Cerebral, cardiac and skeletal muscle stress associated with a series of static and dynamic apnoeas

Antonis Elia1,2 | David R. Woods2,3 | Matthew J. Barlow2 | Matthew J. Lees4 | John P. O’Hara2

1Division of Environmental Physiology, School of Chemistry, Bioengineering and Health, KTH Royal Institute of Technology, Stockholm, Sweden
2Carnegie School of Sport, Leeds Beckett University, Leeds, UK
3Research and Clinical Innovation, Royal Centre for Defence Medicine, Birmingham, UK
4Faculty of Kinesiology and Physical Education, University of Toronto, Toronto, Ontario, Canada

Correspondence
Antonis Elia, Division of Environmental Physiology, School of Chemistry, Bioengineering and Health, KTH Royal Institute of Technology, Stockholm, Sweden.
Email: antonise@kth.se

Funding information
This study was funded by Leeds Beckett University

Purpose: This study sought to explore, for the first time, the effects of repeated maximal static and dynamic apnoeic attempts on the physiological milieu by assessing cerebral, cardiac and striatal muscle stress-related biomarkers in a group of elite breath-hold divers (EBHD).

Methods: Sixteen healthy males were recruited (EBHD = 8; controls = 8). On two separate occasions, EBHD performed two sets of five repeated maximal static apnoeas (STA) or five repeated maximal dynamic apnoeas (DYN). Controls performed a static eupnoeic protocol to negate any effects of water immersion and diurnal variation on haematology (CTL). Venous blood samples were drawn at 30, 90, and 180 min after each protocol to determine S100β, neuron-specific enolase (NSE), myoglobin, and high sensitivity cardiac troponin T (hscTNT) concentrations.

Results: S100β and myoglobin concentrations were elevated following both apnoeic interventions (p < 0.001; p ≤ 0.028, respectively) but not after CTL (p ≥ 0.348). S100β increased from baseline (0.024 ± 0.005 µg/L) at 30 (STA, +149%, p < 0.001; DYN, +166%, p < 0.001) and 90 min (STA, +129%, p < 0.001; DYN, +132%, p = 0.008) following the last apnoeic repetition. Myoglobin was higher than baseline (22.3 ± 2.7 ng/ml) at 30 (+42%, p = 0.04), 90 (+64%, p < 0.001) and 180 min (+49%, p = 0.013) post-STA and at 90 min (+63%, p = 0.016) post-DYN. Post-apnoeic S100β and myoglobin concentrations were higher than CTL (STA, p < 0.001; DYN, p ≤ 0.004). NSE and hscTNT did not change from basal concentrations after the apnoeic (p ≥ 0.146) nor following the eupnoeic (p ≥ 0.553) intervention.

Conclusions: This study suggests that a series of repeated maximal static and dynamic apnoeas transiently disrupt the blood-brain barrier and instigate muscle injury but do not induce neuronal-parenchymal damage or myocardial damage.

Keywords: apnoea, breath-hold, cardiac troponin, diving, hypoxaemia, myoglobin, neuron-specific enolase, S100β
INTRODUCTION

Breath-hold diving continues to gain popularity and recognition as both a competitive and recreational sport. The continued progression of world records is astonishing, particularly given the extreme hypoxaemic hypercapnia and hydrostatic pressures these athletes endure; yet, the continued pursuit of performance raises safety concerns.\(^1\) To date, a breadth of research exists that has delineated the physiological characteristics of breath-hold divers, as well as the responses that occur during and/or following prolonged apnoeic bouts (e.g., the diving response, trigeminocardiac reflex, splenic contractions, erythropoietic responses, etc.).\(^1\) In contrast, there is a paucity of literature concerning the possible health implications associated with exposure to such activities.\(^9\)–\(^14\)

Emerging evidence indicates that a single, maximal static apnoeic attempt is capable of transiently disrupting the blood-brain barrier\(^9,13\) and instigates neuronal-parenchymal damage\(^11\) but is not associated with cardiac injury.\(^10,15\) Specifically, both Andersson et al.\(^9\) and Bain et al.\(^13\) reported significant increases in serum S100 calcium-binding protein \(\beta (S100\beta)\) at the end of a maximal static apnoea (~26%, 335 ± 38 s; ~40%, 307 ± 64 s, respectively), which is indicative of a potential perturbation of the blood-brain barrier. Similarly, in competitive breath-hold divers ~1 h following a single maximal static apnoic attempt (mean ~5 min [range: 2.3–7.6 min]), total tau and amyloid \(\beta 42\) concentrations were significantly increased from basal concentrations.\(^11\)

Furthermore, neuron-specific enolase (NSE), a prognostic indicator of traumatic brain injury,\(^16\) was shown to be significantly elevated (~70%) ~3 h following a combined bout of static and dynamic apnoeas.\(^12\) Taken together, these findings highlight the severity of physiological stress encountered by breath-hold divers during maximal apnoic activity.

To date, only one study has examined the physiological stress imposed by repeated apnoic exposures on bodily organs (e.g., the heart).\(^15\) Specifically, following repeated apnoic dives (i.e., performed over a 5 h spearfishing competition) cardiac troponin I increased (+275%) as did brain natriuretic peptide (+229%), which is notable as both are markers of cardiac stress.\(^15,17\) These increases were not evident following a single maximal static apnoic attempt,\(^15\) nor after a combined bout of static and dynamic apnoeas.\(^12\) However, to the best of our knowledge, no study has investigated the magnitude of physiological stress instigated by a series of repeated maximal static and/or dynamic apnoic bouts on cerebral, cardiac, and striatal muscle markers. Contrary to apnoic competitions (i.e., with the exception of spearfishing competitions), whereby athletes perform one maximal attempt per discipline; during training and spearfishing, breath-hold divers commonly perform a series of repeated sub-maximal and/or maximal attempts. Intermittent hypoxaemia of this nature is capable of upregulating the production of reactive oxygen species,\(^18,19\) exacerbating oxidative stress levels\(^20,21\) as well as inflicting oxidative damage on cells, tissues, and organs. Collectively, these observations reinforce the necessity for further studies to be undertaken to assess the possible health consequences associated with repeated apnoic exposures.

Duly, this study sought to explore the effects of repeated maximal static and dynamic apnoic attempts on the physiological milieu by assessing cerebral (NSE and S100\(\beta\)), cardiac (cardiac troponin T), and striatal muscle (myoglobin) stress-related biomarkers in a group of elite breath-hold divers (EBHD). We hypothesized that both repeated maximal apnoic modalities would be associated with significant physiological stress.

MATERIALS AND METHODS

Participants

Sixteen healthy males volunteered for this study and were differentiated into two groups: EBHD (\(n = 8\)) and controls (\(n = 8\)). All breath-hold divers actively participated in national and/or international competitions (Table 1)

| Variables                  | Elite breath-hold divers (\(n = 8\)) | Control (\(n = 8\)) |
|----------------------------|-------------------------------------|---------------------|
| Age (years)                | 39 ± 7                              | 28 ± 5              |
| Height (m)                 | 1.8 ± 0.1                           | 1.8 ± 0.1           |
| Body mass (kg)             | 84 ± 12                             | 82 ± 11             |
| Body mass index (kg/m\(^2\)) | 26 ± 1.3                           | 25 ± 2.2            |
| Years practicing apnoea (years) | 7 ± 2                               | —                   |
| Personal best static apnoea (s) | 376 ± 39                           | —                   |
| Personal best dynamic apnoea with fins (m) | 193 ± 42                           | —                   |
| Personal best dynamic apnoea without fins (m) | 131 ± 41                           | —                   |

TABLE 1 Mean (± standard deviation [SD]) participant characteristics
and the control group comprised of physically active individuals. Participants were non-smoking, habitual sea-level residents, and provided written informed consent before the study. The study received institutional ethical approval and all experimental procedures were completed in accordance with the Declaration of Helsinki. The study was part of a larger project investigating physiological responses to a series of repeated apneas.5

2.2 | Experimental protocol

During each testing session participants reported to the laboratory at Leeds Beckett University (Leeds, England) after a 12 h fast and refrained from consuming caffeine- and alcohol-containing beverages. Additionally, participants were instructed to avoid physical activity and apnoea-related activities for 24 h prior to and during each testing day.

2.3 | Preliminary resting measurements

Following arrival at the laboratory the participants' height and body mass were assessed (Seca, Vogel & Halke) (Table 1). Thereafter, the participants underwent a 20-min supine resting period and subsequently, two venous blood samples (5 ml) were drawn from a median cubital or basilic vein to assess for resting serum NSE, S100β, myoglobin, and high sensitivity cardiac troponin T (hscTNT) concentrations (BD Vacutainer, 367954).

2.4 | Familiarisation session

Participants underwent a familiarisation session within 24 h of completing the baseline measurements to introduce them to the testing environment, trial conditions and requirements.

2.5 | Apnoic protocols

Within a week from completing the familiarisation session, participants reported to the swimming pool (~28°C) facilities and under the supervision of a qualified safety diver(s) performed, on separate days (i.e, separated by ≥96 h), in a Latin-Square fashion and randomized order (achieved using a computer-generated list of random numbers [https://www.randomizer.org/] ) one of the following protocols: five repeated maximal dynamic apnoeas without fins (horizontal underwater swimming) or two sets of five maximal static apnoic attempts (i.e, two sets separated by a 10 min seated rest; breath-holding performed in a prone/semi-seated position on the water surface) (Figure 1). To control for diurnal oscillations of the measured variables both apnoic protocols were performed at the same time of the day.

Participants were instructed to hold their breath after a deep but not maximal inspiration, without prior hyperventilation or glossopharyngeal insufflation. After each maximal attempt, participants underwent a two-minute resting period whereby they were allowed to relax and breathe normally in a seated position, while remaining immersed in water up to waist height (Figure 1). This procedure was repeated five times per set and performance data (i.e, apnoic duration and/or distance covered) were recorded during each maximal attempt (Figure 1).

2.6 | Control protocol

To control any possible effects of whole-body immersion in water, a control group performed a static eupnoic (normal breathing) protocol. The static eupnoic protocol replicated the water exposure times, resting periods and data collection timepoints of the static apnoea protocol (since the water exposures were longer in the static versus the dynamic apnoea protocol) and replaced apnoeas with normal breathing periods.

Participants reported to the swimming pool facilities at the same time of day as during the apnoic protocols and were immersed in water up to the neck.

2.7 | Post-apnoea blood sample

At completion of the apnoic and control protocols, a cannula was inserted into a suitable median cubital or basilic vein of the participant’s arm and two venous blood samples were drawn at 30, 90, and 180 min after the last apnoic/eupnoic repetition to determine the serum concentrations of circulating NSE, S100β, myoglobin, and hscTNT.

2.8 | Blood sample treatment

Samples were gently inverted, allowed to coagulate at room temperature for 20 min then centrifuged (4000 rpm for 10 min at 4°C; ALC Multispeed Refrigerated Centrifuge, PK131R), and the serum supernatants were frozen at −80°C for subsequent analyses.

2.9 | Blood analyses

Enzyme-linked immunosorbent assay (ELISA) was performed to assess serum concentrations of NSE (R&D
systems, Quantikine IVD ELISA, human enolase 2/neuron-specific enolase immunoassay, DENL20, sensitivity 0.038 ng/ml; intra-assay variability ~2.2%), S100β (R&D Systems, Human S100β Duo Set ELISA, DY1820; intra-assay variability ~4.5%), and myoglobin (Abcam, Human Myoglobin ELISA, ab108652, sensitivity 5 ng/ml; intra-assay variability ~5.4%). hscTNT was quantified by electro-chemiluminescence immunoassay (Cobas Analyzer; Roche Diagnostics) (intra-assay variability ~6%).

2.10 | Statistical analysis

All participants completed the protocols successfully and all data were statistically analysed using the IBM SPSS statistics software (version 21). The Shapiro-Wilk test was used to assess normality, whereas homogeneity was assessed using Levene’s test. Sphericity was evaluated using Mauchly’s test of sphericity; for instances where the assumption of sphericity was violated, the Greenhouse-Geisser correction was applied. Repeated measures ANOVAs with Tukey’s post-hoc tests were used to assess within-group differences for baseline measurements and other timepoints for serum S100β, NSE, myoglobin, and hscTNT concentrations. Two-way ANOVAs were used to assess temporal differences between conditions. Data are reported as mean ± SD, with significance accepted at p < 0.05. Figures were constructed using GraphPad Prism (GraphPad software, version 7.0c).

3 | RESULTS

3.1 | Apnoeic performances

Mean static apnoea duration was 218 s (range 130–350 s) and mean dynamic apnoea distance covered without fins was 71 m (range 46–126 m).

3.2 | S100β

Mean post-apnoeic S100β concentrations were significantly higher than baseline (0.024 ± 0.005 µg/L) following the static (p < 0.001) and dynamic (p < 0.001) apnoea protocols, while no differences were observed during the control protocol (p = 0.348) (Figure 2A). Notably, S100β concentrations were greater than baseline at 30 (static, +149%, 0.059 ± 0.019 µg/L, p < 0.001; dynamic, +166%, 0.061 ± 0.019 µg/L, p < 0.001) and 90 min (static, +129%, 0.055 ± 0.019 µg/L, p < 0.001; dynamic, +132%, 0.054 ± 0.018 µg/L, p = 0.008) after the last apnoeic repetition but not at 180 min (static, +32%, 0.031 ± 0.008 µg/L, p = 0.676; dynamic, +46%, 0.035 ± 0.011 µg/L, p = 0.432). In addition, mean post-apnoeic S100β concentrations were significantly higher than control (static, p < 0.001; dynamic, p = 0.002) at 30 (p < 0.001), 90 (p < 0.001) and 180 min (p ≤ 0.006), whereas no differences were documented between the apnoeic protocols (p = 0.622) (Figure 2A).
3.3 | Neuron-specific enolase

There was no effect of the apnoeic (static, $p = 0.146$; dynamic, $p = 0.836$) or eupnoic ($p = 0.988$) interventions on serum NSE concentrations compared with baseline (EBHD, 2.91 ± 0.55 pg/ml; control, 3.32 ± 0.63 pg/ml). Moreover, there were no between-protocol differences (static vs. dynamic, $p = 0.682$; apnoeic vs. control, $p \geq 0.127$) (Figure 2B).

3.4 | High sensitivity cardiac troponin T

hscTNT concentrations were not significantly different from baseline (EBHD, 5 ± 1 ng/L; control, 6 ± 1 ng/L) neither after the apnoeic interventions ($p \geq 0.224$) nor following the control protocol (6 ± 1 ng/L, $p = 0.553$) (Figure 2C). In addition, there were no between-protocol differences (static vs. dynamic, $p = 0.384$; apnoeic vs. control, $p \geq 0.163$) (Figure 2C).

3.5 | Myoglobin

There was a significant increase in myoglobin concentrations after both the static ($p < 0.001$) and dynamic ($p = 0.028$) apnoea protocols, whereas no significant differences were denoted during control ($p = 0.493$) (Figure 3A). Specifically, myoglobin was significantly higher than baseline (22.3 ± 2.7 ng/ml) at 30 (+42%, 31.2 ± 3.3 ng/ml, $p = 0.040$), 90 (+64%, 36.5 ± 11.5 ng/ml, $p < 0.001$), and 180 min (+49%, 32.7 ± 7.3 ng/ml, $p = 0.013$) following the last static apnoic bout (Figure 3A), whereas during the dynamic apnoea intervention myoglobin concentrations were only elevated at 90 min (+63%, 35.5 ± 16.7 ng/ml, $p = 0.016$) after the last apnoeic repetition.

Resting baseline concentrations were significantly higher in the control group (EBHD, 22.3 ± 2.7 ng/ml; control, 27.5 ± 7.2 ng/ml, $p = 0.047$) (Figure 3A), as such, between-group differences (EBHD vs. control) were compared using delta percentage change (Figure 3B). Myoglobin concentrations were significantly higher in response to the static ($p < 0.001$) and dynamic apnoea ($p = 0.004$) protocols versus control, with no differences observed between the apnoeic protocols ($p = 0.587$) (Figure 3B).
This study examined the effects of repeated maximal apnoeic bouts on the physiological milieu by assessing cerebral, cardiac and skeletal muscle stress-related biomarkers. The primary findings demonstrate that a series of repeated maximal static and dynamic apnoeas incite a significant rise in S100β and myoglobin concentrations without any detectable changes in NSE nor hscTNT. Taken together, our study suggests that a series of repeated maximal apnoeic attempts induce muscle injury and signify a potential, albeit minor blood-brain barrier disruption that appears to occur in the absence of neuronal-parenchymal damage.

S100β was significantly elevated from baseline only following the apnoic interventions (Figure 2A). S100β is a dimeric calcium-binding protein and is predominantly found in brain astrocytes. Molecular communication between blood and the brain is largely prevented by the blood-brain barrier, a highly selective semipermeable border that is primarily composed of microvascular endothelial cells linked by tight and adherent junctions. Evidence suggests that astrocytic proteins extravasate into the serum only when the blood-brain barrier is breached, with a direct correlation reported between the venous concentration of S100β and the magnitude of the blood-brain barrier opening. As such, our study suggests that a series of repeated maximal apnoeas is capable of transiently disrupting the blood-brain barrier. Considering that S100β exerts both neurotrophic and gliotrophic roles, it is presently unclear whether these increases serve a neuroprotective purpose (reactive astrogliosis) or rather represent a more menacing phenomenon (e.g., glial damage).

In spite of never reaching pathological limits (>0.10 µg/L), the transient S100β increases documented in the present study are higher than those previously reported in competitive breath-hold divers following a single, dry maximal static apnoic attempt and after a combined bout of static and dynamic apnoeas. It is currently well accepted that intermittent hypoxaemia has a dose-dependent association (i.e., severity of hypoxaemia, duration of exposure) with an increase in the permeability of the blood-brain barrier. This response is believed to be orchestrated, at least in part, by reactive oxygen species. Interestingly, using a contrast-enhanced magnetic resonance imaging technique, Kanner et al. observed a direct correlation between S100β concentrations and the magnitude of the opening of the blood-brain barrier. It is, therefore, tempting to speculate that the higher post-apnoic S100β concentrations recorded in the present study may relate to the nature of our experimental design which comprised of a series of repeated episodes of hypoxaemia interspersed with short periods of normal breathing.

It is generally accepted in the existing literature that S100β is a peripheral biochemical marker that is implicated in brain damage and neurodegenerative processes. It is also noteworthy that, in contrast to NSE, S100β is expressed in skeletal muscle myofibers and is locally released in response to myofiber damage and degeneration. Moreover, S100β appears to be acutely important for skeletal muscle regeneration, due to its effects on myoblast proliferation through stimulation of extracellular signal-regulated kinase 1/2. Indeed, neutralisation of S100β release from acutely injured wild-type skeletal muscles reduced the population expansion of activated

**FIGURE 3** Absolute (A) and delta percentage change (B) in mean myoglobin from baseline to 180 min after the apnoeic and eupnoeic protocols. Data are presented as mean ± SD. * denotes significant difference (p < 0.05) compared with baseline; † represents significant difference between apnoeic and eupnoeic protocols. ng/ml, nanograms per millilitre
satellite cells, lowered the infiltration of injured tissues with macrophages as well as delayed the transition of macrophages from the M1 (proinflammatory) to the M2 (anti-inflammatory and pro-regenerative) phase\textsuperscript{31,32}; conjointly impairing the regenerative process. However, persistently high S100\(\beta\) levels may compromise the regenerative process by blocking myogenic differentiation\textsuperscript{31} through inhibition of p38 mitogen-activated protein kinase.\textsuperscript{31,32} Therefore, considering also the lack of changes in serum NSE, an alternative explanation for our findings might be that the elevated serum S100\(\beta\) levels may also stem from injured myofibers and represent a transient response to support the regeneration of injured skeletal muscle, by increasing the myoblast population in the local tissue area and preventing precocious myoblast differentiation.

A series of repeated maximal apnoeas did not elicit any changes in circulating NSE concentrations (Figure 2B), attesting against any form of neuronal-parenchymal damage.\textsuperscript{16,22} Our findings are in contrast to those of Kjeld et al.,\textsuperscript{12} whereby a significant rise in plasma NSE (from 14.5 ± 5.3 ng/ml to 24.6 ± 6.4 ng/ml) was documented ~3 h post (i.e, a range of post-apnoea timepoints 94–257 min) a combined maximal bout of static and dynamic apnoeas. It is noteworthy, however, that in the Kjeld et al.\textsuperscript{12} study, nine out of 17 breath-hold divers suffered a blackout episode (loss of consciousness) during their maximal attempts. Liner and Andersson\textsuperscript{33} demonstrated that a blackout episode is associated with disruption of the blood-brain barrier as evidenced by a significant rise in serum S100\(\beta\); increases that persisted for more than a day following the blackout incident. Hence, it is perhaps unsurprising that increases in NSE have been reported by Kjeld et al.\textsuperscript{12} Contrastingly, in our study, none of our participants suffered a blackout nor exhibited any signs associated with loss of motor control (e.g, confusion, postural disturbance, spasms, speech problems, unresponsiveness, etc.). Taken together, our findings suggest that in the absence of a hypoxaemic syncope, a series of repeated maximal apnoeas does not incite neuronal-parenchymal damage.

Myoglobin, a marker of muscle injury, was markedly elevated following both apnoeic interventions, while no changes were detected in the control group (Figure 3). Our findings align with those of Marlinge et al.\textsuperscript{15} who documented a similar rise in myoglobin following a 5 h spearfishing competition. These increases were not evident following a single maximal static apnoeic bout either with\textsuperscript{10} or without glossopharyngeal insufflation.\textsuperscript{15} Repeated maximal apnoeas have been linked with the upregulation of reactive oxygen species production\textsuperscript{18} and aggravation of oxidative stress levels\textsuperscript{20}; undulations that, in the absence of sufficient antioxidant enzyme defenses, are associated with oxidative damage.\textsuperscript{19} Specifically, excessive free radical accumulation instigates muscle cell damage, resulting in dysregulation of sodium-calcium channel functioning, ultimately elevating intracellular free ionized calcium.\textsuperscript{34} This causes a resultant activation of calcium-dependent enzymes, which go on to further metabolise and rupture the sarcolemma.\textsuperscript{34} Consequently, intracellular contents such as myoglobin and creatine kinase are released into the circulation.\textsuperscript{34} Therefore, our study demonstrates that, in a similar manner to repeated apnoeic dives, a series of repeated maximal static and dynamic apnoeas are associated with striatal muscle injury.

The presently recorded myoglobin increases are comparable to those reported following a 90-min cycling exercise bout (power output held at 90W)\textsuperscript{35} and those after eccentric-concentric exercise,\textsuperscript{36} but are substantially lower than those documented following a maximal endurance exercise bout performed under normoxic and hypoxic conditions.\textsuperscript{37} Collectively, the magnitude of myoglobin release denoted following a series of repeated maximal apnoeic epochs is well within the physiological limits (>85 ng/ml), hence, our findings suggest that, at least in EBHD, the risk of sustaining rhabdomyolysis is very low.\textsuperscript{38} However, what might seem surprising is that the post-apnoeic myoglobin concentrations did not differ across our apnoeic interventions (Figure 3), despite significantly lower end-apnoeic SpO\textsubscript{2} levels attained during the dynamic protocol (62 ± 10\% vs. 76 ± 5\%).\textsuperscript{5} It is possible that the greater number of apnoeic repetitions incorporated in our static apnoea protocol (ie, 10 vs. 5 repetitions) may have exacerbated the production of reactive oxygen species, consequently aggravating oxidative damage and promoting a similar release of myoglobin. A measure of oxidative stress would have certainly provided additional insights to the mechanistic basis of this effect.

To evaluate the magnitude of the cardiovascular burden imposed by repeated maximal apnoeic bouts we assessed hscTNT (Figure 2C), a regulatory protein that is expressed in cardiac myocytes and serves as a specific biomarker of myocardial injury.\textsuperscript{17} Interestingly, post-apnoeic cardiac troponin T concentrations did not differ from baseline, suggesting that a series of repeated maximal apnoeic bouts does not incite myocardial injury. Our data align well with earlier studies that showed no changes in cardiac troponin following a static apnoea packing-blackout that included episodes of asystole (cardiac troponin T\textsuperscript{10}), a single dry maximal static apnoeic attempt (cardiac troponin T\textsuperscript{15}) or a combined bout of static and dynamic apnoeas (hscTNT\textsuperscript{12}). They also concur with longitudinal studies that did not unveil any cardiac abnormalities nor morphological alternations in EBHD.\textsuperscript{1,39} Our findings do, however, contrast with those of Eichhorn et al.\textsuperscript{14} who documented a significant rise in hscTNT 4 h following a single, maximal, dry
static apnoic attempt in a group of EBHD. It is noteworthy that this increase was lower (\([\text{pre}]\ 2.2 \pm 1.1 \text{ pg/ml}\) vs. \([\text{post}]\ 3.1 \pm 1.7 \text{ pg/ml}\)) than that recorded in the present study (\([\text{pre}]\ 5.3 \pm 0.5 \text{ ng/L}\) vs. \([\text{3 h post}]\ 7 \pm 1.6 \text{ ng/L}\) and dynamic \(6.9 \pm 2.2 \text{ ng/L}\)). Thus, the current study signifies that a series of repeated maximal apnoic attempts does not evoke myocardial damage.

To conclude, this study suggests that a series of repeated maximal static and dynamic apnoes are associated with a potential, albeit minor, transient disruption of the blood-brain barrier as evidenced by a rise in S100β and instigate muscle injury as evinced by a rise in myoglobin but do not cause any detectable neuronal-parenchymal damage or myocardial damage.

5 | PERSPECTIVE

Considering the growing popularity of breath-hold diving as a competitive and recreational sport, enhancing our understanding of the possible health implications associated with exposure to such activities is paramount from a safety and medical standpoint. In this context, the present study demonstrates that, in EBHD, a series of repeated maximal apnoic bouts do not incite any detectable neuronal-parenchymal damage or myocardial damage but are associated with a potential transient, albeit minor, disruption of the blood-brain barrier and muscle injury. It is presently unclear whether these physiological responses are coherently expressed in non-divers. As such, it is imperative that further research is conducted to evaluate the possible health risks associated with apnoic training.

ACKNOWLEDGEMENTS
We would like to thank all of the participants who volunteered in the present research project.

CONFLICT OF INTEREST
The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT
The datasets presented in this article are not readily available as sharing these will compromise the ethical standards and agreement with the participants.

ORCID
Antonis Elia https://orcid.org/0000-0002-5991-0733
Matthew J. Lees https://orcid.org/0000-0003-1422-0154

REFERENCES
1. Elia A, Gennser M, Harlow PS, Lees MJ. Physiology, pathophysiology and (mal)adaptations to chronic apnoic training: a state-of-the-art review. Eur J Appl Physiol. 2021;121(S):1543-1566. https://doi.org/10.1007/s00421-021-04664-x
2. Taboni A, Fagoni N, Moia C, Vinetti G, Ferretti G. Gas exchange and cardiovascular responses during breath-holding in divers. Respir Physiol Neurobiol. 2019;267:27-34. https://doi.org/10.1016/j.resp.2019.06.002
3. Baković D, Válic Z, Eterović D, et al. Spleen volume and blood flow response to repeated breath-hold apneas. J Appl Physiol. 2003;95(4):1460-1466. https://doi.org/10.1152/japplphysiol.00221.2003
4. Lemaître F, Chowdhury T, Schaller B. The trigeminocardiac reflex - a comparison with the diving reflex in humans. Arch Med Sci. 2015;11(2):419-426. https://doi.org/10.5114/ams.2015.50974
5. Elia A, Barlow MJ, Deighton K, Wilson OJ, O’Hara JP. Erythropoietic responses to a series of repeated maximal dynamic and static apnoes in elite and non-breath-hold divers. Eur J Appl Physiol. 2019;119(11–12):2557-2565. https://doi.org/10.1007/s00421-019-04235-1
6. Elia A, Wilson OJ, Lees M, et al. Skeletal muscle, haematological and splenic volume characteristics of elite breath-hold divers. Eur J Appl Physiol. 2019;119(11–12):2499-2511. https://doi.org/10.1007/s00421-019-04230-6
7. Elia A, Barlow MJ, Wilson OJ, O’Hara JP. Six weeks of dynamic apnoic training stimulates erythropoiesis but does not increase splenic volume. Eur J Appl Physiol. 2021;121(3):827-838. https://doi.org/10.1007/s00421-020-04565-5
8. Elia A, Barlow MJ, Wilson OJ, O’Hara JP. Splenic responses to a series of repeated maximal static and dynamic apnoes with whole-body immersion in water. Exp Physiol. 2021;106(1):338-349. https://doi.org/10.1113/epj088404
9. Andersson JP, Liner MH, Jonsson H. Increased serum levels of the brain damage marker S100B after apnea in trained breath-hold divers: a study including respiratory and cardiovascular observations. J Appl Physiol. 2009;107(3):809-815. https://doi.org/10.1152/japplphysiol.91434.2008
10. Andersson JP, Liner MH, Jonsson H. Asystole and increased serum myoglobin levels associated with ‘packing blackout’ in a competitive breath-hold diver. Clin Physiol Funct Imaging. 2009;29(6):458-461. https://doi.org/10.1111/j.1475-097X.2009.00892.x
11. Gren M, Shahimi P, Lautner R, et al. Blood biomarkers indicate mild neuroaxonal injury and increased amyloid beta production after transient hypoxia during breath-hold diving. Brain Inj. 2016;30(10):1226-1230. https://doi.org/10.1080/02699596.2016.1179792
12. Kjeld T, Jattu T, Nielsen HB, Goetze JP, Secher NH, Olsen NV. Release of erythropoietin and neuron-specific enolase after breath holding in competing free divers. Scand J Med Sci Sports. 2015;25(3):e253-e257. https://doi.org/10.1111/sms.12309
13. Bain AR, Ainslie PN, Hoiland RL, et al. Competitive apnea and its effect on the human brain: focus on the redox regulation of blood-brain barrier permeability and neuronal-parenchymal integrity. FASEB J. 2018;32(4):2305-2314. https://doi.org/10.1007/s00421-021-04664-x
14. Eichhorn L, Doerner J, Luetskins JA, et al. Cardiovascular magnetic resonance assessment of acute cardiovascular effects of voluntary apnoea in elite divers. J Cardiovasc Magn Reson. 2018;20(1):40. https://doi.org/10.1186/s12968-018-0455-x
15. Marlinge M, Coulange M, Fitzpatrick RC, et al. Physiological stress markers during breath-hold diving and SCUBA
16. Cheng F, Yuan Q, Yang J, Wang W, Liu H. The prognostic value of serum neuron-specific enolase in traumatic brain injury: systematic review and meta-analysis. PLoS One. 2014;9(9):e106680. https://doi.org/10.1371/journal.pone.0106680
17. Kemp M, Donovan J, Higham H, Hooper J. Biochemical markers of myocardial injury. Br J Anaesth. 2004;93(1):63-73. https://doi.org/10.1093/bja/aeh148
18. Jouliia F, Steinberg JG, Wolff F, Gavarry O, Jammes Y. Reduced oxidative stress and blood lactic acidosis in trained breath-hold human divers. Respir Physiol Neurobiol. 2002;133(1–2):121-130. https://doi.org/10.1016/s1569-9048(02)00133-7
19. Li C, Jackson RM. Reactive species mechanisms of cellular hypoxia-reoxygenation injury. Am J Physiol Cell Physiol. 2002;282(2):C227-C241. https://doi.org/10.1152/ajpcell.00112.2001
20. Sureda A, Batle JM, Tauler P, et al. Neutrophil tolerance to oxidative stress induced by hypoxia/reoxygenation. Free Radic Res. 2004;38(9):1003-1009. https://doi.org/10.1080/1071576040000984
21. Theunissen S, Sponsiello N, Rozloznik M, et al. Oxidative stress in breath-hold divers after repetitive dives. Diving Hyperb Med. 2013;43(2):63-66.
22. Marchi N, Rasmussen P, Kapural M, et al. Peripheral markers of brain damage and blood-brain barrier dysfunction. Restor Neurol Neurosci. 2003;21(3–4):109-121.
23. Witt KA, Mark KS, Hom S, Davis TP. Effects of hypoxia-reoxygenation on rat blood-brain barrier permeability and tight junctional protein expression. Am J Physiol Heart Circ Physiol. 2003;285(6):H2820-H2831. https://doi.org/10.1152/ajpheart.00589.2003
24. Kapural M, Krizanac-Bengez L, Barnett G, et al. Serum S-100beta as a possible marker of blood-brain barrier disruption. Brain Res. 2002;940(1–2):102-104. https://doi.org/10.1016/S0006-8993(02)02586-6
25. Kanner AA, Marchi N, Fazio V, et al. Serum S100beta: a non-invasive marker of blood-brain barrier function and brain lesions. Cancer. 2003;97(11):2806-2813. https://doi.org/10.1002/cncr.11409
26. Van Eldik LJ, Wainwright MS. The Janus face of glial-derived S100B: beneficial and detrimental functions in the brain. Restor Neurol Neurosci. 2003;21(3–4):97-108.
27. Zongo D, Ribèreau-Gayon R, Masson F, et al. S100-B protein as a screening tool for the early assessment of minor head injury. Ann Emerg Med. 2012;59(3):209-218. https://doi.org/10.1016/j.annemer.2011.07.027
28. Lochhead JJ, McCaffrey G, Quigley CE, et al. Oxidative stress increases blood-brain barrier permeability and induces alterations in occludin during hypoxia-reoxygenation. J Cereb Blood Flow Metab. 2010;30(9):1625-1636. https://doi.org/10.1038/jcbf.2010.29
29. Zehendner CM, Librizzi L, Hedrich J, et al. Moderate hypoxia followed by reoxygenation results in blood-brain barrier breakdown via oxidative stress-dependent tight-junction protein disruption. PLoS One. 2013;8(12):e82823. https://doi.org/10.1371/journal.pone.0082823
30. Sorci G, Rizzi F, Arcuri C, et al. S100B protein in tissue development, repair and regeneration. World J Biol Chem. 2013;4(1):1-12. https://doi.org/10.4331/wjbc.v4.i1.1
31. Rizzi F, Beccafico S, Sagheddu R, et al. Levels of S100B protein drive the reparative process in acute muscle injury and muscular dystrophy. Sci Rep. 2017;7(1):12537. https://doi.org/10.1038/s41598-017-12880-9
32. Donato R, Cannon BR, Sorci G, et al. Functions of S100 proteins. Curr Mol Med. 2013;13(1):24-57.
33. Liner MH, Andersson JP. Hypoxic syncope in a competitive breath-hold diver with elevation of the brain damage marker S100B. Aviat Space Environ Med. 2009;80(12):1066-1068. https://doi.org/10.3357/asem.2554.2009
34. Giannoglou GD, Chatzizisis YS, Misirli G. The syndrome of rhabdomyolysis: pathophysiology and diagnosis. Eur J Intern Med. 2007;18(2):90-100. https://doi.org/10.1016/j.ejim.2006.09.020
35. Suzuki K, Totsuka M, Nakaji S, et al. Endurance exercise causes interaction among stress hormones, cytokines, neutrophil dynamics, and muscle damage. J Appl Physiol. 1999;87(4):1360-1367. https://doi.org/10.1152/jappl.1999.87.4.1360
36. Heckel Z, Atлас T, Těkus É, Köszegi T, Laczkó I, Váčzi M. Monitoring exercise-induced muscle damage indicators and myoelectric activity during two weeks of knee extensor exercise training in young and old men. PLoS One. 2019;14(11):e0224866. https://doi.org/10.1371/journal.pone.0224866
37. Sumi D, Kojima C, Goto K. Impact of endurance exercise on hypoxia on muscle damage, inflammatory and performance responses. J Strength Cond Res. 2018;32(4):1053-1062. https://doi.org/10.1519/jsc.0000000000001911
38. Vangstad M, Bjornaa MA, Jacobsen D. Rhabdomyolysis: a 10-year retrospective study of patients treated in a medical department. Eur J Emerg Med. 2019;26(3):199-204. https://doi.org/10.1097/mej.0000000000000510
39. Doerner J, Eichhorn L, Luetskens JA, et al. Effects of repetitive prolonged breath-hold in elite divers on myocardial fibrosis and cerebral morphology. Eur J Radiol. 2018;103:13-18. https://doi.org/10.1016/j.ejrad.2018.03.020
40. Fitz-Clarke JR. Breath-hold diving. Compr Physiol. 2018;8(2):585-630. https://doi.org/10.1002/cphy.c160008

How to cite this article: Elia A, Woods DR, Barlow MJ, Lees MJ, O’Hara JP. Cerebral, cardiac and skeletal muscle stress associated with a series of static and dynamic apnoeas. Scand J Med Sci Sports. 2022;32:233–241. https://doi.org/10.1111/sms.14067