak et al., 2018). Indeed, the previously reported transient elevations in CBV observed 30 min following a single SCUBA dive were acutely restored by 60 min post-dive (Barak et al., 2016, 2018); such increases in CBV were suppressed with antioxidant administration and were not apparent during exposure to matched duration 60% O_2 breathing experienced during the dive (Barak et al., 2018). Taken together, these consistent findings indicate that subtle increases in intra-cranial CBV following a single SCUBA dive are: (1) transient and normalized within 60 min; (2) unrelated to hyperoxia *per se*; and (3) not sufficient to alter cerebrovascular regulation to hypoxia, hyperoxia or NVC.

The reasons for the alterations in extra-cranial blood flow patterns are not clear but may relate to hyperoxia-induced endothelium-mediated regulation of CBF as evidenced by reductions in ICA and VA shear rate following the SCUBA dive (Attaye et al., 2017; Brueckl et al., 2006). Further, the attenuation in ICA and VA shear rates is likely influenced by the observed 19% decrease in cardiac output; these data are consistent with the 17% reduction in cardiac output 30 min following a single SCUBA dive reported by Dujic and colleagues (2005). Additionally, we observed a small but significant reduction in P_{ETCO_2} at rest following the SCUBA dive (−Δ1.5 mmHg); taken together with the reported hypocapnic cerebrovascular CO_2 reactivity of approximately 3–4% per mmHg reduction in P_{ETCO_2} (Hoiland, Fisher, & Ainslie, 2019), this relative hypocapnia may explain the approximately 6.5% lower global CBF (albeit not statistically significant; Table 1) following the SCUBA dive. This subtle reduction in P_{ETCO_2} post-dive is paradoxical with regard to the observed 4% ICA vasodilatation; however, it may be explained via prevailing elevations

in ROS following a SCUBA dive (Modun et al., 2012; Obad et al., 2010) as ROS-induced pial arteriole dilatation attenuates hypocapnia-evoked cerebrovascular constriction (Leffler et al., 1990; Wei et al., 1985). Further, following resurfacing from a SCUBA dive, reductions in atmospheric pressure provoke bubble-mediated shear stress in addition to hyperoxia-induced oxidative stress and, as such, can elicit inflammatory vascular injuries (Madden et al., 2010; Thom et al., 2012; 2013). Lastly, recent evidence in humans suggests NVC is regulated in part by nitric oxide mediated signalling (Hoiland *et al*., unpublished data); however, conceivably higher oxidative stress following the SCUBA dive (Modun et al., 2012; Obad et al., 2010) was not sufficient to reduce the NVC response in the current study.

4.2 | Stability in functional CBF regulation following a SCUBA dive

Although we observed subtle alterations in CBF regulation at rest, these changes did not translate into any functional changes in cerebrovascular reactivity to hypoxia or hyperoxia, or neurovascular coupling. Conversely, prospective analysis reveals that SCUBA diving may have long-term consequences for CBF regulation (via ^{133}Xe single-photo emission computed tomography) dictated by water temperature, diving frequency and maximal depth (Slosman et al., 2004). Slosman and colleagues (2004) report that repetitive SCUBA participation (e.g. >100 dives per year) in cold water at maximal depths exceeding 40 m sea water has adverse effects on CBF and neuropsychological function (e.g. speed, flexibility, and attention performance), whereas recreational SCUBA diving in warm seas at depths <40 m sea water does not acutely affect CBF regulation (as per current results). Cold-induced peripheral vasoconstriction as well as pressure-induced blood volume centralization can contribute to increased pulmonary capillary pressure during a SCUBA dive (Doubt, 1996; Tetzlaff et al., 2001; Wilmshurst, Nuri, Crowther, & Webb-Peploe, 1989). Although body temperature was not measured in the current study, previous reports indicate that participants engaging in continuous exercise at approximately 45% $\dot{V}_{O_2max}$ are able to effectively regulate normal body temperature in 15–18°C water (Doubt, 1991; Golden & Tipton, 1987; Roberts, Holmes, & Doubt, 1992). As such, the relative hypocapnia observed post-dive in the present study was likely due to exposure to hyperoxia rather than temperature *per se*; this is further supported by the unchanged resting blood pressure post-dive indicating little to no prevailing influence of cold-induced systemic vasoconstriction. Notably, both the ICA and VA shear rate were reduced in the present study following a single SCUBA dive; as such, repeated/prolonged exposure to hyperoxia may contribute to pressure-induced reductions in microvascular endothelial integrity following chronic participation in SCUBA diving (Attaye et al., 2017; Brueckl et al., 2006). Whether prolonged exposure to hyperoxia and hyperbaria during longer, deeper, colder and/or repetitive SCUBA dives would provoke changes to the functional responses of the cerebrovasculature requires further investigation.

4.3 | Experimental considerations

The utility of transcranial Doppler ultrasound to assess CBV as an adequate surrogate of absolute CBF requires that the insonated cerebral vessel diameter does not change (Ainslie & Hoiland, 2014). As the ICA supplies both the anterior cerebral artery (ACA) and MCA, the assumption of unity between $\dot{Q}_{ICA}$ and MCA flow/velocity is contingent on the consistent distributive relationship between the MCA and ACA (Willie et al., 2014; Zarrinkoob et al., 2015). Likewise, the vertebro-basilar circulation is highly anatomically complex; that is, the VA supplies extra-cranial branches of the deep cervical artery and inferior thyroid artery, as well as anterior and posterior spinal arteries, perforating branches to the medulla, and the posterior inferior cerebellar artery before feeding the basilar artery and PCA (Edvinsson & Krause, 2002; Nowinski et al., 2011). The inconsistency between subtle increases in intra-cranial MCAv and PCAv and unchanged extra-cranial $\dot{Q}_{ICA}$ and $\dot{Q}_{VA}$ in the present study is in part explained by the compensatory increases in arterial diameter coupled with reductions in extra-cranial artery blood velocity (Table 1). Notably, cerebrovascular reactivity of both the ICA and MCA to acute poikilocapnic hyperoxia and hypoxia were not different prior to or following a SCUBA dive; therefore, these data indicate that the functional responsiveness of the cerebrovasculature to changes in end-tidal P_{O_2} are related within both the extra-cranial and intra-cranial circulations prior to and following SCUBA, perhaps irrespective of vasomotor regulation at rest. Further, the current experiment did not include a time-control visit; therefore, the observed acute changes in cerebrovascular regulation may not be definitively due to the SCUBA dive intervention. Lastly, this study included healthy male recreational divers; as such, these results may not be generalizable to females or novice divers with less than 4 years of experience. The majority of studies to date investigating the influence of SCUBA diving on vascular function have been conducted in males (Barak et al., 2015, 2016, 2018; Bilopavlovic et al., 2013; Lambrechts et al., 2013; Madden et al., 2010; Obad et al., 2010; Theunissen et al., 2013; Thom et al., 2012, 2013); therefore, the influence of cyclical changes in sex hormones on cerebrovascular function and health following SCUBA diving would be an important follow-up study in females.

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COMPETING INTERESTS

None to declare.

AUTHOR CONTRIBUTIONS

The study was performed at a military base of the Croatian Navy Force in Split, Croatia. P.N.A., O.F.B. and Z.D. conceived and designed the research. R.L.H., O.F.B. and T.M. acquired the data. H.G.C., R.L.H. and J.S.B. analysed the data. H.G.C., R.L.H. and P.N.A. interpreted the data. H.G.C. drafted the manuscript. All authors have read and approved the final version of this 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.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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