Splenic responses to a series of repeated maximal static and dynamic apnoeas with whole-body immersion in water

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Abstract
Splenic contractions occur in response to apnoea-induced hypoxia with and without facial water immersion. However, the splenic responses to a series of static (STA) or dynamic (DYN) apnoeas with whole-body water immersion in non-divers (NDs) and elite breath-hold divers (EBHDs) are unknown. EBHD (n = 8), ND (n = 10) and control participants (n = 8) were recruited. EBHD and ND performed a series of five maximal DYN or STA on separate occasions. Control performed a static eupnoic (STE) protocol to control against any effects of water immersion and diurnal variation on splenic volume and haematology. Heart rate (HR) and peripheral oxygen saturation (SpO2) were monitored for 30 s after each apnoea. Pre- and post-apnoeic splenic volumes were quantified ultrasonically, and blood samples were drawn for haematology. For EBHD and ND end-apnoeic HR was higher (P < 0.001) and SpO2 was lower in DYN (P = 0.024) versus STA. EBHD attained lower end-apnoeic SpO2 during DYN and STA than NDs (P < 0.001). Splenic contractions occurred following DYN (EBHD, −47 ± 6%; ND, −37 ± 4%; P < 0.001) and STA (EBHD, −26 ± 4%; ND, −26 ± 8%; P < 0.01). DYN-associated splenic contractions were greater than STA in EBHD only (P = 0.042). Haemoglobin concentrations were higher following DYN only (EBHD, +5 ± 8g/L, +4 ± 2%; ND, +8 ± 3 g/L, +4.9 ± 3%; P = 0.019). Haematocrit remained unchanged after each protocol. There were no between group differences in post-apnoeic splenic volume or haematology. In both groups, splenic contractions occurred in response to STA and DYN when combined with whole-body immersion. DYN apnoeas, were effective at increasing haemoglobin concentrations but not STA apnoeas. Thus, the magnitude of the splenic response relates to the hypoxemic stress encountered during apnoeic epochs.

KEYWORDS
apnoea, breath-hold, desaturation, diving response, haematocrit, haemoglobin, hypoxia, immersion, lactate, spleen

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INTRODUCTION

The diving reflex is an oxygen conserving mechanism that is activated during the state of apnoea (Gooden, 1994). This reflex is primarily characterized by an initial bradycardic response which slows the depletion of bodily oxygen stores. This process is followed by a selective sympathetically-induced peripheral vasoconstriction in the body’s extremities (arms and legs) and non-vital organs, with oxygenated blood being preferentially redistributed towards the vital organs (brain and heart) (Campbell, Gooden, & Horowitz, 1969; Kyhl et al., 2016; Shamsuzzaman et al., 2014; Sterba & Lundgren, 1988). Therefore, the diving reflex serves a key role in effectively and economically managing bodily oxygen stores, enabling apnoeas to be sustained for prolonged durations until respiration is restored.

It is well accepted that the spleen represents a constitutive part of the sympathetic nervous system (SNS) and serves as an erythrocyte reservoir, with humans storing ~10% of their total erythrocyte volume in their spleens (Bakovic et al., 2013; Hurford et al., 1996; Stewart & McKenzie, 2002). Evidence suggests that hypercapnia and hypoxia/hypoxemia stimulate splenic contractions, with the latter serving as the most effective stimulus (Lodin-Sundström & Schagatay, 2010; Richardson, Engan, Lodin-Sundström, & Schagatay, 2012). To exemplify, following 3-5 repeated maximal static apnoec attempts (i.e. apnoea-induced hypoxemia) the spleen contracts, releasing its stored oxygen-rich erythrocytes (~3.3%) into the systemic circulation (Schagatay, Andersson, Hallén, & Pålsson, 2001). This increases the oxygen reserve and the available oxygen supply to essential tissues, subsequently delaying the physiological breaking point and contributing towards an extended apnoeic duration (Bakovic et al., 2013; Schagatay, Haughey, & Reimers, 2005). Thus, a larger splenic volume with capacity to store a greater amount of erythrocytes is considered advantageous in an apnoeic performance context (Ilardo et al., 2018; Schagatay, Richardson, & Lodin-Sundström, 2012).

To date, the splenic responses to static apnoeas have only been examined under dry conditions (Engan, Richardson, Lodin-Sundström, van Beekvelt, & Schagatay, 2013; Sperlich, Zinner, Pfister, Holmberg, & Michels, 2015) or following face immersion in water (Palada et al., 2007; Schagatay et al., 2001, 2005). However, in a training and competitive context, apnoeas are performed whilst the whole body is fully immersed in water. Thus, the aforementioned studies do not represent the conditions and may not reflect the physiological responses normally experienced by breath-hold divers during bouts of apnoeic training and competition. Whole-body immersion in water (~30°C) has previously been documented to activate the SNS and augment the release of catecholamines (Espersen, Frandsen, Lorentzen, Kanstrup, & Christensen, 2002; Knight & Horvath, 1987; Šrámek, Šimečková, Jansky, Šavilková, & Vybiral, 2000; Weiss, Hack, Stehle, Pollert, & Weicker, 1988). Thespleen’s capacity to contract and regulate its volume is governed by humoral fluctuations (i.e. catecholamines) and mediated via activation of adrenoreceptors (i.e. α1, β1, α2 and β2) located within the spleen’s capsule, vasculature and parenchyma (Ayers, Davies, & Withrington, 1972; Bakovic et al., 2013; Fredén, Lundborg, Vilén, & Kutti, 1978; Kutti, Fredén, Melberg, & Lundborg, 1977; Olsson, Kutti, Lundborg, & Fredén, 1976). It is therefore tempting to speculate that the collective effect of apnoeas and whole-body immersion would stimulate a greater splenic response.

Accordingly, this study examined the effect of a series of repeated maximal static or dynamic apnoeas with whole-body immersion in splenic and systemic haematological responses in EBHD and ND populations. We hypothesized that dynamic apnoeas will induce a
stronger hypoxemic stress compared with static apnoeas and that this will stimulate a greater splenic and systemic haematological response.

2 | METHODS

2.1 | Ethical approval

Ethical approval for this human study was granted by the Leeds Beckett University Research Ethics Committee (52330), and all experimental procedures conformed to the Declaration of Helsinki, expect for registration in a database. All participants provided written informed consent before the study.

2.2 | Participants

Twenty-six, healthy, non-smoking male participants volunteered for this study and were stratified into three groups including, EBHD (n = 8; height, 183 ± 1 cm; body mass, 84 ± 12 kg), ND (n = 10; height, 182 ± 1 cm; body mass, 85 ± 7 kg) and control (n = 8; height, 178 ± 1 cm; body mass, 82 ± 11 kg). All breath-hold divers were national team members (Table 1) and physically active individuals with no prior breath-hold diving experience were randomly assigned to the ND or control group. An independent control group was recruited due to the practical implications and time constraints of the study.

2.3 | Methodology

Participants reported at Leeds Beckett University after a 12 h fast and abstinence from caffeine- and alcohol-containing beverages. In addition, participants were instructed to refrain from physical activity and apnoea-related activities for 24 h prior to and during each testing day (i.e. preliminary measures, apnoeic and eupnoeic protocols).

2.4 | Preliminary measures

Following arrival at the laboratory (~25°C), participant's anthropometric measurements were collected (Seca, Vogel & Halke, Hamburg, Germany). Participants then underwent a 20 min supine resting period followed by measurement of their resting heart rate (HR) and peripheral oxygen saturation (SpO2; Nellcor PM10N, Medtronic, Minneapolis, MN, USA). The participants' splenic volumes were then quantified using a non-invasive ultrasonic portable device (MindRay DP-50, Shenzhen Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, China). Participants were seated vertically while the site for spleen measurements was identified from the dorsal side. Thereafter, three measurements of each triaxial measurement point of the spleen's maximal length (L), thickness (T) and width (W) were determined [coefficient of variation (CV) ~6%], with the mean for each point being used to calculate splenic volume through the use of the Pilström formula \[L_p(W_l - T_l)/3\].

Finger capillary blood samples were collected to assess concentrations of blood lactate (LactatePlus; NOVA Biomedicals, Waltham, MA, USA), haemoglobin (HemoQue Hb 201+; DM System, Ängelholm, Sweden) and haematocrit (Hawksley Micro Haematocrit Centrifuge, London, UK). Plasma and blood volume changes for each post apnoeic time point were determined using the methods of Dill and Costill (1974). Prior to collecting any blood samples, the participant's fingers were cleaned and dried with a towel to avoid any influence of water on the results.

2.5 | Familiarization session

Within 24 h of completing the baseline measurements, participants completed a familiarization session that introduced them to the apnoeic disciplines and testing environment. Participants were familiarized with the trial conditions, requirements and were introduced to the static apnoea position (i.e. seated position immersed up to the neck) and the dynamic apnoea technique (i.e. horizontal underwater breaststroke swimming).

2.6 | Apnoea protocols

Within a week from completing the familiarization session, participants reported at the swimming pool (~28°C). Participants entered the swimming pool without wearing any wet or dry suits and performed, on separate occasions (i.e. separated a week apart), one set of five maximal static or dynamic apnoeas with a 2 min seated rest between each apnoea.

Participants were instructed to hold their breath after a deep but not maximal inspiration, and both hyperventilation and lung packing were prohibited. Participants received a 1 min warning prior to commencing each apnoea, received a nose clip 30 s prior to the apnoea and a 10 s countdown was provided prior to immersing their face underwater and commencing their maximal apnoea attempt. During the static apnoea protocol the participants' heart rate and SpO2 were monitored at 10 s intervals until 30 s post the termination of their maximal apnoeic attempt (Fagoni et al., 2017). During the dynamic apnoea protocol the participants’ heart rate and SpO2 were measured only up to 30 s after the termination of each maximal attempt, due to practical constraints. At the completion of each apnoea

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**TABLE 1** Performance characteristics of elite breath-hold divers

| Characteristics                      | Elite breath-hold divers (n = 8) |
|--------------------------------------|----------------------------------|
| Time practising apnoea (years)       | 6 ± 2                            |
| Personal best static apnoea (s)      | 373 ± 35                         |
| Personal best dynamic apnoea without fins (m) | 133 ± 42 |

Data are mean ± SD.
Peripheral oxygen saturation

**RESULTS**

**Heart rate**

Statistical analysis

Control protocol

Apnoeic performances

Control protocol

A bradycardial response was evident in both groups during the static apnoea protocol, with an earlier response evident in the EBHD group, although this only approached significance (P = 0.067; Table 3). During the static apnoea protocol, the HR$_{\text{min}}$ was significantly lower in the EBHD group compared with the ND group (P = 0.001; Table 3). When static apnoeas were expressed as a biphasic percentage (i.e. beginning of apnoea, 0% and termination of apnoea, 100%) the time to HR$_{\text{min}}$ was not significantly different between groups (P = 0.086). There was a strong negative correlation (r = −0.98, P < 0.001) between the HR$_{\text{min}}$ and the static apnoea duration. There was also a strong negative correlation (r = −0.91, P < 0.001) between the time to HR$_{\text{min}}$ and the static apnoea duration.

For both groups, the end-apnoeic HR for each maximal apnoeic repetition was not different post the static apnoea protocol when compared with baseline (EBHD, P = 0.585; ND, P = 0.179) or when compared between groups (P = 0.585; Table 4). The end-apnoeic HR for maximal dynamic repetition was significantly higher than baseline for both groups post the dynamic apnoea protocol (EBHD, P < 0.001; ND, P < 0.001), however, there was no significant difference between groups (P = 0.342; Table 4). For both groups, end-apnoeic HR was higher post each successive dynamic apnoea attempt compared with the static apnoea protocol (EBHD, P < 0.001; ND, P < 0.001).

Peripheral oxygen saturation

The mean end-apnoeic SpO$_2$ in EBHD was significantly lower in response to the static (P = 0.006) and dynamic (P = 0.006) apnoeic protocols compared with the control protocol (98 ± 1%). In ND, a significantly lower end-apnoeic SpO$_2$ was only observed between
**FIGURE 1**  Mean (± SD) end-apnoeic SpO₂, relative volume of spleen, blood volume and plasma volume for static apnoeas. Abbreviations: EBHD, elite breath-hold divers; ND, non-divers; SpO₂, peripheral oxygen saturation. Significance (P < 0.05) from baseline is denoted as * (P < 0.05), between apnoeic protocol group differences are denoted as † (P < 0.05), and apnoeic versus control protocol differences are denoted as ‡ (P < 0.05).

**TABLE 2**  Participant apnoeic performance characteristics

| Protocol | 1 | 2 | 3 | 4 | 5 |
|----------|---|---|---|---|---|
| STA (s)  | 182 ± 50 † | 60 ± 33 | 187 ± 65 † | 69 ± 54 | 207 ± 68 † | 63 ± 30 | 224 ± 73 † | 72 ± 42 | 248 ± 52 † | 77 ± 36 |
| DYN (m)  | 80 ± 30 † | 25 ± 9 | 72 ± 22 † | 20 ± 5 | 74 ± 22 † | 21 ± 6 | 73 ± 22 † | 22 ± 6 | 75 ± 24 † | 21 ± 7 |

Data are means ± SD. Significant (P < 0.05) between group differences are denoted as †.

**TABLE 3**  Heart rate responses to each successive maximal static apnoeic attempt

| Parameter | Group | Baseline | Static apnoeic repetitions |
|-----------|-------|----------|---------------------------|
| HRₘᵦᵢᵥ (beats min⁻¹) | EBHD | 62 ± 12 | 49 ± 6 † | 45 ± 5 † | 45 ± 7 † | 45 ± 8 † | 43 ± 8 † |
| ND | 71 ± 10 | 64 ± 10 | 60 ± 11 | 60 ± 8 | 60 ± 6 | 58 ± 7 |
| Time to HRₘᵦᵢᵥ (s) | EBHD | – | 77 ± 44 | 81 ± 48 | 81 ± 51 | 80 ± 64 | 84 ± 58 |
| ND | – | 38 ± 10 | 54 ± 40 | 34 ± 23 | 44 ± 18 | 43 ± 18 |
| Time to HRₘᵦᵢᵥ (%) | EBHD | – | 43 ± 17 | 47 ± 27 | 42 ± 26 | 37 ± 25 | 35 ± 26 |
| ND | – | 68 ± 28 | 79 ± 27 | 49 ± 32 | 69 ± 42 | 60 ± 36 |
| Time to bradycardia (s) | EBHD | – | 31 ± 13 | 26 ± 14 | 24 ± 16 | 14 ± 11 | 19 ± 24 |
| ND | – | 44 ± 10 | 45 ± 44 | 40 ± 28 | 51 ± 41 | 33 ± 21 |

Data are means ± SD. Significant (P < 0.05) between group differences are denoted as †.

Abbreviations: EBHD, elite breath-hold divers; HRₘᵦᵢᵥ, heart rate minimum; and ND, non-divers.
### Table 4: Cardiovascular, splenic and haematological responses after each successive maximal static and dynamic apnoeic attempt for both groups

| Parameter                        | Group       | Baseline | Apnoeic repetitions |  |  |  |  |  |  |  |  |  |  |
|----------------------------------|-------------|----------|---------------------|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--}|
3.5 | Spleen

When end-apnoeic splenic volumes were compared between the apnoeic protocols versus control, significantly greater splenic volume reductions occurred in both groups during the dynamic apnoea protocol (EBHD, $P = 0.003$; ND, $P = 0.020$), but not during the static apnoea protocol (EBHD, $P = 0.176$; ND, $P = 0.064$). Significant reductions in splenic volumes were recorded from baseline for both groups during the static apnoea (EBHD, $P = 0.012$; ND, $P < 0.001$) and the dynamic apnoea protocols (EBHD, $P < 0.001$; ND, $P < 0.001$; Figures 1 and 2; Table 4), but no differences were observed between groups (static, $P = 0.954$; dynamic, $P = 0.289$).

When the end-apnoeic splenic volumes of EBHD and ND were compared between the apnoeic protocols, the dynamic apnoea protocol elicited significantly greater splenic contractions compared with the static apnoea protocol in EBHD ($P = 0.042$), but not in ND ($P = 0.228$).

A significant strong, positive relationship ($P < 0.001$, $r = 0.814$) was observed between end-apnoeic splenic volume and end-apnoeic $\text{SpO}_2$ (Figure 3). Similarly, there was a significant strong positive correlation (static, $r = 0.64$, $P = 0.034$; dynamic, $r = 0.50$, $P = 0.006$) across conditions when resting baseline splenic volumes were correlated with apnoeic performances (Figure 3).

3.6 | Haemoglobin

For both groups, end-apnoeic haemoglobin concentrations were significantly higher from baseline during the dynamic apnoea protocol (EBHD, $P = 0.012$; ND, $P = 0.019$), but not during the static apnoea protocol (EBHD, $P = 0.471$; ND, $P = 0.228$; Table 4). Additionally, no differences were observed for either protocol when end-apnoeic haemoglobin concentrations were compared between groups (EBHD, $P = 0.630$; ND, $P = 0.149$), protocols (static, $P = 0.406$; dynamic, $P = 0.102$) or control intervention ($P < 0.992$; Table 4).
FIGURE 3  Relationship between: (a) resting splenic volume and mean best static apnoeic performance for each participant, (b) resting splenic volume and mean best dynamic apnoeic performance for each participant, (c) average end-apnoeic SpO2 and end-apnoeic splenic volume for each apnoeic repetition. Abbreviations: EBHD, elite breath-hold divers; ND, non-divers; and SpO2, peripheral oxygen saturation.

3.7  |  Haematocrit

Haematocrit concentrations were not significantly different from baseline for either group (dynamic, P = 0.853; static, P = 0.735) (Table 4). In addition, no significant differences in mean end-apnoeic haematocrit concentrations were identified when compared between group (EBHD, P = 0.267; ND, P = 0.079) or versus control (static, P < 0.754; dynamic, P < 0.554; Table 4).

3.8  |  Blood lactate

Mean end-apnoeic blood lactate concentrations were significantly higher than baseline for both groups during the static (EBHD, P < 0.001; ND, P < 0.001) and the dynamic apnoea protocols (EBHD, P < 0.001; ND, P < 0.001; Table 4). Significantly higher blood lactate concentrations were attained for both groups during the dynamic apnoea versus static apnoea protocol (EBHD, P < 0.001; ND, P < 0.001), with the EBHD achieving significantly higher lactate concentrations during both protocols compared with the ND (static, P = 0.008; dynamic, P = 0.004; Table 4).

3.9  |  Plasma and blood volume

Plasma volume and blood volume did not change for either protocol or group (P = 0.140; Figures 1 and 2).

4  |  DISCUSSION

This study made the first investigations into the splenic responses to a series of repeated maximal static and dynamic apnoeas with whole-body water immersion in EBHD and ND. The novel findings signify that relative to static apnoeas, dynamic apnoeas induced a stronger hypoxic stress and this was associated with, (i) a higher end-apnoeic HR, (ii) a lower end-apnoeic SpO2, (iii) a higher blood lactate concentration and, (iv) a greater splenic contraction (i.e. in the EBHDs only), but with a similar erythrocyte release. EBHD attained greater apnoeic performances and reached lower SpO2 than ND during both apnoeic protocols, but post-apnoeic splenic responses were similar across groups. These findings demonstrate that the magnitude of the splenic response is largely dictated by the magnitude of the hypoxic stress encountered during apnoeic epochs.

An earlier bradycardic response and a significantly lower HRmin were evident during the static apnoea protocol in the EBHD when compared with the ND. Interestingly, when time to HRmin was reported as a relative biphasic percentage, a faster but not significantly different time to HRmin was observed in the EBHD group compared with ND (Table 3). Our findings are in line with the literature (Ferretti et al., 1991; Lemaitre et al., 2005, 2008) and provide further evidence that apnoeic training augments the magnitude of the apnoea-induced bradycardial response (Joulia et al., 2002, 2003; Schagatay et al., 2000). Additionally, we identified a significant strong negative correlation between static apnoeic durations and HRmin (r = −0.98) and between static apnoeic durations and time to HRmin (r = −0.91), which reinforces the relationship between apnoeic durations and the magnitude of the diving reflex-induced oxygen-conserving mechanism. Collectively, these findings point to a stronger diving reflex response and a more efficient oxygen-conserving mechanism in the EBHD than ND.

A lower end-apnoeic SpO2 was evident in both groups during the dynamic apnoea protocol compared with static apnoeas. During both protocols, EBHD attained lower end-apnoeic SpO2 levels than ND (Table 4). Our findings agree with Overgaard et al. (2006) observations but are contrary to Breskovic et al. (2011) that reported similar end-apnoeic SpO2 post static (two repetitions) and dynamic apnoeas (one bout). These discrepancies might be attributed to the
fundamental differences between the protocols utilised (i.e. number of apnoeic repetitions, pre-apnoeic breathing protocol, resting periods). Additionally, a higher blood lactate concentration was observed in both groups during the dynamic apnoea protocol compared with the static apnoea protocol in our study (Table 4). These findings suggest that the addition of contractile activity during apnoeic attempts upregulates the consumption of bodily oxygen stores and progressively increases the reliance on anaerobic metabolism, evidenced by the concurrent accumulation of lactate. Therefore, our study provides further evidence that maximal dynamic apnoeas induce a greater hypoxic stress compared with maximal static apnoeas.

It is well accepted in the literature that the spleen plays an important role during apnoeic conditions, with its capacity to store oxygen-rich erythrocytes and release them into the systemic circulation during oxygen-deprived conditions (Hurford et al., 1990; Schagatay et al., 2001; Stewart & McKenzie, 2002). We observed a significant positive correlation between apnoeic performance levels and resting splenic volumes, which suggest that a larger splenic volume with capacity to hold a greater amount of erythrocytes is advantageous in an apnoeic context. These data signify that splenic size might serve as a strong predictor of apnoeic capabilities. Moreover, in line with earlier publications we failed to observe any between group differences in resting splenic volumes (Baković et al., 2003; Elia et al., 2019b; Prommer et al., 2007). Ilardo et al. (2018) demonstrated that splenic size is governed by natural selection on genetic variants in the PDE10A gene. Additionally, Bouten et al. (2019) indicated that 8 weeks of static apnoeic training (i.e. five apnoeic bouts per day) was successful in inducing splenic volume expansion (24%) in a non-diving population. Thus, splenic size may be governed by a complex interplay between apnoeic training and genetics.

The present study assessed the collective effect of whole-body immersion and apnoea on the splenic response. Significant splenic contractions were evident across groups and apnoeic protocols, with no effect of the static eupnoea protocol on splenic volume. The magnitude of splenic contractions following maximal static (≤−26%) and dynamic (≤−47%) apnoeas were greater than those documented in the literature after repeated dry static apnoeas (≤−21.8%; Engan et al., 2013; Sperlich et al., 2015), repeated static apnoeas with face immersion in cold water (≤−10°C, ≤−18%; Baković et al., 2003; Schagatay et al., 2001) and repeated static apnoeas at 4 m depth while wearing neoprene wetsuits (28°C; pre, 191 ± 47 mL; post, 144 ± 50 mL; Prommer et al., 2007). Evidence suggest that immersion in water stimulates the SNS which subsequently augments the release of circulating catecholamines (Espersen et al., 2002; Knight & Horvath, 1987; Šrámek et al., 2000; Weiss et al., 1988). Since, humoral fluctuations regulate the spleen’s contractility and volume (Ayers et al., 1972; Bakovic et al., 2013; Fredén et al., 1978; Kutti et al., 1977; Olsson et al., 1976; Stewart & McKenzie, 2002), it is likely that the greater splenic contractions observed in our study are attributable to the combined effect of apnoea-induced hypoxemia and water immersion. Collectively, our findings demonstrate that when apnoeas are coupled with whole-body immersion, a stronger splenic contraction is noticeable.

Splenic contractions developed progressively across apnoeas and reached maximal contractions following 3-5 repeated apnoeas. These observations are in agreement with earlier studies that assessed splenic responses following static apnoeas with or without face immersion in water (Bakovic et al., 2003; Schagatay, 2009). When end-apnoeic splenic volumes were compared between the apnoeic protocols, significantly greater splenic contractions were only observed during the dynamic apnoea protocol in the EBHD. The spleen contains ~98% sympathetic fibres and represents a constitutive part of the SNS (Stewart & McKenzie, 2002). In both mammals and humans, the spleen has been observed to contract in response to sympathetic nervous stimulation and hypoxia-induced increases in sympathetic output (Bakovic et al., 2013; Donald & Aarhus, 1974; Greenway, Lawson, & Stark, 1968; Hoka, Bosnjak, Arimura, & Kampine, 1989; Hurford et al., 1996; Stewart & McKenzie, 2002). Since the degree of hypoxemia is a potent stimulus for evoking splenic contractions, the lower SpO2 attained by the EBHD during the dynamic apnoea protocol compared with ND would have served as a stronger stimulus for evoking splenic contractions. Thus, providing a partial reasoning for the greater splenic volume contractions observed in EBHD in response to the dynamic apnoea protocol.

During maximal apnoeic epochs the human body is subjected to extreme chemoreflex stimulations, with a number of studies noting significant increases in arterial blood pressure and carbon dioxide (CO2) levels (Breskovic et al., 2011; Joulia et al., 2002, 2003; Sieber et al., 2009). In addition, as a consequence of sustaining longer apnoeic durations, EBHD are subjected to a greater degree of hypercapnic stimuli (i.e. higher end-apnoeic arterial CO2 levels) and hypoxic stress (i.e. lower end-apnoeic arterial O2 levels) compared with ND (Breskovic et al., 2012; Joulia et al., 2002; Willie et al., 2015). Interestingly, Richardson et al. (2012) demonstrated that hypercapnia (i.e. pre-breathing 5% CO2 in O2) facilitated a greater degree of splenic contractions during a series of three repeated maximal static apnoeas (−33% from control) compared with hypocapnia (+13%), normocapnia (−9%) and eupneic hypercapnia (+30%) at similar end-apnoeic arterial haemoglobin saturation levels. Accentuating that hypercapnia, acts as an independent stimulus for invoking splenic contractions- likely through interacting with central medullary and peripheral carotid body chemoreceptors (Richardson et al., 2012). Therefore, the greater splenic contractions observed in our EBHD group during the dynamic apnoea protocol (i.e. compared with the static apnoea protocol) may indicate that this group was exposed to a greater magnitude of chemoreflex stress than the ND group, which consequently served as a stronger stimulus for evoking splenic contractions. However, since we did not evaluate end-apnoeic arterial CO2 or blood pressure levels, we are unable to fully elucidate the underlining mechanisms that dictated these group differences and thus further rationalize our findings.

To the best of our knowledge, this is the first study to assess the splenic responses to a series of repeated maximal dynamic
apnoeas performed by either EBHD or ND. Our study demonstrated that dynamic apnoeas elicited, in both groups, splenic contraction and this was associated with a significant increase in haemoglobin concentration. Since no plasma or blood volume changes were reported during the dynamic apnoea protocol, it can be reasoned that the significant increases in haemoglobin concentrations were likely derived from the dynamic apnoea-associated splenic contractions and not evoked by water immersion or haemoconcentration. Interestingly, our EBHD groups’ post-apnoeic haemoglobin increases (+5 g/L, +4%; haematocrit unchanged) are greater than those previously reported in divers by Schagatay, Andersson, and Nielsen (2007) (+3 g/L, +2%; +1.3%, +3%) following repeated near-maximal apnoeas with facial immersion in cold water (10.4 ± 0.7 °C), by Richardson et al. (2005; 2009) (+4 g/L, +2.7%; and +4 g/L, +3%, respectively [haematocrit not assessed]) and by Schagatay et al. (2005) (+3.5 g/L, +2.4%; +0.93%, +2.2%) following repeated dry static apnoeas. However, our values are lower than those reported by Hurford et al. (1990) in Korean Ama divers (+11 g/L, +9.5%; +3.6%, +10.5%) after a routine diving shift (174 ± 46 min, depths of ~5–7 m). An explanation for the higher haemoglobin and haematocrit concentrations reported by Hurford et al. (1990) could be dehydration, hypovolaemia or extravascular volume displacement in connection with prolonged exercise and insufficient hydration (Harrison et al., 1986). Similarly our ND group’s haemoglobin increases (+8 g/L, +4.9%; haematocrit unchanged) were greater than those reported in untrained individuals by Richardson et al. (2005) (skiers, +3.0g/L, +2.1%; untrained +2.1g/L, +1.4% [haematocrit not assessed]), by Schagatay et al. (2001) (healthy untrained, +4.6 g/L, +3.3%; +2.38%, +6.4%) and by Hurford et al. (1990) (untrained Japanese divers, +4 g/L, +3%; haematocrit unchanged). Collectively, our novel findings signify that repeated maximal dynamic apnoeas are successful in stimulating haemoglobin release without affecting haematocrit concentrations. However, due to ethical considerations (i.e. repetitive whole-body immersions in water) we were unable to collect venous blood samples. Thus, the presently recorded post-apnoeic haemoglobin concentrations (i.e. from fingertip sampling) might be an underestimation of the true magnitude of the haematological fluctuations induced by the splenic response.

In conclusion, the present study demonstrated that repeated maximal static and dynamic apnoeas with whole-body immersion are effective in stimulating splenic contractions in both ND and EBHD. Moreover, dynamic apnoeas, in comparison with static apnoeas, elicited greater splenic contractions in EBHD only. In addition, haemoglobin increases were only observed following the dynamic apnoea protocol in both ND and EBHD, whereas haematocrit concentrations were unchanged across groups and apnoeic protocols. Lastly, our findings signify that the magnitude of the apnoea-induced splenic response is largely dictated by the degree of the hypoxic stress experienced during apnoeic epochs.

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**COMPETING INTERESTS**

None declared.

**AUTHOR CONTRIBUTIONS**

All authors contributed towards the research design. A.E. conducted experiments and performed data analysis. All authors wrote and reviewed the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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**REFERENCES**

Ayers, A. B., Davies, B. N., & Withrington, P. G. (1972). Responses of the isolated, perfused human spleen to sympathetic nerve stimulation, catecholamines and polypeptides. *British Journal of Pharmacology, 44*, 17–30.

Baković, D., Pivac, N., Eterović, D., Breskovic, T., Zubin, P., Obad, A., & Dujić, Z. (2013). The effects of low-dose epinephrine infusion on spleen size, central and hepatic circulation and circulating platelets. *Clinical Physiology and Functional Imaging, 33*, 30–37.

Baković, D., Valic, Z., Eterović, D., Vuković, I., Obad, A., Marinović-Terzić, I., & Dujić, Z. (2003). Spleen volume and blood flow response to repeated breath-hold apneas. *Journal of Applied Physiology, 95*, 1460–1466.

Bouten, J., Caen, K., Stautemas, J., Lefevere, F., Derave, W., Lootens, L., … Boone, J. (2019). Eight weeks of static apnea training increases spleen volume but not acute spleen contraction. *Respiratory Physiology & Neurobiology, 266*, 144–149.

Breskovic, T., Lojpur, M., Maslov, P. Z., Cross, T. J., Kraljevic, J., Ljubkovic, M., … Dukic, Z. (2012). The influence of varying inspired fractions of O₂ and CO₂ on the development of involuntary breathing movements during maximal apnoea. *Respiratory Physiology & Neurobiology, 181*, 228–233.

Breskovic, T., Uglesic, L., Zubin, P., Kuch, B., Kraljevic, J., Zanchi, J., … Dukic, Z. (2011). Cardiovascular changes during underwater static and dynamic breath-hold dives in trained divers. *Journal of Applied Physiology, 111*, 673–678.

Campbell, L. B., Gooden, B. A., & Horowitz, J. D. (1969). Cardiovascular responses to partial and total immersion in man. *The Journal of Physiology, 202*, 239–250.

Dill, D. B., & Costill, D. L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of Applied Physiology, 37*, 247–248.

Donald, D. E., & Aarhus, L. L. (1974). Active and passive release of blood from canine spleen and small intestine. *The American Journal of Physiology, 227*, 1166–1172.

Elia, A., Barlow, M. J., Deighton, K., Wilson, O. J., & O’Hara, J. P. (2019a). Erythropoietic responses to a series of repeated maximal dynamic and static apnoeas in elite and non-breath-hold divers. *European Journal of Applied Physiology, 119*, 2557–2565.

Elia, A., Wilson, O. J., Lees, M., Parker, P. J., Barlow, M. J., Cocks, M., & O’Hara, J. P. (2019b). Skeletal muscle, haematological and splenic volume characteristics of elite breath-hold divers. *European Journal of Applied Physiology, 119*, 2499–2511.
Fredén, K., Lundborg, P., Vilén, L., & Kutti, J. (1978). The peripheral platelet count in response to adrenergic alpha- and beta-1-receptor stimulation. *Scandinavian Journal of Haematology, 21*, 427–432.

Gooden, B. A. (1994). Mechanism of the human diving response. *Integrative Physiological and Behavioral Science*, 29, 6–16.

Greenway, C. V., Lawson, A. E., & Stark, R. D. (1968). Vascular responses of the spleen to nerve stimulation during normal and reduced blood flow. *The Journal of Physiology*, 194, 421–433.

Harrison, M. H., Keil, L. C., Wade, C. A., Silver, J. E., Geelen, G., & Greenleaf, J. E. (1986). Effect of hydration on plasma volume and endocrine responses to water immersion. *Journal of Applied Physiology*, 61, 1410–1417.

Hoka, S., Bosnjak, Z. J., Arikura, H., & Kumpel, J. P. (1989). Regional venous outflow, blood volume, and sympathetic nerve activity during severe hypoxia. *The American Journal of Physiology*, 256, H162–H170.

Hurford, W. E., Hochachka, P. W., Schneider, R. C., Guyton, G. P., Stanek, K. S., Zapol, D. G., ... Zapol, W. M. (1996). Splenic contraction, catecholamine release, and blood volume redistribution during diving in the Weddell seal. *Journal of Applied Physiology*, 80, 298–306.

Hurford, W. E., Hong, S. K., Park, Y. S., Ahn, D. W., Shiraki, K., Mohri, M., & Zapol, W. M. (1990). Splenic contraction during breath-hold diving in the Korean ama. *Journal of Applied Physiology*, 69, 932–936.

Ilardo, M. A., Molitke, I., Kornelissen, T. S., Cheng, J., Stern, A. J., Racimo, F., ... Willerslev, E. (2018). Physiological and genetic adaptations to diving in sea nomads. *Cell*, 173, 569–580.e515.

Jouila, F., Steinberg, J. G., Faucher, M., Jamin, T., Ulmer, C., Kissop, N., & Jammes, Y. (2003). Breath-hold training of humans reduces oxidative stress and blood acidosis after static and dynamic apnea. *Respiratory Physiology & Neurobiology*, 137, 19–27.

Jouila, F., Steinberg, J. G., Wolff, F., Gavarry, O., & Jammes, Y. (2002). Reduced oxidative stress and blood lactic acidosis in trained breath-hold human divers. *Respiratory Physiology & Neurobiology*, 133, 121–130.

Knight, D. R., & Horvath, S. M. (1987). Effect of hydrostatic pressure on plasma concentrations of norepinephrine during cold water immersion. *Undersea Biomedical Research*, 14, 1–10.

Kutti, J., Fredén, K., Melberg, P. E., & Lundborg, P. (1977). The exchangeable splenic platelet pool in response to selective adrenergic beta-1-receptor blockade. *British Journal of Haematology*, 37, 277–282.

Kyh, K., Drvis, I., Barak, O., Mijacika, T., Engström, T., Secher, N. H., ... Madsen, P. L. (2016). Organ perfusion during voluntary pulmonary hyperinflation: a magnetic resonance imaging study. *American Journal of Physiology-Heart and Circulatory Physiology*, 310, H444–H451.

Lemaître, F., Bernier, F., Petit, I., Renard, N., Gardette, B., & Jouila, F. (2005). Heart rate responses during a breath-holding competition in well-trained divers. *International Journal of Sports Medicine*, 26, 409–413.

Lemaître, F., Buchheit, M., Jouila, F., Fontanari, P., & Tourry-Chollet, C. (2008). Static apneic effect on heart rate and its variability in elite breath-hold divers. *Aviation, Space, and Environmental Medicine*, 79, 99–104.

Lodin-Sundström, A., & Schagatay, E. (2010). Spleen contraction during 20 min normobaric hypoxia and 2 min apnea in humans. *Aviation, Space, and Environmental Medicine*, 81, 545–549.

Olsson, L. B., Kutti, J., Lundborg, P., & Fredén, K. (1976). The peripheral platelet count in response to intravenous infusion of isoprenaline. *Scandinavian Journal of Haematology*, 17, 213–216.

Overgaard, K., Friis, S., Pedersen, R. B., & Lykkeboe, G. (2006). Influence of lung volume, glossohygeal inflation and $P_{E1}O_2$ and $P_{E1}CO_2$ on apnea performance in trained breath-hold divers. *European Journal of Applied Physiology*, 97, 158–164.

Palada, I., Eterovic, D., Obad, A., Bakovic, D., Valic, Z., Ivancic, V., ... Dujic, Z. (2007). Spleen and cardiovascular function during short apneas in divers. *Journal of Applied Physiology*, 103, 1958–1963.

Prommer, N., Ehrmann, U., Schmidt, W., Steinacker, J. M., Radermacher, P., & Muth, C. M. (2007). Total haemoglobin mass and spleen contraction: A study on competitive apnea divers, non-diving athletes and untrained control subjects. *European Journal of Applied Physiology*, 101, 753–759.

Richardson, M. X., de Bruijn, R., Holmberg, H. C., Björklund, G., Haughey, H., & Schagatay, E. (2005). Increase of hemoglobin concentration after maximal apnealmaximas in divers, skiers, and untrained humans. *Canadian Journal of Applied Physiology*, 30, 276–281.

Richardson, M. X., de Bruijn, R., & Schagatay, E. (2009). Hypoxia augments apnea-induced increase in hemoglobin concentration and hematocrit. *European Journal of Applied Physiology*, 105, 63–68.

Richardson, M. X., Engan, H. K., Lodin-Sundström, A., & Schagatay, E. (2012). Effect of hypercapnia on spleen-related haemoglobin increase during apnea. *Diving and Hyperbaric Medicine*, 42, 4–9.

Schagatay, E. (2009). Predicting performance in competitive apneaa diving. Part I: Static apneaa. *Diving and Hyperbaric Medicine*, 39, 88–99.

Schagatay, E., Andersson, J. P., Hallén, M., & Påltsson, B. (2001). Selected contribution: Role of spleen emptying in prolonging apneas in humans. *Journal of Applied Physiology*, 90, 1623–1629.

Schagatay, E., Andersson, J. P., & Nielsen, B. (2007). Hematological response and diving response during apnea and apnea with face immersion. *European Journal of Applied Physiology*, 101, 125–132.

Schagatay, E., Haughey, H., & Reimers, J. (2005). Speed of spleen volume changes evoked by serial apneas. *European Journal of Applied Physiology*, 93, 447–452.

Schagatay, E., Richardson, M. X., & Lodin-Sundström, A. (2012). Size matters: Spleen and lung volumes predict performance in human apneic divers. *Frontiers in Physiology*, 3, 173.

Schagatay, E., van Kampen, M., Emanuelsson, S., & Holm, B. (2000). Effects of physical and apnea training on apneic time and the diving response in humans. *European Journal of Applied Physiology*, 82, 161–169.

Shamsuzzaman, A., Ackerman, M. J., Kuniyoshi, F. S., Accurso, V., Davison, D., Amin, R. S., & Somers, V. K. (2014). Sympathetic nerve activity and simulated diving in healthy humans. *Autonomic Neuroscience*, 181, 74–78.

Sieber, A., L’Abbate, A., Passera, M., Garbella, E., Benassi, A., & Bedini, R. (2009). Underwater study of arterial blood pressure in breath-hold divers. *Journal of Applied Physiology*, 107, 1526–1531.

Sperlach, B., Zinner, C., Pfister, R., Holmberg, H. C., & Michels, G. (2015). Repeated apnea-induced contraction of the spleen in cyclists does not enhance performance in a subsequent time-trial. *European Journal of Applied Physiology*, 115, 205–212.

Šránek, P., Šimečková, M., Janský, L., Šavliková, J., & Vybíral, S. (2000). Human physiological responses to immersion into water of different temperatures. *European Journal of Applied Physiology*, 81, 436–442.

Sterba, J. A., & Lundgren, C. E. (1988). Breath-hold duration in man and the diving response induced by face immersion. *Undersea Biomedical Research*, 15, 361–375.

Stewart, I. B., & McKenzie, D. C. (2002). The human spleen during physiological stress. *Sports Medicine (Auckland, N.Z.)*, 32, 361–369.
Weiss, M., Hack, F., Stehle, R., Pollert, R., & Weicker, H. (1988). Effects of temperature and water immersion on plasma catecholamines and circulation. *International Journal of Sports Medicine, 9*(Suppl 2), S113–S117.

Willie, C. K., Ainslie, P. N., Drvis, I., MacLeod, D. B., Bain, A. R., Madden, D., ..., Dujic, Z. (2015). Regulation of brain blood flow and oxygen delivery in elite breath-hold divers. *Journal of Cerebral Blood Flow and Metabolism, 35*, 66–73.

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