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HEALTH SCIENCES

Heart rate variability, thyroid hormone concentration, and neuropsychological responses in Brazilian navy divers: a case report of diving in Antarctic freezing waters

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Abstract: Open-water diving in a polar environment is a psychophysiological challenge to the human organism. We evaluated the effect of short-term diving (i.e., 10 min) in Antarctic waters on autonomic cardiac control, thyroid hormone concentration, body temperatures, mood, and neuropsychological responses (working memory and sleepiness). Data collection was carried out at baseline, before, and after diving in four individuals divided into the supporting (n=2) and diving (n=2) groups. In the latter group, autonomic cardiac control (by measuring heart rate variability) was also assessed during diving. Diving decreased thyroid-stimulating hormone (*effect size* = 1.6) and thyroxine (*effect size* = 2.1) concentrations; these responses were not observed for the supporting group. Diving also reduced both the parasympathetic (*effect size* = 2.6) and sympathetic activities to the heart (ES > 3.0). Besides, diving reduced auricular (*effect size* > 3.0), skin [i.e., hand (*effect size* = 1.2) and face (*effect size* = 1.5)] temperatures compared to pre-dive and reduced sleepiness state (*effect size* = 1.3) compared to basal, without changing performance in the working memory test. In conclusion, short-term diving in icy waters affects the hypothalamic-pituitary-thyroid axis, modulates autonomic cardiac control, and reduces body temperature, which seems to decrease sleepiness.

Key words: autonomic regulation, cold, parasympathetic, sympathetic, thyroid-stimulating hormone (TSH), thyroxine (T4).

INTRODUCTION

The permanence in the Antarctic environment, characterized by the acronym ICE (isolation, confinement, and extreme condition) (Olson 2002), represents a significant challenge to the human body. During Antarctic expeditions, Brazilian military navy divers need to submerge to maintain the ship (e.g., inspecting the hull) and perform regular training. Also, navy personnel partially immerse themselves during boat transportation of people and materials between the vessel and Antarctic beaches (near research stations and campsites). Diving in these freezing waters (i.e., water temperatures about -1.7 ºC; Milne & Thomson 1994) is likely a stressful condition for these military subjects that we investigated in the present study.

Autonomic cardiac control is markedly altered during a dive in the Arctic freezing waters, as indicated by the heart rate variability (HRV) measurements (Lundell et al. 2020). Whole-body immersion, including the face, initially triggers the diving reflex, a protective oxygen-conserving response (Kane & Davis 2018). During the diving reflex, trigeminal-brainstem-vagal pathways inhibit respiration and stimulate cardiac-vagal motoneurons (Jungmann et al. 2018, Lemaitre et al. 2015, Khurana et al. 1980). After that, parasympathetic activity decreases within the first five to ten minutes of diving. Moreover, diving demands moderate-intensity physical effort (Buzzacott et al. 2014, Pollock 2007), and icy waters impose a thermoregulatory burden due to the increased risk of hypothermia. Previous studies have shown that, even with the improved technology and training, considerable body heat loss and its psychophysiological impacts seem inevitable when diving in extreme environments (Lundell et al. 2020, Mantoni et al. 2006). Either cold exposure or physical exertion stimulates sympathetic and reduces parasympathetic efferent activity (Lundell et al. 2020, Harinath et al. 2005, Christensen & Galbo 1983). Therefore, the combined effects of diving in cold waters and exercising can provoke an exaggerated autonomic outflow, favoring the occurrence of arrhythmias and sudden death (Lundell et al. 2020, Kane & Davis 2018, Buchholz et al. 2017).

Reductions in body temperature may cause thermal discomfort and activate endocrine responses that augment thermogenesis (Mullur et al. 2014). In particular, thyroid hormones influence key metabolic pathways modulating heat production (Mullur et al. 2014). Experimental evidence indicates that cold exposure increases the concentrations of thyroid-stimulating hormone (TSH) and thyroxine (T4) (Mullur et al. 2014, Canali & Kruel 2001), depending on the exposure duration and intensity (Kovaničová et al. 2020, Iwen et al. 2017, Leppäluoto et al. 1988). Besides, physical exertion stimulates TSH (Canali & Kurel 2001) and induces the conversion of T4 into triiodothyronine (T3), thus boosting metabolism (Canali & Kurel 2001); however, whether a dive in Antarctica changes the concentration of these hormones is still unknown.

Cold exposure induces neurobehavioral changes, including increased negative mood states (Palinkas & Suedfeld 2008, Angus et al. 1979), impaired cognition (Lundell et al. 2020, Sandal et al. 2006, Le Scanff et al. 1997, Coleshaw et al. 1983, Davis et al. 1975), and reduced performance in tasks requiring sustained attention (Romeijn et al. 2012). As stated earlier, cold exposure also changes skin temperatures that, in turn, can modify alertness (Romeijn et al. 2012). Thus, integrated psychophysiological changes can take place in individuals during a dive session.

This case study aimed to evaluate the effects of diving on autonomic cardiac control and thyroid hormone concentration (i.e., TSH and T4). The temperature changes in exposed skin induced by diving in icy Antarctic waters and the subsequent psychophysiological aspects (i.e., mood, cognition, and wakefulness) were investigated. We hypothesized that short-term 10-min diving in Antarctic cold water would influence the body temperature, peripheral vascular tonus, and autonomic cardiac responses, modifying thyroid hormones and impacting neurobehavior.

MATERIALS AND METHODS

Ethics

This study followed the regulations of the Conselho Nacional de Saúde (CNS) do Brasil (resolution 466/2012) and was approved by the Research Ethics Committee of the Universidade Federal de Minas Gerais (Ref. 79278517.3.0000.5149). The volunteers were informed about the research objectives and experimental procedures before signing an informed consent form.

Subjects

Four Brazilian military divers participated in the study. The participants' anthropometric characteristics are presented in table I.

Experimental design

The divers took part in an expedition onboard Brazil's Navy polar ship "Almirante Maximiano" (number of tack H-41) from Rio de Janeiro (Brazil) to Antarctica. At the destination, we evaluated a dive in the vicinity of the Comandante Ferraz Antarctic Station (Admiralty Bay, King George Island). Our experiment was conducted in November 2018 during the Antarctic summer season. The four divers were divided into the Supporting group (n = 2, military divers who, at the time of the dive, remained on the boat) and the Diving group (n = 2, military divers who submerged). The Supporting group performed the same tasks as the Diving group, except the dive. More specifically, the Supporting group handled and checked the diving equipment (together with the Diving group) and helped the divers to enter the water (Figure 1a and 1b). The Supporting group entered the water for a brief period (only to assist the Diving group in the immersion) and returned to the boat less than five minutes later. Also, the Supporting group wore protective clothing and did not immerse their heads (body area that allows significant heat exchange with the environment). For both groups, data collection was carried at different time points (baseline, pre-dive, and post-dive). For the Diving group, autonomic cardiac control was also assessed during submersion in the icy water.

Data collection design

On the data collection day, the experimental procedures were carried out in the morning, between 09:00 h and noon. Blood sample collection (for TSH and T4 measurements), thermoregulatory responses (body temperatures and thermal sensation and comfort), HR and HRV recordings, and application of self-reported questionnaires (mood and sleepiness assessment) were conducted at baseline [on the ship, 09:00 h; ambient temperature of 18.6 ± 0.1° C and relative humidity (RH) of 29.5 ± 1.0% measured with a thermo-hygrometer (K29-5070H, Kasvi®, Brazil)], pre-dive (field, 11:30 h; 7.9 ± 0.6° C and 28.5 ± 5.7% RH), and post-dive (on the ship, noon; 18.3 ± 0.5° C and 29.8 ± 0.5% RH). HR and HRV were also recorded during diving (from 11:45 to 11:55 h; 7.9 ± 0.6° C and 28.5 ± 5.7% RH) in icy waters [-1.7° C ± 0.1° C, measured using an infrared sensor (566, Fluke®, USA) positioned 10 cm from the water)]. Baseline and post-dive measures were obtained on the ship and consisted of the cognitive test application, the 10-min HR and HRV recording, and the questionnaire filling; the volunteers remained seated in a chair inside a reserved room. During the cognitive test, the volunteer remained with his back turned to the researcher and with his hands in a standardized position to ensure

Table I. Heart rate variability (HRV) parameters measured at four time points (baseline, pre-dive, diving, and post-dive) in the Diving group (n=2).

	Age (years)	Height (cm)	Body mass (kg)	Body fat (%)	BMI ($kg.m^{-2}$)	ΣSkinfold (mm)	Systolic and diastolic blood pressure (cmHg)
Mean ± SD	39 ± 3	174 ± 3	82.5 ± 7.5	18.8 ± 4.1	27.1 ± 1.5	149 ± 14	12 ± 1 / 8 ± 1
Range	36 – 42	174 – 177	81.3 – 91.0	15.3 – 23.3	25.3 – 29.0	133 – 159	11 – 13 / 7 – 8

The data are expressed as means ± SD and range of variation (from minimum to maximum). Abbreviation: body mass index (BMI).

similar conditions between baseline and post-dive conditions.

Blood samples were obtained in both the Supporting and Diving groups. HR and HRV measures, thermoregulatory responses, questionnaires, and the cognitive test were recorded/obtained only in the Diving group. The timeline describing the data collection time points and variables measured is presented in figure 2.

The volunteers were oriented to refrain from ingesting alcohol and maintain their routine (i.e., habitual physical activity, sleep, and feeding) 24 h before data collection. The urine specific gravity was measured using reagent strips (Uriquest Plus I, Labtest, Lagoa Santa, Minas Gerais, Brazil). The subjects were considered euhydrated (urine specific gravity < 1.030; Armstrong 2000) before (1.021 ± 0.008) and after (1.020 ± 0.009) the dive.

Control data collection in the warmer waters of Rio de Janeiro

The effects of a dive in Rio de Janeiro (n=2 divers) were also evaluated, using similar procedures to those used in Antarctica. Physical characteristics of divers (one of whom also dived in Antarctica) and additional data are presented as Supplementary Material (Tables SI-SV). The measures were conducted at baseline (on the ship, 09:00 h; ambient temperature of 20.0° C and 40% RH), pre-dive (field, 11:00 h; 28.0° C and 68% RH), and post-dive (on the ship, noon; 29.0°

Figure 1. **Divers entering the icy waters in Antarctica. The moment when the Supporting group helped the divers to enter the water (a), and the moment immediately before submersion in the water (b).**

C and 64% RH). HR and HRV were also recorded during diving (from 11:13 to 11:19 h; 28° C and 65% RH) in the warmer waters [24.0°C, measured using an infrared sensor (566, Fluke®, USA)].

Procedures

Anthropometric characteristics

Body mass (digital balance HBF-214LA, Omron, Japan) was measured with volunteers wearing shorts, and their self-declared height was recorded. Skinfold thickness was measured on the right side using a skinfold caliper (MI, Lange®, USA) with a 1-mm accuracy at nine different sites (subscapular, triceps, biceps, pectoral, mid-axilla, supra-iliac, abdominal, mid-thigh, and calf). The same individual measured these skinfolds in triplicate, and the average value was recorded. The nine measures were summed to determine the Σskinfolds. Body fat was calculated according to the protocol proposed by Jackson & Pollock (1978). Body mass index (BMI) was calculated using the equation proposed by Quetelet (1869).

Blood pressure

Blood pressure was measured by an aneroid sphygmomanometer (Premium, Accumed, Rio de Janeiro, Brazil) in the volunteers' left arm after resting for at least 5 min.

Blood measures

A blood drop was collected from the digital pulp for glucose dosage (Accu-chek Active, Roche®, Switzerland). Five blood drops were collected on a filter paper (Whatman™ 903 Neonatal Screening Cards, Life Sciences, GE Healthcare, US) for the dosage of thyroid hormones. Each filter paper was dried and then placed in a separate envelope in plastic bags with silica. The envelopes were maintained away from light exposure and hot temperature until the samples were analyzed. TSH and T4 dosages were performed in duplicate using two disks (each dried blood spot had 3 mm in diameter). The circles were plated, diluted in europium buffer solution, and analyzed by time-resolved two-site fluoroimmunoassay using the direct double-sandwich technique (AutoDELFIA®

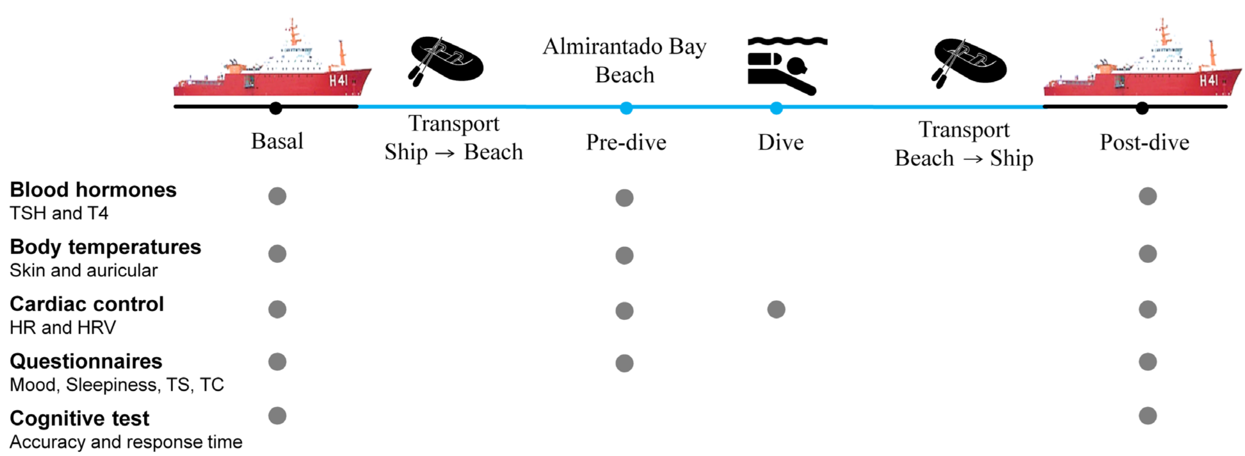

Figure 2. Timeline of data collection.
Data were collected at the following moments: 'basal', on the ship (at 9:00 am), 'pre-dive, in the Admiralty Bay Beach (at 11:30 am), 'dive', during immersion in open waters (from 11:45 am to 11:51 am), and 'post-dive, on the ship (at noon). The displacements between the ship and the dive site were carried out by boat. The gray circles represent the measurements made at each time point. Abbreviations: thyroid-stimulating hormone (TSH); thyroxine (T4); heart rate variability (HRV); heart rate (HR); thermal sensation (TS); thermal comfort (TC).

Neonatal hTSH and AutoDELFIA® Neonatal T4; WallacOy, Finland), as previously reported in similar population (Manousou et al. 2020, Moraes et al. 2020, Magalhães et al. 2017). TSH sensitivity is typically better than 2µ U/mL, and T4 sensitivity is typically better than 1.5 µg/dL. There is no relevant cross-reactivity to report (as only T4 has cross-reactivity with D-Thyroxine (30%), an isomer of thyroxine) (B032-312 AutoDELFIA Neonatal hTSH. Instructions for use 2016, B065-112 AutoDELFIA Neonatal Thyroxine (T4). Instructions for use 2016).

HRV and HR measures

HRV and HR were determined via recording the R-R intervals by a chest strap heart rate monitor (V800, Polar®, Finland). Data were exported to the Polar Flow web service for subsequent analysis, performed in Kubios HRV® Standard version 3.1.0 free software (Kubios Oy, Kuobios, Finland). All tachograms were visually inspected, and artifacts and ectopic heartbeats were excluded with a *very low* filter (which did not exclude more than 2% of the recorded data) (Camm et al. 1996). Data analysis was performed using 6-min intervals containing continuous recordings.

The following time-domain parameters were calculated: mean RR interval; the square root of the mean of the sum of the squares of differences between adjacent normal-to-normal (NN) intervals (RMSSD); the standard deviation of NN intervals (SDNN); the number of interval differences of successive NN intervals greater than 50 ms (NN50); the percentage of adjacent NN intervals with a time difference greater than 50 ms (pNN50). The following frequency-domain parameters were also calculated: the high frequency (HF) power band (0.15 to 0.40 Hz); the low frequency (LF) power band (0.04 to 0.15 Hz); the ratio between low and high-frequency components (LF / HF), and overall autonomic activity (total power; i.e., the sum of LF, HF, very low frequency, and ultra-low frequency bands).

The mean RR interval, RMSSD, and SDNN reflect the cardiac parasympathetic activity, predominantly influenced by the vagus nerve (Akselrod et al. 1981). HF reflects the parasympathetic influence and is related to the respiratory sinus arrhythmia (Akselrod et al. 1981), whereas the LF power band is assumed to have a dominant sympathetic component (Reyes del Paso et al. 2013) and to represent baroreflex activity as well (Goldstein et al. 2011). Finally, the LF / HF ratio represents the sympathovagal balance (Goldstein et al. 2011).

Skin and auditory canal temperatures

Body skin temperatures (Tsk) were measured at seven sites (i.e., $T_{FOREHEAD}$, T_{NOSE}, T_{CHEEK}, T_{CHIN}, T_{CHEST}, T_{HAND}, and T_{FOOT}) using an infrared sensor (566, Fluke®Corporation, OH, USA) positioned 10 cm from the skin for measurements of $T_{FOREHEAD}$, T_{NOSE}, T_{CHEEK}, $T_{CHIN,}$ and using Tsk probes (400A Series, Yellow Springs Instruments, Yellow Springs, OH, USA) connected to a thermometer (4600 Series, Yellow Springs Instruments) for measurements of T_{CHEST}, T_{HAND}, and T_{FOOT}. Mean face temperature (T_{FACE}) was determined by calculating the arithmetic mean of $T_{FOREHEAD}$, T_{NOSE}, T_{CHEEK}, and T_{CHIN}. The auditory canal temperature (T_{AUC}) was measured using a digital thermometer with an infrared sensor (G-TECH, model IR1DB1, Accumed). All measures were performed on the right side of the body.

Thermoregulatory scales

The subjects reported their thermal comfort (TC) using a numerical scale ranging from -10 (“extremely uncomfortable”) to 10 (“extremely comfortable”). Similarly, thermal sensation (TC) was reported using a numerical scale ranging from -10 (“unbearably cold”) to 10 (“unbearably hot”) (adapted from Nakamura et al. 2013).

Mood questionnaire

The mood states were assessed using the 24-item Brunel Mood Scale (BRUMS). The BRUMS has six dimensions (i.e., anger, confusion, depression, fatigue, tension, and vigor), with each dimension being composed of four items. Each item is preceded by the question “How do you feel right now?” and should be answered on a 5-point scale (from 0 to 4). Therefore, the total score for each dimension ranges from 0 to 16. The sum of the following feelings – anger, confusion, depression, fatigue, and tension – was used to determine the negative mood dimension.

Cognitive test

Working memory was assessed using the Match to Sample cognitive test provided by an application (Psych Lab 101, Presentation, USA) using a tablet with a 9.6-inch screen (Samsung Galaxy Note 8, South Korea). This test consists of a delayed match-to-sample test that measures working memory capacity (Daniel et al. 2016). In each trial, the volunteers first saw a pattern of red and blue squares (termed as boxes) within a larger square (termed as a grid). They should memorize the distribution pattern of the blue and red boxes within the grid, which disappeared after 1 s. After a delay (1 or 5 s), two grids reappeared: one with the previous distribution and the other with another distribution. The volunteers should select the grid corresponding to the previous distribution; they completed 32 attempts, with a random sequence consisting of 16 attempts with a 1-s delay and 16 attempts with a 5-s delay. The participants were instructed to perform this task as fast as possible with their hands in a standard position. Two parameters were measured: (i) percentage of correct hits in the 32 attempts (accuracy); and (ii) the average response time to the attempts. The application provides these parameters according to the delay (i.e., 1-s or 5-s delay). Before performing the test, the volunteers completed a trial to familiarize themselves with the task, both at the baseline and post-dive measurements.

Sleepiness

The Karolinska Sleepiness Scale (KSS; Åkerstedt & Gillberg 1990) was used to assess the volunteers’ level of alertness/sleepiness. They answered the question “How are you feeling now?” on a 9-point scale, ranging from 1 (“extremely alert”) to 9 (“very sleepy, great effort to keep awake, fighting sleep”).

Statistical analyses

Since there were few divers on the Brazilian ship, the number of subjects was limited in the present study. We then calculated Cohen’s *d* effect-size (*ES*) to understand our findings by assessing the magnitude of differences between data collection time points. The *ES* was calculated as follows: one mean value was subtracted from the other, and then the resulting difference was divided by a combined standard deviation of the data. The *ES* values were classified as trivial ($ES < 0.2$), small ($ES = 0.2 – 0.6$), moderate ($ES = 0.6 – 1.2$) or large ($ES \geq 1.2$) (Cohen 1988). All data are shown as means ± SD and as individual values of each volunteer. Only large effect sizes were considered relevant differences, and these large effects were only observed when all participants responded in the same direction. Data were normalized relative to the baseline values for the presentation concerning TSH, T4, and T4/TSH (e.g., normalized value for pre-dive = pre-dive / baseline value). The baseline value was set at 1. Finally, a Spearman Rank Order correlation was used to assess the strength of the association between thermoregulatory responses and sleepiness.

RESULTS

Blood measures

At baseline, the Diving group had higher TSH (0.8 ± 0.0 μ U.mL^{-1} vs. 0.5 ± 0.2 μ U.mL^{-1}; ES = 1.7) and T4 (8.0 ± 1.0 μ U.mL^{-1} vs. 7.0 ± 0.1 μU.mL^{-1}; ES = 1.4) concentrations than the Supporting group. Therefore, the pre- and post-dive data concerning the thyroid hormones were normalized to baseline values. There was no difference in the T4/TSH ratio between the two groups (Supporting: 13.9 ± 5.1 AU vs. Diving: 10.2 ± 1.2 AU; ES =1.0).

Compared to baseline, the Diving group presented an increase in TSH at the pre-dive time point (ES > 3.0), while there was no change in the Supporting group (ES = 0.6). Diving decreased TSH compared to pre-drive (ES = 1.6), with only a moderate effect compared to baseline (ES = 1.0). In contrast, no difference was observed in the Supporting group when comparing the TSH concentrations at the end of the experiment with baseline (ES = 0.3, Figure 3a) and pre-drive (ES = 0.4).

The Diving group presented an increase in T4 concentration at the pre-dive compared to the baseline (ES = 1.5), which was not observed for the same comparison in the Supporting group (ES = 0.6). Diving decreased T4 (ES = 2.1), and this response was not observed in individuals that did not submerge (Figure 3b). Interestingly, diving in tropical waters did not change TSH or T4 concentration (Table II).

Regarding the T4/TSH ratio, no difference was found between the Diving and Supporting group at the pre-dive relative to the baseline time point (ES = 0.6 and 0.5, respectively). There was no intergroup difference at the post-dive measurements, which were similar to baseline values in both groups (Figure 3c).

There was a reduction in blood glucose at the pre-dive relative to the baseline time point

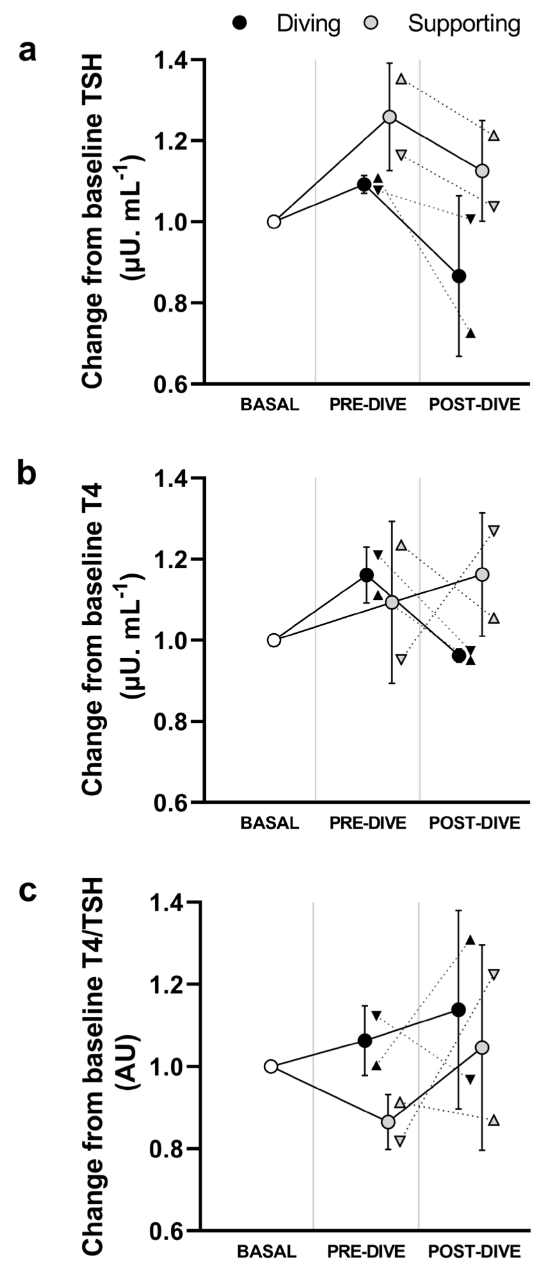

Figure 3. Blood hormonal concentrations measured at three time points: baseline (on the ship, 09:00 h), pre-dive (field, 11:30 h), and post-dive (on the ship, noon) in the two groups (n = 4). a) thyroid-stimulating hormone (TSH). b) thyroxine (T4). c) the T4/TSH ratio. The pre-and post-dive data were normalized to the baseline data, which were set at 1. The data are expressed as means ± SD for the Diving (black circle) and Supporting (grey circle) groups. The dots represent the individual datum of the Diving group - volunteer one (▼) and volunteer two (▲) - and the Supporting group - volunteer three (▲) and volunteer four (▼).

Table II. Psychophysiological changes induced by diving in tropical waters (Rio de Janeiro, Brazil).

Variables	Diving in tropical waters				Cohen's *d*		
	Basal	Pre-dive		Post-dive	Pre-dive vs Basal	Post- vs Pre-dive	Post-dive vs Basal
Thyroid hormones							
Change from baseline TSH (µU. mL^{-1})	1	1.50 ± 0.60 1.93 – 1.08		1.50 ± 0.88 2.12 – 0.87	0.5	0.4	0.3
Change from baseline T4 (µU. mL^{-1})	1	1.03 ± 0.44 1.33 – 0.72		0.92 ± 0.02 0.94 – 0.91	0.1	0.4	0.3
Skin temperatures							
T_{FACE} (°C)	33.3 ± 0.8 32.7 – 33.9	34.0 ± 0.1 33.9 – 34.1		29.8 ± 1.3 30.7 – 28.9	1.2	1.3	1.3
T_{HAND} (°C)	32.3 ± 0.4 32.6 – 32.0	32.6 ± 0.8 33.2 – 32.0		28.3 ± 0.7 28.8 – 27.8	0.4	> 3.0	> 3.0
Variables	**Basal**	**Pre-dive**	**Diving**	**Post-dive**	**Pre-Dive vs Basal**	**Diving vs Pre-dive**	**Post-dive vs Diving**
HR and HRV							
Mean HR bpm)	84 ± 13 71 – 96	95 ± 10 85 – 106	124 ± 8 116 – 133	99 ± 7 92 – 107	1.0	> 3.0	> 3.0
RMSSD (ms)	40 ± 2 42 – 38	24 ± 7 17 – 31	31 ± 13 44 – 18	46 ± 23 69 – 23	2.9	0.7	0.8
LF (ms^2)	3567 ± 1139 2428 – 4706	1931 ± 1112 819 – 3043	754 ± 545 1299 – 209	1504 ± 99 1405 – 1603	1.4	1.3	1.9
HF (ms^2)	688 ± 70 758 – 617	226 ± 120 106 – 346	118 ± 50 168 – 68	291 ± 4 294 – 287	> 3.0	1.2	4.8
LF/HF	5.4 ± 2.2 3.2 – 7.6	8.3 ± 0.5 7.7 – 8.8	5.4 ± 2.3 7.7 – 3.1	5.2 ± 0.4 5.6 – 4.8	1.8	1.8	0.1
Total power (ms^2)	4550 ± 1134 3416 – 5684	2244 ± 1189 1055 – 3432	1105 ± 807 1912 – 298	1842 ± 78 1764 – 1920	1.0	2.0	0.9

for the Diving (82 ± 3 $mg.dL^{-1}$ vs. 99 ± 6 $mg.dL^{-1}$; ES > 3.0) but not for the Supporting group (80 ± 1 $mg.dL^{-1}$ vs. 86 ± 13 $mg.dL^{-1}$; ES = 0.6). An increase of glucose concentration toward the baseline values was observed at the post-dive time point compared to pre-dive values for the Diving group (98 ± 6 $mg.dL^{-1}$; ES > 3.0), and for Supporting group (86 ± 1 $mg.dL^{-1}$; ES > 3.0).

Heart rate and heart rate variability

HR (mean, min and max) increased during the dive relative to the pre-dive and baseline time points; this HR increase was followed by a reduction in the post-dive period (Table III).

The HRV variables addressed in the time domain (mean RR interval, RMSSD, SDNN, NN50, and pNN50) were reduced during the dive relative to the pre-dive and baseline time points and then presented a subsequent increase in the post-dive period. The HRV variables in the frequency domain (LF, HF, and total power) presented a similar response over time as the response observed for time-domain variables. The LF/HF ratio decreased during the pre-dive relative to baseline though no difference was observed during the dive compared to the pre-dive time point (Table III).

An increased HR and reduced frequency power bands were also observed when diving in tropical waters; however, the reduction in the frequency power bands had a lower magnitude than that registered in Antarctica (Table II).

Thermoregulatory responses

T_{FACE}, $T_{FOREHEAD,}$ T_{HAND}, T_{NOSE}, T_{CHEEK}, T_{CHIN}, T_{FOOT}, and T_{CHEST} reduced at the pre-dive relative to the baseline time point (except T_{FOOT} and $T_{CHEST,}$ moderate reductions). Diving resulted in further reductions in T_{AUC} and $T_{HAND,}$ no differences in T_{FOOT} and T_{CHIN}, while all face skin temperatures increased (i.e., T_{FACE}, $T_{FOREHEAD}$, T_{NOSE}, and T_{CHEEK}) (Table IV). As expected, staying outdoors before diving decreased thermal comfort and thermal sensation, which remained reduced after diving (Table IV).

After diving in tropical waters, the T_{FACE} and T_{HAND} decreased; however, the skin temperatures were still at levels higher than those recorded after the Antarctic dive (Table II).

Mood questionnaire, cognitive test, and sleepiness

There were no differences in mood status during the experimental timeline, except a reduction in tension at the post-dive relative to the pre-dive time point. Likewise, there was no difference in cognitive parameters (either accuracy and response time) between the post-dive and baseline time points. In contrast, the divers' sleepiness reduced; i.e., the alert state increased in the post-dive relative to baseline (Table V), moving from a “rather alert” to an “extremely alert” state (modification of 3 points on the Karolinska sleepiness scale, 57% increase). This reduction in sleepiness was correlated with the reduction in T_{CHEST} (p = 0.002; r = 0.97) and T_{CHIN} (p = 0.01; r = 0.73), with no significant correlation with T_{HAND} (p = 0.160; r = 0.52) (data not shown in the table).

Discussion

The evaluation of psychophysiological responses during diving in Antarctic waters is paramount to understand how to prevent detrimental impacts of exposure to extreme water temperature on the human body. This information is relevant because polar waters induce unique risk factors for divers. For example, one of the novel findings was the observation that diving under freezing Antarctic waters changes the concentrations of thyroid hormones. Also, we showed a myriad of effects of diving on the autonomic cardiac control, mood states, and thermoregulatory and perceptual responses.

Table III. Heart rate variability (HRV) parameters measured at four time points (baseline, pre-dive, diving, and post-dive) in the Diving group (n=2).

Variables	Diving in Antarctic waters				Cohen's *d*		
	Basal	Pre-dive	Diving	Post-dive	Pre-dive vs Basal	Diving vs Pre-dive	Post-dive vs Diving
Mean HR (bpm)	82 ± 0 82 – 81	87 ± 11 98 – 75	129 ± 3 126 – 131	93 ± 3 96 – 89	0.6	> 3.0	> 3.0
Min HR (bpm)	65 ± 3 62 – 68	67 ± 6 73 – 61	103 ± 7 97 – 110	68 ± 0 69 – 68	0.5	> 3.0	> 3.0
Max HR (bpm)	102 ± 3 105 – 98	115 ± 4 119 – 111	143 ± 3 140 – 147	114 ± 2 116 – 112	> 3.0	> 3.0	> 3.0
Mean RR interval (ms)	733 ± 4 729 – 736	705 ± 90 615 – 795	467 ± 10 477 – 457	649 ± 24 625 – 673	0.4	> 3.0	> 3.0
RMSSD (ms)	34 ± 4 39 – 30	46 ± 15 32 – 61	7 ± 1 6 – 8	50 ± 17 34 – 67	1.1	> 3.0	> 3.0
SDNN (ms)	56 ± 8 64 – 48	57 ± 5 62– 51	14 ± 0 14 – 13	55 ± 4 50 – 59	0.1	> 3.0	> 3.0
NN50	47 ± 5 51 – 42	90 ± 57 33 – 146	2 ± 2 3 – 0	97 ± 61 36 – 158	1.1	2.2	2.2
pNN50 (%)	9.6 ± 0.8 10.3 – 8.8	19.0 ± 13.4 5.7 – 32.4	0.2 ± 0.2 0.4 – 0.0	18.0 ± 11.7 6.3 – 29.7	1.0	2.0	2.1
LF (ms^2)	2619 ± 1125 3745 – 1494	1916 ± 462 2378 – 1455	104 ± 1 103 – 105	2177 ± 606 2783 – 1571	0.8	> 3.0	> 3.0
HF (ms^2)	379 ± 88 466 – 291	930 ± 486 444 – 1416	23 ± 6 16 – 29	610 ± 162 448 – 772	1.6	2.6	> 3.0
LF/HF	6.6 ± 1.4 8.0 – 5.1	3.2 ± 2.2 5.4 – 1.0	5.0 ± 1.4 6.4 – 3.6	4.1 ± 2.1 6.2 – 2.0	1.8	1.0	0.5
Total power (ms^2)	3256 ± 1179 4435 – 2077	3289 ± 223 3066 – 3512	145 ± 2 143 – 148	3007 ± 380 3387 – 2627	0.1	1.2	1.7

Diving in Antarctica decreased blood TSH concentration. Although a previous study showed that severe cold exposure induced by swimming in ice water (using swimsuits, with no thermal protection) increased TSH (Kovaničová et al. 2020), mild cold conditions decreased or did not change circulating TSH concentration (Kovaničová et al. 2020, Iwen et al. 2017, Leppäluoto et al. 1988). Therefore, it is possible that the environment itself has provided an initial stimulus for releasing TSH, as showed by the substantial increase in pre-dive TSH compared to baseline in the diving group. In contrast, the stress of diving in extremely cold waters may have reduced TSH concentration, considering the inhibitory effect of cortisol on TSH secretion (Van der Spoel et al. 2021, Re et al. 1976). It is worth noting that TSH is highly responsive to different stressors due to its pulsatile secretion and short half-life (Van der Spoel et al. 2021), which supports the hypothesis of stress-mediated effects on

Table IV. Body temperatures and perceptual parameters measured at three time points (baseline, pre-dive, and post-dive) in the Diving group (n=2).

Variables	Diving in Antarctic waters			Cohen's *d*		
	Basal	Pre-dive	Post-dive	Pre-dive vs Basal	Post- vs Pre-dive	Post-dive vs Basal
T_{AUC} (°C)	35.5 ± 0.2 35.7 – 35.4	31.1 ± 3.8 28.4 – 33.8	27.2 ± 0.1 27.3 – 27.2	1.6	1.4	> 3.0
T_{FACE} (°C)	32.3 ± 0.8 32.9 – 31.8	16.5 ± 0.4 16.8 – 16.2	19.0 ± 2.4 20.7 – 17.3	> 3.0	1.5	> 3.0
$T_{FOREHEAD}$ (°C)	34.1 ± 0.9 34.8 – 33.5	18.4 ± 4.0 21.3 – 15.6	23.8 ± 4.2 26.8 – 20.9	> 3.0	1.3	> 3.0
T_{HAND} (°C)	35.9 ± 6.1 40.3 – 31.6	27.7 ± 2.8 25.7 – 29.6	23.9 ± 3.5 26.4 – 21.4	1.7	1.2	2.4
T_{FOOT} (°C)	34.4 ± 5.5 38.3 – 30.5	30.9 ± 2.9 33.0– 28.9	28.8 ± 1.6 30.0 – 27.7	0.8	0.9	1.4
T_{CHEST} (°C)	34.4 ± 1.5 33.2 – 35.5	32.0 ± 3.6 29.4 – 34.6	31.8 ± 3.5 29.3 – 34.8	0.9	0.1	1.0
T_{NOSE} (°C)	31.3 ± 2.5 33.1 – 29.5	17.2 ± 1.9 18.6 – 15.9	21.3 ± 0.7 20.8 – 21.8	> 3.0	2.8	> 3.0
T_{CHEEK} (°C)	33.0 ± 2.7 34.9 – 31.1	15.2 ± 1.4 14.2 – 16.2	18.9 ± 1.6 20.1 – 17.8	> 3.0	> 3.0	> 3.0
T_{CHIN} (°C)	30.9 ± 3.0 28.8 – 33.0	15.1 ± 3.0 12.9 – 17.2	11.9 ± 4.6 15.2 – 8.7	> 3.0	0.8	> 3.0
TS	1 ± 1 2 – 0	- 6 ± 3 - 4 – - 8	- 6 ± 0 -6 – -6	> 3.0	0.0	> 3.0
TC	3 ± 4 6 – 0	- 4 ± 3 - 2 – - 6	- 7 ± 1 - 6 – - 8	1.9	1.3	> 3.0

the hypothalamic-pituitary-thyroid axis caused by diving. However, considering our small sample and the literature's divergences, further investigations about the TSH response to diving in polar waters are still warranted.

We also observed that diving in Antarctica reduced T4 levels, as reported for swimmers during immersion in icy waters, with no thermal protection (Kovaničová et al. 2020). T4 is considered a prohormone, and T4 transformation into T3 occurs via the intracellular enzyme iodothyronine deiodinase D2 (thyroxine-5'-deiodinase II) (Tsibulnikov et al. 2020, Bianco et al. 2005). Cold exposure induces D2 activity; thus, T4 reduction may also reflect increases in T4 tissue uptake and in T4-to-T3 conversion (Mullur et al. 2014, Silva 2001), which together enhance heat production through mitochondria

Table V. Mood, sleepiness, and working memory measured at three time points (baseline, pre-dive, and post-dive) in the Diving group (n=2).

Variables	Diving in Antarctic waters			Cohen's *d*		
	Basal	Pre-dive	Post-dive	Pre-dive vs Basal	Post- vs Pre-dive	Post-dive vs Basal
Mood states						
Anger	0.5 ± 0.7 1 – 0	1.5 ± 2.1 3 – 0	0.5 ± 0.7 1 – 0	0.6	0.6	0.0
Confusion	0 ± 0 0 – 0	0 ± 0 0 – 0	0 ± 0 0 – 0	0.0	0.0	0.0
Depression	1.0 ± 1.4 2 – 0	0.5 ± 0.7 1 – 0	0.0 ± 0.0 0 – 0	0.4	1.0	1.0
Fatigue	0.5 ± 0.7 1 – 0	0.5 ± 0.7 1 – 0	0.5 ± 0.7 0 – 1	0.0	0.0	0.0
Tension	2.5 ± 3.5 5 – 0	4 ± 1.4 3 – 5	0.0 ± 0.0 0 – 0	0.6	> 3	1.0
Negative mood categories	0.9 ± 1.6 1.8 – 0.0	1.3 ± 1.8 1.6 – 1.0	0.2 ± 0.4 0.2 – 0.2	0.2	0.9	0.6
Vigor	12 ± 3 14 – 10	14 ± 3 16 – 12	13 ± 3 16 – 11	0.7	0.2	0.5
Sleepiness						
Sleepiness	3.5 ± 2.1 2 – 5	2.0 ± 1.4 1 – 3	1.5 ± 0.7 1 – 2	0.8	0.5	1.3
Working memory						
Accuracy for 1 second (%)	69 ± 18 56 – 81	NA	66 ± 31 44 – 88	NA	NA	0.1
Accuracy for 5 second (%)	69 ± 35 44 – 94	NA	69 ± 18 56 – 81	NA	NA	0.0
RT for 1 second (s)	1.8 ± 1 2.5 – 1.1	NA	1.9 ± 1.3 2.9 – 1.0	NA	NA	0.1
RT for 5 second (s)	1.9 ± 0.8 2.5 – 1.3	NA	2.0 ± 1.3 2.9 – 1.1	NA	NA	0.1

calorigenic effect (Tsibulnikov et al. 2020). The impact of cold on our volunteers is evidenced by reduced T_{AUC}, T_{HAND}, and thermal comfort after 10 min of cold water immersion, compared to the pre-dive moment. Finally, the TSH and T4 responses after diving in Antarctica were not observed after diving in tropical waters (Rio de Janeiro, Brazil), which reinforces the possible influence of water temperature on the results obtained.

The lowest T_{HAND} and T_{AUC} values were observed after the dive, when the divers were already in a sheltered environment (i.e., onboard the ship). Thus, the reduction in the temperature of body extremities was likely even more intense during the dive; in this sense, we suggest future studies should investigate body temperatures across time points while an individual is diving. The lower T_{HAND} is a consequence of peripheral vasoconstriction, an autonomic defense for conserving body heat. The auricle is supplied with blood from both superficial and deep sources (Taylor et al. 2014); thus, the lower T_{AUC} may indicate a core temperature reduction reflecting a combination of reduced skin temperatures in the head, in the air within the ear canal, and in the deeper body temperature transmitted by tympanic temperature, as well as a lower heat radiated from the inner canal wall (Childs et al. 1999, Greenleaf & Castle 1972).

Another significant thermoregulatory response observed was low T_{FACE} values corresponding to 16°C before diving. Cold exposure results in cutaneous vasoconstriction by increasing sympathetic vasoconstrictor tone. Additionally, severe skin cooling (i.e., when skin temperature reduces to ~17°C) stimulates non-adrenergic and non-neuronal mechanisms that reduce cutaneous blood flow, including inhibited NO-mediated vasodilator response and increased vasoconstriction via activation of Rho-kinase-mediated pathways (Alba et al. 2019). After the intense vasoconstriction to avoid hypothermia during diving, the volunteers were exposed to the ship's sheltered environment. Under the latter conditions, the withdrawal of the direct cold stimulus may have reduced sympathetic vasoconstrictor tone and inhibited vasoconstriction-induced intracellular changes, resulting in reflex vasodilation and warming of the face.

Interestingly, an opposite response occurred after diving in warmer waters; the skin temperature was likely reduced due to enhanced heat dissipation from the face through evaporative and convective means, resulting in decreased T_{FACE}. However, it should be noted that, although thermoregulatory responses in the face have occurred in different directions after diving in polar or tropical waters, the difference between conditions was still substantial after diving, thus indicating the determinant role of ambient temperature on skin temperature (Romanovsky 2018). Specifically, in Antarctica, the T_{FACE} increased from approximately 16 to 19°C (i.e., it remained cold), whereas, in Rio de Janeiro, the T_{FACE} decreased from 34 to 30°C (i.e., it remained at "normal" levels).

HR increased during diving alongside a reduction in time-domain parameters associated with parasympathetic activation (i.e., RMSSD and NN50) and a reduction in the frequency-domain parameters associated with both the parasympathetic (HF) and sympathetic (LF) activities. However, the sympathovagal balance increased during dive relative to the pre-dive time point. The reduction in parasympathetic activity aligns with a previous study conducted with navy divers in Arctic waters. Lundell et al. (2020) suggested a quick loss of the trigeminocardiac part of the diving reflex, leading to a vagal response within 5 to 10 min after diving has started. Considering that we evaluated 10-min diving and that HRV analysis did not include the first 2 to 3 min (due to the inaccurate determination of the exact moment of submersion), our results reinforce the mechanism proposed by Lundell et al. (2020).

Reducing parasympathetic and sympathetic activities may represent a counter-regulatory mechanism to maintain sympathovagal balance at a physiological level (White & Raven 2014). Despite an activity reduction in both branches of the autonomic nervous system, the LF/HF ratio increased comparatively to pre-dive, in agreement with the observed increase in HR. Also, intrinsic heart regulation may have contributed to increasing HR. During immersion, the greater venous return due to the redistribution of blood from the extremities to the central circulation (McCally 1964, Harrison et al. 1986) stretches the atrial wall receptors that can reflexively increase HR in a process termed as the 'Bainbridge reflex' (White & Raven 2014, Barbieri et al. 2002, Kappagoda et al. 1972). Although similar responses of the frequency power bands occurred during a dive in tropical waters (Rio de Janeiro, Brazil), their magnitude was lower than during a dive in polar waters. Unfortunately, we could not directly compare the dives in polar and tropical waters. Further studies should use a repeated-measures design and control the exact moment of immersion to better investigate the differences in autonomic control while diving in different water temperatures.

Regarding mood status, an increase in tension was observed in compliance with the demands of preparing for the dive (e.g., checking materials and weather conditions); this augmented tension observed before diving was reduced after the dive. The dive also reduced sleepiness, which possibly resulted from the reduction in body temperatures (Romeijn et al. 2012), as indicated by the association between the reductions in sleepiness and T_{CHIN} or T_{CHEST}. However, these changes did not influence the cognitive parameters evaluated.

Despite the exciting findings reported, our study has limitations. The first limitation is the restricted sample size that consisted of only two divers. However, a small number of volunteers is a common feature of field studies under extreme conditions (Lundell et al. 2020, Hattersley et al. 2019, Moraes et al. 2018, Bridgman 1990, 1991, Dick 1984); notably, some patterns in hormonal and autonomic cardiac changes have emerged, even with the limited number of divers tested. Second, it is necessary to consider the present results according to the diving time, depth, and the mixture of gases used. Differences in physiological adjustments can arise due to prolonged exposure to extreme conditions, the use of diverse gas mixtures (e.g., enriched air nitrogen and enriched air helium TRIMIX mixtures), and the effects of inhalation of a gas inert at sea level but neurotoxic at high atmospheric pressures. Also, it should be considered that our volunteers were Brazilian military personnel that lived and trained in tropical locations, with average ambient temperatures of 28°C and 76% RH in summer and 22°C and 79% in winter (Time and Date 2021). These individuals underwent similar training and work routine, and the classification between the 'Diving' and 'Supporting' groups was determined based on the work performed on the day of the measurements (those that immersed: 'Diving'; those that assisted immersing divers: 'Supporting'). Therefore, we do not expect differences in acclimatization status between these groups. However, further experiments are necessary to assess the acclimatization status of Brazilian military personnel diving in polar waters.

In conclusion, the present results show that a short-term immersion in icy waters affects the hypothalamic-pituitary-thyroid axis by reducing thyroid hormones (TSH and T4) concentration, plausibly due to stress-mediated inhibition of TSH secretion alongside increased T4 peripheral uptake, leading to cold-induced thermogenesis. Besides, our results reinforce the effect of diving in modulating autonomic cardiac control, possibly resulting from the diving reflex. Finally, diving in Antarctic freezing waters and the apparent reduction in body temperatures seem to reduce sleepiness.

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SUPPLEMENTARY MATERIAL

Tables SI-SV

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Author contributions

Each author contributed individually and significantly to the development of this study and approved the final version submitted for publication. RSB, MMM, ASRH, SPW, and RMEA: designed research; RSB: performed data collection; RSB, MMM, RVPL: analyzed data; RVPL, CNE, SPW, and RMEA: provided laboratory support; RSB, MMM, SPW, and RMEA: wrote the paper. RSB, MMM, YATM, ASRH, RVPL, CNE, SPW, RMEA: edited the paper and approved the submitted version.

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