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RESEARCH PAPER

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 eupnoeic (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 (SpO_2) 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 SpO_2 was lower in DYN ($P = 0.024$) *versus* STA. EBHD attained lower end-apnoeic SpO_2 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

1 | 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 apnoeic 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, Janský, Šavlíková, & Vybíral, 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. α_1, β_1, α_2 and β_2) 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.

New Findings

- **What is the central question of this study?**
  Splenic contractions occur in response to apnoea-induced hypoxia with and without face immersion in water. However, the splenic responses to a series of static or dynamic apnoeas with whole-body water immersion in non-divers and elite breath-hold divers are unknown.
- **What is the main finding and its importance?**
  Static and dynamic apnoeas were equally effective in stimulating splenic contractions across non-divers and elite breath-hold divers. These findings demonstrate that the magnitude of the splenic response is largely dictated by the degree of the hypoxemic stress encountered during voluntary apnoeic epochs.

To the best of our knowledge, there are no reports of the splenic responses to a series of dynamic apnoeas performed by either non-divers (ND) or elite breath-hold divers (EBHD). The physiological demands imposed by static and dynamic apnoeas differ substantially (Elia et al., 2019a; Overgaard, Friis, Pedersen, & Lykkeboe, 2006). The addition of contractile activity during the state of dynamic apnoea imposes a significant challenge to the diving reflex, where myocardial and skeletal muscle oxygen consumption is increased, and blood flow is redistributed to meet the competing needs of both the vital organs and recruited striated muscle. In addition, apnoea-induced physiological responses (e.g. the magnitude of the bradycardial response, erythropoietin release) vary across trained and untrained populations (Elia et al., 2019a; Joulia, Steinberg, Wolff, Gavarry, & Jammes, 2002; Lemaitre et al., 2005; Lemaitre, Buchheit, Joulia, Fontanari, & Tourny-Chollet, 2008). Evidence suggests that these differences across diving and non-diving populations are, at least in part, the result of a training-induced stimulus (Joulia et al., 2003; Richardson et al., 2005; Schagatay, van Kampen, Emanuelsson, & Holm, 2000). However, it is currently unknown whether apnoea-induced splenic responses differ across trained and untrained populations. Therefore, to what extent the execution of a series of dynamic apnoeas results in greater splenic contractions and the concomitant increases in circulating erythrocytes differ between static and dynamic apnoeas and populations (EBHD *versus* ND) warrants further investigation.

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

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.

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 (SpO_2; 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 [$L\pi(WT - T^2)/3$].

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 apnoeic attempt. During the static apnoea protocol the participants' heart rate and SpO_2 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 SpO_2 were measured only up to 30 s after the termination of each maximal attempt, due to practical constraints. At the completion of each apnoea

the participants' splenic volumes were assessed and a finger capillary blood sample was collected for the identification of haemoglobin, haematocrit and blood lactate concentrations. After each apnoea the participants underwent a two-minute resting period during which they were allowed to relax and breathe normally in a seated position whilst remaining immersed in water up to the waist. This procedure was repeated five times for each protocol, with apnoeic duration (static and dynamic protocols) and distance swam (dynamic protocol) measured during each maximal apnoeic attempt.

2.7 | Control protocol

To control against any possible effects of whole-body immersion in water and diurnal variation on splenic volume and haematology, a control group performed a static eupnoeic (normal breathing) protocol. The static eupnoeic protocol replicated the water exposure times, resting periods and data collection time points of the static apnoea protocol and replaced apnoeas with normal breathing periods. The static apnoea protocol was chosen to construct the static eupnoeic protocol as the water exposure periods were longer compared with the dynamic apnoea protocol.

Participants reported to the swimming pool testing site, at same time period as for the apnoea measurements and were immersed up to the neck level.

2.8 | Statistical analysis

All participants completed the protocols successfully, and all data were statistically analysed using the SPSS statistics software v.21 (IBM, NY, USA). The Shapiro–Wilk test was used to assess normality, whereas homogeneity was assessed using Levene's test. Sphericity was assessed using Mauchly's test of sphericity; where the assumption of sphericity was violated, the Greenhouse–Geisser correction was applied. Repeated-measures ANOVA with Bonferroni *post hoc* contrast comparisons were used to assess differences between and within groups for baseline measurements and other collection time points for splenic volume, haemoglobin, haematocrit, blood lactate, HR, heart rate minimum (HR_{min}), SpO_2, plasma volume and blood volume. Time to HR_{min} was expressed as a relative percentage time course of static apnoeas for both groups (e.g. beginning of apnoea, 0% and termination of apnoea, 100%) and were compared using repeated-measures ANOVA with *post hoc* contrast comparisons to assess differences between and within groups. MANOVAs were used to assess differences in collection time points between groups (EBHD *versus* ND) and conditions (static *versus* dynamic). Pearson's correlation was used to assess for relationships between splenic volumes, performance levels, HR_{min} and SpO_2. Data were reported as mean ± SD. and significance was accepted at $P < 0.05$, and $P = 0.000$ was reported as $P < 0.001$. GraphPad Prism v.7.0c (GraphPad Software, CA, USA) was used to construct figures.

3 | RESULTS

3.1 | Control protocol

No significant difference was observed in SpO_2 ($P = 0.850$), splenic volume ($P = 0.229$), haemoglobin ($P = 0.141$) and haematocrit ($P = 0.664$) concentrations when compared with resting baseline values for the control group (Figure 1).

3.2 | Apnoeic performances

The EBHD attained significantly longer (68%) static apnoeic durations during each successive maximal attempt ($P < 0.001$; Table 2). A significant difference between groups in the distance travelled during each dynamic apnoeic bout was observed ($P < 0.001$), with EBHD covering significantly greater distances (67%) at all time points compared with ND (Table 2).

3.3 | Heart rate

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_{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_{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_{min} and the static apnoea duration. There was also a strong negative correlation ($r = -0.91$, $P < 0.001$) between the time to HR_{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, $P < 0.001$).

3.4 | 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

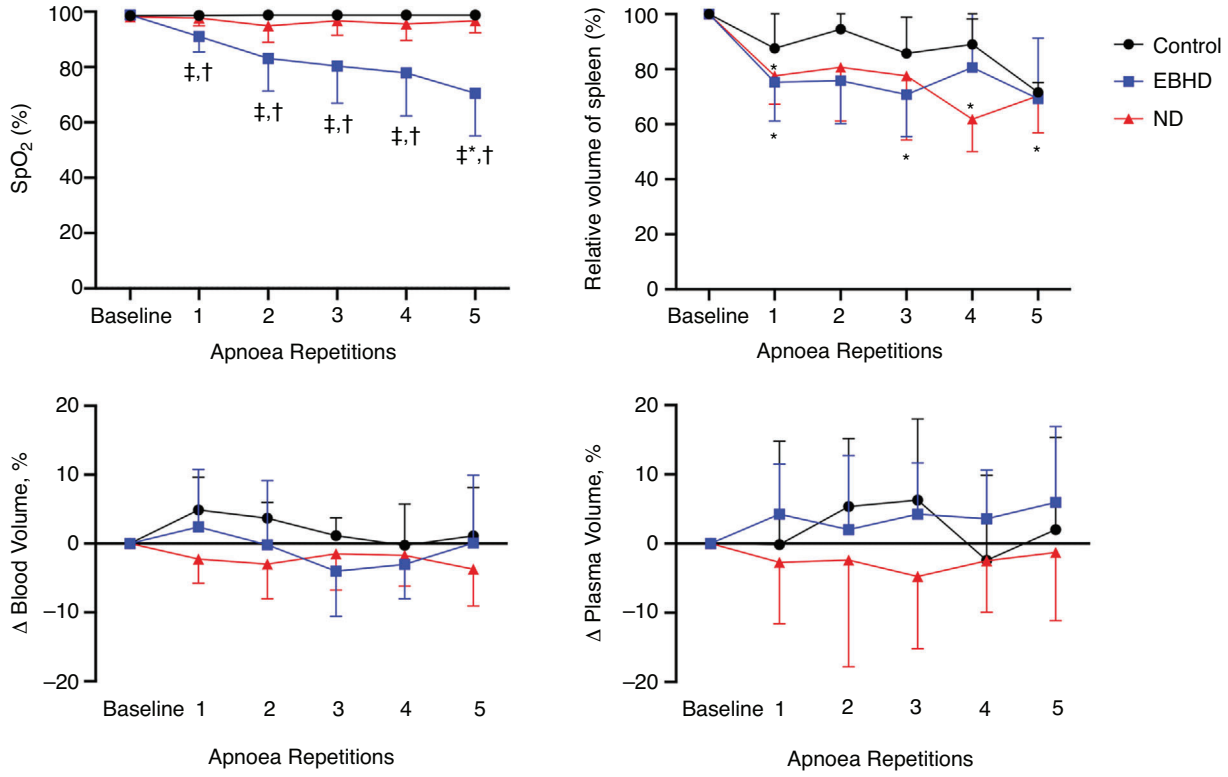

FIGURE 1 Mean (±SD) end-apnoeic SpO_2, relative volume of spleen, blood volume and plasma volume for static apnoeas. Abbreviations: EBHD, elite breath-hold divers; ND, non-divers; SpO_2, peripheral oxygen saturation. Significance ($P < 0.05$) from baseline is denoted as * ($P < 0.05$), between apnoeic 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

	Apnoeic repetitions									
	1		2		3		4		5	
Protocol	EBHD	ND	EBHD	ND	EBHD	ND	EBHD	ND	EBHD	ND
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 *.
Abbreviations: DYN, dynamic apnoea; EBHD, elite breath-hold divers; ND, non-divers; STA, static apnoea.

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

			Static apnoea repetitions				
Parameter	Group	Baseline	1	2	3	4	5
HR_{min} (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 HR_{min} (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 HR_{min} (%)	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; HR_{min}, 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

			Apnoeic repetitions									
			1		2		3		4		5	
Parameter	Group	Baseline	STA	DYN	STA	DYN	STA	DYN	STA	DYN	STA	DYN
Heart rate (beats min^{-1})	EBHD	62 ± 12	58 ± 11	107 ± 6*§	61 ± 19	113 ± 8*§	59 ± 19	116 ± 6*§	65 ± 28	116 ± 9*§	68 ± 32	115 ± 6*§
	ND	71 ± 10	66 ± 10	111 ± 13*§	62 ± 11	109 ± 15*§	65 ± 9	118 ± 16*§	63 ± 6	122 ± 15*§	64 ± 9	118 ± 15*§
	Control	–	–	–	–	–	–	–	–	–	–	–
SpO_2 (%)	EBHD	99 ± 1	91 ± 6‡	71 ± 10*†‡§	83 ± 12‡	67 ± 5*†‡§	80 ± 13‡	60 ± 16*†‡§	78 ± 16‡	59 ± 15*†‡§	71 ± 15*†‡	51 ± 18*†‡§
	ND	98 ± 1	97 ± 3	87 ± 8*§	95 ± 6	88 ± 4*†§	97 ± 5	84 ± 7*†§	96 ± 6	84 ± 6*†§	97 ± 5	85 ± 6*†§
	Control	98 ± 1	99 ± 1	–	99 ± 1	–	99 ± 1	–	98 ± 1	–	98 ± 1	–
Splenic volume (mL)	EBHD	307 ± 127	220 ± 76	163 ± 48	213 ± 88	157 ± 50	211 ± 76	152 ± 46	226 ± 74	138 ± 46†	206 ± 93	131 ± 53*†
	ND	308 ± 83	239 ± 76*	203 ± 63	250 ± 90	197 ± 78	226 ± 37	187 ± 63†	186 ± 42*	172 ± 54*†	217 ± 77*	199 ± 81*†
	Control	257 ± 136	210 ± 67	–	212 ± 112	–	231 ± 157	–	236 ± 147	–	186 ± 106	–
Splenic volume (%)	EBHD	100	75 ± 14*	60 ± 21*†§	74 ± 21	56 ± 21*†§	71 ± 15*	55 ± 18*†§	81 ± 25	49 ± 20*†§	69 ± 22	45 ± 15*†§
	ND	100	78 ± 10*	67 ± 16*†	81 ± 19	65 ± 23	78 ± 23	62 ± 17*†	62 ± 12*	57 ± 18*†	70 ± 13*	64 ± 18*†
	Control	100	88 ± 18	–	82 ± 10	–	86 ± 15	–	89 ± 10	–	72 ± 4	–
Haemoglobin (g/L)	EBHD	148 ± 6	146 ± 12	151 ± 9	150 ± 12	147 ± 6	155 ± 6	149 ± 9	153 ± 11	162 ± 11*	149 ± 14	153 ± 10
	ND	149 ± 4	152 ± 5	160 ± 11	154 ± 8	148 ± 13	151 ± 8	164 ± 12*	152 ± 6	154 ± 14	155 ± 8	158 ± 5*
	Control	159 ± 10	144 ± 18	–	154 ± 11	–	158 ± 10	–	160 ± 11	–	158 ± 8	–
Haematocrit (%)	EBHD	45 ± 2	46 ± 6	46 ± 5	46 ± 4	47 ± 3	49 ± 4	44 ± 5	48 ± 5	46 ± 6	47 ± 4	46 ± 5
	ND	45 ± 2	45 ± 5	48 ± 4	45 ± 5	47 ± 4	43 ± 5	50 ± 5	45 ± 4	49 ± 5	46 ± 4	48 ± 2
	Control	46 ± 3	44 ± 6	–	47 ± 3	–	47 ± 4	–	45 ± 8	–	46 ± 6	–
Lactate (mmol/L)	EBHD	0.51 ± 0.1	1.59 ± 0.4*	3.84 ± 0.8*‡§	1.69 ± 0.4*	4.56 ± 0.6*‡§	1.86 ± 0.3*‡	5.69 ± 1.3*‡§	1.93 ± 0.5*‡	5.88 ± 1.1*‡§	2.26 ± 0.6*‡	6.19 ± 1*‡§
	ND	0.66 ± 0.1	1.21 ± 0.5	2.69 ± 1.1*§	1.48 ± 0.5*	3.79 ± 1.6*§	1.33 ± 0.2*	3.71 ± 1.2*§	1.48 ± 0.4*	3.89 ± 1*§	1.49 ± 0.3*	4.23 ± 1.2*§
	Control	–	–	–	–	–	–	–	–	–	–	–

Data are mean ± SD. Significant ($P < 0.05$) difference from baseline is denoted as *, between apnoeic protocol differences ($P < 0.05$) are denoted as §, between apnoeic group differences ($P < 0.05$) are denoted as ‡, and apnoeic versus control protocol differences ($P < 0.05$) are denoted as †. Abbrevations: DYN, dynamic apnoea; EBHD, elite breath-hold divers; NDs non-divers; SpO_2, peripheral oxygen saturation; STA, static apnoea.

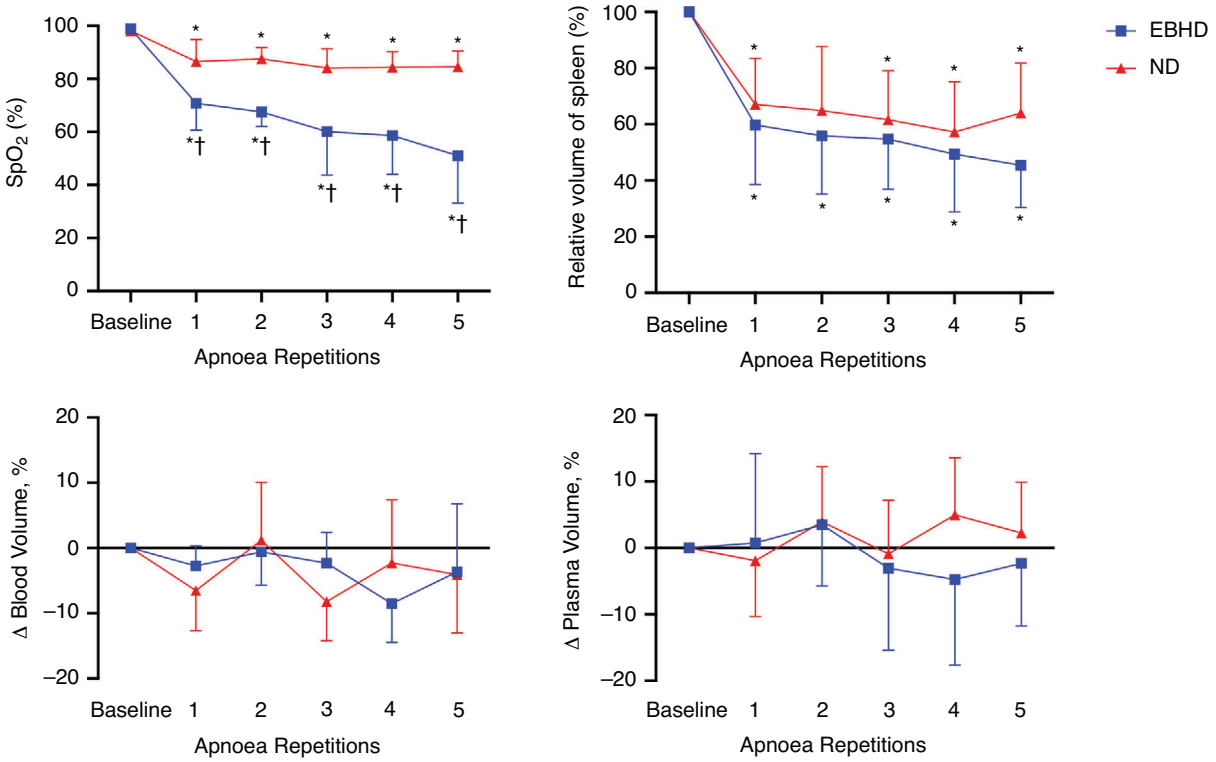

FIGURE 2 Mean (±SD) end-apnoeic SpO_2, relative volume of spleen, blood volume and plasma volume for dynamic apnoeas. Abbreviations: EBHD, elite breath-hold divers; ND, non-divers; and SpO_2, peripheral oxygen saturation. Significance ($P < 0.05$) from baseline is denoted as *, between group differences are denoted as † ($P < 0.05$)

the dynamic apnoea protocol *versus* control ($P < 0.001$) but not between the static apnoeas (statics *versus* control) ($P = 0.231$). The mean SpO_2 was significantly lower than baseline during each static apnoeic repetition in the EBHD group ($P = 0.002$), but not in the ND group ($P = 0.176$; Table 4). EBHD reached significantly lower SpO_2 levels at all static apnoeic repetitions than the ND group ($P = 0.002$; Table 4). Dynamic apnoeas induced a significant decrease in mean end-apnoeic SpO_2 from baseline in both groups (EBHD, $P < 0.001$; ND, $P < 0.001$), with the EBHD reaching lower SpO_2 at all apnoeic repetitions when compared with the ND group ($P < 0.001$; Table 4). When the end-apnoeic SpO_2 of EBHD and ND were compared between the apnoeic protocols, the dynamic apnoea protocol elicited significantly lower SpO_2 in EBHDs ($P = 0.004$) and ND ($P < 0.001$), respectively.

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 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).

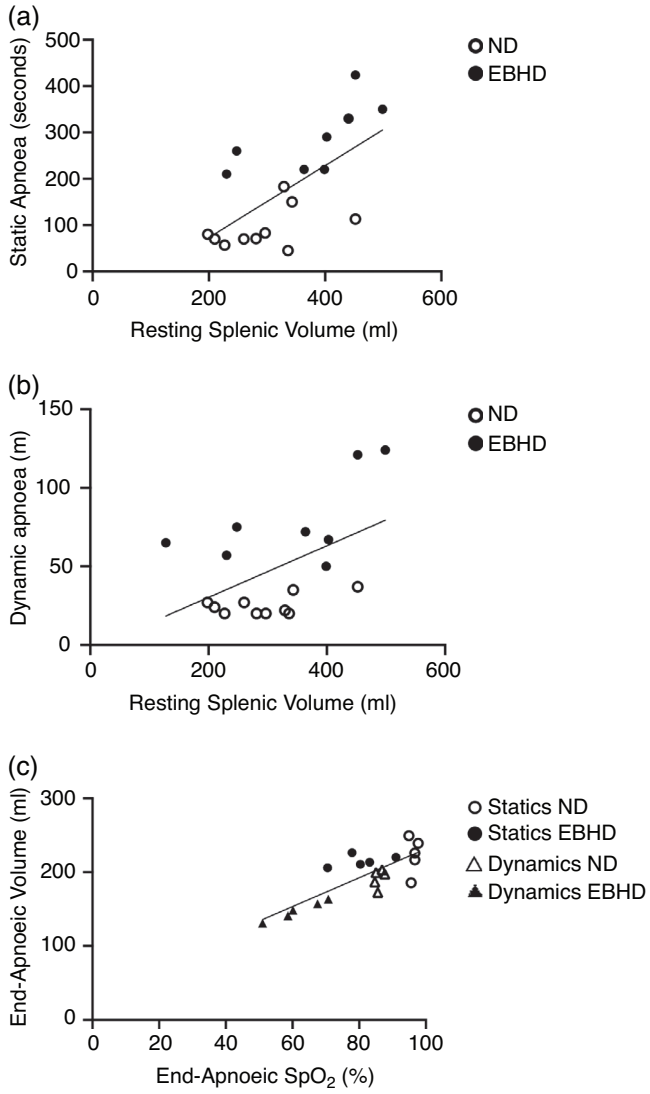

FIGURE 3 Relationship between: (a) resting splenic volume and mean best static apnoeic performance for each participant, (b) resting splenic volume and mean best dynamic apnoeic performance for each participant, (c) average end-apnoeic SpO_2 and end-apnoeic splenic volume for each apnoeic repetition. Abbreviations: EBHD, elite breath-hold divers; ND, non-divers; and SpO_2, 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 hypoxemic stress and this was associated with, (i) a higher end-apnoeic HR, (ii) a lower end-apnoeic SpO_2, (iii) a higher blood lactate concentration and, (iv) a greater splenic contraction (i.e. in the EBHDs only), but with a similar erythrocyte release. EBHDattained greater apnoeic performances and reached lower SpO_2 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 hypoxemic stress encountered during apnoeic epochs.

An earlier bradycardic response and a significantly lower HR_{min} were evident during the static apnoea protocol in the EBHD when compared with the ND. Interestingly, when time to HR_{min} was reported as a relative biphasic percentage, a faster but not significantly different time to HR_{min} 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 HR_{min} ($r = -0.98$) and between static apnoeic durations and time to HR_{min} ($r = -0.91$), 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 SpO_2 was evident in both groups during the dynamic apnoea protocol compared with static apnoeas. During both protocols, EBHD attained lower end-apnoeic SpO_2 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 SpO_2 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 hypoxemic 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 SpO_2 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 (CO_2) 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 (i.e. higher end-apnoeic arterial CO_2levels) and hypoxemic stress (i.e. lower end-apnoeic arterial O_2 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% CO_2 in O_2) 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 CO_2 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, +4.90%; 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 hypoxemic 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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