COPING WITH EXTREME ENVIRONMENTS: A PHYSIOLOGICAL/ PSYCHOLOGICAL APPROACH

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COPING WITH EXTREME ENVIRONMENTS: A PHYSIOLOGICAL/PSYCHOLOGICAL APPROACH

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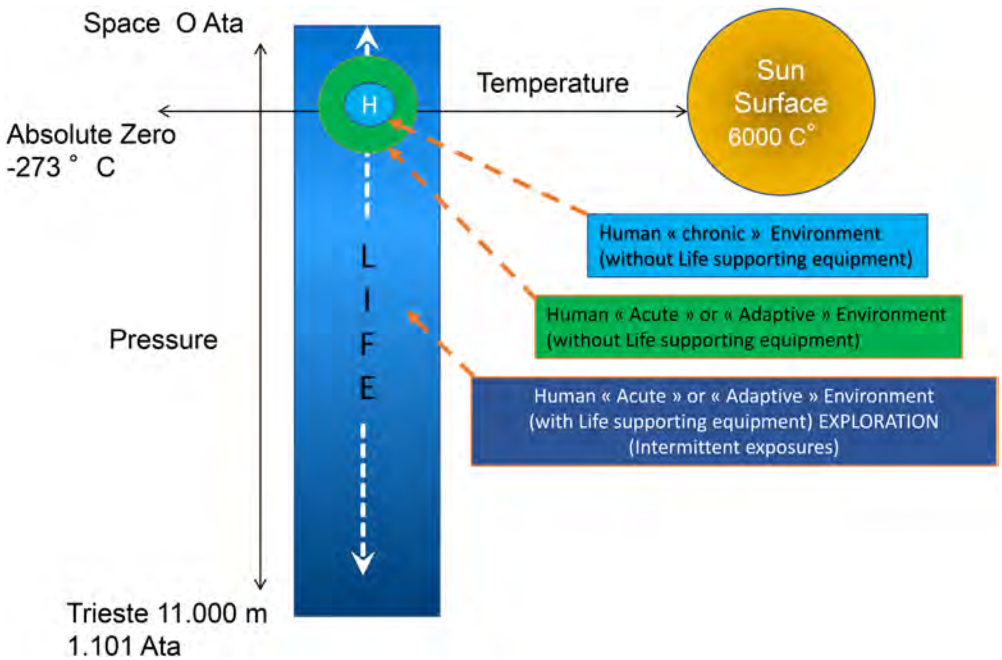

Schematic representation of human challenges and coping possibilities in a chronic or intermittent setting.
Human Environments in Perspective (Modified from: Piantadosi CA. (2003). *The biology of human survival : Life and death in extreme environments*. Oxford University Press, New York.

Understanding how humans cope in extreme environments has expanded our knowledge of the physiological and psychological challenges involved and helped us to quit our comfortable paradigms built on "steady states". Furthermore, measuring our reactions to intermittent stressors and determining the oscillations of our coping mechanisms has led us to unexpected understandings. This methodology has also directly improved our translational or multidisciplinary approach to the subject. Studying healthy individuals in extreme environments could improve our understanding of patients with impaired physiological capacities (who are coping with an environment that becomes extreme to them) and also improve our understanding of physiology and psychology in the elderly.

This eBook collects articles that address this translational multidisciplinary approach in an integrative way. As a whole, this Research Topic aims to better understand human/animal physiology and psychology.

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EDITORIAL
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Editorial: Extreme Environments in Movement Science and Sport Psychology

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Editorial on Research Topic

Extreme Environments in Movement Science and Sport Psychology

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If we want simply to depict what extreme environments are, we can consider them as primarily depending on two parameters: temperature and pressure. Gravitational and radiation changes can both also be added.

As a matter of fact, both dimensions are also well-linked together. Depending on those two parameters, hydration, gas partial pressures, effort, work of breathing, metabolism, gene expression and many other essential "ingredients" of human life and performance can vary widely.

Human studies in extreme environments (altitude hypoxia, microgravity, hyperbaric, and terrestrial extreme climatic conditions) over the last decades have expanded knowledge in physiology, highlighting new routes of regulation, breaking previous old concepts, and offering new models of some physiopathological troubles in patients (Trivella et al., 2017; Burtscher et al., 2018).

Some years ago, on the physiological side, the two parameters that characterize extreme environments were identified to elicit the production of two particular elements: Hypoxia inducible factors and heat shock proteins. Surprisingly, these two elements can be triggered by either hypobaric/hypoxic or hyperbaric/hyperoxic environments. The reason is that, in biologym what is mostly being sensed is the fluctuation rather than absolute values.

The two are ubiquitous and essential to cellular life. The first is a factor that triggers around 200 genes responsible for vascular, cellular, and metabolic homeostasis as well as apoptosis. In fact, its beneficial actions on the fight against cancer cells have recently been advocated (De Bels et al., 2011; Khalife et al., 2018). The second is a family of proteins acting as chaperones for other proteins and resetting impaired proteic structures (Kopecek et al., 2001; Gjovaag and Dahl, 2006; Hageman et al., 2011).

Some psychological aspects have been explored independently or sometimes combined with physiology. However, little is known about cognition and neuronal plasticity in extreme environments, although adaptation to extremes is an integrative matter that the body and brain have to solve conjointly. How do peripheral body signals, homeothermic regulation, energy expenditure, and psychological and cognitive functions interact with each other? New insights on how extreme external factors may change emotional and cognitive functions of self-perception and the perception of the surrounding environment, and what impact this has on decision-making processes, are matters of interest.

It has long been established that a general law applies to humans in extreme environments: the Yerkes-Dodson Law (Calabrese, 2008). The relationship between arousal and performance is known and discussed (Balestra et al., 2018), but we seldomly know up to what point the positive effect on performance/coping exists in extreme environments (Mair et al., 2011; Rietschel et al., 2011).

Not so long ago it has been shown that environments are also able to interact with the genome. In fact, epigenetics seems to be a major point in extreme environments, especially when partial oxygen pressure changes are involved (Lautridou et al., 2017b; Kiboub et al., 2018b), but remains poorly investigated.

The proposed research topic has addressed most of the psychological and physiological reflexions needed in extreme environments, opening for future research and progress.

New challenges are also important in changing gravity environments. Although physiological and psychological parameters have been widely investigated, cognitive functions during long term missions in space remain to be evaluated. For example, spatial cognition, including the self-perception, orientation and navigation required during 3D robotic arm control, rendez-vous docking and extra-vehicular activities are all affected by the loss of gravity-related sensors. Koppelmans et al. (2013) consequently, the next challenging step is understanding how decision making, spatial cognition, emotional aspects, as well as cortical sensory integration supporting self-bodily perception and orientation are influenced by and during extreme short or prolonged missions. The ways humans have adapted ancestrally and how we will adapt to strong and fast environmental and climatic changes on Earth require an integrative approach at the frontiers between cognition, psychology, and physiology. Reviews, reports, and the most recent data will support the preparation for human solar system exploration, firstly to Mars. Understanding how humans cope with extreme environmental or physiological/psychological challenges has helped us to leave our comfortable paradigms built on stable "steady states" (Balestra, 2012). Today's measurement systems allow us to analyze our reactions to intermittent stressors and follow the oscillations of our coping mechanisms. This new approach has led us to unexpected understandings (Lautridou et al., 2017a) since most of the results expressed in this research topic are unexpected or even counterintuitive.

This methodology has also directly improved our translational or multidisciplinary (integrative) approach as well as the idea that studying humans in good health at extremes could help us to understand both patients (Khalife et al., 2018; Kiboub et al., 2018a) with impaired physiological capacities coping with our environment (which) becomes extreme to them), or better understanding physiology/psychology of the elderly, or to better prepare people working in constraining environments.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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ORIGINAL RESEARCH
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Acute Anxiety Predicts Components of the Cold Shock Response on Cold Water Immersion: Toward an Integrated Psychophysiological Model of Acute Cold Water Survival

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Introduction: Drowning is a leading cause of accidental death. In cold-water, sudden skin cooling triggers the life-threatening cold shock response (CSR). The CSR comprises tachycardia, peripheral vasoconstriction, hypertension, inspiratory gasp, and hyperventilation with the hyperventilatory component inducing hypocapnia and increasing risk of aspirating water to the lungs. Some CSR components can be reduced by habituation (i.e., reduced response to stimulus of same magnitude) induced by 3–5 short cold-water immersions (CWI). However, high levels of acute anxiety, a plausible emotion on CWI: magnifies the CSR in unhabituated participants, reverses habituated components of the CSR and prevents/delays habituation when high levels of anxiety are experienced concurrent to immersions suggesting anxiety is integral to the CSR.

Purpose: To examine the predictive relationship that *prior* ratings of acute anxiety have with the CSR. Secondly, to examine whether anxiety ratings correlated with components of the CSR *during* immersion before and after induction of habituation.

Methods: Forty-eight unhabituated participants completed one (CON1) 7-min immersion in to cold water (15°C). Of that cohort, twenty-five completed four further CWIs that would ordinarily induce CSR habituation. They then completed two counter-balanced immersions where anxiety levels were increased (CWI-ANX) or were not manipulated (CON2). Acute anxiety and the cardiorespiratory responses (cardiac frequency [f_c], respiratory frequency [f_R], tidal volume [V_T], minute ventilation [$\dot{V}_E$]) were measured. Multiple regression was used to identify components of the CSR from the most life-threatening period of immersion (1st minute) predicted by the anxiety rating *prior to* immersion. Relationships between anxiety rating and CSR components *during* immersion were assessed by correlation.

Results: Anxiety rating predicted the f_c component of the CSR in unhabituated participants (CON1; $p < 0.05$, $r = 0.536$, $r^2 = 0.190$). After habituation immersions (i.e., cohort 2), anxiety rating predicted the f_R component of the CSR when anxiety levels were lowered (CON2; $p < 0.05$, $r = 0.566$, $r^2 = 0.320$) but predicted the f_c component of the CSR ($p < 0.05$, $r = 0.518$, $r^2 = 0.197$) when anxiety was increased suggesting different drivers of the CSR when anxiety levels were manipulated; correlation data supported these relationships.

Discussion: Acute anxiety is integral to the CSR before and after habituation. We offer a new integrated model including neuroanatomical, perceptual and attentional components of the CSR to explain these data.

Keywords: drowning prevention, cold water, "float first", cold-water survival, open water safety

INTRODUCTION

A conservative estimate suggests that approximately 375,000 people unintentionally enter in to water and drown each year (World Health Organization, 2014) although the true figure may be four or five times higher (Bierens et al., 2016). Consequently, death by drowning is the second most common cause of accidental death in adults and the third most common cause in children in most countries (Bierens et al., 2002). If the water is cold, the physiological responses evoked during the first few minutes of whole body cold water immersion (CWI) are life threatening (Tipton, 2003) and are strongly implicated in this drowning statistic (Tipton, 1989) even in strong swimmers or those with basic survival skills (Golden et al., 1986; Bowes et al., 2016). The responses evoked by CWI, known collectively as the cold shock response (CSR Tipton, 1989), include an "inspiratory gasp," (Burke and Mekjavic, 1991) hyperventilation, a resultant hypocapnia, tachycardia, peripheral vasoconstriction and hypertension (Keatinge and Nadel, 1965; Cooper et al., 1976). The hyperventilatory component of the CSR significantly decreases maximum breath hold time in the majority of participants, thus increasing the chances of involuntarily aspirating water and drowning (Hayward and French, 1989; Tipton and Vincent, 1989); this represents a further hazard to that posed by the high cardiovascular strain (Tipton et al., 1994). The current behavioral recommendation to survive acute accidental CWI is to "float first" (Barwood et al., 2011) and for those who are unable to float without aid to "float first and kick for your life" thereby providing some further buoyancy (Barwood et al., 2016). The CSR subsides after the initial peak at 60–90 s and swimming to safe refuge or executing a survival strategy may become possible (Golden et al., 1986; Bowes et al., 2016).

For those at risk individuals (e.g., pilots, naval personnel, persons recreating on/near water), protective steps should be taken to reduce the magnitude of CSR on water entry. For example protective clothing may mitigate the rapid skin cooling that evokes the CSR (Tipton, 1991; Power et al., 2016) but this is not always feasible, especially if immersion is unexpected. An alternative may be to induce an habituation of the CSR which can be achieved by undergoing a minimum of four short CWIs following which the CSR is significantly blunted (Tipton et al., 1998; Barwood et al., 2007); habituation is defined as reduced response to a stimulus of the same magnitude (Zald, 2003). The consequence of habituation is a significantly reduced cardiorespiratory response to CWI which may be retained for 7 months and partially present up to 14 months later (Tipton et al., 2000). Reducing the CSR may confer some benefit to defending the airway in the emergency scenario as the hyperventilatory drive seen in unhabituated participants is significantly reduced (Tipton et al., 1998), although large variability in the habituation of the response is often seen (Barwood et al., 2007).

Part of the variability between individuals in the CSR could be accounted for by differences in psychological state both prior to, and during a CWI (e.g., Barwood et al., 2006, 2007, 2013, 2014). Indeed, it has been shown that there are salient moderating influences on the extent of the CSR which are, at least in part, caused by high in contrast to low levels of anxiety (Glaser et al., 1959; Barwood et al., 2017). It has also been shown that components of the CSR can be influenced positively by psychological training thereby inducing an 80% improvement in maximal breath hold time on CWI (Barwood et al., 2006). Moreover, familiarity with the immersion scenario, thereby reducing the associated anxiety with immersion also has a beneficial effect. We showed that repeatedly experiencing the immersion sequence (i.e., repeated thermoneutral water immersion; 35°C) in the absence of a repeated cold-water stimulus leads to a small but significant reduction in respiratory tidal volume on subsequent CWI (Barwood et al., 2014). Accordingly, we concluded that repeated immersion in thermoneutral water induces a perceptual habituation of the threat posed by imminent immersion and this confers some benefit even when the water temperature is cold. Most recently we have shown that the concurrent experience of high levels of acute anxiety throughout a series of habituation immersions prevents or delays CSR habituation (Barwood et al., 2017). We suggested that the concomitant experience of anxiety disinhibits the transmission of thermal afferent information such that it magnifies the CSR response or prevents habituation; a mechanism first suggested by Glaser et al. (1959). Most importantly we suggested that the high levels of anxiety prevented or delayed the learned control of ventilation that we believe occurs during habituation since ventilation is under greater voluntary control than cardiac components (Barwood et al., 2017). Given that respiratory impairment is the primary threat to otherwise healthy individuals on CWI (Tipton, 2003), the consequences of impaired respiratory control caused by high anxiety levels in the emergency scenario may increase the risk of death by drowning.

Clearly the valence of the psychological experience prior to and during CWI is a potential driver of the physiological response that is seen; it is no longer appropriate to consider the CSR as solely a physiological phenomenon. The observations above question the reliability and potential practical value of inducing habituation to defend against the CSR in the emergency scenario. They may suggest that anxiety levels could underpin at least some of the variation in the CSR that is evident before and after habituation and suggest that the CSR should be considered as an integrative psychophysiological experience. If the extent of anxiety is an important mediator in the resultant CSR then the CSR should be altered by the experience of high(er) by contrast to low(er) levels of anxiety (Barwood et al., 2017).

Accordingly, from our database of recent immersions ($n = 48$), the present study examined the predictive relationship that prior ratings of acute anxiety have with components of the CSR on the first minute of immersion; the period widely accepted as critical in determining survival chances and thought to be under minimal voluntary influence (Tipton, 1989). Subsequently, we examined whether the anxiety we recorded during immersion correlated with components of the CSR before and after habituation of the CSR. Lastly, we examined whether these relationships changed when we experimentally induced increases in anxiety when contrasted to low(er) anxiety levels. We tested the experimental hypothesis that, if anxiety levels were an important mediator of the CSR, they would predict components of the CSR (H_1). Secondly, we hypothesized that prior to a series of habituation immersions (i.e., on the first CWI) high levels of anxiety would predict the cardiac component of the CSR as learned control of ventilation was yet to occur (H_2). Subsequently, we hypothesized that after repeated CWIs low(er) levels of anxiety would predict respiratory components of the CSR as low levels of anxiety would be permissive of voluntary respiratory control whereas high(er) levels of acute anxiety would predict cardiac components of the CSR which are under lesser voluntary control; thereby inferring the directional nature of the anxiety response.

MATERIALS AND METHODS

Participants

The University of Portsmouth Science Faculty Research Ethics Committee provided ethical approval for the studies from which the data are drawn. The study was conducted in accordance with the Declaration of Helsinki and all participants provided written informed consent following a verbal and written briefing. The onward use of data, as in the case in the present analysis, was included in the consent procedure. The participants were non-smokers and were not cold water habituated, i.e., had not been exposed to cold water in the preceding year. They abstained from alcohol and caffeine consumption for 24 h before each test and from undertaking any exercise on the day of the test. The participants included in the present tests were pooled from our previous studies (Barwood et al., 2013, 2014, 2017) culminating in a maximum of 48 participants to choose from. To address our research questions we considered two cohorts; some of the participants were common between cohorts. Cohort 1 included forty-eight unhabituated participants (34 male, 14 female; mean[SD] age 20[2] years, height 1.75[0.1]m, mass 76.2[16.7]kg); these data were used to examine the relationships between the anxiety ratings and the CSR before habituation (i.e., during one of their first two immersions and when anxiety levels were not manipulated). Cohort 2 included 25 (16 male, 9 female; age 20[2] years, height 1.75[0.1]m, mass 77.9[17.2]kg) participants who completed further CWIs.

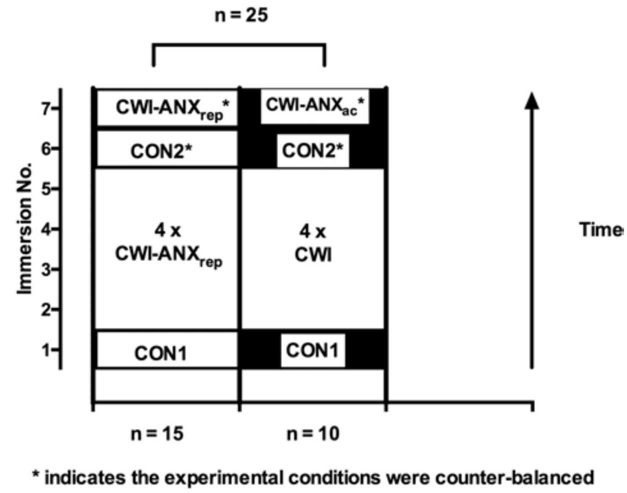

FIGURE 1 | Order of experimental conditions and manipulations for participants in cohort 2; note: the participants were combined from two studies to make a total of 25 participants for analysis after the habituation immersions were complete.

Experimental Design

Participants were recruited on the basis of undertaking a within participant, repeated measures, experimental design and acted as their own control; the experimental designs and manipulations in the respective studies are reported in detail elsewhere (Barwood et al., 2013, 2014, 2017). Briefly, all participants completed an initial CWI where anxiety levels were not manipulated but were expected to be naturally high due to novelty and unfamiliarity with CWI; forming cohort 1 (CON1; $n = 48$). In two of our four previous studies, participants underwent four further CWIs which would be sufficient to induce a habituation forming cohort 2 (see **Figure 1**; $n = 25$); albeit with different manipulations of anxiety levels during these immersions. Participants in cohort 2 completed two further counter-balanced CWIs during which time their anxiety levels were manipulated to be increased (CWI-ANX$_{ac}$ or CWI-ANX$_{rep}$ described as CWI-ANX hereafter) or were not manipulated (CON2). The order of CWIs and manipulations of anxiety therein are described in **Figure 1**. All immersions included in the present analysis were conducted at the same time of day within-participant.

Immersion Protocol

Following arrival at the Extreme Environments Laboratory, each participant's height (m) and mass (kg) was recorded using a

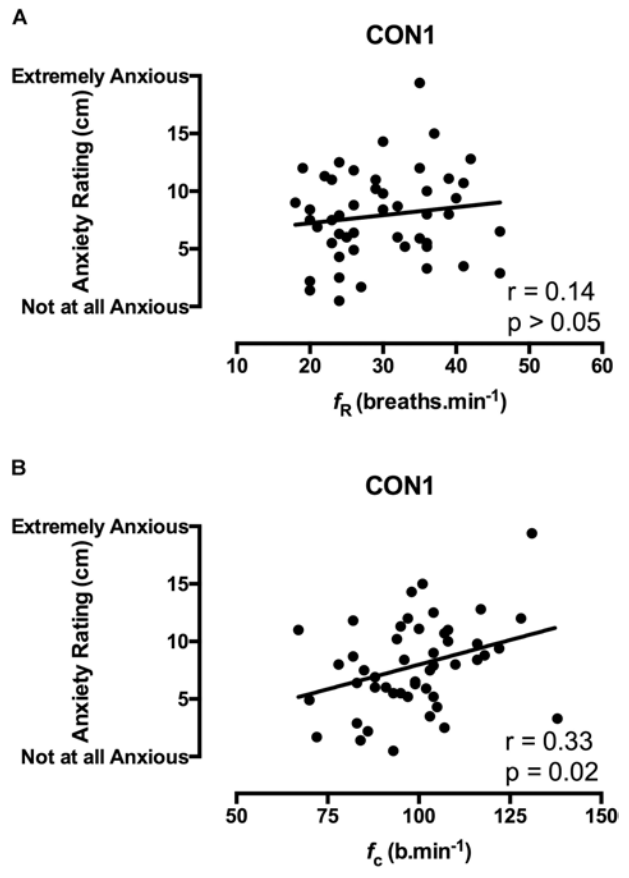

FIGURE 2 | Correlation coefficients between acute anxiety ratings in the 1st minute of immersion and the f_R **(A)** and f_c **(B)** components of the CSR in unhabituated participants (*n* = 48).

stadiometer (Bodycare Stadiometer, Leicester, United Kingdom) and calibrated weighing scales (OHAUS digital weighing scales, Parsippany, NJ, United States). Each participant changed into their swimming costume. Males wore swimming trunks and females wore a swimsuit; the same swimming costume was worn by each participant on each occasion. Participants were then instrumented with a 3-lead ECG (HME Lifepulse, England) and entered an ambient temperature (T_a) controlled laboratory. They sat on an immersion chair attached to an electrical winch (CPM, F1-8; 2-8; 5-4, Yale, Shropshire, United Kingdom) with a seat belt fastened around their waist to counteract buoyancy on immersion. The participant inserted a two-way mouthpiece (Harvard, United States) and attached a nose clip. The mouthpiece was connected to a spirometer (spirometric transducer module, KL Eng. Co, Northridge, CA, United States) by respiratory tubing in order to measure the respiratory responses to immersion. The participant was winched above the immersion tank to rest for 1-min. Thirty seconds into the 1-min rest period participants provided a rating of their state anxiety on a visual analog scale (Cella and Perry, 1986); they were familiarized with the scale in advance of the study. Toward the end of the 1-min period a 10-s verbal countdown preceded the participant being lowered at a reproducible rate (8 m min^{-1}) until immersed to the clavicle in stirred water. Participants remained seated and with their limbs stationary during the immersion. After 1, 3, 5, and 7-min of immersion they again reported their anxiety rating, following which they were winched from the immersion tank. They then had a hot shower, re-dressed and left the laboratory. Each immersion was standardized immersing the participant to the same depth, at the same rate and in to stirred 15°C cold water.

Anxiety Manipulations

In cohort 2 (i.e., see **Figure 1**), in either their penultimate or final immersion the participants were told by an independent researcher 2 min prior to immersion that the water temperature would be 5°C colder than their first immersion but in reality it was unchanged. This was achieved by slightly different means (i.e., perceived 1°C reductions across 4 immersions or one perceived 5°C reduction) to meet the experimental aims of the particular study (see Barwood et al., 2013, 2017); the cohorts are being grouped here to improve the statistical power of our observations.

Measurements

Water temperature (T_w) and T_a were measured and recorded using a calibrated thermistor [Grant Instruments (Cambridge) Ltd, Shepreth, United Kingdom) secured to the wall of the immersion tank and a Wet Bulb Globe Thermometer station respectively, both attached to a data logger [1000 series, Squirrel Data Logger, Grant Instruments (Cambridge) Ltd, Shepreth, United Kingdom]. Average T_w was closely matched within participant (±0.2°C) between CON1, and the sixth and seventh immersions (i.e., CON2, CWI-ANX). During the habituation immersions (i.e., immersions 2–5) the T_w was ± 0.5°C of 15°C.

Cardiorespiratory Responses

The ECG and spirometer were interfaced with a digital data acquisition system (16SP PowerLab, Castle Hill, NSW, Australia) which captured data continuously throughout the rest and immersion periods. Chart analysis software (Chart version 6, AD Instruments Ltd, Oxford, United Kingdom) was used to automatically identify R-waves from the ECG and calculate cardiac frequency (f_c); movement artifacts were visually identified and excluded from analysis. The spirometer was calibrated using a syringe of known volume (3 L syringe, Harvard Instruments, Harvard, United States). Respiratory frequency (f_R) was recorded by Chart analysis software using auto-recognition of the peak after inspiration. The peak value after the onset of inspiration was recorded as tidal volume (V_T) and multiplied by the calculated f_R to generate minute ventilation ($\dot{V}_E$).

Anxiety Perceptual Responses

The state anxiety response to immersion was quantified using a 20 cm visual analogue scale (VAS) with descriptive phrases ranging from 0 cm (*not at all anxious)* to 20 cm

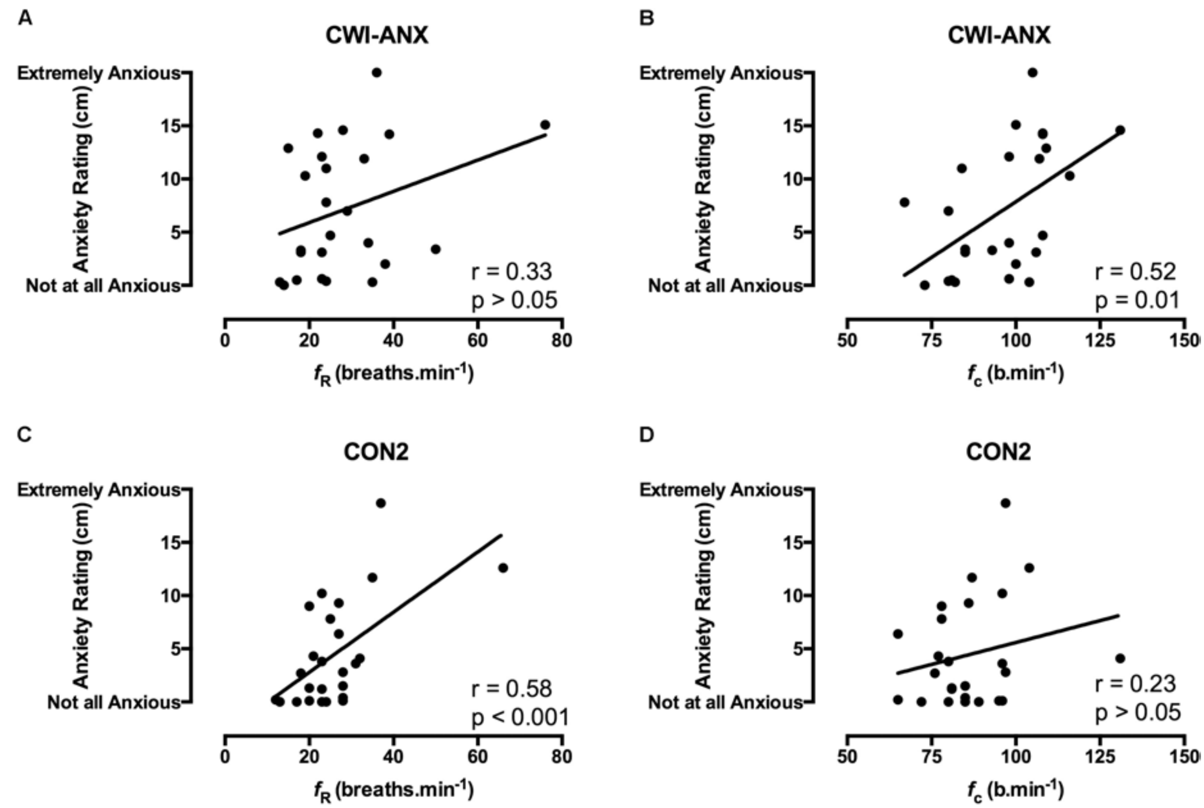

FIGURE 3 | Correlation coefficients between acute anxiety ratings in the 1st minute of immersion and the f_R and f_c components of the CSR in habituated participants where anxiety levels were experimentally increased (CWI-ANX; **A,B**; $n = 25$) and anxiety levels were not manipulated (CON2; panels **C,D**; $n = 25$).

(*extremely anxious*; Cella and Perry, 1986. Participants reported their anxiety by drawing a horizontal line on the vertical scale (see example in **Figures 2**, **3**, y axes) that corresponded to their feeling of anxiety with the distance between points in centimeters providing a numerical value for the measure.

Statistical Analysis

In order to examine the possible predictive relationship between *prior* anxiety levels on the resultant CSR *during* immersion, stepwise multiple regression analyses were undertaken between the anxiety rating recorded prior to immersion and the components of the CSR recorded in the first minute of immersion (i.e., the most life threatening period of acute immersion when the CSR peaks); comparisons were made to 1-min averages for f_c, f_R, V_T and $\dot{V}_E$. This analysis was also undertaken for cohort 2 for immersions 6 and 7; i.e., the counter-balanced immersions after habituation when anxiety levels were manipulated to be high(er) or low(er).

In order to examine how the relationship between anxiety rating and components of the CSR developed throughout each immersion before and after completion of the habituation immersions, Pearson's moment correlation coefficients were calculated across the 1st, 3rd, 5th, and 7th minutes of CON1, CON2 and CWI-ANX immersions. Analyses were undertaken using SPSS *v*22 and Prism 6.0 to an alpha level of 0.05.

RESULTS

Multiple Regression Analysis

Anxiety rating prior to immersion was predictive ($p < 0.05$). The f_c component of the CSR was predicted in the unhabituated cohort (CON1; $r = 0.536$, $r^2 = 0.190$, $n = 48$). After habituation immersions the prior anxiety rating predicted different components of the CSR depending on whether anxiety rating was high, where f_c was again predicted in the CWI-ANX condition ($r = 0.518$, $r^2 = 0.197$, $n = 25$) or low where f_R was predicted in CON2 ($r = 0.566$, $r^2 = 0.320$, $n = 25$). Collectively, the anxiety rating prior to immersion predicted different components of the CSR before and after habituation immersions had taken place dependent upon whether anxiety ratings were high or low. In higher anxiety conditions (e.g., CWI-ANX and CON1 where in the latter case anxiety rating was high due to novelty), the anxiety rating predicted the f_c component of the CSR. When anxiety levels were low the anxiety rating predicted the f_R component of the CSR. The strength of the predictive relationship was far stronger when anxiety levels were low.

Correlation Analysis

Correlation data support the directional relationships suggested by the regression model. In CON1 when anxiety levels were high the anxiety rating was correlated with f_c but not f_R in the 1st minute of immersion (see **Figures 2A,B**). In the 3rd and 5th minute of immersion the anxiety rating was correlated with f_R; see **Table 1**. An identical set of results was evident after habituation immersions (see **Figures 3A,B** for 1st minute data) when anxiety levels were manipulated to be higher (i.e., CWI-ANX) although this did not extend in to the 5th minute of immersion. Collectively the *r*-values indicated weak to moderate strength relationships (r = 0.29–0.52); see **Table 2**.

DISCUSSION

We hypothesized that if anxiety levels were an important mediator of the CSR they would predict components of the CSR prior to immersion; we support our hypothesis (H_1). Our data support the idea that high levels of anxiety, which occurred instinctively before an initial immersion (i.e., CON1) and were manipulated to be increased on one occasion after habituation immersions (i.e., CWI-ANX), would be predictive of the cardiac component of the CSR. By contrast, when anxiety levels were allowed to fall after habituation immersions, the low(er) levels of anxiety were predictive of the respiratory frequency component of the CSR; this finding infers a mediating role for anxiety level. Given that first minute of immersion is the most life-threatening as it is when the CSR peaks (Tipton, 1989), it seems prudent that interventions to aid those at risk of CWI should additionally aim to reduce anxiety prior to and on immersion.

We also examined the relationships that were evident between components of the CSR and acute anxiety ratings *during* immersions. The results in the first minute of immersion support the predictive relationship evident with our regression model with high levels of anxiety associated with priming the cardiac component of the CSR and low(er) levels associated with driving the respiratory frequency component of the response. The clarity of this relationship is not sustained in to the 3rd, 5th, and 7th minutes of immersion with the resultant effect of high anxiety levels not necessarily exclusively associated with one or other component of the CSR thereafter (see **Tables 1**, **2**). However, it is clear that the significant relationships seen with high(er) levels of anxiety (i.e., CON1 and CWI-ANX) by contrast to low(er) levels (i.e., CON2) temporally oppose one another (view **Tables 1–3**). Moreover, when anxiety levels were not manipulated to be increased after habituation immersions (i.e., CON2), the anxiety rating is less variable, more consistently and strongly associated with the respiratory frequency component (i.e., it approached a significant relationship in the 3rd minute of immersion and was significant in the 1st and 5th). Although correlation does not confer causation, the observation from our regression model provides some quantifiable evidence of this effect with approximately 20% of the variance in f_c explained when anxiety levels are high whereas 32% of the variance is explained by f_R when anxiety levels are allowed to fall. Clearly, some of the evident variation in CSR seen in previous studies must be accounted for by differing levels of anxiety about impending immersion and it is also clear that the predictability of the CSR is improved when anxiety levels are lowered (see **Figures 2**, **3**); a further reason to target lowering anxiety levels by way of preparatory training for those at risk. It is also possible that this benefit would extend to improving breath-hold time on immersion which may be a requirement in some situations (e.g., ditched helicopter egress).

The protective benefit that habituation would provide in the real life scenario is now questionable given that: we have shown previously that habituation is prevented/delayed when anxiety levels are not concurrently reduced during repeated immersions (Barwood et al., 2017); habituation is partially reversed (cardiac component only) when subsequent high levels of anxiety are experienced (Barwood et al., 2013); conversely and as shown in the present study, low levels of anxiety which are less plausible in the emergency scenario are permissive of respiratory control. Consequently, we contend that the specificity of the habituation stimulus (also see Leblanc and Potvin, 1966) in reflecting the real-world scenario plays an important role in the response that is evoked. Therefore, our habituation techniques (e.g., survival training) in preparing at risk individuals should be as reflective of the true stimulus as is possible. The resultant effects of anxiety on different components of the CSR are also important given that the primary risk on accidental CWI is caused by a loss of respiratory control and the associated increased risk of aspirating water to the lungs (Tipton, 1989). It has been speculated that this mechanism accounts for a significantly larger proportion of sudden immersion deaths with the remainder (∼10%) accounted for by a sudden cardiac event (Tipton, 2003). Hence a significantly increased heart rate would be of a lesser concern than a raised respiratory rate on immersion in an otherwise healthy individual. Our previous finding in unhabituated participants that high levels of anxiety augment both the ventilatory and cardiac components of the CSR probably applies to the majority of those who are accidentally immersed (Barwood et al., 2013). Collectively, this suggests that anxiety level should be added to our future measurements of the CSR and an integrated psychophysiological model is required to consider the multifactorial CSR drivers. Accordingly we offer some novel insights on potential neuroanatomical, perceptual and attentional components that may be responsible for our observations.

Neuroanatomy of the Stress Response

Concurrent to an activated thermal neural network, we suggest that emotion (e.g., panic and anxiety), attentional processing and behavior could all influence respiratory motor output as has been observed in studies examining respiratory responses to panic and fear (Masaoka and Homma, 2001). Areas of the hypothalamus, forebrain, limbic and cortical structures have been implicated in the biological systems that process information from the external environment resulting in stimulation of spinal respiratory motor neurones thereby increasing respiratory rate (f_R) but not ventilatory depth (V_T; Masaoka and Homma, 2001, 2004); we also saw no change in V_T. These anatomy also share a common anatomical connection with the spinal lamina I neurons which convey the thermoafferent volley triggered by sudden skin cooling (see Craig, 2002) which also evoke the CSR (for reviews see Tipton, 1989; Datta and Tipton, 2006; Tipton et al., 2017). Small afferent Lamina I neurons are part of the Lamina I

TABLE 1 | Correlation between acute anxiety rating and components of the CSR in the 1st, 3rd, 5th, and 7th minutes of the first immersion (n = 48); *denotes significant correlation ($p < 0.05$).

		CSR Component			
Immersion period	**Correlation result**	f_R **(breaths·min^{-1})**	f_c **(bt·min^{-1})**	V_T **(L·min^{-1})**	$\dot{V}_E$ **(L·min^{-1})**
1st min	*r*-value	0.14	0.33	−0.10	−0.02
	p-value	0.36	0.02*	0.52	0.89
3rd min	*r* value	0.30	0.24	0.09	0.28
	p-value	0.04*	0.10	0.53	0.06
5th min	*r*-value	0.29	0.18	0.03	0.21
	p-value	0.04*	0.21	0.84	0.16
7th min	*r*-value	0.10	0.26	0.25	0.32
	p-value	0.50	0.08	0.09	0.03*

TABLE 2 | Correlation between acute anxiety rating and components of the CSR in the 1st, 3rd, 5th, and 7th minutes of the anxiety inducing immersions (i.e., immersion 6 or 7; CWI-ANX) after habituation immersions (n = 25); *denotes significant correlation ($p < 0.05$).

		CSR component			
Immersion period	**Correlation result**	f_R **(breaths·min^{-1})**	f_c **(bt·min^{-1})**	V_T **(L·min^{-1})**	$\dot{V}_E$ **(L·min^{-1})**
1st min	*r*-value	0.33	0.52	−0.25	0.05
	p-value	0.11	0.01*	0.23	0.82
3rd min	*r*-value	0.40	0.28	−0.28	−0.01
	p-value	0.04*	0.18	0.18	0.97
5th min	*r*-value	0.32	0.25	−0.01	0.18
	p-value	0.11	0.22	0.97	0.40
7th min	*r*-value	0.33	0.11	−0.01	0.24
	p-value	0.11	0.61	0.96	0.25

*In contrast, in the 1st minute of the CON2 (n = 25) immersion acute anxiety levels were positively correlated with f_R but not f_c (see **Figures 3C,D**); a relationship also evident in the 5th minute of immersion. Anxiety ratings were correlated with the f_c component of the CSR in the 3rd minute of immersion; see **Table 3**. Collectively the r-values more consistently indicated moderate strength relationships (r = 0.40–0.58).*

TABLE 3 | Correlation between acute anxiety rating and components of the CSR in the 1st, 3rd, 5th, and 7th minutes of the control immersion (i.e., immersion 6 or 7; CON2) after habituation immersions (n = 25); *denotes significant correlation ($p < 0.05$).

		CSR Component			
Immersion period	**Correlation result**	f_R **(breaths·min^{-1})**	f_c **(bt·min^{-1})**	V_T **(L·min^{-1})**	$\dot{V}_E$ **(L·min^{-1})**
1st min	*r*-value	0.58	0.23	−0.09	0.45
	p-value	0.00*	0.28	0.68	0.02
3rd min	*r*-value	0.36	0.40	0.06	0.14
	p-value	0.08	0.05*	0.78	0.51
5th min	*r*-value	0.49	0.37	0.16	0.49
	p-value	0.01*	0.07	0.44	0.01
7th min	*r*-value	0.37	0.21	−0.09	0.11
	p-value	0.07	0.32	0.67	0.61

spinothalamocortical pathway that relay afferent information to the main homeostatic integration sites in the brainstem (Todd et al., 2005). The brainstem projects to the insula cortex, which also receives afferent input from the somatosensory cortex (Craig, 2002) both of which we suggest are important in producing the sensory experiences prior to and on immersion. When immersion is planned as in the present studies we suggest that, based on the ideas of Craig et al. (2000) model of interoception, the dorsolateral (DL)PFC provides the insula with corollary discharge which predicts the expected sensory consequences of the immersion. On receiving the afferent feedback the insula compares the predications with the actual afferent information in order to generate a current awareness state (Craig, 2002; Gu et al., 2013). We speculate that discrepancies between the actual and expected afferent signals, magnified by our high compared to low anxiety conditions, may produce an altered physiological response and differential activation of f_c in the high anxiety condition and f_R in the low anxiety condition. Craig (2002) and others (Medford et al., 2010) suggest that other brain areas including the anterior cingulate cortex (ACC), medial (m)PFC and DLPFC are important in generating this awareness state with the insula cortex and ACC also including neural connections to the amygdala (Shi and Cassell, 1998) which may account in part for the emotional effects found in our previous research (Barwood et al., 2013, 2017).

Thermal and Stress Habituation

In understanding the likely interaction between the repeated thermal and perceptual stress induced by our experiments it is important to consider the central site and mechanism by which thermal stimulation may be consolidated and habituation mediated. Historically, studies of CWI habituation have linked the frontal cortex to habituation of the CSR (Glaser and Griffin, 1962; Griffin, 1963). More recently, the sub-division of the cerebral cortex, the prefrontal cortex (PFC) has been linked with behavioral and cognitive flexibility (i.e., the process by which environmental feedback is used to modify behavior) the normal function of which is absent in stress related disorders (Campeau et al., 2011). Animal studies involving cold stress have been shown to impact the orbitofrontal subregion of the PFC whereas the prelimbic and infralimbic cortices are more specifically linked to responses to chronic psychological stressors (Campeau et al., 2011). In line with the mechanism outlined above, the spinal Lamina I pathway includes projections to the orbitofrontal sub-region of the mPFC, which consists of Brodmann's areas 10, 11, and 47 and is thought to play a major role in cold stress interpretation and the associated hedonic tone (i.e., pleasant or unpleasant nature) of a given event (Kringelbach, 2005). This is consistent with the idea that the orbitofrontal cortex encodes the outcome expectations of a given situation (Schoenbaum et al., 2009) which were manipulated in the present study in the high compared to low anxiety conditions. This region has also been found to play a major role in risk avoidance (Brown and Braver, 2007). The mPFC, in turn, sends ascending projections to the DLPFC which, following habituation, is responsible for re-configuration of predictions of sensory effects that it will pass to the insula cortex in the form of corollary discharge (Kringelbach, 2005) during future immersion situations.

Perceptual and Attentional Demands of CWI

We have previously hypothesized that a model of stress and coping may prevail in the emergency scenario that may ultimately increase or decrease the perceptual component and resultant anxiety response depending upon the primary and secondary appraisal of the stimulus (Barwood et al., 2013, 2014, 2017) thereby stimulating or mitigating the activity of the multiple neural networks involved on CWI. Accordingly, anxiety level may be increased if a victim is confronted with a highly novel and important stimulus (primary appraisal) which may be compounded by the perception that coping resources are limited or absent to deal with this situation (i.e., secondary appraisal) thereby resulting in a perceived threat (Lazarus and Folkman, 1984). Anxiety levels may be reduced if the stimulus were appraised as familiar, consequently less important and when accompanied by a perceived high level of coping resource, as might be the case in those who receive survival training or basic survival skills for the immersion scenario. Models of attentional processing are required to explain the real time attentional demands of accidental immersion given its critical nature. One proposition is that, when under duress, the brain has a finite processing capability that is narrowed by increased arousal levels as might be expected in the emergency scenario (Rejeski and Ribsl, 1980). Evidently, filtering multiple relevant environmental and behavioral cues (i.e., 'floating first,' waiting for CSR to subside, keeping the airway above the water; Barwood et al., 2011, 2016) whilst ignoring the irrelevant cues may not be feasible when attentional capacity is limited. Similarly, a processing efficiency theory would contend that, under stress, working memory is taken up with worry, anxiety and intrusive thoughts that consume limited working memory capacity and deny ressources for processing important task-relevant information (Eysenck and Calvo, 1992). Both theories suggest that processing capacity would be limited in the acute, accidental immersion scenario whereby survival training or basic survival skills could be used to guide behavior at a time when attentional demand is high and the resultant decisions are critical. The concurrent decline in cerebral blood flow on immersion (Mantoni et al., 2007; Button et al., 2015) may compound any decrement to cognitive performance and increase the risk of drowning. One such possibility to improve any cognitive decrement on accidental immersion is to integrate a form of psychological skills training (PST) technique into survival training techniques or safety behavior messages. Such techniques have been shown to extend maximal breath-hold duration by up 80% on CWI in unhabituated participants (Barwood et al., 2006) and 120% (of that seen in air) in habituated participants (Barwood et al., 2007). Safety statements such as 'float first' include important procedural and environmental behavioral cues, similar to the embedded cognitive principles of PST, that convey important information in a succinct manner.

Limitations

Clearly it is not ideal that we are considering the resultant effects of high(er) compared to low(er) acute anxiety after habituation immersions which included different experimental manipulations. Observations from animal studies (i.e., Kelly et al., 2011) may suggest that participants experiencing CWI concurrent to psychological stress (i.e., CWI-ANX_{rep} see **Figure 1**) would experience independent, and potentially additive, serotonergic drivers of the stress response resulting in different or absent habituation (see Barwood et al., 2017). Yet, irrespective of whether anxiety is manipulated or not during habituation immersions, it is experimentally very difficult to entirely remove the anxiety associated with impending and ensuing immersion. The tentative findings we show here with our combined analysis require subsequent verification; as is the case with all novel experimental findings. Yet we acknowledge that the neural mechanisms we outline above may have been stimulated differently in some of our participants *during* the habituation immersions despite experiencing an identical thermal stimulus. However, most importantly, the high vs. low level anxiety conditions we feature in the present study clearly showed a separation in the predictive and related components of the CSR despite the manipulations in the habituation immersions; hence we have been able to interrogate our hypotheses.

The water temperature used in the present experiments and our indices of the CSR may allow a mediating role for acute anxiety when none would be evident. In the case of our CSR indices, it is possible that a better index of respiratory drive than the one used here (e.g., mouth occlusion pressure at 100 ms of inspiration; P0.1) would share a different relationship with anxiety level than respiratory frequency does. P0.1 has been shown to more closely track the thermally induced neural drive to breathe during immersion whilst ventilation was shown to plateau (Mekjavic et al., 1987; Burke and Mekjavic, 1991). Hence the contribution of anxiety may not be truly reflected in our chosen index of respiratory drive after ventilation has plateaued. Given that the most critical period of the CSR is the first 60–90 s during which ventilation has yet to plateau, the issue of misrepresentation of respiratory drive by respiratory frequency is more likely to be a factor later in the 7-min immersion period we have studied here. In the case of water temperature, the CSR is suggested to be maximally evoked at water temperatures between 5°C and 15°C (Goode et al., 1975; Tipton et al., 1991) although nocioceptors may be activated at temperatures below 10°C. Indeed, studies that examine the cardiorespiratory response to pain in anesthetized patients (Eger et al., 1972; Borgbjerg et al., 1995) and decerebrate cats (Waldrop et al., 1984) suggest that neural pain networks transmit nocioceptive information to the bulbar respiratory nuclei without involving higher cortical centers. Hence, nocioceptors and thermoreceptors share a relatively direct and uncomplicated neural pathway to the respiratory centers the former of which may have an additive effect on the CSR that is seen. However, it must be noted that the *anticipation* of pain does stimulate higher cortical centers and result in an increase in respiratory frequency (Willer, 1975). In the present study, a water temperature of 15°C reduces the possibility that pain networks are also driving the response. A theoretical maximum CSR must exist for each immersed individual and we speculate this lies at the saturation point of the sympathetic branch of the autonomic nervous system with thermal, pain and perceptual stimuli.

Lastly, the gender imbalance in favor of males may also contribute to our findings and studies sub-divided by gender may be worthy future line of enquiry. Similarly, the rate of immersion in the present study was carefully controlled but does not reflect the rapid rate that would be evident on accidental immersion. It is known that cutaneous thermoreceptors respond to the rate and summation of a thermal stimulation culminating in the centrally integrated thermoafferent signal (Hensel and Schafer, 1984). Clearly both the rate and the summation of thermoreceptor stimulation would be higher on falling in to water as opposed to staged immersion. Other researchers have achieved more ecologically valid means of water entry whilst studying the CSR in the laboratory (Croft et al., 2013) although our methods do demonstrate a consistently administered and controlled thermal stimulus.

CONCLUSION

Acute anxiety prior to immersion predicts different components of the CSR on immersion before and after habituation. This suggests that safety training and behavioral advice to survive accidental CWI should consider interventions that can also reduce acute anxiety about impending immersion particularly in at risk individuals. The CSR should be considered as an integrative psychophysiological response which may include the activation of multiple neural networks. We offer a new integrated model of the CSR and CSR habituation to explain our observations. Further studies are required to test this model.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Cardiac Autonomic Modulations and Psychological Correlates in the Yukon Arctic Ultra: The Longest and the Coldest Ultramarathon

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Studies on human physical performance in extreme environments have effectively approached the investigation of adaptation mechanisms and their physiological limits. As scientific interest in the interplay between physiological and psychological aspects of performance is growing, we aimed to investigate cardiac autonomic control, by means of heart rate variability, and psychological correlates, in competitors of a subarctic ultramarathon, taking place over a 690 km course (temperatures between +5 and −47°C). At baseline (PRE), after 277 km (D1), 383 km (D2), and post-race (POST, 690 km), heart rate (HR) recordings (supine, 15 min), psychometric measurements (Profile of Mood States/POMS, Borg fatigue, and Karolinska Sleepiness Scale scores both upon arrival and departure) were obtained in 16 competitors (12 men, 4 women, 38.6 ± 9.5 years). As not all participants reached the finish line, comparison of finishers (FIN, $n = 10$) and non-finishers (NON, $n = 6$), allowed differential assessment of performance. Resting HR increased overall significantly at D1 (FIN +15.9; NON +14.0 bpm), due to a significant decrease in parasympathetic drive. This decrease was in FIN only partially recovered toward POST. In FIN only, baseline HR was negatively correlated with mean velocity [r −0.63 (P.04)] and parasympathetic drive [pNN50+: r −0.67 (P.03)], a lower HR and a higher vagal tone predicting a better performance. Moreover, in FIN, a persistent increase of the long-term self-similarity coefficient, assessed by detrended fluctuation analysis (DFAα2), was retrieved, possibly due to higher alertness. As for psychometrics, at D1, POMS Vigor decreased (FIN: −7.0; NON: −3.8), while Fatigue augmented (FIN: +6.9; NON: +5.0). Sleepiness increased only in NON, while Borg scales did not exhibit changes. Baseline comparison of mood states with normative data for athletes displayed significantly higher positive mood in our athletes. Results show that: the race conditions induced early decreases in parasympathetic drive; the extent of vagal withdrawal, associated to the timing of its recovery, is crucial for success; pre-competition lower resting HR predicts a better performance; psychological profile is reliably depicted by POMS, but not by Borg fatigue scales. Therefore, assessment of heart rate variability and psychological profile may monitor and partly predict performance in long-duration ultramarathon in extreme cold environment.

Keywords: cold, exercise performance, extreme environments, fatigue, heart rate variability, mood, subarctic ultramarathon, ultra-endurance

INTRODUCTION

Human physiology is characterized by continuous reactive adaptation to internal and external conditions and stressors (Ramirez et al., 1999; Hawley et al., 2014). Subjects exposed to extreme conditions and environments display astounding adaptive potential, which ultimately ensures optimal adjustment to current organismic demands and external stress (Kälin et al., 2012; Gunga, 2014). Assessment of autonomic cardiac modulation by means of heart rate variability (HRV) has shown to be a reliable tool to evaluate not only physiological changes (Taralov et al., 2015; Kobayashi et al., 2016), but also psychological aspects of human reactive adaptation to different stressors (Souza et al., 2013). Therefore, HRV assessment may describe human resilience, as it represents a bridge between physiology and psychology, and, by integrating these two aspects, it mirrors human adaptive ability (Thayer et al., 2009; Spangler and Friedman, 2015). Particularly in endurance athletes, training effects, performance level and physical wellbeing may be contextualized through HRV assessment (Atlaoui et al., 2007; Plews et al., 2013a; Buchheit, 2014; Bellenger et al., 2016a). Successful adaptation to increased training load, resulting in improved performance, is associated with increased HRV, as well as enhanced parasympathetic predominance at rest (Plews et al., 2013b; Stanley et al., 2015; Lucini et al., 2017). Assessment of autonomic cardiac modulation conducted directly post-exercise or after competitions, demonstrated a decrease in HRV and a parasympathetic withdrawal (Bricout et al., 2010; Buchheit et al., 2010; Bellenger et al., 2016a), which, however, was effectively recovered depending on the intensity of the preceding exercise (Martinmäki and Rusko, 2008; Manzi et al., 2009; Stanley et al., 2015), and on the individual's training status (Bricout et al., 2010; Buchheit et al., 2010; Bellenger et al., 2016a). This has been vastly evidenced in endurance exercisers (Buchheit et al., 2010; Plews et al., 2012; Da Silva et al., 2014; Kiviniemi et al., 2014), and investigations of cardiac autonomic function in response to extreme endurance exercise, such as ultramarathon, display similar findings (Gratze et al., 2005; Scott et al., 2009; Foulds et al., 2014), even though specific studies related to cardiac autonomic modulation during ultramarathon, are still scarce in comparison. On the other hand, in ultra-endurance athletes, physical exertion has been commonly associated with mental fatigue and increased mood disturbance (Anglem et al., 2008; Siegl et al., 2017), especially in participants who experience adverse incidents, then perform poorly or are forced to prematurely withdraw (Parry et al., 2011; Joslin et al., 2014).

Ultramarathon is mostly defined by course lengths exceeding marathon distance and is characterized by the combination of extremely challenging highly intensive exercise (e.g., track lengths >300 km or great elevation gains), often under strenuous environmental conditions, with concurrently impaired possibilities to recover. The Yukon Arctic Ultra (YAU) is considered to be one of the world's toughest ultramarathons (Coker et al., 2017), as it combines the great course distance of 690 km with extreme environmental conditions typical of a subarctic winter. Except for several in-race checkpoints, there are no indoors sleeping vacancies, so that competitors have to camp on the race-course and experience complete environmental exposition. Therefore, YAU competitors are challenged by a *three-folded stress stimulus* of (i) long-term strenuous exercise, (ii) extreme cold exposure, and (iii) impaired resting conditions, due to in-race camping. So far, among studies on ultra-endurance exercise, research objectives mostly diverge from evaluation of autonomic cardiac function in ultramarathon runners (Degache et al., 2014; Hurdiel et al., 2015; Mrakic-Sposta et al., 2015; Wüthrich et al., 2015; Tonacci et al., 2017), which, to our knowledge, was only implemented in three previous studies (Gratze et al., 2005; Scott et al., 2009; Foulds et al., 2014). However, these investigations differed regarding (i) the race length (e.g., 160 km ultramarathon, or Ironman competition with a total distance of 226.35 km), (ii) study protocol (i.e., pre- vs. post-race comparison only), (iii) environmental conditions (mild climate, summer), and (iv) terrain characteristics (e.g., mountain, large altitude variation, etc.). Therefore, this is the first study to assess cardiac autonomic modulation in competitors of an extremely long (i.e., 690 km) ultramarathon on a mostly flat course, in subarctic climate, which may provide essential insights into the adaptive capacity, as, aside from exercise, HRV is associated with numerous external and internal factors (Rajendra Acharya et al., 2006; Shaffer et al., 2014).

With outdoor temperatures ranging from +5 to −47°C and the air humidity accounting for up to 100%, YAU competitors face extreme subarctic weather conditions. Comparable scientific knowledge is, however, insufficient. Autonomic balance has been observed to shift toward greater parasympathetic predominance during Antarctic stays (Farrace et al., 2003; Harinath et al., 2005), but these results were obtained in expeditioners confined to indoors housing. Moreover, a significant interplay between autonomic cardiac regulation and psychological wellbeing has been observed (Sakuragi et al., 2002; Karavidas et al., 2007; Sgoifo et al., 2015), so that psychometric assessment may also serve to contextualize findings about HRV (Bellenger et al., 2016b; Flatt et al., 2017b). Increased performance and greater parasympathetic drive in cardiac autonomic regulation are associated with increased psychological wellbeing (Cervantes Blásquez et al., 2009; Bisschoff et al., 2016). Conversely, fatigued states and increased mood disturbance have been related to decreased indexes of total HRV, as well as parasympathetic tone (Nuissier et al., 2007; Leti and Bricout, 2013; Schmitt et al., 2013; Flatt et al., 2017a). In this context, impaired resting conditions present another vital influence on cardiac autonomic regulation. Assessment under concurrent sleep-deprivation, which itself is again related to impaired both cognitive and physical performance (Marcora et al., 2009; Fullagar et al., 2015), shows decreased HRV indexes in the parasympathetic domain (Dettoni et al., 2012; Glos et al., 2014; Tobaldini et al., 2014).

To our knowledge, exposition to such a particular combination of stress-stimuli as presented by competition in the YAU has never been investigated regarding cardiac autonomic function and psychological profile. Therefore, we assessed autonomic cardiac regulation in terms of HR/HRV, as well as psychometric measurements including mood states, indicators of sleepiness, exertion and recovery, to investigate adaptation to extreme conditions and performance, by analyzing cardiac autonomic control and its interplay with mood and fatigue. We hypothesized that higher performing competitors, compared to less successful athletes, would exhibit differential profiles of autonomic cardiac regulation associated with optimal psychometric profile, overall characterized by higher adaptability and greater resilience to the extreme challenges of the three-folded stress stimulus.

MATERIALS AND METHODS

Subjects and Study Implementation

This study is part of a larger investigation regarding "*Physiological changes of participants of the Yukon Arctic Ultra - an ultramarathon in extremely cold climate*," where it is planned to assess a variety of different physiological parameters and their interplay.

From a total number of 78 athletes partaking in the 690 km foot-race category of the YAU during the years 2013, 2015, and 2017, 27 (20 men, 7 women) volunteers enrolled in the study (8 in 2013, 9 in 2015 and 10 in 2017). Due to issues related to data collection, from the 27 participants, only 16 (ALL: 12 men, 4 women) were included in the data analysis (see section Statistics). The majority ($n = 15$) were of Caucasian descent and one was of Asian origin. Their anthropometric data are presented in **Table 1**.

The recruitment for this study was conducted with the support of the event organizers. A call for participants, with a brief description of the study and planned measurements, was transmitted to the athletes who had enrolled in the 690 km foot-race category. The organizers were encouraged to predominantly contact experienced athletes, who had a long history of completed endurance events and/or who had completed the YAU before. Athletes who were interested in the study contacted the principal investigator via e-mail and received further detailed information. The potential study participants had several weeks to ask questions via e-mail and to decide whether to partake in the study or not. There were no further inclusion or exclusion criteria: all athletes enrolled in the 690 km foot-race category were eligible to enter the study. All athletes were required to present to the event organizers a health certificate issued by their home physician, in order to be enrolled in the race. During a meeting in Whitehorse, Yukon Territory, Canada, 4–5 days before the race start, the potential study participants met with the investigators in person, had the chance to ask further questions and to finally give their informed written consent to partake in the study. The study was approved by the Charité Ethics Board (review number EA4/109/12), and all measurements and procedures complied with the Declaration of Helsinki (54th Revision 2008, Korea)[1] regarding the treatment of human subjects.

TABLE 1 | Subject demographics.

Group	Gender	*n*	Age, years mean (S.D.)	Weight, kg mean (S.D.)	Height, cm mean (S.D.)	BMI, kg/m^2 mean (S.D.)
FIN	Men	7	42 (10)	80 (9)	176 (6)	25.7 (3.0)
	Women	3	38 (10)	61 (2)	168 (10)	21.7 (3.4)
	All	10	40 (9)	74 (12)	174 (8)	24.5 (3.5)
NON	Men	5	33 (7)	79 (12)	179 (10)	24.7 (1.7)
	Women	1	51 (0)	58 (0)	170 (0)	20.1 (0)
	All	6	36 (10)	76 (14)	177 (10)	23.9 (2.4)
ALL	Men	12	38 (10)	80 (10)	177 (7)	25.2 (2.5)
	Women	4	41 (11)	60 (2)	169 (9)	21.3 (2.9)
	All	16	39 (10)	75 (12)	175 (8)	24.3 (3.1)

Subject demographics at baseline for all participants (ALL) and in subgroups (FIN and NON). No significant differences between groups.

All study participants included in the final analysis had completed either one marathon (9.6 ± 24.4) or ultramarathon (14.4 ± 24) prior to their study-participation. The mean longest ultramarathon distance completed by the athletes before their YAU participation was 380 ± 220 km. In addition, seven of the study participants had previously participated in the YAU foot-race in various distance categories, with a mean longest completed distance of 478 ± 219 km. Thus, the study participants were experienced endurance athletes, which is also reflected by their self-reported sedentary HR of 52.6 ± 7.3 bpm. From one participant, this background data was not made available.

The Yukon Arctic Ultra: The Longest, the Coldest Ultramarathon

The Montane® YAU ultra-endurance race takes part in the beginning of February, covering a 690 km distance between Whitehorse and Dawson City in the Canadian Yukon Territory. Besides the foot-race, the YAU also allows the competition in cross-country-skiing and mountain-biking. The first and last sections of the trail account for elevations between 500 and 700 m, however, especially in the last 200 km, the terrain along the Yukon river partly exhibits great elevation gains (up to 1,000 m). The YAU is not an orientation race, as the trail is marked and prepared with snow-mobiles. Via GPS devices, athletes can be tracked on the course and have the possibility to call for assistance in case of an emergency. To

[1]https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/

further increase their safety, the time until the race has to be completed is limited to 14 days and additionally, medical screenings are administered at the 10 checkpoints which are located (mostly about 50 km apart) along the route. Despite these partly indoor vacancies (otherwise, tents were provided), during the race, competitors face complete exposition to the subarctic environment, with outdoor temperatures in February ranging between +5°C (highest temperature measured in February 2013 in Whitehorse) and −47°C (lowest temperature measured in February 2015 in Dawson City). Additionally, the extremely high air humidity (up to 100% as measured in February 2013 in Whitehorse) contributes to the possible onset of frostbite, which, along with other (medical) concerns, may lead to immediate disqualification. Importantly, the weather conditions between editions were not significantly different (**Figure 1**), detailed information on weather conditions can be assessed in respective weather archives[2].

Notably, participants walked between 12 and 15 h per day whilst pulling their gear on a sled-like pulk (accounting for 30–40 kg; additionally, participants were allowed up to three drop bags) and, apart from the checkpoints, had to eat, rest and make toiletry arrangements in the outdoor conditions of the Yukon Territory.

More detailed information about the Montane® YAU is provided on the official website of the event[3].

Experimental Protocol and Measurements

Experimental protocol details are depicted in **Figure 2**. At two out of ten in-race checkpoints, we respectively implemented two in-race assessments, so that, in summary, measurements were performed: (1) at baseline during the 3 days preceding the race in Whitehorse (PRE), (2) at the Carmacks in-race checkpoint at 277 km (During 1, D1), (3) at the Pelly Crossing in-race checkpoint at 383 km (During, D2) and (4) immediately after completion of the race in Dawson City at 690 km (POST).

The in-race checkpoints had to be selected for measurement implementation due to essential practical concerns. They had to be indoors facilities buildings with sufficient space, comfortable ambient temperature and low noise in order to perform measurements under controlled conditions, as well available electricity and that it was accessible by car for the investigators. Exemplarily, several of the race checkpoints were mere tents that did not meet these criteria and therefore, the study checkpoints were chosen as they were. Thus, the distance between the race start (i.e., PRE) and the first in-race assessment (D1) accounted for over a third (277 km) of the entire race-course and additionally, in this period, athletes would face the most strenuous weather conditions (which tend to ameliorate toward the second half of the month; see **Figure 1**). As the second assessment was performed at 383 km (D2), the distance between D1 and D2 (as well as the time to cover it, which accounted for only 30 h in some subjects) was the shortest between the measurements and, in fact, more than 50% less than the other two distances. Conversely, the distance between D2 and POST was again very high (307 km) and additionally, the terrain in the last third of the course accounted for the greatest elevation gains (see section The Yukon Arctic Ultra: The Longest, the Coldest Ultramarathon).

Baseline Assessment

During the three race-preceding days (PRE), baseline anthropometric data (age, weight and height) were obtained. Weight was measured using a calibrated scale (Seca® GmbH, Hamburg, Germany) and height was taken from the participants' interview.

Fifteen minutes baseline recordings of beat-to-beat HR to assess HRV were collected with a HR monitor (RS800CX Polar Electro Oy, Kempele, Finland), which is widely used and validated for HRV assessment (Wallén et al., 2012). HR recordings were performed in supine position upon awakening (between 5 and 10 a.m.) directly after participants had slept 6–8 h the previous night. The athletes had not consumed food, beverages or stimulants (e.g., coffee) in the 2 h before the recording and were instructed to breathe normally, avoid speaking and moving during the data collection. Additionally, it was ensured by the investigators that subjects would not fall asleep. With the limitations of this specific in-field study, special attention was devoted to performing data collection sessions in a quiet and comfortable setting, with participants lying in a bed or on a sleeping sleeping mattress, ambient temperatures between 17 and 23°C, and reduced light.

On the morning of the race start before departure, additionally, psychometric assessment was performed (see **Figure 2** and section Psychometric Assessment).

In-Race and POST Assessment

Upon arrival at the in-race checkpoints, as well as at the race finish, psychometric scales were administered according to physiological needs and conditions of arriving competitors. Participants then had a few hours of rest (ranging from 4 up to 6–8 h) and upon awakening (at morning, between 5 and 10 a.m.), HR data was collected, as at baseline, to assess HRV. Afterwards, before departure, psychometric assessment again took place (see **Figure 2** and section Psychometric Assessment).

Indoors ambient conditions between the different measurement facilities were comparable, with special attention dedicated to a quiet and comfortably warm setting with reduced light exposure.

Moreover, throughout the entire race, participants were continuously (day and night) monitored by means of a heart rate monitor (RS800CX Polar Electro Oy, Kempele, Finland - sample rate 15 s).

Data Analysis

Performance Assessment and Heart Rate Continuous Recordings

The official time at the end of the race for each participant who reached the finish line was collected, together with the times and the respectively completed distance for each participant who had to withdraw. Subsequently, the mean running velocity of the race

[2] http://climate.weather.gc.ca/historical_data/search_historic_data_e.html.
[3] http://arcticultra.de/en.

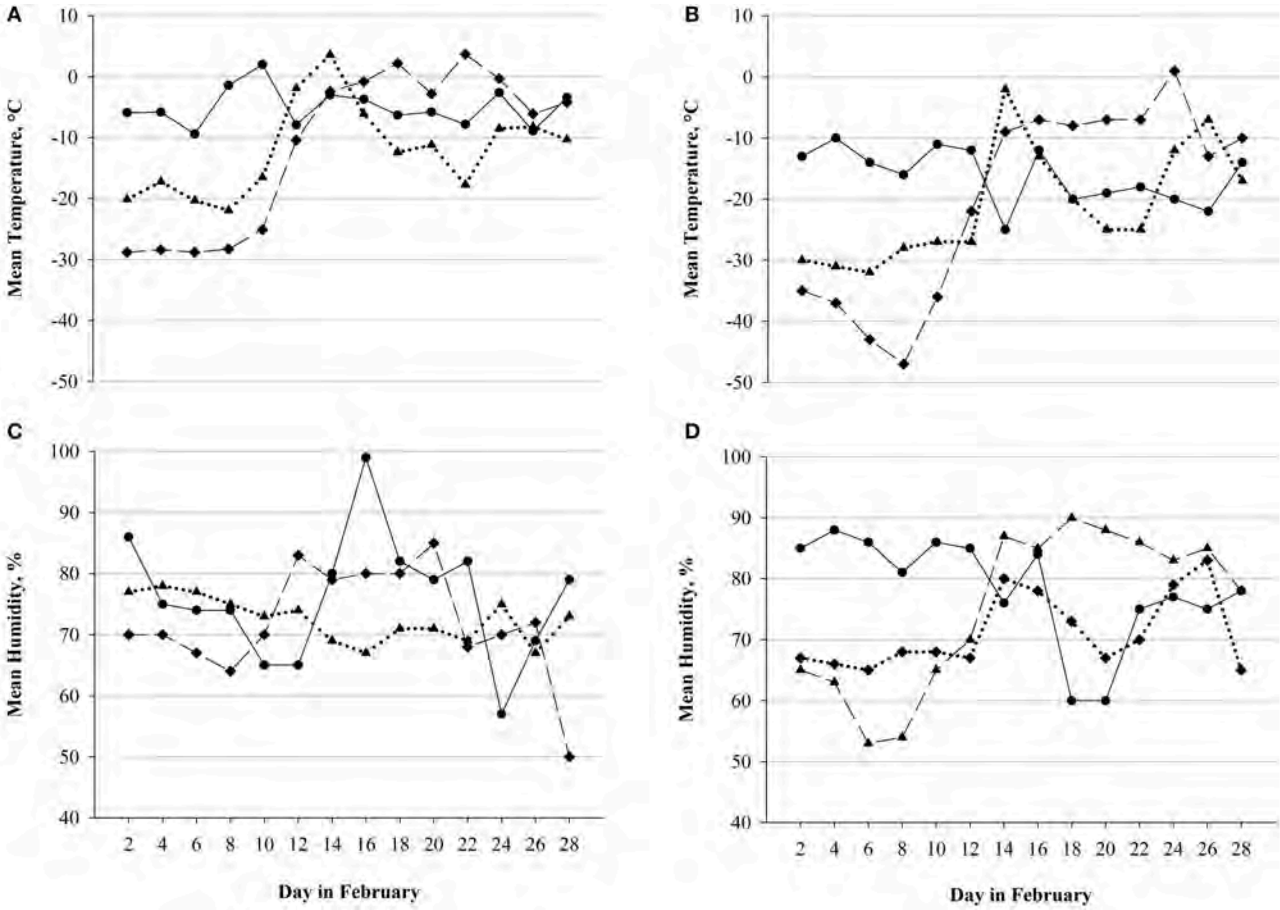

FIGURE 1 | Weather conditions across editions. Mean temperature and mean humidity as measured in Whitehorse and Dawson City in February 2013 (solid line), February 2015 (long dashed line), and February 2017 (dotted line). **(A)** Mean temperature in Whitehorse. **(B)** Mean temperature in Dawson City. **(C)** Mean humidity in Whitehorse. **(D)** Mean humidity in Dawson City. No significant differences between editions or locations[4].

was calculated from the total recorded time and the total distance covered [*time* (*h*)/*space* (*km*) = *velocity* (*km/h*)]. By collection of in- and out-going times at in-race measurement points for each participant, both split times and velocities could be computed, allowing detailed assessment of performance. Additionally, continuous HR measurements served to determine exercise intensity, as well as resting quality (in respect to HR expressed as a percentage of calculated maximal HR). The continuous HR recordings, collected during the race, were screened for quality (no more than 3% signal lost/disturbed). The average, maximum and minimum HR were determined per each selected race period, and the values were normalized with respect to the individual age-related maximal HR (HR_{max}) (Tanaka et al., 2001). This provided further information about exercise intensity and quality of rest. Specifically, data were divided into four time-segments, according to the selected period of recording: (i) HR recorded in the first 36 h following the race start (D1a), (ii) during the 24 h before arriving at D1 (D1b), (iii) during the 24 h before arriving at D2 (D2a), and (iv) HR recorded during the last 24 h before finishing the race (D2b). Collected HR data were then expressed as a percentage of the HR_{max}, for average exercise intensity (ExHR) and average resting HR (RestHR). This approach was selected to allow comparison with parameters assessed at checkpoints (i.e., psychometric and HRV analysis) and, by classifying data, served to better interpret findings.

Heart Rate Variability Assessment

An expert operator visually inspected the R–R interval series, and with the support of a dedicated software (Kubios HRV ver. 2.1, Kuopio, Finland), premature beats or artifacts were removed. The filter threshold was set at the "low" level (Tarvainen et al., 2014) and only files 15-min long and including less than 0.3% of beats recognized as artifacts were considered; then the last 10 min were selected for HRV assessment, to better standardize the analysis. After providing the normal-to-normal (NN) interval series, HRV was assessed as validated indices of autonomic cardiac modulation, based on time-domain, frequency-domain,

[4]Data taken from https://www.timeanddate.com/weather/canada/whitehorse/historic, https://www.timeanddate.com/weather/canada/dawson-city/historic and http://climate.weather.gc.ca/historical_data/search_historic_data_e.html (last accessed December 13, 2017).

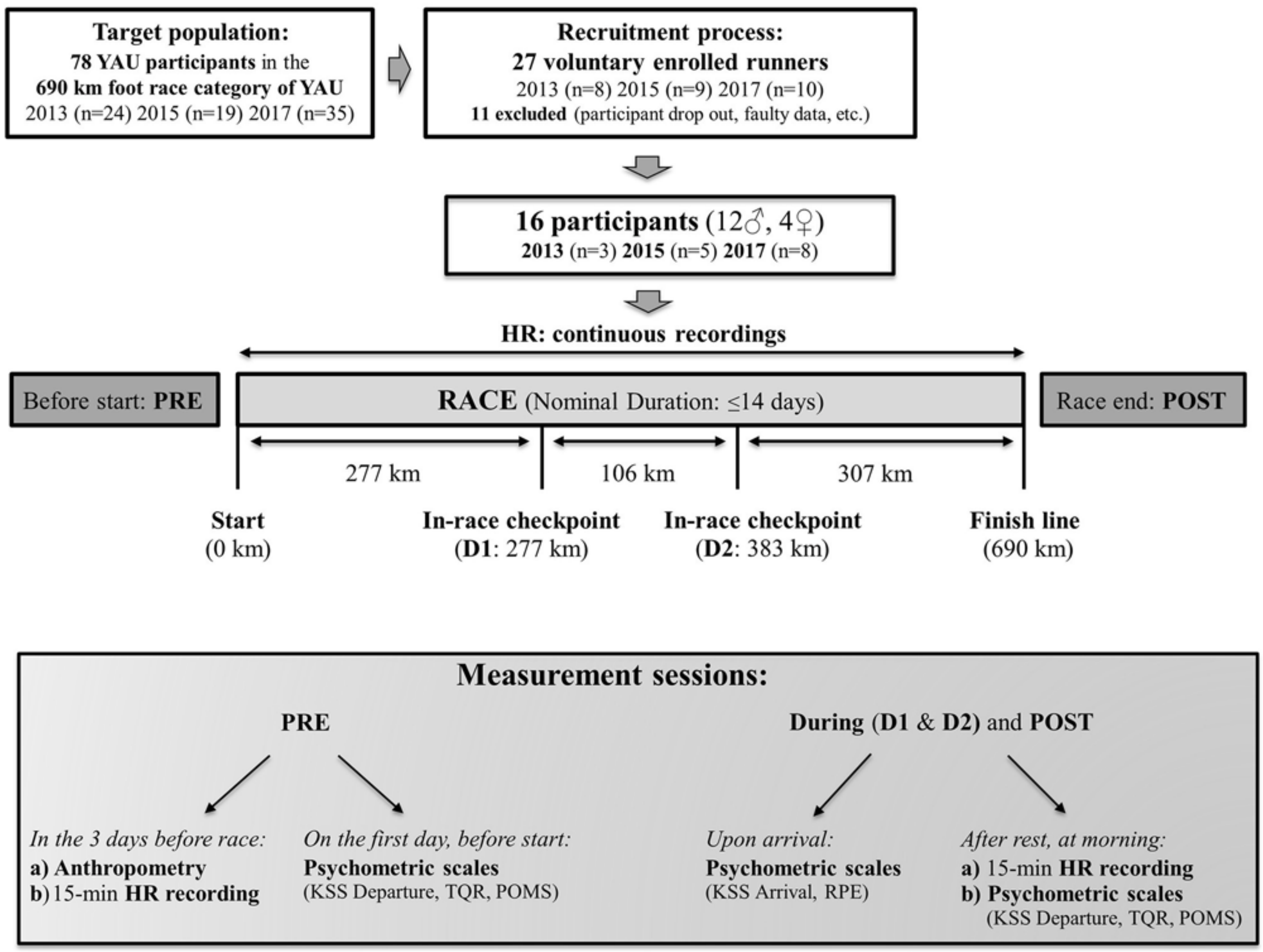

FIGURE 2 | Experimental protocol. Schematic representation of the study protocol and experimental procedure employed in all three YAU races of 2013, 2015, and 2017, except for psychometric assessment (included only in the 2015 and 2017 races).

and complexity (European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Specifically, as for time-domain analysis, the root mean square of the successive RR differences (RMSSD), an indirect index of vagal activity, was calculated. Furthermore, NN50 statistics were computed, specifically, the hourly number of increases (NN50+) or decreases (NN50−) between consecutive NN intervals larger than 50 ms (Ewing et al., 1984), as well as the percentage of such differences with respect to the total number of NN intervals (pNN50+ and pNN50−) (Bigger et al., 1988; Merati et al., 2015). The NN50 statistics may reflect the rate of "vagal bursts," as bursts of vagal outflow are producing NN intervals greater than 50 ms (Ewing et al., 1984). In the frequency domain, the total spectral power density (TP) was assessed together with its components: (i) high frequency (HF) band (0.15–0.40 Hz), which depends mainly on parasympathetic activity and is synchronous with the respiratory sinus arrhythmia; (ii) low frequency (LF) band (0.04–0.15 Hz), which depends on both parasympathetic and sympathetic activity; and (iii) LF/HF ratio, which is currently considered a marker of sympathovagal balance (Ewing et al., 1984). In the non-linear domain, as for complexity analysis, the following indices were assessed: (i) the HR sample entropy (SampEn), which measures the level of irregularity of the NN interval series and mirrors vagal activations or sympathetic deactivations (Porta et al., 2008); (ii) the short-term self-similarity coefficient (α1) and long-term self-similarity coefficient (α2) of NN intervals, as assessed by detrended fluctuation analysis (DFA), mentioned here respectively as DFAα1 and DFAα2 (Peng et al., 1995). Both indices may be affected by parasympathetic tone, whereas, for example, higher DFAα1 is associated with sympathovagal balance increase or vagal tone decrease (Penttilä et al., 2003). The significance of DFAα2 has not yet been completely elucidated, as there is indeed only scarce evidence within the literature. However, it seems to be associated with alertness (Ivanov et al., 1999) and may be influenced by sleep stages, being higher in awake states and REM sleep with respect to light and deep sleep (Schumann et al., 2010).

All HRV indices, except for NN50 statistics (manually calculated), were assessed by means of the Kubios HRV software, ver. 2.1 (Kuopio, Finland), a free available software to assess HRV, widely used in the scientific literature, especially in the field of sport sciences (Tarvainen et al., 2014).

Psychometric Assessment

Karolinska Sleepiness Scale

The Karolinska Sleepiness Scale (KSS) (Kaida et al., 2006), which has been highly validated to sensitively depict objective sleepiness (Kaida et al., 2006; Sallinen et al., 2008), was administered both after rest (before departure in the morning: KSS Departure) and upon arrival (KSS Arrival) (see **Figure 2**). The athletes were asked to rate their subjective sleepiness on a numerical scale ranging from 1 to 10. Specifically, 1–6 are assigned to an "active state" of alertness (1 corresponding to "extremely alert" and 6 to "some signs of sleepiness") and 7–10 to a "sleepy state" (7 corresponding to "sleepy, but no difficulty remaining awake" and 10 to "falling asleep all the time").

Borg Scales

After rest (before in-race departure and at the finish), subjects were administered the Borg Total Quality of Recovery (TQR) questionnaire (Kenttä and Hassmén, 1998). The Borg TQR has been demonstrated to sensitively represent the individual recovery status (Freitas et al., 2014), whereas the use of recovery and wellness indicators has exhibited important validity in the monitoring of athletes (Buchheit, 2015; Bisschoff et al., 2016). It consists of a 6–20 numerical scale, with 6 being equivalent to "very, very poor recovery" and 20 to "very, very good recovery," so that the obtained score allows determination of the athlete's subjectively evaluated quality of recovery.

Upon arrival at checkpoints or the finish (**Figure 2**), participants were administered the Borg Rating of Perceived Exertion (RPE) scale (Borg, 1982; Scherr et al., 2013), which is commonly used in athletes to monitor exertion and also the current subjective workload, additionally, in association with cardiac autonomic regulation (Parry et al., 2011; Thorpe et al., 2016; Siegl et al., 2017) and performance (Suzuki et al., 2006). It again consists of a 6–20 numerical scale, 6 being "very, very light" and 20 "very, very hard," the individual score indicating the athlete's degree of subjectively perceived exertion.

Profile of Mood States

At morning, after rest, mood states in the YAU participants were investigated through the Profile of Mood States questionnaire in the short-form (POMS-SF, here referred to as POMS) (Curran et al., 1995). This extensively validated tool is commonly used in athletic monitoring (Hedelin et al., 2000; Leti and Bricout, 2013; Bisschoff et al., 2016) and has been variously observed to be associated with both HRV and performance (Hedelin et al., 2000; Leti and Bricout, 2013; Comotto et al., 2015; Bisschoff et al., 2016). The POMS required participants to state the extent of emotions currently experienced during the last hours (respectively operationalized as "not at all" providing a subscore of 1; up to "extremely," providing a subscore of 5). Analysis of individual subscores in emotional subcategories subsequently provided an individual raw score representing the 6 main mood states Depression, Vigor, Fatigue, Tension, Confusion and Anger, as well as a total sum score of mood disturbance (POMS Total).

The psychometric assessment of mood states in our subjects was further analyzed by comparison with normative data for an athletic sample (Terry and Lane, 2000). This data had been obtained in mixed general athletic samples as well as, amongst others, subgroups of athletes at different competition levels and situations (pre- or post-competition, etc.) (Terry and Lane, 2000). In accordance with Terry, raw scores were transformed to a normalized T-Score (using the individual raw score, group mean and group standard deviation) through the formula: $T\text{-}Score = 50 + [10 + (raw\ score - group\ mean)]/SD$. This transformation converted raw scores to normalized scores on a standard scale with a mean of 50 and a standard deviation of 10, so that individual results could be compared with normative sample data. POMS normative scores of athletes from various sport disciplines, plotted against college student norms originally obtained by McNair in 1971 (McNair et al., 1971), show a distinctive pattern of mood states in athletes compared to sedentary populations, which is referred to as the *Iceberg Profile* (**Figure 9A**). Specifically, athletes have been found to account for significantly higher Vigor, whereas all other (negative) dimension scores remain below mean values for non-athletes, i.e., "under the surface." This distinctive profile has been proposed to indicate greater mental health and reduced mood disturbances in athletic subjects compared to sedentary populations. By plotting individually obtained values against normative data, this specific pattern of higher positive mood and mental health in our participants compared to normative data of sedentary subjects could therefore be assessed.

Moreover, by analyzing result scores of the administered psychometric scales, the so-called *psychological wellbeing* (Scully et al., 1998; Johnston et al., 2015; Saw et al., 2016) was evaluated. A higher psychological wellbeing would correspond to an overall low score both for POMS Total (i.e., higher Vigor, lower Fatigue, Tension, Confusion, Anger and Depression scores) and for fatigue scales (i.e., Borg RPE and KSS), and inversely higher scores for Borg TQR.

Statistics

Data are reported as means ± standard deviations (m ± SD), if not otherwise stated. From 27 enrolled competitors, we included 16 in the statistical analysis (due to early dropouts before D1, as well as related to HRV-data availability and quality). As a result of the extreme conditions of the competition, several participants withdrew before course completion (see section Performance). Therefore, after the race, the entire sample of all participants (ALL) was divided into two subgroups: finishers (FIN: measurements throughout the race until POST) and non-finishers (NON: measurements until D1) (see **Table 1**). Normal distribution was tested with

Shapiro-Wilk and variance with the Equal-Variance-Test. A log-transformation was applied to frequency domain indices to attain normal distribution (Castiglioni et al., 2011). According to the distribution, the variance of HRV parameters, exercise intensity and rest quality and psychometric measurements over the entire race-course in FIN was tested with one-way repeated measures analysis of variance (One-Way RM ANOVA) and *post-hoc* Student's-Newman-Keuls-Test, or Friedman ANOVA on ranks with *post-hoc* assessment through Tukey's Test (i.e., RMSSD, pNN50+, POMS Depression, Borg TQR). In addition, differences in weather conditions between editions were assessed with One-Way RM ANOVA. As all participants reached the first checkpoint, we could implement a direct comparison between the two groups regarding PRE and D1 by applying two-way repeated measures ANOVA (Two-Way RM ANOVA), after normality was passed. Psychometric assessment of mood states was further analyzed through comparison with normative data for athletic samples. In accordance with Terry (Terry and Lane, 2000), raw values of mood states were conversed to standardized T-Scores. Hence, T-Scores could be plotted against the athletic sample mean in order to assess expression of the *Iceberg Profile*, which represents specific mood profiles in athletic subjects. Unpaired Student's *t*-Test was used on raw values, as well as computed T-Scores, to allow comparison of significant differences between YAU participants and normative data for mixed athletic samples. In order to further analyze mood states in YAU competitors, comparison of baseline values (as individual raw scores) with normative data obtained in athletes directly pre-competition, as well as with normative data obtained in athletes post-competition, was performed by application of unpaired Student's *t*-Test. Correlations between HRV indices and psychometric measurements, as well as correlations between HRV indices or psychometric measurements and performance were assessed with Pearson Product-Moment-Correlation or, if normality was not passed, Spearman Correlation. All statistical analyses were performed using SigmaPlot 12.3 (Systat Software, San José, CA, USA). The significance level was set at $p < 0.05$.

RESULTS

Performance

Of the 16 participants included in the statistical analysis (ALL), 10 successfully completed the course (FIN). Due to general fatigue, cardiovascular distress or gastrointestinal problems, as well as injuries (e.g., sprained ankle), 6 withdrew from the competition at earlier points (NON). Baseline anthropometric characteristics of the two subgroups, based on the completion of the race, are presented in **Table 1**. Details of performance are depicted in **Table 2**. The official times recorded among FIN ranged between 225 and 312 h. Specifically, analysis of split times displayed that FIN accounted for 82 ± 15 h (velocity 3.6 ± 0.6 km/h) to reach D1, whereas for NON it took 91 ± 17 h (moving at a velocity of 3.2 ± 0.8 km/h). For FIN, 41 ± 6 h were required to reach D2 (velocity 2.7 ± 0.4 km/h) and 125 ± 21 h to reach the finish line (at a velocity of 2.5 ± 0.4 km/h), so that the overall total finish time (i.e., excluding resting time at checkpoints) was 248 ± 36 h (velocity 2.8 ± 0.3 km/h). A positive correlation between the split times PRE-D1 and D2-POST with respect to the finish time was retrieved (r 0.83, p 0.01 and r 0.93, p 0.001, respectively). This shows that the participants, who were the fastest both in the first and in the last part of the race, also accounted for the best overall time at the end of the race. Such correlations were not found for the split time between D1 and D2.

TABLE 2 | Performance data.

Group	Gender	Distance, km mean (S.D.)	Finish Time, h mean (S.D.)	Velocity, km/h mean (S.D.)
FIN	Men	690.0 (0.0)	254.6 (21.8)	2.7 (0.2)
	Women	690.0 (0.0)	300.2 (18.8)	2.3 (0.2)
	All	690.0 (0.0)	268.3 (29.7)	2.6 (0.3)
NON	Men	384.3 (107.4)	139.3 (60.0)	3.1 (1.2)
	Women	278.4 (0.0)	92.0 (0.0)	3.0 (0.0)
	All	366.7 (105.4)	131.4 (57.0)	3.1 (1.0)
ALL	Men	350.3 (106.4)	206.6 (71.4)	2.9 (0.8)
	Women	358.3 (124.3)	248.1 (105.2)	2.5 (0.4)
	All	352.3 (106.7)	217.0 (79.3)	2.8 (0.7)

Performance data of subgroups (FIN and NON), as well as of all participants (ALL). The overall completed distance and total time to cover it are reported, as well as the overall mean velocity (including resting times). No significant differences between groups.

Heart Rate Continuous Recordings

A total of 185 recordings fulfilled the criteria for analytic inclusion. They were obtained in 15 participants, as one competitor of the 2017 race belatedly volunteered to participate in the study and could not be equipped with the measuring device anymore. As reported above, recorded HR was used as a marker of exercise intensity, by normalizing absolute values with respect to the calculated HR_{max}. A similar approach was used to define the quality of rest during the actual race, i.e., the time spent at checkpoints was excluded. Results of ExHR (mean HR during exercise as percentage of HR_{max}) and RestHR (mean HR during rest in between checkpoints, as a percentage of HR_{max}), classified according to the 4 segments mentioned above, i.e., D1a (36 h after race start), D1b (24 h before arriving at D1), D2a (24 h before reaching D2), D2b (24 h before arrival at the finishing line), are displayed in **Table 3**.

Heart Rate Variability

For all 16 participants, a total of 53 R-R interval recordings were available and exhibited sufficient quality for analysis and assessment of HRV. 16 recordings corresponded to PRE, 13 to D1, and 10 to both D2 and POST. Morning HR pre-, post- and in-race (at checkpoints) is depicted in **Figure 3** and HRV results are depicted in **Figures 4**, **5**. **Figure 4** shows the significant decrease of parasympathetic tone in both groups at D1 compared to PRE, and in the following in-race time-points as for FIN only. **Figure 5** depicts sympathovagal balance indices, where a significant decrease at D1 in both groups of log LF lead to no variations of log LF/HF, whereas in FIN a significant increase of DFAα2 across all time-points was retrieved, and in NON

TABLE 3 | Average exercise intensity and rest quality.

Group	*n*	HR rec. time-point	ExHR, % mean (S.D.)	RestHR, % mean (S.D.)
FIN	9	D1a	70.9 (5.3)	47.4 (8.3)
	8	D1b	62.0 (3.9)*	38.4 (4.4)*
	8	D2a	62.1 (2.6)*	40.0 (3.6)*
	7	D2b	59.1 (4.4)*	37.0 (4.7)*
NON	6	D1a	66.9 (5.6)	44.6 (4.9)
	6	D1b	59.1 (5.8)*	40.0 (3.6)
ALL	14	D1a	69.5 (5.6)	46.4 (7.2)
	14	D1b	60.7 (4.8)	39.1 (4.0)
	10	D2a	60.9 (3.5)	39.6 (3.7)
	8	D2b	57.0 (7.1)	36.2 (4.9)

*Average HR during exercise (ExHR) and at rest (RestHR) across time-points. Data are presented as a percentage of the maximal HR (HRmax) for mean HR during exercise (ExHR) and mean HR during rest periods (RestHR). D1a: HR recorded in the first 36 h; D1b: HR recorded during the 24 h before arriving at D1; D2a: HR recorded during the 24 h before arriving at D2; D2b: HR recorded during the last 24 h before reaching the finish line. Data for the whole sample (ALL) are also reported. *p < 0.05 vs. PRE within subgroup (One-Way RM ANOVA).*

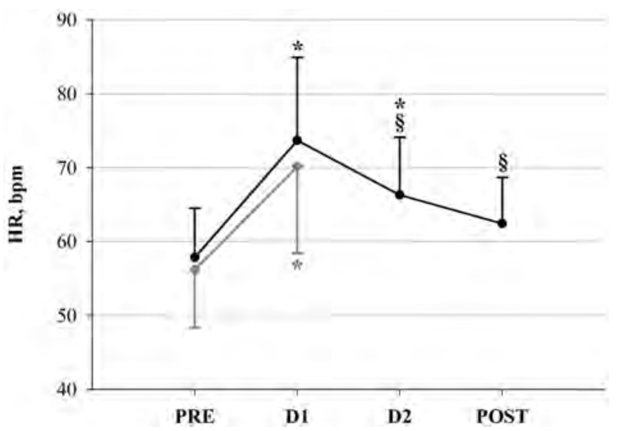

FIGURE 3 | Heart Rate (HR) in FIN (black line) and in NON (gray line) across time-points. *$p < 0.05$ vs. PRE within subgroup (One-Way RM ANOVA). $^{§}p <$ 0.05 vs. D1 (One-Way RM ANOVA).

only a significant increase at D1 as for DFAα1. Between PRE and D1, an overall decrease in TP could be observed in both groups (FIN: −1,964.7 ms^2 and NON: −3,699.6 ms^2), but the decrease was only significant (*p* 0.02) in NON. In fact, in NON the decrease in parasympathetic drive was to some extent greater when compared to FIN, as indicated by DFAα1, which was significantly higher at D1 only in NON (**Figure 5**), and by the difference between PRE and D1 in values of RMSSD: −34.1 ms in NON (*p* 0.01) and −18.1 ms in FIN (ns) and log HF: −0.8 ms^2 (*p* 0.04) in NON and −0.5 ms^2 (ns) in FIN.

Only in FIN, a significant negative correlation between HR and mean running velocity, as well as between HR and pNN50+, was detected at PRE (**Figure 6**). The negative correlation between HR and vagal tone indices was observed also at D1, as for pNN50- (*r* −0.82 *p* 0.02), and NN50- (*r* −0.82, *p* 0.02) with respect to HR. This could not be detected in NON.

Psychometric Scales

Psychometric measurements were performed in competitors of the 2015 and 2017 races, so that a total of 45 assessments (13 in PRE, 13 in D1, 10 in D2 and 9 in POST) were included in the statistical analysis. Results of the POMS questionnaire revealed significant decreases in POMS Vigor and Tension, associated with an increase in Fatigue and POMS Total scores (**Figure 7**). No changes in POMS Depression, Confusion and Anger scores were observed, nor significant differences between FIN and NON at D1. As for fatigue scales, i.e., Borg RPE, Borg TQR and KSS, results are depicted in **Figure 8**. In NON, values of KSS Departure scores were significantly higher at D1 than at PRE. However, as for the other psychometric scores, no significant between-group differences could be detected.

Nevertheless, at PRE, positive correlations between several indices of vagal tone (RMSSD: *r* 0.86, *p* 0.03; NN50+: *r* 0.90, *p* 0.01; NN50–: *r* 0.89, *p* 0.02; pNN50+: *r* 0.87, *p* 0.03; pNN50–: *r* 0.87, *p* 0.03; log HF: *r* 0.87, *p* 0.03) and Borg TQR were detected in NON only, so the higher the vagal tone, the higher the TQR score. Moreover, at PRE, again in NON only, a negative correlation between POMS Fatigue and pNN50+ (*r* −0.82, *p* 0.04) was observed, which indicates that the lower the vagal tone, the higher POMS Fatigue. On the other hand, in FIN at D1, a negative correlation was observed between HR and KSS Departure (*r* −0.85, *p* 0.02), indicating that the lower the HR, the higher the KSS Departure score; this was associated with a positive correlation between both pNN50– (*r* 0.84, *p* 0.02) and NN50– (*r* 0.84, *p* 0.02) and KSS Departure, which confirmed that a lower HR and a higher vagal drive were coupled with higher sleepiness; additionally in FIN at D1, there was a positive correlation between Borg TQR and DFAα2 (*r* 0.81, *p* 0.03): the higher the TQR score, so the quality of recovery after rest, the higher the DFAα2.

POMS T-Scores of YAU participants were plotted against normative data to provide the above-mentioned *Iceberg Profile* (Terry and Lane, 2000), which depicts POMS Vigor to be significantly higher and all other (negative) dimension scores to be significantly lower compared to mean values for a sedentary population. Analysis of variance between YAU participants and normative athletic data revealed distinctive differences, which are depicted in detail in **Figure 9B**. In comparison to the athletic sample, raw and T-Scores at baseline were significantly lower in ALL regarding POMS Depression, Anger, Fatigue and Confusion, but also Vigor. This was similarly observed when plotting YAU scores against normative data for athletes before and after competition. Compared to pre-competition normative data, YAU subjects at PRE displayed significantly lower Depression, Anger, Confusion and Fatigue, whereas Tension and Vigor were not different (**Figure 9C**). At POST, there were no significant differences between normative data for athletes in post-competition situations and the YAU subjects, except for significantly lower Vigor in YAU (**Figure 9D**).

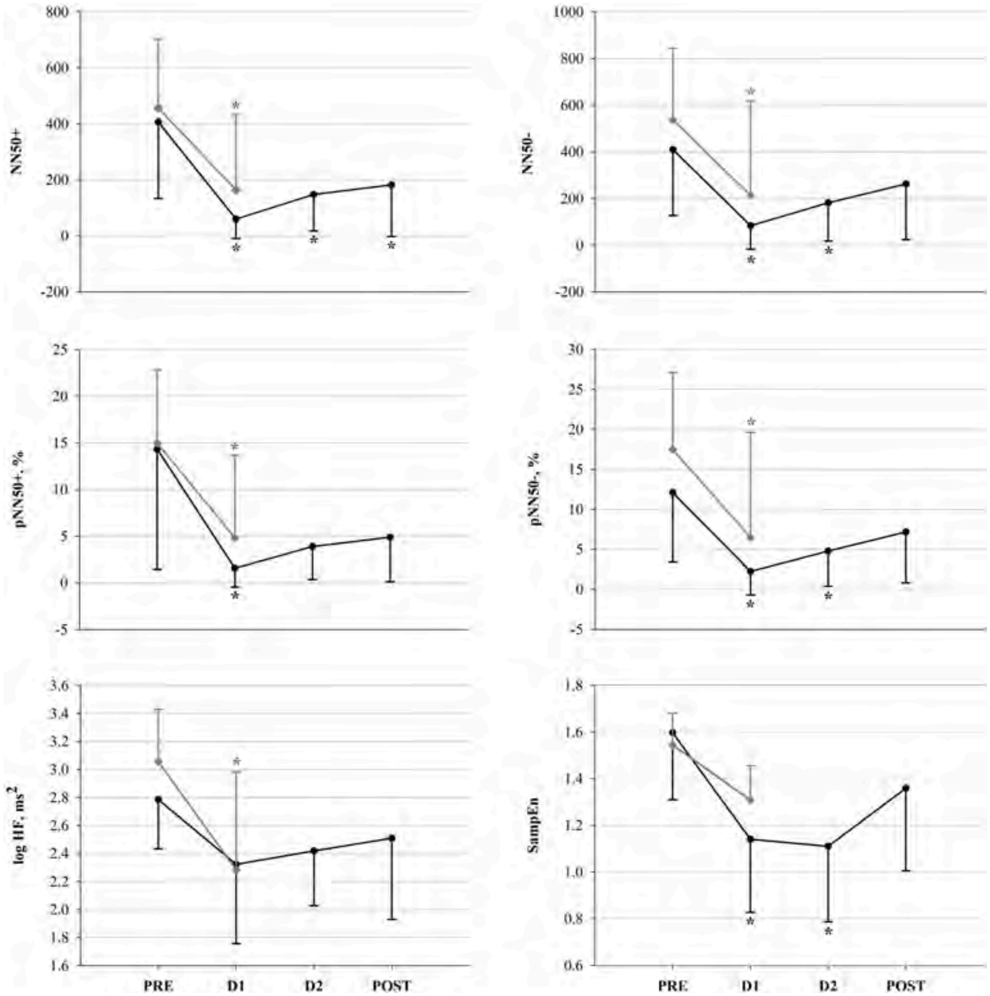

FIGURE 4 | Vagal modulation indices in FIN (black line) and in NON (gray line) across time-points. *$p < 0.05$ vs. PRE within subgroup (all variables: One-Way RM ANOVA; pNN50+: Friedman ANOVA).

DISCUSSION

To our knowledge, this is the first study investigating cardiac autonomic modulation and psychological correlates during ultramarathon in a subarctic environment. This setting provided the unique combination of three extreme environments: (i) ultra-endurance exercise performance (Perini and Veicsteinas, 2003; Scott et al., 2009), (ii) arduous environmental circumstances (Maughan et al., 2007), such as severe continuous cold exposition, and (iii) sleep deprivation/disturbances, induced by the condition of outdoor living during the race (Stein and Pu, 2012). The interplay of each single component of this *three-folded stress stimulus*, amplifies and affects several physiological and psychological aspects, which may be reflected in physical performance outcomes. Regarding race results, 10 of 16 subjects successfully completed the 690 km ultramarathon. Taking into account characteristics and conditions of the competition (see section The Yukon Arctic Ultra: The Longest, the Coldest Ultramarathon), the distribution of measurements sessions (see section Experimental Protocol and Measurements and **Figure 2**) holds great importance to interpret observations. In fact, the greatest effect on autonomic cardiac modulation, mood and fatigue was observed in the race segment between PRE and D1 (i.e., more than one third of the entire race); this may have been related to: (i) the initial stress of entering the race (i.e., performance demands coupled with environmental conditions), (ii) the different characteristics of the three parts of the race (see section Experimental Protocol and Measurements), and (iii) the running strategy of successful participants. Indeed, by analyzing the split times between measurement sessions, a positive correlation between the first and the final split time was found. This indicates that the fastest competitors in PRE-D1 (and D2-POST), were also the fastest finishers. The challenge of this first part of the race would be significantly underlined by this observation, and it provided ulterior evidence of the central role of the ability to cope with in-race demands at

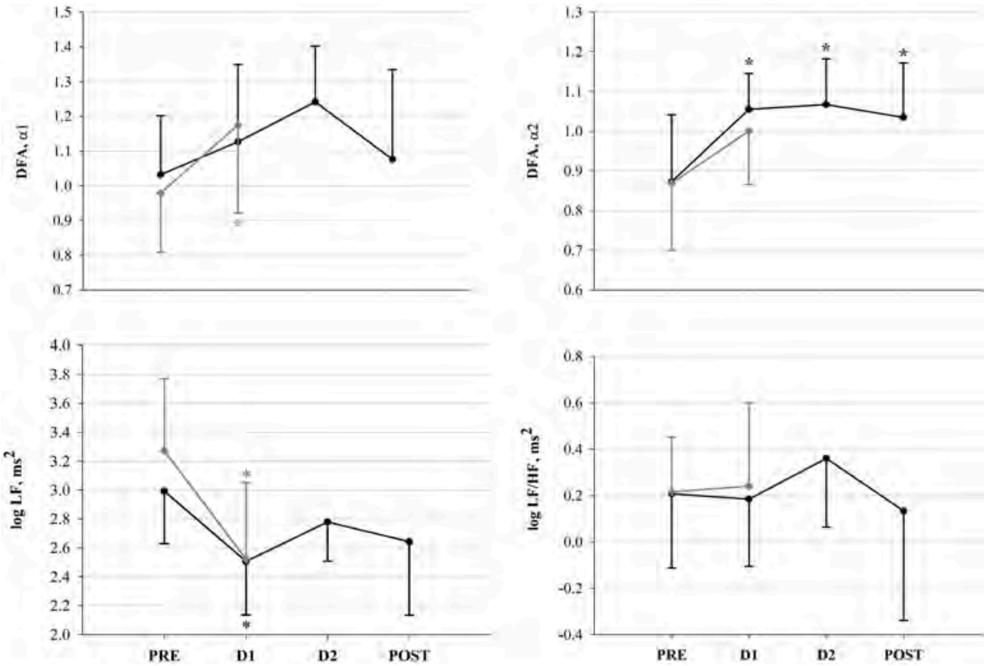

FIGURE 5 | Sympathovagal balance indices in FIN (black line) and in NON (gray line) across time-points. *$p < 0.05$ vs. PRE within subgroup (One-Way RM ANOVA).

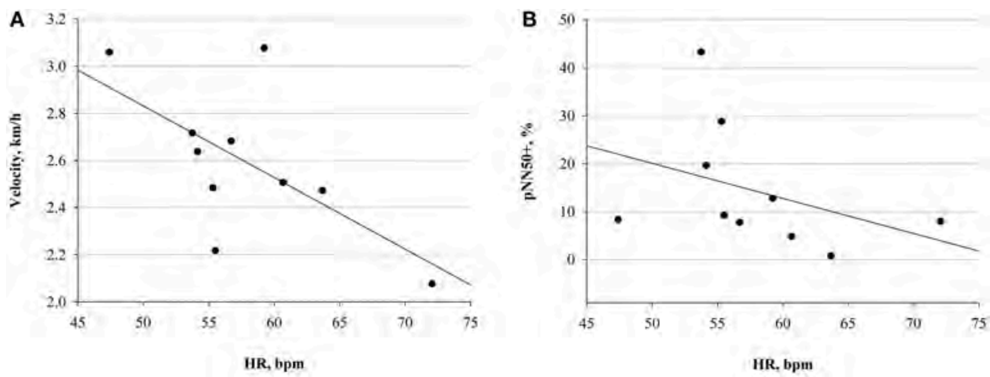

FIGURE 6 | Correlations at PRE in FIN group. **(A)** Correlation between HR and mean velocity ($r = -0.63$; $p = 0.04$). **(B)** Correlation between HR and pNN50+ ($r = -0.67$; $p = 0.03$).

very early time-points (i.e., directly after entering the race) for optimal adaptation. Therefore, during the first kilometers, successful competitors could already be recognized. This is in line with previous investigations on early recognition of successful competitors by their initial pacing strategies (Renfree et al., 2016; Bossi et al., 2017). Specifically, Lambert et al. observed more successful competitors in a 100 km ultramarathon to display higher velocities than lower performing athletes in the early race stages (Lambert et al., 2004). Moreover, the continuous significant decrease in ExHR in FIN, associated to a concomitant decrease of RestHR ($p < 0.05$ across all time-points vs. PRE), reflected decrements in performance, while the need of rest increased. On the other hand, the continuous decrease in RestHR may also indicate higher quality of recovery (Waldeck and Lambert, 2003; Silvani and Dampney, 2013), demonstrating that successful competitors had higher recovery potential, as they attained a higher quality of rest. Indeed, by comparing FIN and NON, we found a significant decrease of RestHR between PRE and D1 in FIN only.

In line with Millet (Millet and Millet, 2012), our observations show that ultramarathon might be an excellent model to test the adaptive potential under extreme conditions. However, in our case, we have to take into account not only potential effects of ultraendurance exercise, but the interplay of three factors (i.e., *three-folded stress stimulus*) on influencing

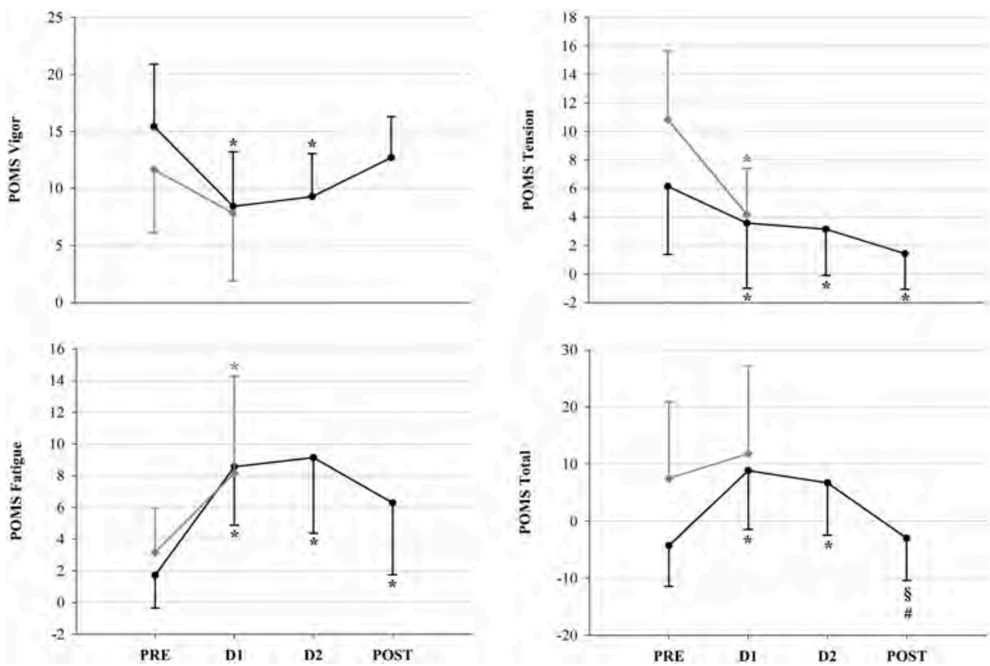

FIGURE 7 | POMS mood state scores in FIN (black line) and in NON (gray line) across time-points: *$p < 0.05$ vs. PRE within subgroup; §$p < 0.05$ vs. D1; #$p < 0.05$ vs. D2 (One-Way RM ANOVA).

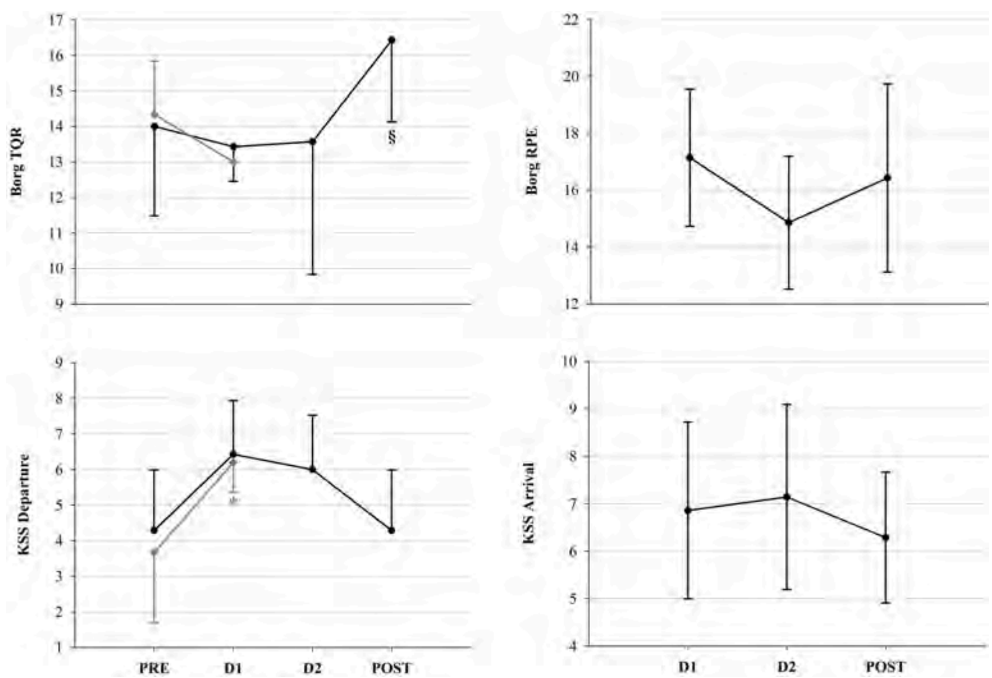

FIGURE 8 | Fatigue scale scores. Borg and KSS scores in FIN (black line) and in NON (gray line) across time-points. *$p < 0.05$ vs. PRE within subgroup; §$p < 0.05$ vs. D1 (One-Way RM ANOVA).

autonomic cardiac modulation and psychometric aspects: strenuous exercise, living outdoors in subarctic winter and sleep deprivation/disturbances.

Autonomic Cardiac Control and Endurance

An overall reduction of vagal drive, as well as total HRV, has been widely observed during acute exercise and competition (Perini

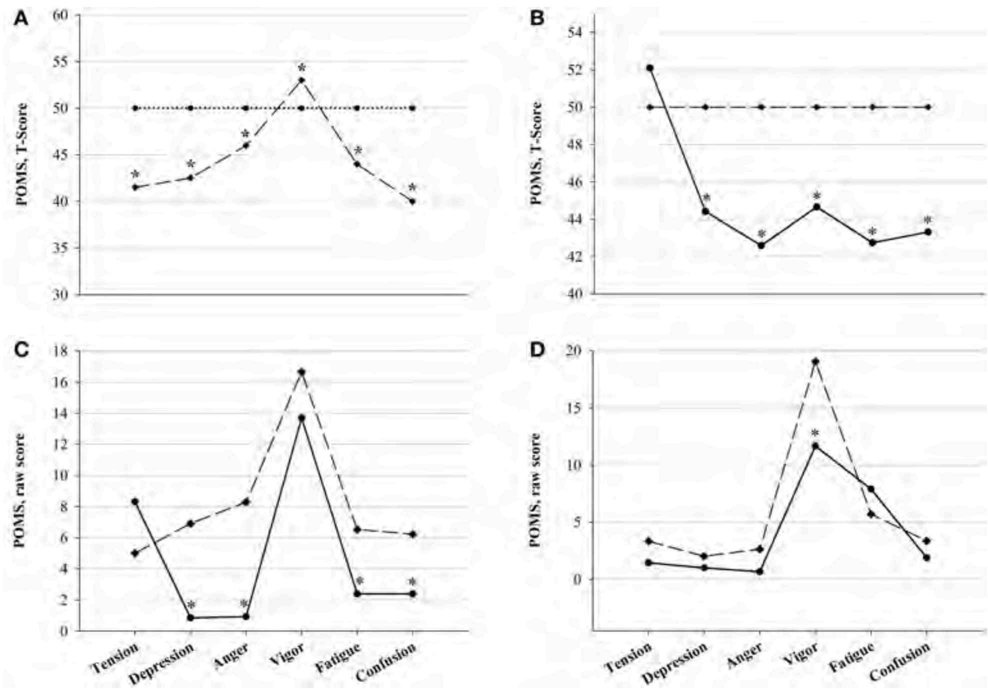

FIGURE 9 | Comparison of POMS results with normative data (Unpaired Student's t-Test). **(A)** *Iceberg profile* of mood states in an athletic sample (dashed line) plotted against sedentary subject norms (dotted line), **(B)** Mood states in all YAU subjects (ALL, solid line) plotted against normative data for mixed athletic samples (dashed line), **(C)** Mood states in ALL at PRE (solid line) plotted against normative data for pre-competition assessment (dashed line), **(D)** Mood states in ALL at POST (solid line) plotted against normative data for post-competition assessment (dashed line): $^{*}p < 0.05$ between groups.

and Veicsteinas, 2003; Buchheit et al., 2010). After cessation of exercise, parasympathetic predominance is gradually restored, depending on the preceding intensity, the training status and the quality of recovery, as reported also in previous studies on ultramarathon (Gratze et al., 2005; Scott et al., 2009; Foulds et al., 2014), and this is in line with our results. However, due to the very long distance of YAU we did observe already a vagal tone recovery before the end of the race, in successful participants (**Figure 4**). Considering that our HR recordings were collected after several hours of rest, early at morning during the race, the significant increase in HR (**Figure 3**) clearly describes the inability for participants to recover completely, as HR remained significantly higher at both D1 and D2. Nevertheless, at D2, HR decreased again with respect to D1 (−7.8 bpm, p 0.03), remaining, however, significantly above baseline values. At POST, HR was significantly lower than at D1 (−11.3 bpm, p 0.004) and not different from PRE. This would demonstrate that successful competitors (i.e., FIN) were able to positively adapt by recovering toward baseline conditions. This specific trend of HR can also be compared with observations in functional overreaching training interventions, where, initially, the increased training stimulus promotes a decrease in vagal drive (i.e., increase in HR), but, due to optimal adaptation, this is subsequently recovered (Buchheit, 2015; Bellenger et al., 2017). Indeed, in our participants, the increased HR between PRE and D1 was modulated via a significant attenuation in vagal tone in both groups (**Figure 4**). This, associated with a significant concomitant decrease of log LF, lead to an overall reduced HRV (see result section for TP). Additionally, evidence of reduced parasympathetic drive at D1 was further underlined by significant decreases in SampEn in FIN (**Figure 4**). In line with previously investigated implications of reduced SampEn values, this suggest lower responsiveness to environmental stimuli under attenuated entropy (Sassi et al., 2015). Interestingly, our findings indicate that in unsuccessful participants (i.e., NON), this parasympathetic drive decrease was to some extent greater than in FIN. This was shown firstly by the significantly higher DFAα1 at D1 in NON only (**Figure 5**), which has been associated to vagal tone decrease (Penttilä et al., 2003), and secondly by the difference in values of RMSSD and log HF for NON vs. FIN between PRE and D1 (see results section). Taking into account that in athletic subjects, stress has been associated to lower HRV and depressed parasympathetic drive (Nuissier et al., 2007; Cervantes Blásquez et al., 2009), this suggests that successful participants were able to efficiently relax and therefore fall asleep. The stronger decrease of vagal tone in NON, indeed, may indicate that these participants, ultimately unable to complete the race, were characterized by an impaired ability to cope with the in-race demands, already at early points of the competition, which may be reflected by lower quality of recovery (i.e., sleep quality impairment), whereas FIN displayed higher recovery potential. In turn, this supports the hypothesis that in such extreme conditions, vagal tone modulations may mirror the individual's ability to adapt, showing in resilient individuals earlier and efficient increase of parasympathetic tone, after the large initial decrement. Between PRE and D1, we also observed the typical reduction of overall HRV, which normally occurs during exercise, as well as a significant reduction of log LF in both groups, thus leading to non-significant changes in log LF/HF ratio (**Figure 5**). Only in FIN, DFAα2 was significantly higher at D1 compared to baseline and remained higher at both D2 and POST. As the exact implications of this non-linear HRV index have not been elucidated, this is an interesting finding. Previous investigations have reported that DFAα2 would decrease after the application of clonidine, an imidazoline-derived centrally-acting α2-adrenergic agonist and hypothalamic inductor of hypotension, which affects the overall sympathetic activity by resetting it to a lower setpoint (Castiglioni et al., 2011). Conversely, increased DFAα2 values have been reported in subjects who were awake compared to when asleep, but have also been linked to sleep stages, being higher in awake states and REM sleep than during light and deep sleep (Schumann et al., 2010). These findings allow us to hypothesize a link between increased DFAα2 and hyperarousal or enhanced alertness and vigilance, which in this case would be driven by the sympathetic branch of the vegetative nervous system. As we reported a significant increase in DFAα2 during the race, which persisted up until POST, this interpretation concurs very well with our observations (**Figure 5**). Higher values of DFAα2 could have been induced by an increased need for vigilance (i.e., sleeping outdoors during the subarctic winter in the Yukon Territory), leading to sleep impairment and/or deprivation, and to a general acute stress response promoting hyperarousal. Furthermore, the negative correlation between HR and velocity, paired with the negative correlation between pNN50+ and HR at PRE only in FIN (**Figure 6**), indicated that a lower HR in association with a higher vagal tone would predict a better performance. In FIN, the persistency of the above-mentioned negative correlation of vagal indices with HR at D1 (see Results section), demonstrated how, in our study, the observed increase in HR was specifically driven by a decrease of parasympathetic tone. This mechanism was mirrored mainly by time domain indices of vagal drive, i.e., NN50 and pNN50 statistics, which are linked to mean HR. Indeed, while pNN50+ quantifies the rate of HR decelerations (increase in successive R–R intervals), NN50– quantifies the rate of HR accelerations (decrease in successive R–R intervals) (Merati et al., 2015). At PRE, our data showed a negative correlation between HR and pNN50+ (i.e., rate of successive HR decelerations) and at D1, HR correlated negatively with both pNN50– and NN50– (i.e., rate of successive HR accelerations). Differences in the distribution of HR decelerations and accelerations have been associated with the enhanced presence of sympathetic modulations, whereas the HR decelerations have been identified as a better marker of vagal activity (Merati et al., 2015). This further demonstrates that at the beginning of the race, between PRE and D1, the reduction of vagal tone determined the increase in HR. Indeed, at D2 and POST, no correlation between vagal indices and HR could be detected. During this second in-between measurement section, HR decreased with respect to D1. Concomitantly, a slight increase of vagal tone was observed (**Figures 3**, **4**). After D1, the correlation between HR and vagal tone disappeared, indicating that the increase of parasympathetic tone was not able to elicit *per se* the decrease in HR. Instead, it would be suggested that the observed HR reduction also occurred due to other concurrent factors as for example psychological states. In fact, at D2, a significant reduction of POMS Total scores (indicating increased negative mood states or disturbance) with respect to D1 was found (**Figure 7**). This was associated with a decrease of Borg RPE at D2 (although not significant), suggesting that psychological factors were involved in recovering overall wellbeing, and thus were associated with reducing the HR (see section Psychological Wellbeing).

Psychological Wellbeing

We observed an overall decrease of psychological wellbeing across the whole ultramarathon (**Figures 7**, **8**). Interestingly, the POMS Tension item exhibited significantly higher values already at PRE with respect to D1 in both groups, in particular in NON (+6.7 vs. +2.6 in NON vs. FIN). This may reflect pre-competition anxiety. In fact, during the subsequent race, POMS Tension decreased significantly across all time-points. The concurrent increase in Vigor from D2 onwards (as POST values were no longer significantly lower in respect to PRE), may be related to the recovering process of positive mood, but also to the fact that participants were succeeding in the race and the finishing line was getting closer. Between PRE and D1, a significant reduction of positive mood items (lower POMS Vigor and higher Fatigue as well as POMS Total scores) had been observed. Therefore, we can infer that psychometric measurements sensitively reflected the impact of this extremely demanding competition on different subgroups, more strongly affecting those subjects that were unable to cope with the in-race demands. Nevertheless, after D1, it was possible to recognize a particular pattern in FIN, who recovered their wellbeing and positive mood. Indeed, not only POMS Tension scores continuously decreased, but also Vigor again attained values comparable to baseline at POST. Enhanced positive mood or motivation may have furthermore contributed to the observed recovery of vagal tone. In fact, previous investigations have demonstrated associations between enhanced parasympathetic drive in the frequency domain and POMS Vigor, as well as energy index (i.e., the POMS Vigor/Fatigue ratio) (Bisschoff et al., 2016), and the Vigor subscale has been proposed as a marker of the overall autonomic nervous system modulatory activity (Nuissier et al., 2007).

As mentioned above, this finding could be related to the fact that completion of the race was approaching. On the other hand, we found significantly lower POMS Total scores (indicating reduced mood disturbance) paired with higher Borg TQR values at D2 compared to D1 (even if not reaching statistical significance). This reflects a trend of increase in psychological wellbeing. As at POST, POMS Total was similar to PRE values, but significantly higher than at D1 and D2, successful recovery of mood disturbance in FIN is accentuated.

Moreover, during the first part of the race, as mentioned above, the increased HR depicted the inability of participants to recover completely. However, this event was not reported by data of the Borg RPE scale (**Figure 8**), which, although in-race values had decreased, did not exhibit any significant changes across the race. In this sense, it is likely that in the case of the YAU competitors, the Borg RPE failed to detect the perceived exertion.

Results of Borg TQR in NON showed a significant correlation between vagal indices and TQR scores at PRE, which may suggest that the higher the parasympathetic tone, the higher the perceived quality of recovery, underlining previous findings about the effect of parasympathetic tone on perceived fatigue in athletes (Bisschoff et al., 2016). However, this correlation was not found at D1. Instead, only in NON, KSS Departure scores were higher at PRE compared to D1. As the KSS has been extensively validated to depict objective sleepiness (Kaida et al., 2006), this subjective measurement indicates greater sleepiness, probably due to impaired rest and insufficient recovery in NON compared to FIN.

At PRE, no correlation between psychometric scales and HRV indices was found in FIN. Nonetheless, at D1, KSS Departure correlated negatively with the HR and positively with vagal indices in FIN, i.e., the lower the HR and the higher the vagal tone, the higher the subjective sleepiness upon departure. On the other hand, the concomitant positive correlation between Borg TQR and DFAα2 could suggest that subjects with higher recovery and better sleep quality, were also in a state of enhanced vigilance and alertness, ready to continue on the trail. Nevertheless, we must admit that as we recorded HR early in the morning, just after awakening, and DFAα2 has been reported to be higher in awake states and REM sleep than in light and deep sleep (Schumann et al., 2010), our observations could also be influenced by the circumstances of the measurement sessions, immediately after waking up. The high adaptive potential in our FIN subjects promoting recovery of initially increased mood disturbance, exertion and sleepiness, paired with a concurrently re-increasing subjective recovery status, presents several implications. Possibly, lower sleepiness and therefore higher alertness would yield essential importance for coping with the environmental challenges of the YAU competition. Moreover, sleepiness and fatigue have been associated with impaired cognitive, as well as physical, function and performance (Fullagar et al., 2015). Therefore, the ability to recover from attenuated psychometric wellbeing in our high-achieving FIN once more underlines the importance of adaptability.

Finally, comparison of mood states with normative data for athletic samples (Terry and Lane, 2000) generally displayed lower mood disturbance in our competitors (**Figure 9**). At baseline, POMS Depression, Fatigue, Confusion and Anger were lower, with Vigor conversely being higher compared to normative scores. Further comparison with normative data for pre- and post-competitive assessment again confirmed the great mental health in our participants, who had significantly higher positive mood than compared to pre-competitive normative values. During the first stages of the race, mood disturbance significantly increased under the exhausting demands, but recovered. Therefore, at POST, mood states in YAU participants (except for Vigor scores) did not significantly differ from normative data in post-competition assessment. To conclude, the high adaptive capacity in our subjects, who attained recuperation of gravely impacted mood states after enduring the extreme in-race conditions and stress stimuli, is again underlined.

Practical applications of these findings are related to training methods, highlighting the importance of high and/or fast increasing vagal tone, and of mood states: the "mind," i.e., mood state and motivation, plays a crucial role, especially with respect to such a long-lasting and highly demanding competition. In fact, successful competitors were able to perform greatly also in the second part of the race, where the decrease of HR was not coupled directly with higher vagal drive (as instead was in the first part of the race for FIN only), and the intervention of psychological aspects could be hypothesized (see above). All in all, assessment of HRV and psychological profile may contribute to monitor and partly predict performance in such extreme environments.

LIMITATIONS

Given that this is an in-field study in extreme environments, a number of possible limitations must be addressed.

First of all, the sample size of 16 may appear small, however, considering that a total number of only 78 athletes competed in the three investigated editions and 27 of them enrolled in our study, we regard this number to be quite considerable and sufficient under these specific conditions.

Moreover, there is a substantial difference in the distance between the in-race checkpoints (i.e., D1, D2) selected to perform measurement sessions and a study protocol over three equispaced checkpoints may have been favorable. However, the choice of measurement implementation was due to essential practical concerns, as previously mentioned (see section Experimental Protocol and Measurements). These concerns also held essential importance for standardizing as much as possible measurement conditions, (i.e., indoors facility, comfortable setting regarding space, temperature, noise, and light exposure), especially regarding HR beat-to-beat recordings.

Furthermore, we aimed to allow comparison of additional data from HR continuous measurements with HRV and psychometric parameters obtained at measurement points. Therefore, continuous HR recording data were clustered and were split up in the above-mentioned four sections (see section Performance Assessment and Heart Rate Continuous Recordings).

CONCLUSION

The main findings of this study are: (i) the extent of the early vagal withdrawal, associated to the timing and potential of its recovery, is crucial for success in this specific competition, (ii) a pre-competition lower resting HR, coupled with a higher vagal tone, would predict a better performance, as already reported in the literature for endurance sports (Gratze et al., 2005; Buchheit, 2014), and (iii) psychological profile and wellbeing is reliably depicted by mood state assessment with the POMS questionnaire, but not by Borg fatigue scales, and again associated with autonomic cardiac modulation. Successful ultramarathoners were coping better already in early stages of the competition, which allowed recovery of cardiac autonomic balance and positive mood, thus associated with higher athletic achievement. Therefore, assessment of HRV and psychological profile may contribute to monitor and partly predict performance in such extreme long-duration competitions in extremely cold environments.

AUTHOR CONTRIBUTIONS

LR and MM contributed equally to the study by writing the manuscript and analyzing the data. MS designed, planned and implemented the study, secured funding sources, and performed measurements and data collection. AS assisted with the measurements and data collection. AR-R, LR, and MM performed statistical analyses. RC and H-CG contributed to the study design, provided expertise and feedback. LR formatted, and, with assistance of MM and MS, revised the manuscript.

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Physiological and Psychological Responses during Exercise and Recovery in a Cold Environment Is Gender-Related Rather Than Fabric-Related

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We evaluated gender-specific effects of two types of undergarments on exercise-induced physiological and psychological stress and subsequent recovery in cold conditions for male and female participants. Ten healthy men and eleven healthy women (25.0 ± 1.5 versus 23.4 ± 1.2 years old, respectively) completed the experimental session twice with two different types of undergarments: polyester or merino wool leggings and long-sleeve tops; specifically, merino fabric had greater thermal resistance and water absorbency, and less water vapor as well as air permeability than polyester. Experimental sessions involved performing 1 h of exercise on a cycle ergometer at 8°C ambient temperature and 55% relative humidity, holding at 70–80 revolutions per minute and 60% of each participant's predetermined maximal power output (assessed by maximal oxygen uptake test), followed by 1 h recovery in the same environment. Every 5 min during exercise and every 10 min during recovery, rectal temperature, heart rate, subjective ratings for thermal, shivering/sweating and clothing wetness sensations, and clothing next-to-skin and outer side surface temperature and humidity on the chest, back and thigh were recorded. All participants experienced high physiological stress (assessed by physiological strain index) during exercise. No significant gender differences were found in core temperature or heart rate changes during exercise, but women cooled down faster during recovery. Next-to-skin humidity was similar between genders and different garment sets during exercise and recovery, but such temperatures at the chest during exercise and at the thigh during exercise and recovery were lower in women with both sets of garments. Subjective thermal sensations were similar in all cases. In the last 20 min of cycling, women started to feel wetter than men ($P < 0.05$) for both garment sets. Shivering was reported as stronger in women in the last 10 min of recovery. Most of the changes in the garment microclimates during exercise and recovery in the cold were associated with gender-related differences rather than with fabric-related differences.

Keywords: polyester, merino wool, undergarment, hyperthermia, physiological strain index, psychological stress

INTRODUCTION

While exercising in a cold environment, the elevation of body core temperature (Sawka and Young, 2006) causes an increase in sweating (Sato, 1993). Mainly because of heat transfer by water, which is 25 times greater than by air alone (Toner and McArdle, 2011), heat loss in a cold ambient temperature (T_a) 5°C has been found to be twice as high when skin and clothing are wet than when dry (Kaufman and Bothe, 1986). Heat production during high-intensity exercise prevents a decrease in core temperature, so warm clothing is unnecessary, but this clothing might not protect the wearer after completion of the exercise, or after being forced to stop exercise because of fatigue or injury (Sawka and Young, 2006). In such health-threatening conditions, an important feature of garments is to evaporate sweat as quickly as possible from the body surface through high water vapor permeability; rather than retaining evaporated sweat, which normally condenses on the outer side of garments with a knitted fabric structure. Garments should also keep the body as warm as possible with low air permeability.

Synthetic garment materials such as polyester (PES) (Gonzalez, 1987) or wool fiber are widely used during physical activity. PES is characterized by poor absorbency, but with organic additives comprising hollow fibers with an inner hydrophobic layer and an outer hydrophilic surface, PES wicks sweat away from the skin better than the more traditional cotton fibers (Watkins, 1984). PES garments resulted in a lower heart rate (HR) and core body rectal temperature (T_{re}) during 1 h cycling at a T_a of 23°C (Zhang et al., 2001), but during moderate walking and following recovery at a T_a of 2°C, participants felt wetter with PES compared with cotton garments (Ha et al., 1996). In general, wool fibers are both hydrophobic (repelling water) and hygroscopic (absorbing moisture when dry), so they can absorb or give off moisture. They also have better thermal insulating properties than cotton or PES (Holcombe and Hoschke, 1983; Hearle and Morton, 1986). Such properties of wool can help regulate the skin temperature and the microclimate of temperature and relative humidity (RH) in the space between skin and the garment, which in turn can keep the wearer more comfortable under a range of conditions (Li et al., 1992). Among the different wool fibers, merino wool (MER) has been shown to have the greatest amount of crimp and the maximum density of scales, and these two characteristics contribute to its superior thermal insulating capability (Holmér, 1985).

For next-to-skin garments, some physiological benefits (i.e., lower HR and later onset of a sweating response) in wearers have been reported for upper-body single layer MER garments compared with PES garments while exercising. This consisted of 30 min running at 70% of maximal oxygen consumption (VO_{2max}), 10 min walking at 40% of VO_{2max} in a T_a 8°C with an air flow of ∼11 km/h during running and ∼6 km/h during walking (Laing et al., 2008). However, these benefits disappeared when an air flow of ∼7.2 km/h was applied during the same exercise and T_a conditions (MacRae et al., 2014). These two studies revealed no differences for any perception of wearer responses between first-layer garments (MER vs. PES). Exercise-induced analgesia persisting to the recovery period (up to 30 min) (Beaumont and Hughes, 1979) seems likely to have blunted the wearers' perceptions of any differences between the undergarment types. Moreover, wool can easily absorb up to 30% of its weight in moisture without feeling damp or clammy, and that helps keep a layer of dry air next to the skin which, in turn, helps to hold in body heat. Therefore, the 3–4 g of moisture absorbed by MER and PES garments during exercise (MacRae et al., 2014) might have failed to negate any impact of the clothing type. However, to test this garment-specific response hypothesis, a study involving a longer exercise-induced sweat release and post-exercise recovery in a T_a 8°C (to induce a shift from heat to cold thermogenesis) (Gavit et al., 2001; Brazaitis et al., 2010) is necessary.

In general, manufacturers do not separate sportswear materials for women and men. Men have higher sweating rates (Keatisuwan et al., 1996) and predominantly show evaporative heat loss (Inoue et al., 2005) and commonly have a greater total lean body mass than women (Anderson, 1999). In contrast, women generally have a greater body surface area (BSA)-to-mass ratio and higher percentage of body fat (Anderson, 1999), and lose more heat by convection (Inoue et al., 2005). Nevertheless, in the present study we questioned whether garments from the same sportswear materials and knit structure could be equally physiologically and/or psychologically efficient for male and female subjects while exercising and recovering in the cold. It has been demonstrated that whole body cooling in cold water (2°C) after exercise-induced hyperthermia (T_{re} 39.5°C) had a greater cooling rate in female than in male subjects (Lemire et al., 2009), but this gender-specific difference disappeared when an acute cold stress was induced by immersion in 14°C water from normothermic conditions (Solianik et al., 2014, 2015). Moreover, women are more sensitive to thermal stimuli and experience greater thermal discomfort related to temperature changes than men (Lautenbacher and Strian, 1991; Golja et al., 2003; Gerrett et al., 2014). Hence, there is a question as to whether whole body cooling after exercise-induced hyperthermia in cold air has a similar gender-specific response on cooling rate to that found in cold water, and whether wearing MER or PES full-length first layer garments can modulate or prevent these health threatening responses.

In this study, we aimed to evaluate gender-specific effects on exercise-induced physiological and psychological stresses followed by recovery in cold ambient conditions in subjects wearing two different types of undergarments. Microclimates created by the different first layer of garment sets were analyzed without adding extra microclimates created by second layers, which could blunt the true response (MacRae et al., 2014). We expected that MER fibers compared with PES fibers might have a greater capacity to absorb moisture and release heat to/from surrounding air or skin, in a temperature- and RH gradient-dependent manner (Shin et al., 2016). We hypothesized that both genders, when carrying out similar intensity exercise regimens, would reach a lower T_{re} and HR while wearing the MER garment set compared with the PES set because of higher water absorption in the former. The high-intensity exercise used in this study aimed to induce a high level of physiological stress [assessed by physiological strain index (PSI), Moran et al., 1998], which might blunt sensitivity to wearing (Beaumont and Hughes, 1979) the different garment types in both genders. During the post-exercise resting period in T_a 8°C conditions, hyperthermic women might cool down faster than hyperthermic men and feel higher thermal discomfort (Gerrett et al., 2014) and heat loss while wearing the PES garment set compared with the MER set.

MATERIALS AND METHODS

Participants

After being informed of the purpose, experimental procedures and known risks of the study, 10 men and 11 women volunteered and signed a written informed consent to participate to this study. They were considered healthy and physically active with (1) age 20–30 years; (2) homogeneous relative VO_{2max} ($ml \cdot min^{-1} \cdot kg^{-1}$); (3) no excessive sport activities, i.e., <3 times per week; (4) no involvement in any temperature-manipulation program or extreme temperature exposure for ≥3 months; (5) non-smokers; and (6) no medications that could affect natural thermoregulation and/or tolerance to fatigue. Subjects with Raynaud's syndrome, asthma, neurological pathology, or conditions that could be worsened by exposure to cold environment or by high intensity exercise were excluded from this study.

All procedures were approved by LUHS Kaunas Region Biomedical Research Ethics Committee.

Experimental Design

Rationale for the Experiment

The experiment was designed to induce whole-body moderate hyperthermia (T_{re} ∼38.5°C; Lucas et al., 2015) and heat stress-induced sweat release (0.8–1.4 L/h; Armstrong et al., 1993) in subjects performing sustained high-intensity aerobic exercise (60 min) and thereafter induce a thermoregulatory shift from heat to cold thermogenesis during their post-exercise recovery phase (60 min) in a T_a 8°C, and to investigate the physiological and psychological gender-specific responses in male and female subjects wearing full-length first-layer garments made of MER or PES.

Experimental Garments

The physical characteristics of the experimental clothing sets are given in **Table 1**. All garments were made by the same manufacturer (Omniteksas, Raudondvaris, Lithuania). Each subject was provided with two types of garments, consisting of a long-sleeved shirt and full-length leggings. Subjects were asked for their typical garment size, and the sizes for the two garment sets for each subject were the same. Each set was worn only once. For each subject, the order in which the garment sets were tested was randomized. For permeability to air we used the LST EN ISO 9234:1997 testing standard, for water vapor permeability the cup method STP-1:2014, for absorption ISO 18696:2006 and for thermal resistance the LST EN ISO 11092:2015 testing standard. At 24 h before study, all garments were placed at a thermally neutral T_a of 23°C and RH 30–40%.

Preliminary Procedures

Each participant visited the laboratory three times. The first time was approximately 1 week before the experiment for assessing maximal oxygen uptake (VO_{2max}) (Stasiule et al., 2014). The load for the experimental protocol was calculated based on this capacity. Increasing ramp cycling load (ICL) was performed on an electronically braked cycle Ergometrics–800S ergometer (Ergo Line, Medical Measurement Systems, Binz, Germany) at a pedal cadence of 70–80 RPM. The test was started by 3 min of baseline pedaling at 20 W and was increased by 2 W every 5 s until the intensity of cycling could not be maintained at the required level for longer than 10 s. The seat and handlebar positions on the cycle ergometer were adjusted for each subject prior to the initial exercise test and maintained in that position for subsequent tests. VO_{2max} was assessed using a mobile spirometry system (Oxygen Mobile, Jaeger/VIASYS Healthcare, Hoechberg, Germany). VO_{2max} was used to determine the ICL setting for work intensity based on the highest value of VO_2 reached during 15 s of exercise. The relative VO_{2max} was calculated by dividing the absolute VO_2 per min with the body mass of the subject.

Experimental Protocol

Each participant completed the experimental session twice, each time with a different garment set (the order of testing was randomized), but at the same time of the day, and with sessions at least 5 days apart. Participants were asked to avoid strenuous exercise within 40 h before, food within 3 h, and to avoid any eating or drinking during all experiment sessions (from the first to second weighing). First, the subject's nude body was weighed. After that, the participant inserted the T_{re} thermocouple by themselves, as mentioned above, dressed in the relevant test garment set and then entered the climatic chamber (Design Environmental Ltd., Gwent, South Wales, United Kingdom). In the climatic chamber, the subject sat at rest for 10 min. Then, all measured parameters (T_{re}, HR, subjective ratings, and temperature and humidity of the clothing microclimates) were recorded (set as time t_0). They then underwent 1 h exercise at a T_a of 8°C and RH 55% on a cycle ergometer holding at 70–80 RPM and at 60% of the subject's predetermined maximal power output. Every 5 min, T_{re}, HR, subjective ratings, and clothing surface temperature and humidity were recorded. After the test session, each participant had a recovery period. They sat on a stool for 1 h in the same environment as when they performed the exercise. Every 10 min, all measured parameters were recorded. At the end of experiment each subject was weighed in the nude.

Experimental Measurements

Physical Characteristics of the Participants

Each subject's anthropometric characteristics (Tanita UK Ltd., Philpots Close, United Kingdom, accuracy ± 0.1 kg) were estimated. The subject's body surface area (BSA, m^2) was calculating using the following equations: $BSA = 128.1 \times weight^{0.44} \times height^{0.60}$ (m^2) for men and $BSA = 147.4 \times weight^{0.47} \times height^{0.55}$ (m^2) for women (Tikuisis et al., 2001). The body's sweat loss was calculated by

TABLE 1 | Physical characteristics of the experimental clothing fabrics.

Fabric	Rib	Air permeability (mm/s)	Water vapor permeability (g/m^2 per 24 h)	Water absorbed (%)	Thermal resistance, R_{ct} (m^{2}K/W)
Merino wool (MER)	1 + 1	2411.5	4177	86.2	0.053
Polyester (PES)	1 + 1	2769.1	4439	53.5	0.027

subtracting the body mass measured after the experiment from that measured before the experiment. Skinfold thickness (in mm) was measured using a skinfold caliper (SH5020, Saehan, Masan, South Korea) at 10 body regions (chin, subscapular, pectoral, suprailiac, midaxillary, abdomen, triceps, anterior thigh, medial collateral ligament and medial calf). Mean skinfold thickness was calculated from these 10 skinfold sites (McArdle et al., 1992).

Measurements of Core Body Temperature and Cardiovascular Responses

The T_{re} was measured using a rectal thermocouple (Rectal Probe, Ellab, Hvidovre, Denmark; accuracy ± 0.01°C) inserted to 12 cm past the anal sphincter. This was inserted by each participant. The HR was measured (S-625X, Polar Electro, Kempele, Finland) throughout the testing and then consecutive 5 s average HR was used for the analysis. T_{re} and HR values were recorded after 10 min sitting at rest in the climatic chamber (time t_0) with an T_a of 8°C and RH 55%; every 5 min during the exercise and every 10 min during recovery. To assess physiological heat stress, we calculated the PSI as described by Moran et al. (1998):

$$PSI = 5(T_{ret} - T_{re0}) \times (39.5 - T_{re0})^{-1} + 5(HR_t - HR_0) \times (180 - HR_0)^{-1}$$

The T_{re0} and HR_0 measurements were taken before exercise; T_{ret} and HR_t measurements were taken after 60 min of exercise. This index was scaled to a range from 1 (no heat stress) to 10 (very high heat stress) within the limits of the following values: $36.5 \leq T_{re} \leq 39.5$°C and $60 \leq HR \leq 180$ beats min^{-1}.

Subjective Ratings

The method described by Ha et al. (1996) and adapted by Brazaitis et al. (2010) was used to measure subjective ratings for thermal, shivering/sweating and clothing wetness sensations. Thermal sensation ratings ranged from 1 (very cold) to 9 (very hot), with 5 being neutral. Shivering/sweating ratings ranged from 1 (heavily sweating) to 7 (vigorously shivering), with four being neutral. Reported sensations of clothing wetness ranged from 1 (dry) to 6 (dripping wet). During exercise, participants also were asked about perceived exertion using a Borg rating scale (Borg, 1970) ranging from 6 (no exertion at all) to 20 (maximal exertion). Subjective ratings were recorded at the same time points as the T_{re} and HR measurements.

Temperature and Humidity of Clothing Microclimates

The clothing surface temperature and humidity were measured from six points: three between the skin and the experimental clothing (defined as 'next-to-skin') and three points from the outer sides of the garments. However, sweat rates on the chest, back and thigh are greater in men than in women (Ichinose-Kuwahara et al., 2010; Gagnon and Kenny, 2012), so thermistors and humidity sensors (HMP-35A, Vaisala, Helsinki, Finland) were placed on the chest, back and one thigh of all participants. The temperature and humidity of the clothing were recorded every 5 min during the exercise, and every 10 min during the recovery period after exercise (at the same time points as T_{re}, HR and subjective ratings).

Statistical Analysis

Data was analyzed using IBM SPSS statistics. The data are presented as the mean ± standard error of the mean (SEM). The data were tested for normality of distribution using the Kolmogorov–Smirnov test. Repeated-measures analysis of variance (ANOVA) considering time × garment material (PES vs. MER) × gender was used to analyze the differences in thermophysiological responses of T_{re}, HR and in fabric properties (microclimate temperature and next-to-skin and outside humidity values). Where a significant main effect was found, a *post hoc* test with Šidák correction was applied to clarify significant differences. Changes during the exercise and recovery were evaluated separately, using the same statistical analysis. Paired-sample Student's *t*-tests were used to estimate differences in mean PSI and body sweat loss. The non-parametric Wilcoxon signed-rank exact test was used to compare changes in subjective ratings of perceptions (thermal, shivering/sweating and clothing wittedness sensations) and perceived exertion levels. The changes were calculated between genders with the same garment sets and between different garment sets, but for the same gender during exercise and recovery. For all statistical analyses, $P < 0.05$ was considered significant.

RESULTS

Physical Characteristics of the Participants

The female group compared with male group had significantly lower height, body mass, body mass index and BSA, but they had a higher body fat percentage and greater BSA-to-mass ratio ($P < 0.05$) (**Table 2**). The groups did not differ in age, mean skinfold thickness and relative VO_{2max}.

Physiological Variables

Exercise Intensity

All participants performed exercise at the same relative intensity. The 60% load of maximal power output for women was 138.25 ± 6.15 W; for men, it was 174.55 ± 6.90 W.

TABLE 2 | Physical characteristics of the participants.

	Male ($n = 10$)	Female ($n = 11$)
Age, y	25.0 ± 1.5	23.4 ± 1.2
Height, m	1.84 ± 0.01	1.69 ± 0.02*
Mass, kg	78.67 ± 1.9	60.07 ± 1.7*
Body mass index, kg·m^{-2}	23.31 ± 0.49	20.96 ± 0.41*
Body fat, %	14.49 ± 0.70	20.02 ± 1.27*
Body surface area, m^2	2.00 ± 0.03	1.69 ± 0.03*
Surface to mass ratio, cm^2·kg^{-1}	254.14 ± 3.17	284.16 ± 3.04*
Mean skinfolds thickness, mm	10.42 ± 0.96	12.33 ± 1.03
VO_2 max, ml·min^{-1}·kg^{-1}	42.26 ± 1.8	39.49 ± 2.5

*Values are shown as the mean ± SEM. *P < 0.05 for women compared with men.*

Rectal Temperatures

T_{re} values (all in °C) increased significantly during exercise in all cases (**Figures 1A,B**). Women started performing exercise with significantly higher absolute T_{re} values (37.4 ± 0.1 for PES and 37.5 ± 0.1 for MER) than men (37.2 ± 0.1 for PES and 37.1 ± 0.1 for MER) and reached significantly higher T_{re} values at the end of exercise (38.7 ± 0.1 for PES and 38.7 ± 0.1 for MER) compared with men (38.4 ± 0.1 for PES and 38.3 ± 0.1 for MER). Both genders with both sets of garments reached moderate hyperthermic conditions at the end of exercising. There were no significant differences between the two fabrics or between genders for changes in T_{re} during exercise.

During the recovery phase, men reached their initial T_{re} faster with PES garments (within 30 min, to 37.4 ± 0.06) than with MER (within 40 min, to 37.2 ± 0.07). The men's T_{re} did not fall below their initial temperature during the remaining recovery period. Women cooled down to their initial T_{re} within 30 min of recovery and, at the end of recovery, their T_{re} was significantly lower ($P < 0.05$) than their initial T_{re} value; there was no difference between the PES and MER garment sets. With the MER set, women had significantly lower ($P < 0.05$) T_{re} values than did men.

Heart Rate

Changes in HR during exercise and recovery for the PES and MER garment sets are shown in **Figures 1A,B**. Augmentation of HR (all in beats min^{-1}) was significant ($P < 0.05$) during exercise in all cases (PES 99.90 ± 3.31 and MER 102.30 ± 4.62 for men; PES 103.36 ± 3.12 and MER 102.09 ± 2.74 for women), but there were no significant main group effects. The HR recovered to initial values only in men with the MER garments (within 50 min of recovery). In all other cases, the HR stayed significantly higher than the pre-exercise values. Overall, there were no significant differences in the changes in HR between clothing conditions or genders.

Physiological Stress

All participants experienced high physiological stress (increased PSI) during exercise (**Table 3**). There were no significant

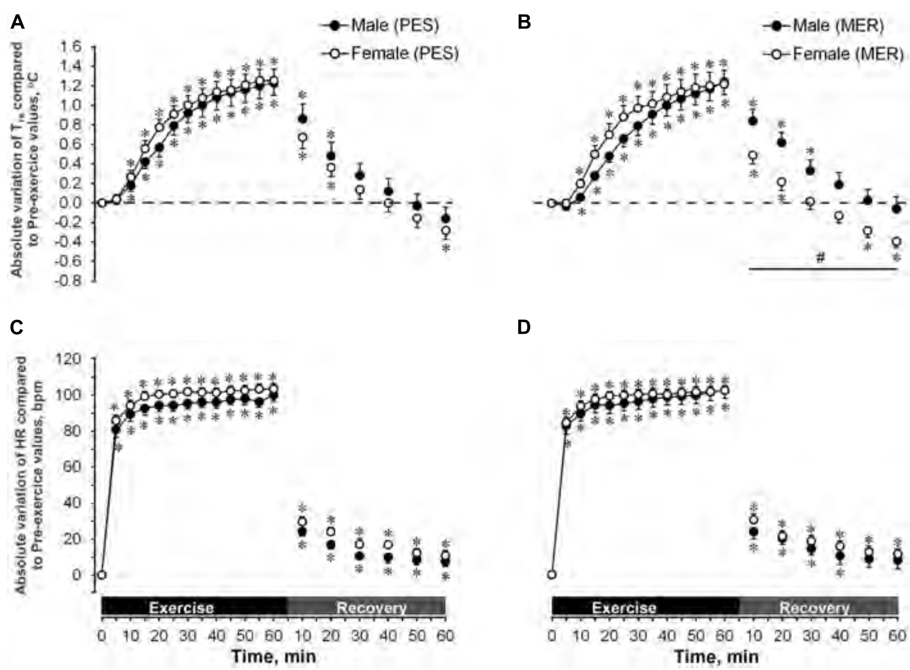

FIGURE 1 | Absolute variation of rectal temperature (T_{re} in °C) and heart rate (HR in b·min^{-1}) of individuals wearing polyester (PES) or merino wool (MER) as 'first layer' garment sets during exercise and recovery. Pre-exercise value (t_0) is taken as reference for variation calculation. T_{re} values in women, 37.4 ± 0.1 for PES **(A)** and 37.5 ± 0.1 for MER **(B)**; in men, 37.2 ± 0.1 for PES **(A)** and 37.1 ± 0.1 for MER (B). HR values in women, 72.2 ± 3.3 for PES **(C)** and 74.3 ± 2.7 for MER **(D)**; in men, 66.0 ± 3.2 for PES **(C)** and 63.9 ± 3.3 for MER **(D)**. Values are shown as the mean ± SEM (10 men, 11 women). *$P < 0.05$ compared with pre-exercise values; #$P < 0.05$ between genders.

main group effects seen when comparing genders and clothing conditions.

Sweat Loss

Absolute sweat loss in women was significantly ($P < 0.05$) lower than in men (PES 0.69 ± 0.08 kg and MER 0.74 ± 0.63 kg for men; PES 0.53 ± 0.04 kg and MER 0.57 ± 0.05 kg for women), but the ratio of sweat loss to BSA (**Table 3**) was similar between genders. Both men and women had significantly higher ($P < 0.05$) relative sweat losses when wearing the MER garment sets than with the PES sets.

Perceptual Variables

Perceived Exertion

Subjective ratings of perceived exertion increased significantly within 15 min of exercise and by the end of exercise reached a rating of 'very hard' (**Figure 2**). No significant main group effects were found.

Thermal Sensation

Figures 3A,B display the responses in thermal sensation during exercise and following recovery in PES and MER clothing conditions for both genders. There was a significant main effect for time ($P < 0.05$), but there were no significant differences between the genders or clothing conditions. Men rated themselves as between 'warm' and 'hot'; women between 'hot' and 'very hot' in the end of exercise. During recovery, there was a shift from a heat-generated response to cold stress and all participants started to rate themselves as being between 'cool' and 'cold.'

Sweating/Shivering Sensation

Figures 3C,D display the responses in sweating/shivering sensation during exercise and following recovery in PES and MER clothing conditions for both genders. During exercise there was a significant main effect for time ($P < 0.05$), but there were no significant differences between genders or clothing conditions. All participants rated themselves as being between 'moderately sweating' and 'heavily sweating' at the end of exercise. During recovery, after shifting from heat to cold stressors, women wearing the MER set reported a significantly higher shivering rating than men with the MER set (between 'slightly' and 'moderately shivering' vs. between 'not at all' and 'slightly shivering,' respectively) in the last 10 min of recovery.

TABLE 3 | Physiological strain index (PSI) and relative changes in body mass (sweat loss/BSA).

	PSI	Sweat loss/BSA, kg/m^2
Male PES	7.21 ± 0.22	0.88 ± 0.09
Male MER	7.08 ± 0.33	0.94 ± 0.08*
Female PES	7.60 ± 0.34	0.89 ± 0.08
Female MER	7.62 ± 0.31	0.96 ± 0.09*

*Values are shown as the mean ± SEM (10 men, 11 women). BSA, body surface area in m^2. *$P < 0.05$ for wearing different garment fabrics in the same gender.*

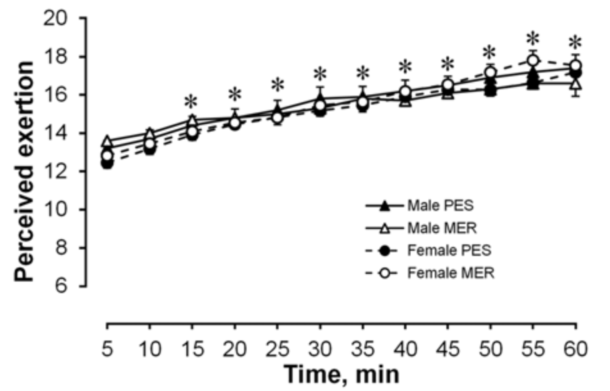

FIGURE 2 | Subjective ratings of perceived exertion. Values are shown as the mean ± SEM (10 men, 11 women). *$P < 0.05$ compared the fifth and subsequent minutes.

Wetness Sensation

Figures 3E,F display the responses in wetness sensation during exercise and post-exercise recovery in PES and MER clothing conditions for both genders. There was a significant main effect for time ($P < 0.05$), but there were no significant differences between the clothing conditions. For both PES and MER garment sets, wetness sensation was greater in women than in men after 45 min of exercise. At the end of exercise, women rated themselves as between 'wet' and 'dripping wet' when wearing the PES set, and between 'sticky' and 'wet' when wearing the MER set; men rated themselves as between 'sticky' and 'wet' when wearing the PES set, and between 'damp' and 'sticky' when wearing the MER set.

Garment Microclimates

Humidity Transfer

There were no differences in next-to-skin humidity between genders or types of garment (**Figure 4**). All changes involved differences in humidity outside the garments. A comparison of temporal changes (**Figure 5**) showed that sweat permeability was significantly higher ($P < 0.05$) in men than in women at the back for both garment sets and at the thigh when wearing the MER set during exercise. Sweat permeability at the back differed significantly ($P < 0.05$) between the PES and MER garment sets in men during recovery. Garment humidity returned to the initial values at the thigh in all cases during the recovery phase, but did not reach the initial values at the chest or back.

Temperature

The next-to-skin temperature (**Figure 6**) and differences in temperature between the next-to-skin microclimate and the outer side of garment (**Figure 7**) was significantly ($P < 0.05$) higher in men at the chest and thigh and did not differ at the back (compared with women) during exercise, for both PES and MER garment sets. The next-to-skin temperature was significantly higher ($P < 0.05$) at the thigh in men than in women during recovery for both clothing sets. The differences in temperature

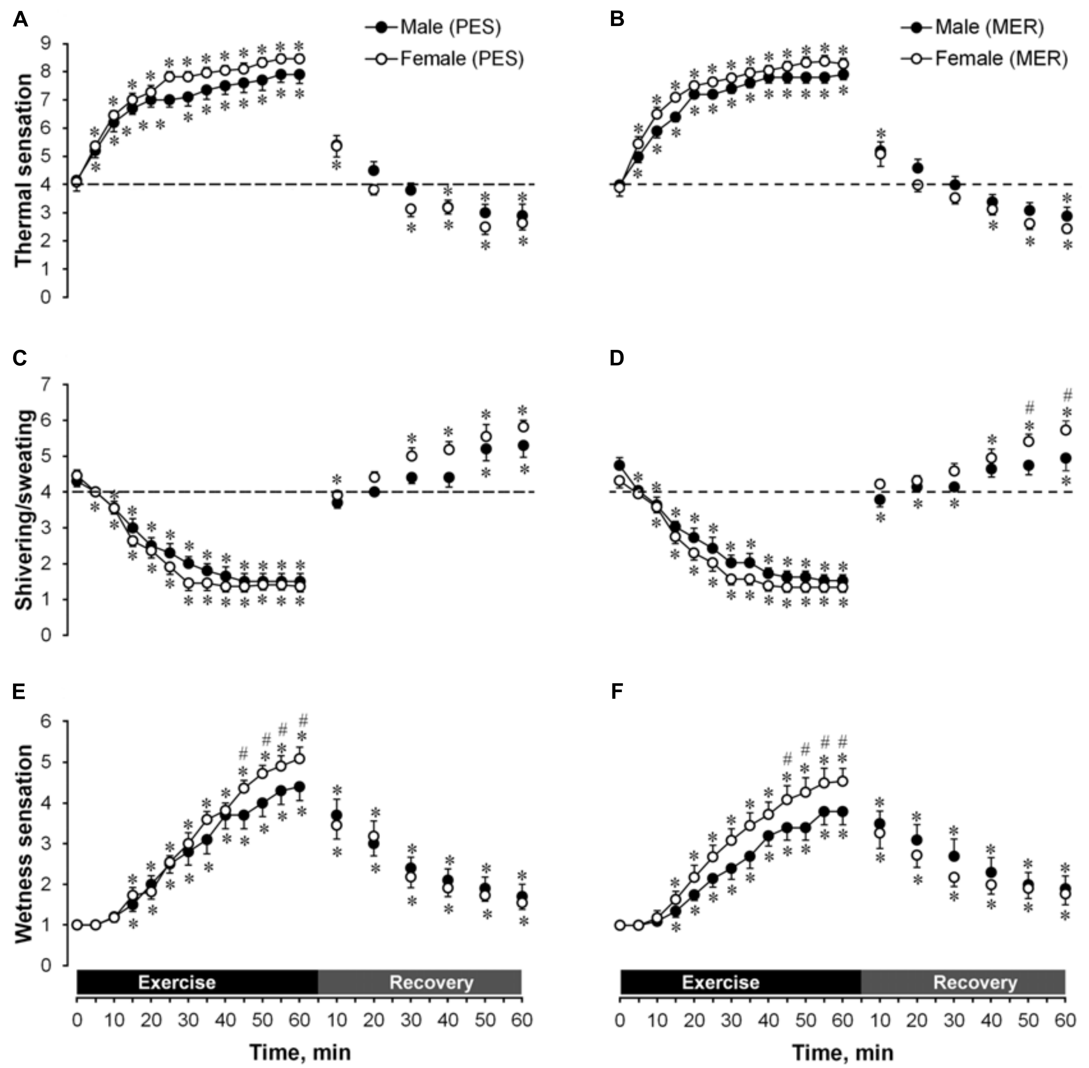

FIGURE 3 | A comparison of temporal changes in thermal sensation wearing **(A)** polyester (PES) or **(B)** merino wool (MER); shivering/sweating sensations wearing **(C)** polyester (PES) and **(D)** merino wool (MER); and wetness sensations wearing **(E)** PES and **(F)** MER garment sets. Values are shown as the mean ± SEM (10 men, 11 women). *$P < 0.05$ compared with pre-exercise values; #$P < 0.05$ between genders.

between the next-to-skin microclimate and the outer side of garment was significantly ($P < 0.05$) higher in men at chest and thigh (compared with women), and significantly ($P < 0.05$) higher in men at thigh compared PES and MER.

DISCUSSION

This study aimed to determine whether high-intensity aerobic exercise followed by prolonged recovery (60 min) in a cold environment (8°C) would induce gender-specific physiological or psychological changes and whether wearing MER (natural) vs. PES (synthetic) full-length first layer garments could modulate such responses. In this study, we observed that although both genders experienced similar levels of exercise-induced physiological stresses (increased PSI), which accompanied similar perceived sensations of exertion, women had a greater post-exercise recovery cooling rate (decrease in T_{re}) than men in cold air. These data agree with those of Lemire et al. (2009) who showed that women had an approximately 1.7-fold greater rectal cooling rate than men when recovering from exercise-induced hyperthermia in cold water (2°C). They also suggested that the BSA-to-mass ratio and body adiposity did not influence core (rectal) cooling rates in previously hyperthermic individuals, and attributed the differences in cooling rates to gender-specific physical differences in lean body mass (Lemire et al., 2009). In our study, men also had significantly higher mean lean body mass (67.3 kg) than women (47.0 kg). Interestingly, there were no gender differences in the rates of core cooling when normothermic participants were immersed in 14°C water (Solianik et al., 2014, 2015), suggesting that differences in lean body mass alone do not fully explain the differences in cooling

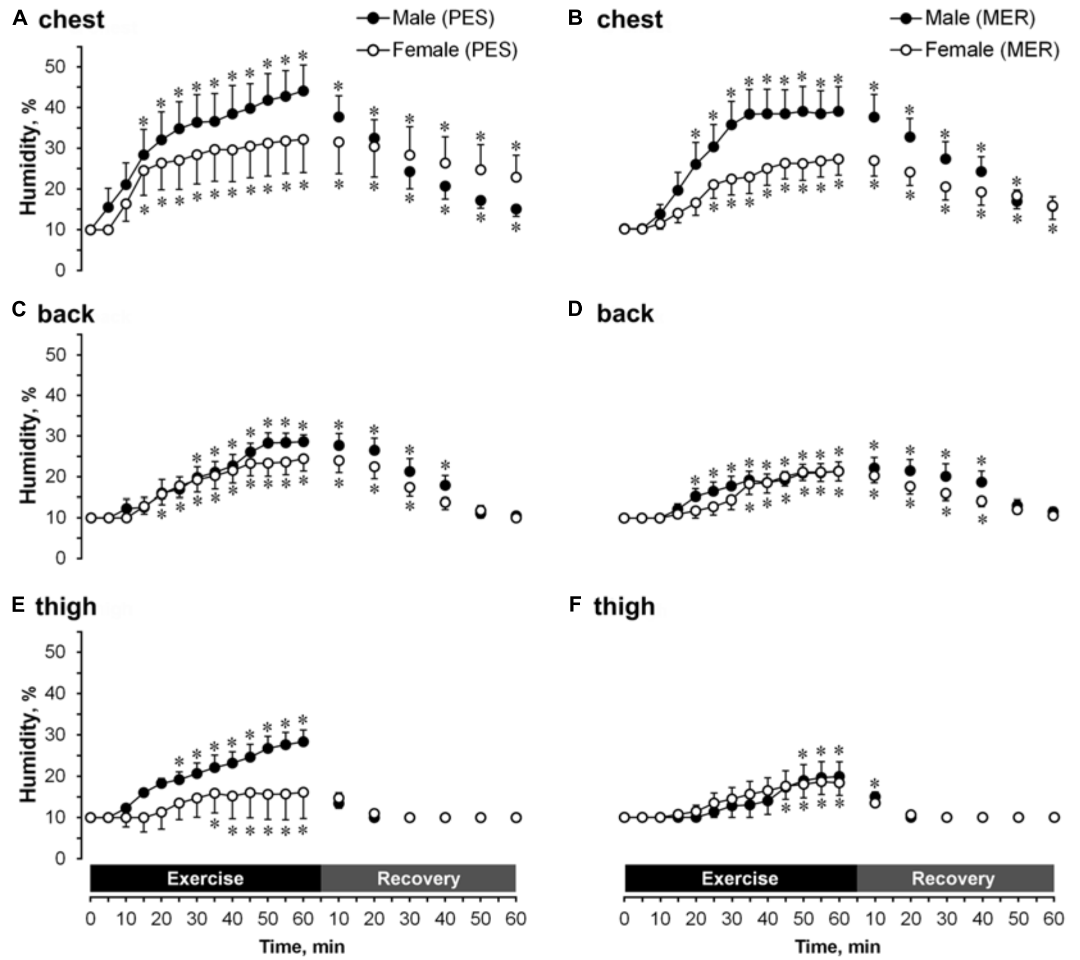

FIGURE 4 | A comparison of temporal changes in garment microclimate humidity next-to-skin values at the chest wearing **(A)** polyester (PES) and **(B)** merino wool (MER) garment sets; at the back wearing **(C)** PES and **(D)** MER garment sets; at the thigh wearing **(E)** PES and **(F)** MER garment sets. Values are shown as the mean ± SEM (10 men, 11 women). *$P < 0.05$ compared with pre-exercise values.

rates. The greater cooling rates in hypothermic women might be affected by their dominant cutaneous vasodilation heat loss mechanism (Inoue et al., 2005). It is known that women lose more heat by convection than by evaporation, whereas men predominantly show evaporative heat loss (Inoue et al., 2005). Women have a significantly greater BSA-to-mass ratio than men (**Table 2**), and this is one of the indicators for a higher cooling rate (McArdle et al., 1984). Furthermore, it has been demonstrated that temperatures in active and inactive muscles in women stay higher for a longer time after dynamic exercise compared with men (Kenny and Jay, 2007), suggesting a larger blood circulation in skin and muscles recovering from exercise (Halliwill, 2001). Thus, it seems likely that in our study cutaneous vasodilation, heat convection and low ambient temperature played a major role for the greater heat loss seen in hyperthermic women than in hyperthermic men during their recovery in cold conditions.

According to Nielsen and Endrusick (1990) the perception of humidity is mainly correlated with skin dampness. In our study, despite the similar next-to-skin humidity of garments in all measured locations (comparing genders and different fabrics), women felt more wetness than men. Filingeri et al. (2013) showed that humans could mistakenly have a sensation of local skin wetness when in contact with a cold dry surface producing skin cooling rates of 0.14–0.41°C/s. In our case, women had a lower next-to-skin temperature at the chest and thigh during exercise, which could create a sensation of dampness. Humans do not have humidity receptors (Clark and Edholm, 1985) and how we feel 'wetness' is still unknown. Ackerley et al. (2012) suggested that humans feel this sensation from complex somatosensory interactions integrating temperature and mechanical inputs at different anatomical levels (Cappe et al., 2009; Ackerley et al., 2012). Wet clothing in a cold environment feels colder then dry clothing. Low temperatures perceived through thermoreceptors such as small myelinated Aδ and unmyelinated C-fibers could play an important role in the perception of local skin wetness (Campero and Bostock, 2010; Filingeri et al., 2013). Wet clothing is also heavier then dry, so it creates higher friction and gives a

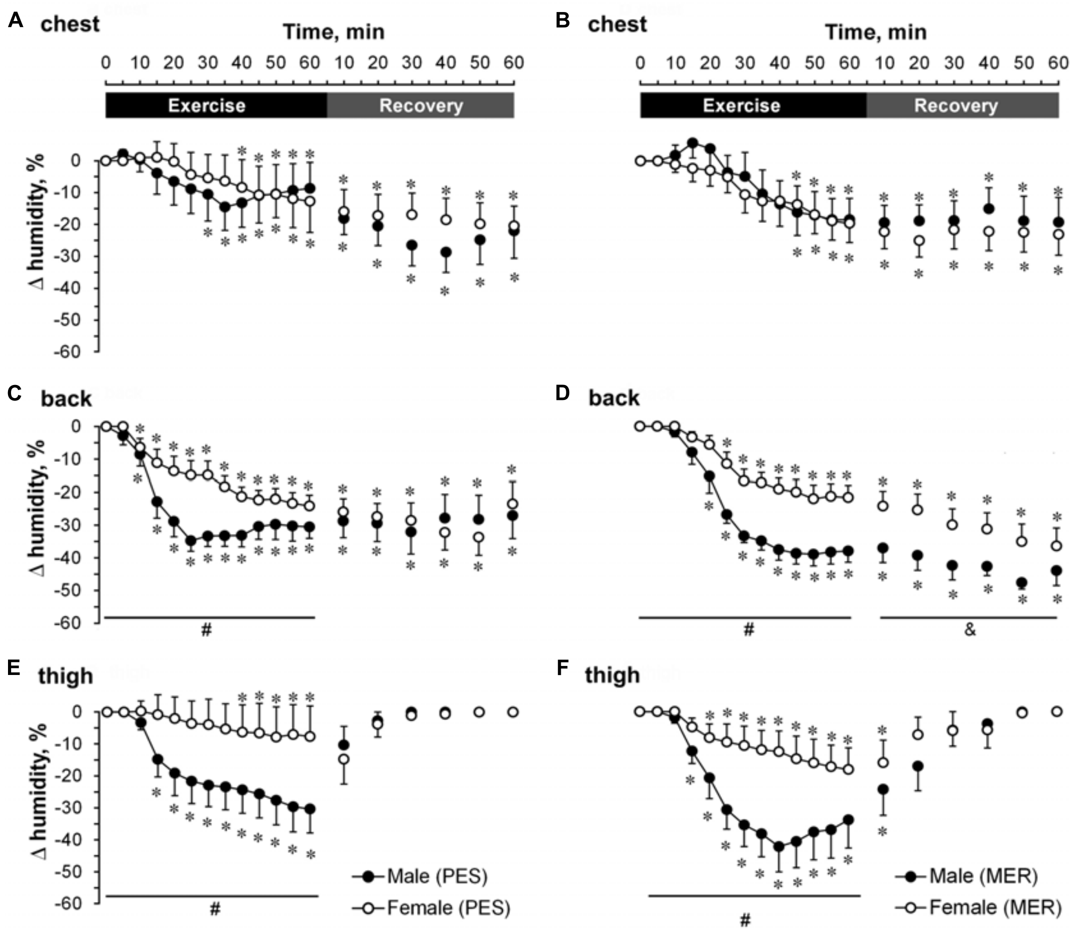

FIGURE 5 | A comparison of temporal changes in garment humidity values at the chest wearing **(A)** polyester (PES) and **(B)** merino wool (MER) garment sets; at the back wearing **(C)** PES and **(D)** MER garment sets; at the thigh wearing **(E)** PES and **(F)** MER garment sets. Results are shown as differences in humidity between next-to-skin values and the outer side of garments. Values are shown as the mean ± SEM (10 men, 11 women). $^{*}P < 0.05$ compared with pre-exercise values; $^{\#}P < 0.05$ between genders; $^{\&}P < 0.05$ significant difference between men when wearing different garment fabrics.

stronger stimulus to tactile receptors than a dry garment (Nielsen and Endrusick, 1990). Women respond to acute stressors with more intensely negative effects than men because of their greater activity in brain regions that translate stress responses to subjective awareness. This greater activity is found particularly in limbic regions dense with gonadal hormone receptors (Ordaz and Luna, 2012). Therefore, we speculate that the feeling of humidity affects higher central nervous system levels, which are more sensitive in women than in men.

We show that, despite the lower next-to-skin temperature in women, thermal sensation did not differ between genders. We probably feel hot while exercising in the cold by integrating information about increased core temperature, and because the skin receives warmer blood from the body's center via peripheral vasodilatation. The body core and dermis sense temperature through spino-reticulo-hypothalamic pathways, which project to the preoptic anterior hypothalamus and induce autonomic thermoeffector responses. Two other pathways project to the insula via the spino-thalamo-cortical route and cause thermal and sweating sensations. During post-exercise recovery, when we are sensitive to temperature changes, epidermal thermoreceptors react to decreased heat production in combination with low ambient temperature through the spino-thalamo-cortical pathways, and trigger cold sensations and a shivering response (Romanovsky, 2007; Nakamura and Morrison, 2008). In our study, all participants showed high PSI values during exercise. All stressors affect the activation of specific cognitive processes (Feldman et al., 1995). Thus, exercise-linked stress causes exercise-induced analgesia (EIA) as described by Beaumont and Hughes (1979). They showed that performing exercise with an intensity greater than 50% of VO_{2max} for longer than 10 min elicits EIA associated with activation of the endogenous opioid system via neural and hormonal changes during exercise. This has similar effects to morphine and reduces sensitivity to subjective perceptions (Ouzzahra et al., 2012). Therefore, it seems likely that in our study sensitivity to thermal stimuli was suppressed via such EIA and even higher absolute T_{re} values in women did not influence stronger sensations of

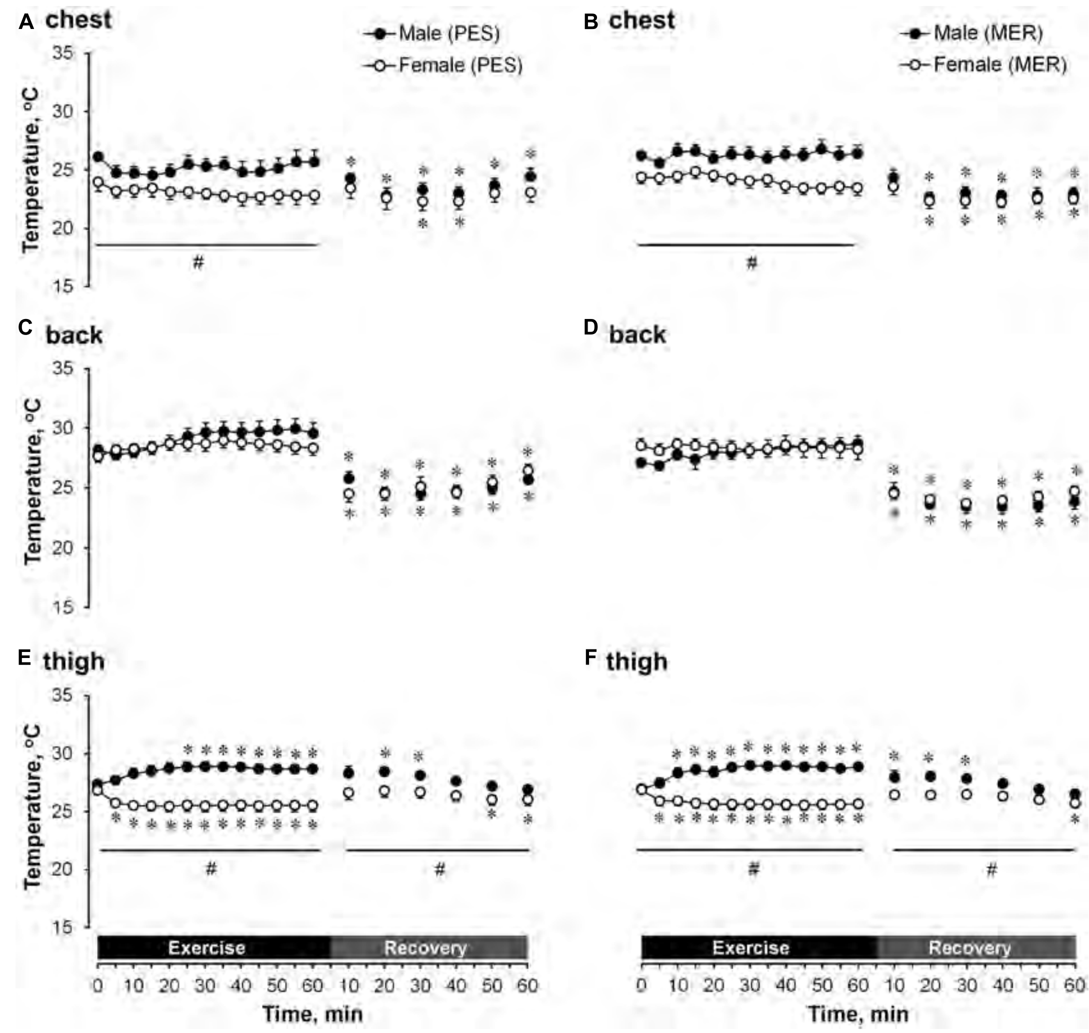

FIGURE 6 | A comparison of temporal changes in garment microclimate next-to-skin temperature at the chest wearing **(A)** polyester (PES) and **(B)** merino wool (MER) garment sets; at the back wearing **(C)** PES and **(D)** MER garment sets; at the thigh wearing **(E)** PES and **(F)** MER garment sets. Values are shown as the mean ± SEM (10 men, 11 women). $^{*}P < 0.05$ compared with pre-exercise values; $^{\#}P < 0.05$ between genders.

warmth. In post-exercise recovery, despite the women's greater shivering ratings while wearing the MER garment set, subjective ratings of thermal sensations did not differ between genders. Hoffman et al. (2004) showed that EIA-reduced pain ratings persisted into a 30-min recovery phase after exercising at 75% VO_{2max} for 30 min. Here, we observed that during post-exercise recovery in a cold environment, heat-induced stressors changed to cold-induced stressors (**Figures 1**, **3**). This suggests that EIA together with a shift from heat-induced thermogenesis to cold-induced thermogenesis had no gender-specific effect on thermal perception during post-exercise recovery in a cold environment.

Proper selection of clothing layers is an important and effective strategy in enabling the wearer to withstand prolonged exposure to cool and cold environmental conditions (MacRae et al., 2014). In general, the effectiveness of clothing as an insulator is mainly because of the entrapment of air (a poor conductor) between the body and garment, within the fabric itself, and bound to body and garment surfaces. There is experimental evidence showing that, compared with synthetic fibers, wool can better mitigate the accumulation of free moisture between fibers and yarns in a fabric (Schneider et al., 1992), and buffer changes in both temperature and humidity under transient conditions (Li et al., 1992). Considering this, in our study we expected that the MER garment set with lower thermal permeability than the PES fabric, together with its higher thermal resistance, and specifically containing both hydrophobic and hygroscopic properties would demonstrate superior microclimate responses for humidity and temperature transfer during exercise and recovery in the cold. However, we found that for most of the variables measured in our conditions both the PES and MER garment sets performed similarly in terms of garment microclimate, and the subjects' physiological and psychological responses. Moreover, variability in clothing microclimate during exercise and recovery highlights that overall

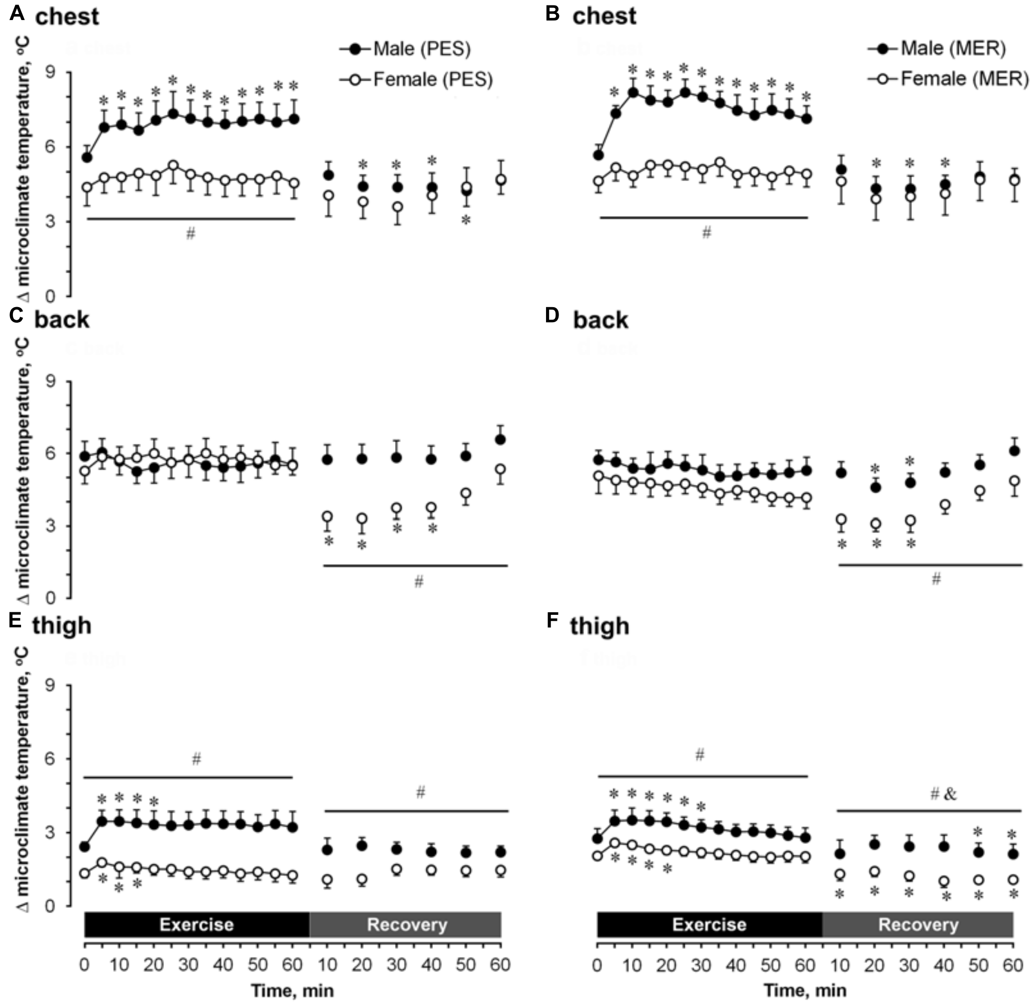

FIGURE 7 | A comparison of temporal changes in garment temperatures at the chest wearing **(A)** polyester (PES) and **(B)** merino wool (MER) garment sets; at the back wearing **(C)** PES) and **(D)** MER garment sets; at the thigh wearing **(E)** PES and **(F)** MER garment sets. Results are shown as differences in temperature between the next-to-skin microclimate and the outer side of garment. Values are shown as the mean ± SEM (10 men, 11 women). $^{*}P < 0.05$ compared with pre-exercise values; $^{\#}P < 0.05$ between genders; $^{\&}P < 0.05$ significant difference between women wearing different garment fabrics.

clothing performance is governed by multiple factors in the context of dynamic and realistic wearing conditions. This finding provides further evidence that the performance of clothing systems during exercise is complex, and comparisons based on fiber or fabric characteristics alone are unlikely to reflect this complexity (MacRae et al., 2014). In addition, changes in garment microclimate during exercise and recovery in cold environments had a gender-specific response in our study. Experimental evidence has shown that men have higher local sweat rates on the back, chest (Havenith et al., 2008) and thigh (Inoue et al., 2014) than women. Consistent with this, we observed greater local sweat release in men than women on warmer skin surfaces, which wet both fabrics and then spread out and evaporated. Thus, our results indicate that sweat was properly adjusted by transfer from next-to-skin to the outer side in both garment sets. However, the lack of statistical significance between the two garment types might have been because of insufficient sweat was released and the high variabilities in humidity and temperatures measured within subjects.

Despite the similar microclimates in terms of humidity and temperature, it seems that MER garments, rather than PES fabrics, induced relatively greater sweating in the cold for both genders. It has been suggested that fabrics with poorer absorptive capacity retain moisture on the skin and inhibit further sweat stimulation, thereby reducing fluid losses (Laing et al., 2008). Ha et al. (2010) found a similar tendency in that local sweat rates were greater in cotton clothing with high moisture absorption and high air retention than in polyester clothing with low moisture absorption and high air permeability. Presumably, some sweat was absorbed by the MER fabric in our study because of merino yarn's ability to absorb liquids, but poor capacity to wick sweat away, as for PES fabrics. MER garments accumulate sweat within the fabric, and in combination with long lasting cutaneous vasodilation after exercise in women, MER rather than PES garments were associated with greater cooling rate and shivering sensations during recovery. According to Moyen et al. (2014), increases in RH also increase sweating rates, so we hypothesize that the MER garments, which cover the body with absorbed sweat, induced an effect similar to a humid environment. Because of that, the relative sweating rates were higher when wearing MER garment sets during exercise compared with the PES sets.

Limitations

The complexity of garment end performance during wear is often not reflected adequately in comparisons based upon fabric properties alone (MacRae et al., 2014). In agreement with this, we observed high variabilities together with lack of statistical significance between the microclimate of two garment types measured within subjects during wear while exercising and recovering in a cold environment. Conceivably, some of our results might have reached significance with a larger sample size. The present study is not statistically underpowered; that said, power analysis indicated a power of 0.80–1.00 for significant variables with low sample size (10 men and 11 women). Moreover, our environmental conditions were carefully controlled, which makes it difficult to transfer conclusions to real-life varying conditions. Thus, inferences about garment characteristics drawn from fabric properties should be made in cognisance of these limitations. Another point to consider is that reproductive hormones in females may influence the thermoregulatory system (Brooks et al., 1997; Farage et al., 2008), mood (Miller and Miller, 2001), perception (Gescheider et al., 1984), mental and physical function (Farage et al., 2008) during exercise and recovery in the cold. Estrogen and progesterone levels are reported to alter baseline core temperature (Brooks et al., 1997; Farage et al., 2008). In the present study, there were no significant differences in baseline T_{re} in female subjects between two experimental visits, suggesting similar menstrual cycle condition (i.e., similar hormone level). Moreover, effects of different menstrual cycle phases on investigated physiological and psychological responses in our study were out of scope and thus not evaluated. Another limitation of our study is that weaker thermoregulatory, perception and neuromuscular system response to exercise and temperature conditions in children and older people (Kenney and Munce, 2003; Gorianovas et al., 2013; Brazaitis et al., 2017) suggest that the results of the present study may not be directly applicable to children or older people.

CONCLUSION

Despite the similar level of relative loss in body mass, similar sweating sensations and similar next-to-skin humidity values in women and men, women felt wetter at the end of the exercise sessions in cold air in this study. However, the lower next-to-skin temperature in women than in men did not affect thermal sensations differently. Post-exercise recovery in cold air induced a greater T_{re} cooling rate in hyperthermic women to below the initial level. In contrast, in hyperthermic men the T_{re} value returned to initial levels for both sets of garments. However, it is remarkable that although the MER garment set induced greater sweating in both genders than did PES garments, the T_{re} cooling rate and greater shivering sensations were more pronounced in hyperthermic women than in hyperthermic men when wearing the former. Most of the changes in the garment microclimates during exercise and recovery in the cold were associated with gender-related differences rather than with fabric-related differences. This is the first study to find evidence supporting the idea of gender-specific differences for the proper selection of fabrics when exercising and recovering in cold environments, and will be relevant for manufacturers who construct sportswear garments for men and women.

AUTHOR CONTRIBUTIONS

The author MB contributed to the design of the work. The authors MC, NB, NE, LD, and MB performed the experiments. The authors MB and MC contributed to the analysis and interpretation of data for the work. The authors MB and MC drafted the work for important intellectual content. The authors MC, NB, NE, LD, SK, and MB finally approved the version to be submitted. The author MB contributed to the revision of this work. All the authors agreed 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.

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Effect of Passive Hyperthermia on Working Memory Resources during Simple and Complex Cognitive Tasks

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The aim of this study was to verify the hypothesis that hyperthermia represents a cognitive load limiting available resources for executing concurrent cognitive tasks. Electroencephalographic activity (EEG: alpha and theta power) was obtained in 10 hyperthermic participants in HOT (50°C, 50% RH) conditions and in a normothermic state in CON (25°C, 50% RH) conditions in counterbalanced order. In each trial, EEG was measured over the frontal lobe prior to task engagement (PRE) in each condition and during simple (One Touch Stockings of Cambridge, OTS-4) and complex (OTS-6) cognitive tasks. Core (39.5 ± 0.5 vs. 36.9 ± 0.2°C) and mean skin (39.06 ± 0.3 vs. 31.6 ± 0.6°C) temperatures were significantly higher in HOT than CON ($p < 0.005$). Theta power significantly increased with task demand ($p = 0.017$, $\eta^2 = 0.36$) and was significantly higher in HOT than CON ($p = 0.041$, $\eta^2 = 0.39$). The difference between HOT and CON was large ($\eta^2 = 0.40$) and significant ($p = 0.036$) PRE, large ($\eta^2 = 0.20$) but not significant ($p = 0.17$) during OTS-4, and disappeared during OTS-6 ($p = 0.87$, $\eta^2 = 0.00$). Those changes in theta power suggest that hyperthermia may act as an additional cognitive load. However, this load disappeared during OTS-6 together with an impaired performance, suggesting a potential saturation of the available resources.

Keywords: EEG, hyperthermia, cognitive tasks, task complexity, overload

INTRODUCTION

Exposure to heat stress leads to the development of hyperthermia when the prevailing ambient conditions become uncompensable. When hyperthermic, individuals stimulated with cold report feelings of pleasure, whereas displeasure is expressed when heat stress is further increased (Cabanac, 1987). Along with influencing the perception of pleasure, heat stress has been shown to influence cognitive function. Indeed, marked increases in core and/or skin temperature have been demonstrated to impair complex cognitive task performance (Hancock, 1986; Simmons et al., 2008). Recently, a hypothesis was developed linking this impairment to the alliesthesial change accompanying compensatory physiological responses to hot environmental conditions (i.e., strain related to thermoregulation) (Gaoua et al., 2012). More specifically, increases in temperature during heat exposure generated unpleasant stimuli, as measured by the Positive and Negative Affect Schedule (PANAS), which could be considered as a 'cognitive load.' It was proposed that this load might reduce the available resources for concurrent cognitive tasks. Interestingly, this could explain why reducing thermal discomfort, by cooling the head, for example, can restore some complex cognitive function in a hot environment (Gaoua et al., 2011b).

It has been suggested that performance of cognitive tasks under heat stress deteriorates when the total cognitive resources are insufficient for both the task and the thermal stress (Hocking et al., 2001). However, these findings have not been demonstrated empirically. Electroencephalography (EEG) measures recorded during cognitive tasks carried out in hot environments could provide insight into this process. Most EEG studies have focused on fluctuations in the theta (3–8 Hz) and alpha (8–12 Hz) power bands (Klimesch, 1999; Smith et al., 2001, 2004), as this allows discrimination between tasks having different workloads, under both simulated and actual working conditions (Wilson and Russell, 2003). Changes in alpha power are inversely related to cognitive processing with Lang et al. (1988) reporting decreased alpha activity when performing a concept formation task. Several other reports have also shown decreased alpha activity in association with increased task difficulty and the highest working memory loads during several cognitive tasks (Earle and Pikus, 1982; Gundel and Wilson, 1992). Conversely, an increase in theta power relative to rest has been reported during working memory (Gevins et al., 1997; Ishii et al., 1999; Mizuhara et al., 2004) and concentration tasks (Gevins et al., 1997; Ishii et al., 1999; Aftanas and Golocheikine, 2001; Jensen and Tesche, 2002). Such an increase in theta power over the frontal lobe is suggested to indicate an increase in the workload and demand on working memory (Kahana et al., 1999; Bastiaansen et al., 2002). Mean theta activity has also been shown to increase toward the end of difficult task sessions (Gevins et al., 1997) and when subjects are tired, but attempting to remain vigilant (Paus et al., 1997; Caldwell et al., 2003). An increase of theta power has also been reported at the frontal midline sites of the scalp during working memory and mental arithmetic tasks in the heat (Gevins et al., 1997; Ishii et al., 1999; Mizuhara et al., 2004). The elevated theta power is associated with an increase in concentration and heightened attention (Ishii et al., 1999; Aftanas and Golocheikine, 2001; Jensen and Tesche, 2002). This suggests that theta activity is not strictly related to the amount of information being manipulated, but to the level of mental effort being expended to cope with the task. As such, theta oscillations may be the best indicator of mental workload (Smith et al., 2001) and cognitive fatigue (Smith et al., 2004).

Therefore, the aim of this study was to determine whether cognitive resources are overloaded during passive hyperthermia by investigating the EEG responses to tasks of varying complexity. It was hypothesized that hyperthermia would represent a load and, as such, limit the resources available for performing cognitive tasks. This load would be characterized by a decrease in alpha activity and increase in theta activity under thermal strain, while an 'overload' during complex cognitive tasks would lead to an impairment in performance.

MATERIALS AND METHODS

Participants

A total of 10 healthy males (35 ± 3 years, 79 ± 11 kg, 175 ± 5 cm; for age, weight, and height, respectively) volunteered for the study. Participants were asked to avoid all vigorous physical activity for the 24 h preceding the experiment. They were also asked to avoid caffeine and nicotine intake, as well as maintain their sleeping habits in the 24 h preceding each trial. The Institutional Human Ethics Committee approved the study, which was conducted in accordance with the Helsinki Declaration.

General Procedure

After a familiarization trial, participants completed two experimental trials: one in a hyperthermic state in hot conditions (HOT: 50°C and 50% relative humidity) and another in a normothermic control state in temperate (CON: 25°C and 50% relative humidity) condition, separated by 4–7 days, in a counterbalanced design. Both experimental trials were conducted at the same time of day in an environmental chamber (Tescor, Warminster, PA, United States), with constant noise, light (212 lx), and ventilation (0.5–0.6 ms^{-1}). During both trials, participants wore shorts and a T-shirt. In order to avoid the confounding effects of dehydration, water was provided *ad libitum* throughout both experimental trials.

Familiarization Session

Commencing the experimental trials 1 week before, participants completed a familiarization session during which they performed the complete cognitive testing protocol and were accustomed to EEG procedures. In addition, the cognitive testing software (testing battery described below) provided a brief familiarization that was repeated before each test.

Experimental Sessions

Before the experimental sessions, participants provided a urine sample for the measurement of urine-specific gravity (Pal-10-S, Vitech Scientific Ltd., West Sussex, United Kingdom) and were then weighed (nude body mass). After 20 min of rest for EEG electrode placement, they entered the environmental chamber. Participants initially walked for 10 min on a motorized treadmill (T170, COSMED, Rome, Italy) at 4 $km.h^{-1}$. This procedure was done to minimize the initial decrease in core temperature (T_{core}) related to the peripheral vasodilation. This protocol has been employed by previous studies to promote heat production without causing fatigue (Racinais et al., 2008). After walking, subjects sat resting in the upright position inside the environmental chamber for 35 min (CON) or until the target T_{core} of 39°C (HOT) was reached. This target T_{core} was selected based on previous studies showing decrements in cognitive performance from 38.7°C (Gaoua et al., 2011a) and to avoid subjects reaching too high temperatures by the end of the cognitive task. At this time, a planning task (OTS: One Touch Stockings of Cambridge) with on-going EEG recording was conducted. Prior to the cognitive testing, EEG recordings with eyes open were collected for 30 s in HOT or CON conditions.

Temperature Recording

Core and skin temperatures were monitored using the VitalSense® system (precision ± 0.01°C, Mini Mitter, Respironics, Herrsching, Germany). A wireless Jonah™ ingestible thermometer pill, swallowed 5–7 h before the testing session, was used to measure T_{core}. The validity of ingestible thermometer pills for monitoring T_{core} has been confirmed during both rest and exercise, making them a viable substitute for more invasive methods (Casa et al., 2005). Wireless XTP dermal adhesive temperature patches were used to measure chest (T_{chest}), hand (T_{hand}), and calf (T_{calf}) skin temperatures. Both internal and external sensors sent data by telemetry to a single data logger every 60 s. Mean skin temperature (T_{skin}) was calculated using Burton's (1934) weighted formula: 0.5 T_{chest} + 0.14 T_{hand} + 0.36 T_{calf}.

Cognitive Testing

The OTS test was performed upon reaching 39°C in HOT or after 35 min of seated rest in CON. This test has been used in previous studies investigating the effect of hot environmental conditions on cognitive performance and was shown to be a valid tool to differentiate the effects of heat on simple and complex tasks (Gaoua et al., 2011a, 2012). This test was also used because instead of categorizing different tasks as simple and complex, it manipulates the complexity within the same task (Gaoua, 2010). Hence, the mechanism required to perform the task and the brain area being assessed remain constant, but the cognitive load required to successfully complete the task is manipulated.

The OTS was performed during each trial using Cantab software (CANTABeclipse, Cambridge Cognition, Cambridge, United Kingdom) and hardware (a tactile screen and a touch pad). Subjects were shown two displays containing three colored balls. The displays were presented in such a way that they could be perceived as stacks of colored balls held in stockings suspended from a beam. Along the bottom of the screen, there was a row of numbered boxes. Subjects were initially shown how to move the balls in the lower display to copy the pattern in the upper display. The experimenter completed one demonstration problem, where the solution required one move, following which the subjects completed three further practice problems, one each of two, three, and four moves before starting the test. For the test itself, subjects were shown further problems, requiring 2, 3, 4, 5, or 6 moves. Four of each of the task complexities was randomly presented to the participants. Participants had to mentally calculate the minimum number of moves required to solve the problems, and then to touch the corresponding box (1–6) at the bottom of the screen to indicate their response. The outcome measures were the number of problems solved on the first attempt, the latency to first responses (whether correct or wrong) and the latency to correct responses. Measures were analyzed for two different levels of complexity requiring either 4 (OTS-4, simple) or 6 moves (OTS-6, complex). Each measure was calculated by averaging the scores obtained over 4 trials.

EEG Recordings

Continuous EEG data was recorded using the NicoletOne LTM system (Viasys Healthcare, Madison, WI, United States). Genuine gold cup electrodes (10 mm diameter, Grass Technologies, West Warwick, RI, United States) were affixed to the scalp with conductive paste (EC2, Grass Technologies, West Warwick, RI, United States) and secured with a small gauze pad. A lightweight hairnet was used to prevent the electrodes from moving. The primary recording electrode was placed at the Fz position and recorded with a paired mastoid reference and grounding electrode at the Fpz position according to the International 10–20 System (Homan et al., 1987). The frontal midline area has been shown to be a primary activity region within the brain during working memory tasks (Gevins et al., 1997; Ishii et al., 1999; Mizuhara et al., 2004). All electrode impedances were maintained below 10 kOhm. EEG data was sampled at 256 Hz, low pass filtered at 0.3 Hz, high pass filtered at 35 Hz, and stored on a computer hard disk for subsequent analysis. A Fast Fourier Transformation (FFT) was calculated with 2-s bins using a Hanning window with 75% overlap to yield the absolute power values for the theta (3–8 Hz) and alpha (8–12 Hz) frequency bands. These signal frequencies have been previously shown to have very high test–retest reliability when measured in the context of working memory tasks (McEvoy et al., 2000). Each measure was obtained by averaging the values from consecutively recorded 2-s data segments preceding correct responses during all OTS-4 and OTS-6 tasks. Digital markers were applied during data acquisition to represent the start and end (correct answer) of each task. When correct answers were given in less than 2 s, they were not used for analysis due to the limitations of the FFT analysis (i.e., at least 2 s of data were required for analysis). For the purpose of this study, measures at rest just before the cognitive tests (PRE) during the OTS-4 and the OTS-6 were analyzed.

Thermal Perception

Thermal comfort and thermal sensation were recorded on visual analogic scales ranging from very comfortable (0) to very uncomfortable (20, white to black scale) and from very cold (0) to very hot (20, blue to red scale). The scores ranging from 0 to 20 were on the reverse sides of both scales and only visible to the researcher. Higher scores represented feeling less comfortable and hotter for thermal comfort and thermal sensation, respectively.

Statistical Analysis

We used Shapiro–Wilk test and confirm that all data were normally distributed. Data were coded and analyzed in SPSS Version 17 software (SPSS, Chicago, IL, United States). A one-way within-subjects ANOVA was performed to study the effect of condition (CON, HOT). In addition, a two-way within-subjects ANOVA was performed to analyze the effect of condition as well as the effect of task (i.e., PRE, OTS-4, and OTS-6) and potential interaction on EEG data. All variables were tested using Mauchly's procedure for sphericity. If a significant condition × task interaction was found, pairwise comparisons using a Bonferroni correction were used to compare the effect of condition at each time interval. The level of statistical significance was set at $p < 0.05$. Moreover, effect-sizes are described in terms of partial eta-squared (η^2; with $\eta^2 \geq 0.06$ representing a moderate effect and $\eta^2 \geq 0.14$ a large effect, Cohen, 1969, pp. 278–280).

RESULTS

Temperature and Thermal Perception

The T_{core} during the cognitive tasks was significantly higher in HOT (39.1 ± 0.3°C) than in CON (36.9 ± 0.2°C; $p < 0.05$, $\eta^2 = 0.97$). T_{skin} was also significantly higher in HOT (39.5 ± 0.5°C) than in CON (31.6 ± 0.6°C; $p < 0.05$, $\eta^2 = 1$). Participants reported a higher thermal sensation in HOT (16.2 ± 2.2) compared with CON (9.2 ± 1.5; $p < 0.05$, $\eta^2 = 0.95$), as well as higher thermal discomfort in HOT (12.7 ± 5.3) relative to CON (5.8 ± 2.7; $p < 0.05$, $\eta^2 = 0.80$). Body mass did not change from the start to the end of the CON trial (+0.1%; $p > 0.05$); however, a 0.4% body mass loss did occur during the HOT trial ($p < 0.05$). Urine-specific gravity prior to the experimental sessions was within the normal range for both HOT and CON (1.011 ± 0.007 vs. 1.016 ± 0.008 g/ml).

Cognitive Function

During the OTS-4, latency to first response was shorter in HOT than in CON ($p = 0.018$, $\eta^2 = 0.48$; **Table 1**). There were no differences between conditions in the latency to the correct answer ($p = 0.38$, $\eta^2 = 0.09$; **Table 1**) and the number of problems solved on first choice (i.e., accuracy) ($p = 0.59$, $\eta^2 = 0.03$; **Figure 1**). For OTS-6, accuracy was significantly reduced in HOT compared with CON ($p = 0.003$, $\eta^2 = 0.64$; **Figure 1**). The difference in the latency to the first response did not reach significance ($p = 0.058$), however, presented a large effect ($\eta^2 = 0.34$; **Table 1**). Moreover, latency to the correct response was longer in HOT than in CON ($p = 0.07$, $\eta^2 = 0.57$; **Table 1**).

EEG Responses

Theta power significantly increased with task demand ($p = 0.017$, $\eta^2 = 0.36$) and was significantly higher in HOT than CON ($p = 0.041$, $\eta^2 = 0.39$; **Figure 2**). The difference between HOT and CON was large ($\eta^2 = 0.40$) and significant ($p = 0.036$) PRE, large ($\eta^2 = 0.20$) but not significant ($p = 0.17$) during OTS-4, and disappeared during OTS-6 ($p = 0.87$, $\eta^2 = 0.00$). Alpha power tended to decrease with task engagement with higher alpha power PRE (2.06 ± 0.8 μV^2) compared to OTS-4 (1.4 ± 0.5 μV^2; $p = 0.102$, $\eta^2 = 0.28$; **Figure 3**), but did not further decrease with task complexity during the OTS-6 (1.4 ± 0.6 μV^2). Changes in alpha power were not associated with T_{core} ($p = 0.68$, $\eta^2 = 0.02$).

TABLE 1 | Latency to first choice and to the correct response for the OTS-4 and OTS-6 in CON and HOT presented in mean ± SEM.

		CON	HOT
OTS-4	Latency to first choice (s)	9.28 ± 2.56	7.10 ± 1.76*
	Latency to correct (s)	10.13 ± 4.04	11.65 ± 3.52
OTS-6	Latency to first choice (s)	24.70 ± 14.27	15.93 ± 5.85
	Latency to correct (s)	21.92 ± 7.21	35.32 ± 9.72*

*Significant difference between conditions, $p < 0.05$.

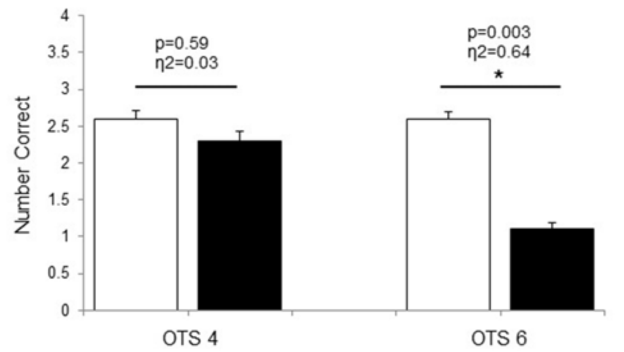

FIGURE 1 | Number of problems solved on first choice during OTS-4 and OTS-6 in CON (white bars) and HOT (black bars) environments. Values are mean ± SEM. *Significant difference between HOT and CON conditions.

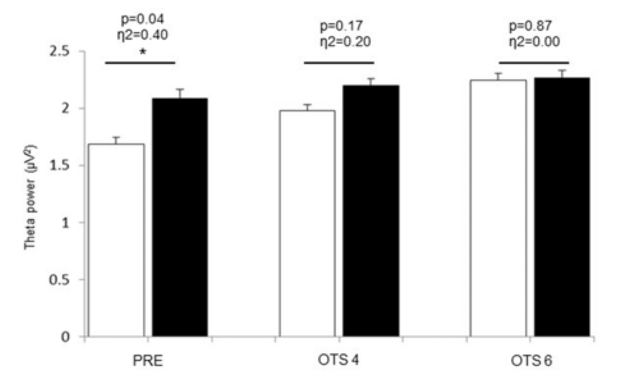

FIGURE 2 | Theta power values obtained at PRE, during OTS-4 and OTS-6 in CON (white bars) and HOT (black bars) environments. Values are mean ± SEM. *Significant difference between HOT and CON conditions.

DISCUSSION

The aim of this study was to determine whether cognitive resources are overloaded during passive hyperthermia by investigating the EEG response (i.e., alpha and theta power) in the frontal lobe during simple and complex cognitive tasks. In accordance with previous studies, our data indicate that accuracy during complex cognitive tasks decreases in hot environments with and without an increase in T_{core} (Hancock, 1986; Racinais et al., 2008; Gaoua et al., 2011a,b). This decrease was previously associated with a dynamic change in T_{core} (Hancock, 1986; Gaoua et al., 2011b). Our study shows a similar decrease with a non-compensable but stable increase in T_{core} (≃39°C).

In the current study, hyperthermia was associated with a reduction in accuracy in the number of problems solved on first choice during the OTS-6 as well as an increase in the latency to the correct response (**Table 1**). For the first time,

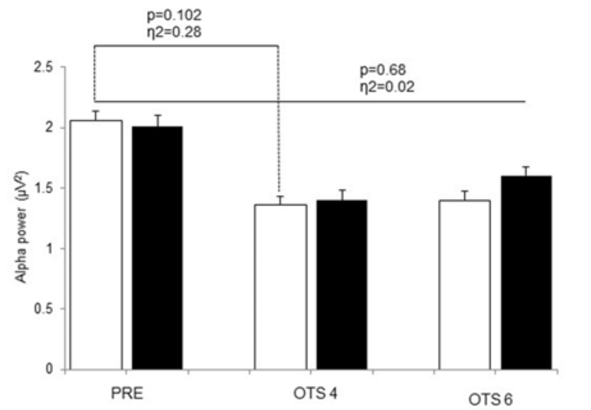

FIGURE 3 | Alpha power values obtained at PRE, during OTS-4 and OTS-6 in CON (white bars) and HOT (black bars) environments. Values are mean ± SEM.

our data provide some EEG insight to explain these results. Indeed, EEG theta power was significantly elevated prior to task engagement (PRE, **Figure 2**), suggesting that hyperthermia imposed a cognitive load possibly related to the significant increase in thermal discomfort. Despite this, the simple task was successfully completed (**Figure 1**), but at a higher theta activity (OTS-4, **Figure 2**). However, it appears that theta power reached a threshold during the complex task beyond which it was not possible to allocate additional cognitive resources (OTS-6, **Figure 2**) to successfully complete the task, hence performance decreased (**Figure 1**).

It was previously suggested that theta power increases with greater memory demands (Gevins et al., 1997; Bastiaansen et al., 2002). The current results confirm that theta power significantly increases with task demand, as observed during the complex cognitive task in CON (**Figure 2**). However, the current data further shows that theta power also increases with hyperthermia. This increase in theta power could be related to the impact of physiological responses during heat stress on cognitive function. It is interesting to note that at this time subjects were hyperthermic but not actively engaged in any task (PRE). Accordingly, heat stress may represent a load that drains cognitive resources as in a dual task paradigm (Gaoua et al., 2011a,b).

Previous studies from Dubois et al. (1980, 1981) demonstrated a general slowing of EEG activity in clinical patients suffering from fever with a T_{core} of 38–40°C in association with an increase in theta power (Dubois et al., 1980, 1981). In the current study, the rise in T_{core} to ∼39°C induced an increase in theta power, which was higher both PRE and during the OTS-4 in HOT than in CON, despite there being no difference in performance. Similar results were observed in a study using steady-state visual evoked potentials (Hocking et al., 2001). This study demonstrated that with increasing T_{core}, the potentials increased in amplitude and decreased in latency in the frontal lobe for working memory tasks and in occipito-parietal regions for vigilance tasks, with no significant difference in task performance compared to control conditions (Hocking et al., 2001). This indicates that despite changes in the underlying theta activity supporting task performance during hyperthermia, OTS-4 accuracy was not negatively impacted (**Figure 1**).

According to the multiple-resource theory, tasks using separate resources may be performed simultaneously without interference and, in the presence of resource conflict, the required resource can allocate part of its processing time to each task (Navon and Gopher, 1979). However, in the current experiment, hyperthermia was an ongoing factor during the cognitive task (i.e., concurrent processing time) and may have used similar cerebral resources as the cognitive task (i.e., frontal lobe resource conflict). This may have had an additive effect on cerebral resources in the area involved, rather than involving new brain areas (Adcock et al., 2000). The current data suggest that when performing a simple task in a hot environment (e.g., OTS-4), the cognitive load of the task and of the heat stress cumulate and lead to a higher load, as indicated by the higher theta values (**Figure 2**). Hence, working memory resources during the OTS-4 were increased to maintain task performance. In accordance with previous studies (Gaoua et al., 2011b), the speed of response to the first choice during the OTS 4 was higher in HOT compared to CON (**Table 1**) possibly in relation to an increase in nerve conduction velocity and in impulsivity, as previously observed in similar tasks performed in a hot environment (Racinais et al., 2008; Gaoua et al., 2011b). However, the latency to correct is a measure of both the time to process the information and the time to register the response on the screen. The absence of a difference between conditions in the latency to correct response may indicate that when hyperthermic, mental processing for a given task takes longer. The current data show that during the more complex OTS-6 task, speed of response was not different between conditions, but that more mistakes were made in HOT. This result is different from previous studies that have observed an improvement in reaction time during complex cognitive tasks in the heat (Simmons et al., 2008; Gaoua et al., 2012), and may relate to additional efforts being made to mobilize greater mental resources during the complex task. This premise is supported by the increase in theta power noted during OTS-6 compared to OTS-4 in CON.

Interestingly, our data showed that performance during the complex task (i.e., OTS-6) was impaired in HOT. This may be due to interference between two concurrent tasks requiring activation of the same part of the neural cortex (Klingberg, 1998). Indeed, interference has been observed between two cognitive tasks (Jaeggi et al., 2003), two motor tasks (Wenderoth et al., 2005), during the combination of a cognitive and a motor task in a temperate environment (Lorist et al., 2002), and during exercise-induced fatigue in a hot environment (Hocking et al., 2001). Our data suggest that heat stress also interferes with complex cognitive task performance and that cognitive resources may reach a critical threshold and become overloaded during hyperthermia, resulting in a decrease in performance. This supports the idea that there is a single pool of cognitive resources one can withdraw from (Kahneman, 1973) and that cognitive performance is impaired when combined with heat stress, but not when it is completed in normothermic conditions. In this case, the absence of a dual-task decrement during the OTS-4 can be explained by single resource theory on the assumption that the combination of tasks, or in the current study the combination of the OTS-4 and hyperthermia, does not exceed the upper threshold on the available resources (i.e., the task can be completed without interference) (Kahneman, 1973). It is worth noting that participants in the current study were passively exposed to heat stress with no option for behavioral thermoregulation, other than hydration. Hence, the decrement in resources could only influence the cognitive task (OTS-6).

Several reports have shown decreased alpha activity in the occipital and parietal regions of the brain in association with increased task difficulty and the highest working memory loads during several cognitive tasks (Earle and Pikus, 1982; Gundel and Wilson, 1992). Our study shows that this decrease in alpha activity also occurs in the frontal area with task engagement (OTS-4 and OTS-6, **Figure 3**). Higher alpha power is associated with reduced cortical activity and has been described as cortical idling, with a greater availability of resources for engagement in cognitive tasks (Klimesch, 1999). Interestingly, in the HOT condition alpha power appeared to be slightly higher during the OTS-6 than the OTS-4 (**Figure 3**). However, this task-related increase in alpha power during working memory tasks has been observed elsewhere (Jensen et al., 2002; Busch and Herrmann, 2003; Cooper et al., 2003; Sauseng et al., 2005, 2009). This paradoxical response in alpha activity during task engagement has been suggested to reflect the inhibition of task-irrelevant/interfering processes (Klimesch et al., 2011), such as the environmental and physiological heat stress in our experiment. Thus, we conclude that despite the attempt to manage the cognitive load associated with hyperthermia, there was no re-allocating of additional working memory resources as seen by no further increase in theta activity.

This study is not without limitations. Despite using a familiarization session and randomizing the trials in HOT and CON, it is possible that some other factors may have influenced cognitive performance and the associated EEG responses. These factors may include differences in motivation, fatigue, and arousal across trials. In addition, the sweat during HOT trial may have influenced the conduction of the electrodes and therefore EEG results. This would have been minimal as conductive paste was used to fix the electrodes to the scalp. Finally, only male participants were recruited for the study and the results may not be generalized to female populations. Future studies may consider including female participants to investigate gender differences. In fact, differences were previously suggested in a variety of psycho-behavioral and physiological factors including thermoregulatory responses and brain functions and structures that may influence the additional load imposed by hyperthermia.

In summary, the current data show that EEG theta power in the frontal area was significantly elevated PRE in HOT ambient conditions, suggesting that hyperthermia may in itself impose a cognitive load. Moreover, alpha power decreased during both simple and complex cognitive tasks. However, the simple task was successfully completed at the cost of an increase in mental load in the frontal area. Hence, during the complex task in hyperthermia, cognitive function may have reached a threshold beyond which it was not possible to allocate additional resources to successfully complete the cognitive task, and as a result performance declined.

ETHICS STATEMENT

This study was carried out in accordance with the declaration of Helsinki and was approved by 'Aspetar Orthopaedic and Sport Medicine Hospital Ethics Committee' with written informed consent obtained from all subjects.

AUTHOR CONTRIBUTIONS

All authors have contributed to the manuscript. NG and SR developed the research design and protocol. NG, SR, and CH performed the experiment. NG prepared the first draft of the manuscript. JP critically reviewed it. All authors contributed to the final manuscript and gave final approval of the version to be published.

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Gravity Cues Embedded in the Kinematics of Human Motion Are Detected in Form-from-Motion Areas of the Visual System and in Motor-Related Areas

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The present study investigated the cortical areas engaged in the perception of graviceptive information embedded in biological motion (BM). To this end, functional magnetic resonance imaging was used to assess the cortical areas active during the observation of human movements performed under normogravity and microgravity (parabolic flight). Movements were defined by motion cues alone using point-light displays. We found that gravity modulated the activation of a restricted set of regions of the network subtending BM perception, including form-from-motion areas of the visual system (kinetic occipital region, lingual gyrus, cuneus) and motor-related areas (primary motor and somatosensory cortices). These findings suggest that compliance of observed movements with normal gravity was carried out by mapping them onto the observer's motor system and by extracting their overall form from local motion of the moving light points. We propose that judgment on graviceptive information embedded in BM can be established based on motor resonance and visual familiarity mechanisms and not necessarily by accessing the internal model of gravitational motion stored in the vestibular cortex.

Keywords: biological motion, gravity, functional MRI, motor resonance, form-from-motion perception

INTRODUCTION

Earth's gravity is an important factor that influences visual perception. Psychophysical experiments demonstrated that several spatiotemporal characteristics of a visual scene are estimated employing implicit knowledge about the effects of gravity on moving objects in the physical world. For instance, visual gravity cues contribute to the perception of size, distance and flight time of falling objects (Watson et al., 1992; Huber and Krist, 2004; Brouwer et al., 2006; Moscatelli and Lacquaniti, 2011). Motion naturalness of a freely swinging pendulum is also established judging violations of the natural relation between period and length imposed by gravitational acceleration (Pittenger, 1990). Furthermore, manual interception of falling objects under microgravity is not accurately timed given the lack of object acceleration (McIntyre et al., 2001; Senot et al., 2012). Based on the fact that the visual system is quite poor at estimating image accelerations (Werkhoven et al., 1992), the above predictive behaviors in visual perception and interceptive responses involving knowledge about gravity were in favor of the existence of an internal model of gravitational motion internalized in the human brain. Functional magnetic resonance imaging (fMRI) experiments tested this hypothesis and demonstrated that an internal model implemented within a vestibular neural network, including the posterior insula, the retroinsular cortex and the temporoparietal junction, transforms the gravity vector into an abstract representation accessible by the visual system to establish judgments on gravitational motion of objects (Indovina et al., 2005; Lacquaniti et al., 2013). Likewise, Indovina et al. (2013) showed that a similar network is engaged during vertical self-motion coherent with natural gravity.

There is also evidence that gravity cues are critical for the visual perception of biological motion (BM) as presented with point-light displays (Jokisch and Troje, 2003; Shipley, 2003; Troje and Westhoff, 2006). Such displays, first described by Johansson (1973), convey a vivid impression of figures in motion, which is already decoded by young infants (Fox and McDaniel, 1982). The rudimentary information contained in point-light displays of BM is sufficient even to solve sophisticated recognition tasks, including identity and gender recognition (Cutting and Kozlowski, 1977; Kozlowski and Cutting, 1977; Pollick et al., 2005), emotion recognition (Pollick et al., 2001), and understanding of social interactions (Centelles et al., 2011). Interestingly, the detection and recognition of BM from point-light displays are disrupted once they are turned upside down (Sumi, 1984; Pavlova and Sokolov, 2000). An explanation for this 'inversion effect' would be that the novel orientation of the display makes the form of the stimuli unfamiliar so that individuals are no longer able to extract form and then determine the action (Reed et al., 2003). Nevertheless, it has been demonstrated that even when form information is disrupted, perception of the displays is still subjected to an inversion effect, which in turn likely results from the violation of the familiar (earth-based) spatiotemporal relations between body joints specified by the kinematics (Shipley, 2003; Troje and Westhoff, 2006). Therefore, the visual perception of BM from point light displays involves picking-up dynamic information from the kinematics of body movements that relies on the natural gravity.

At the brain level, a sensitivity to the inversion effect (as obtained by contrasting intact and inverted point-light displays) was found in several regions belonging to the BM perception network (Saygin et al., 2004), especially the occipito-temporal cortex and regions in the parietal (i.e., intraparietal sulcus) and frontal (i.e., caudal part of the middle/inferior frontal gyrus) lobes (Grezes et al., 2001; Grossman and Blake, 2001; Pavlova et al., 2004; Peuskens et al., 2005). With respect to the occipito-temporal cortex, data from Maffei et al. (2015) suggest that activity changes induced by displays with a non-normal gravitational kinematics is related to backward modulatory influences from regions that internalize the effects of Earth gravity on visual motion in general, namely the insula and the temporoparietal junction (Indovina et al., 2005, 2013; Lacquaniti et al., 2013). Thus, modulation of activity in the occipito-temporal cortex would signal mismatches (errors) between incoming and expected stimuli as predicted by the internal model of gravity stored in the vestibular cortex, meaning that predictive coding of gravity effects contributes to BM interpretation. However, there is no evidence that activation gradients in the previously mentioned posterior parietal and frontal regions when inverting BM displays constitute prediction errors that relate to the internal model of gravitational motion. It can only be argued that these regions are commonly involved during the execution and the observation of movements (Rizzolatti and Craighero, 2004; Dinstein et al., 2007; Kilner et al., 2007; Chong et al., 2008; Kilner et al., 2009; Saygin et al., 2012), or otherwise are core nodes of the mirror-neuron system (MNS) whose activation is often interpreted within the framework of motor resonance, whereby an observed action is understood through mapping onto the observer's own motor representation. In this framework, interpreting gravitational cues embedded in BM would rely on a mechanism that 'judges' the compliance of the observed BM with naturalistic (Earth-based) BM stored in the observer sensorimotor repertoire, that is a sort of implicit coding of gravity effects that may not require a predictive code from the internal model of gravity.

Relevant to this explanatory framework is the fact that activity within the MNS was found to be sensitive to human kinematic invariants during action/motion observation (Dayan et al., 2007; Casile et al., 2010). In particular, these experiments reported that compliance of the moving stimuli with a natural law of motion (i.e., the two-thirds power law) was reflected in stronger activation in certain areas of the MNS, especially motor-related areas (e.g., ventral premotor cortex). Therefore, the more plausible the kinematics of the observed action, the stronger the resonance of the MNS. Alternatively, studies also examined the hypothesis that regions of the MNS, including motor-related and parietal areas, should not be active during the observation of biomechanically impossible movements that are not part of the observer's motor repertoire (Stevens et al., 2000; Costantini et al., 2005). Although findings by Stevens et al. (2000) suggest that motor and parietal cortices are selectively activated to process movement that conforms to the capabilities of the observer, Costantini et al. (2005) showed that premotor areas coded movement regardless of whether it is biologically possible or impossible while parietal areas coded for movement plausibility (i.e., an activation gradient between possible and impossible movements). Overall, despite certain discrepancies, all these studies tend to demonstrate that violations of the physical laws that apply on Earth in displayed movements is inferred using motor resonance, with the sensory inputs being mapped onto one's own body motor repertoire and thus coding the possibility of actually performing the same movements.

The present fMRI study investigated the cortical regions responsible for detecting graviceptive information during visual perception of BM. Gravity cues were manipulated by presenting point-light displays depicting a person moving under normogravity or microgravity, the displays having been recorded during parabolic flights (see Materials and Methods for details). The displayed avatars executed the same movements in both normogravity and microgravity so that shape characteristics changed only marginally between the two conditions but with different kinematic characteristics. We expected that coding of gravitational content in BM displays engages motor resonance, which should be reflected in a larger activity in regions of the MNS (i.e., a larger motor resonance) for normogravity BM displays given the closer match between the observed action and the observers' own sensorimotor representations.

MATERIALS AND METHODS

Participants

Twenty healthy right-handed volunteers (mean ± SD [range] age: 36 ± 8 [27–45] years; 9 females) participated in the study. All the participants were naïve as to the purpose of the study and never experienced microgravity. This study was reviewed and approved by the local Ethics Committee "CPP Sud-Méditerranée 1." Before the study, all participants provided written informed consent. This study was carried out in accordance with the Declaration of Helsinki.

Stimuli

The stimuli were three-second silent point-light displays of human movements. The displays were created by videotaping actors (1 woman and 1 man) who performed various movements of everyday life, including standing-up from or sitting on a chair, crouching, moving the arms/legs in isolation or in combination, touching the floor from a sitted position, stepping aside, and tilting forward or backward. Specifically, the displacements of 22 markers (15 mm in diameter) taped onto major body parts (top of the head, acromions, elbows, wrists, metacarpophalangeal joint of the index fingers, manubrium, xiphoid process, navel, hips, greater trochanters, knees, ankles, toes) of the actors were sampled at a rate of 120 Hz with a four cameras SMART-E motion analysis system (BTS, Milan, Italy). Accordingly, each actor was depicted by a set of white dots moving against a black background (**Figure 1**).

The recordings took place onboard the French Airbus A300-Zero G (Novespace) during three parabolic flights. In parabolic flight, the aircraft is put into a suborbital trajectory (30 parabolas per flight) that provides free-fall. Each parabola includes a pull-up phase and a pull-out phase, each 20–22 s long, where occupants are subjected to around 1.75 times the force of gravity, and a microgravity phase in the middle that lasts about 20 s where gravity is close to zero (0.02 ± 0.018 g). Each parabola is followed by 2 min of normogravity (1 g). Therefore, we recorded the actors' movements during the microgravity and normogravity phases. In the microgravity phase, the actors always had at least one foot attached on the floor of the aircraft, so that they were not free-floating. This ensured that form characteristics of the point-light displays were comparable under both microgravity and normogravity for similar movements. Furthermore, the starting positions of the different movements depicted in the displays were standardized, the actors having either executed the movement starting from a standing upright position or sitting on a chair. Besides, the viewpoints could differ from one point-light display to another depending on the movement performed and each point-light display was presented in two different viewpoints to increase the number of stimuli. The set of stimuli was composed of 84 point-light displays in total, including 42 normogravity displays and 42 microgravity displays. The 42 displays per condition corresponded to 21 different movements multiplied by the two viewpoints.

Experimental Design

The participants, lying inside the fMRI scanner, had to watch the point-light displays and indicate whether the movements were performed under normogravity or microgravity (**Figure 1**). The task consisted of three runs of 84 trials each. Each experimental run lasted approximately 11 min. A trial included a point-light display (3 s), followed by an instruction display asking whether the movement was performed on Earth (i.e., normogravity) or in Space (i.e., microgravity). The participants had 2 s maximum to respond as accurately as possible with either of two buttons on a keyboard corresponding to a green or red rectangle on the screen, with the former rectangle coding for a movement performed on Earth and the latter rectangle for a movement performed in Space. A fixation cross was then displayed during the inter-trial interval for an average of 3 s (range 1–12 s), obtained from exponential distribution (Hagberg et al., 2001). The order of the point light displays, including 42 normogravity and 42 microgravity displays per run, and the left-right locations of the rectangles on the screen were randomized across participants and across the three runs.

Stimuli were back-projected onto a semiopaque screen placed at the back end of the MRI tunnel. Participants viewed the displays through tilted mirrors placed over their eyes. They responded with their right index and middle fingers using an MRI-compatible response box. Responses (accuracy) were recorded using a custom software developed using LabVIEW (National Instruments, Austin, TX, United States). Before scanning, the participants had been instructed and had performed a few practice trials on a computer outside the scanning room to ensure understanding of the task.

fMRI Data Acquisition

The experiment was performed using a 3-T fMRI scanner (Medspec 30/80 AVANCE, Bruker, Ettlingen, Germany). EPI BOLD images were acquired over the three runs (i.e., three fMRI time series) with a T2* weighted gradient echo-planar imaging sequence [repetition time: 2133.3 ms; echo time: 30 ms; flip angle: 79.5°; 3 mm isotropic voxel size; reco matrix: 64 × 64; 32 interleaved axial slices with 1 mm gap; field of view (FOV): 192 mm × 192 mm]. The scanning planes were parallel to the anterior commissure/posterior commissure and covered the whole brain from the top of the cortex down to the base of the cerebellum. Structural MRI data was acquired using a standard T1-weighted scanning sequence of 1 mm^3 resolution (MPRAGE; repetition time: 9.4 ms; echo time: 4.424 ms; inversion time: 800 ms; FOV: 256 mm × 256 mm × 180 mm, reco matrix: 256 × 256 × 180) to allow anatomical localization of brain activation.

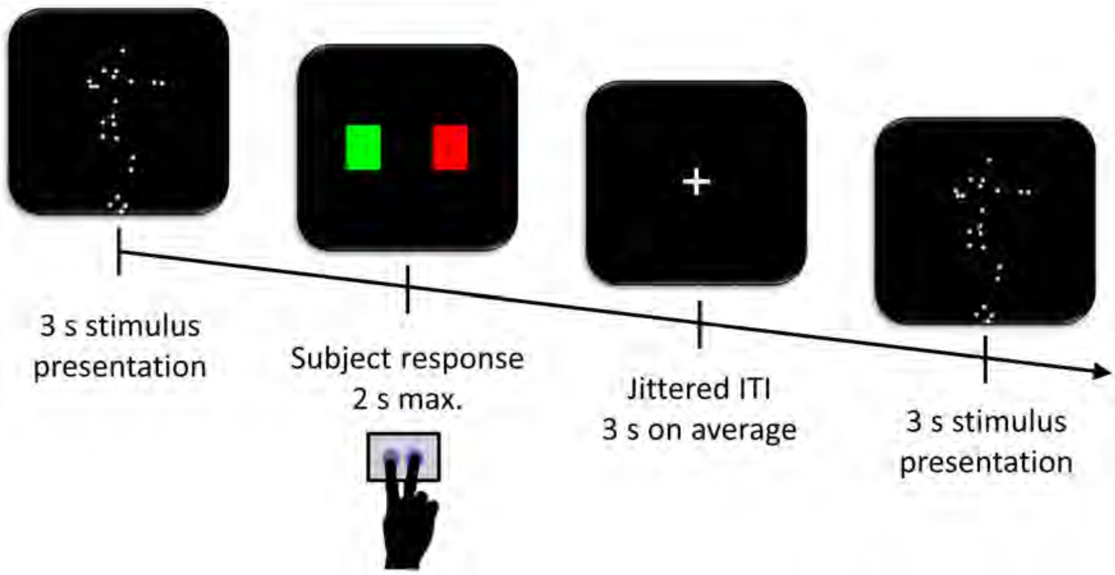

FIGURE 1 | Task procedure. Participants observed the display (either normogravity or microgravity) on the screen for 3 s and had 2 s afterward to indicate whether the movement was performed on Earth (green rectangle) or in Space (red rectangle). Trials were separated by a jittered inter-trial interval (ITI) with a mean duration of 3 s during which a fixation cross was displayed at the center of the screen.

fMRI Data Preprocessing and Analysis

Data preprocessing was conducted following the standard SPM8 (Wellcome Department of Imaging Neuroscience, London, United Kingdom[1]) workflow for fMRI (Friston et al., 1995; Henson et al., 1999). Each run consisted of 317 scans, including six dummy images of magnetic field saturation that were discarded before analysis. The remaining images were slice-time corrected. After discarding the last two volumes, these images were realigned to the first image of the time series (6-parameter rigid body) to correct for head movement between scans, and a mean realigned image was created. The realigned images were also "unwarped" to reduce residual movement-related variance (Andersson et al., 2001). Each structural MRI was co-registered to the corresponding mean realigned image, and normalized to a template in the stereotactic space of the Montreal Neurological Institute (MNI) by matching gray matter with a priori gray matter template (Ashburner and Friston, 2005). Normalization was then applied to the functional images before smoothing with a 6 mm × 6 mm × 6 mm Gaussian kernel. The absence of gross normalization errors was visually confirmed by an experienced operator for all participants. No excessive head motion were observed (i.e., cumulative translation and rotation <3 mm and 3° and mean point-to-point translation and rotation <0.15 mm and 0.1°).

Statistical analysis of the fMRI time series was based on the general linear model (GLM) approach (Friston et al., 1994, 1995). The GLM design matrix included the two gravity conditions (i.e., normogravity and microgravity), the instruction, and the fixation cross, which were modeled as boxcar regressors and were convolved with the canonical hemodynamic response function of SPM8. Furthermore, the design matrix also included the participant's realignment parameters, to regress out residual movement-related variance. Low-frequency drifts were removed from images using high-pass filtering (1/128 Hz). Contrasts of interest were defined at the first level of analysis (i.e., participant-level) to reveal areas coding for: (i) the perception of human movement in point-light displays (i.e., voxels where parameter estimates of the normogravity and microgravity regressors were significantly greater than baseline; labeled "normogravity > baseline" and "microgravity > baseline"); and (ii) gravity information embedded in the displays (i.e., voxels where parameter estimate of the normogravity regressor was significantly greater than that of the microgravity regressor, and inversely; labeled "normogravity > microgravity" and "microgravity > normogravity"). For contrasts (i), active voxels common to both normogravity and microgravity effects were identified by using the contrast "microgravity > baseline" as an inclusive mask of the contrast "normogravity > baseline." Exclusive masking was also conducted to reveal areas that might have been specifically activated by normogravity or microgravity, although results were insignificant (see Results section). Contrasts (ii), i.e., "normogravity > microgravity" and "microgravity > normogravity," were masked inclusively with the contrast "normogravity > baseline" to discard any voxel whose activation was unrelated to gravity information (i.e., voxel that can be considered as false positive). Individual contrast maps were then entered into a second level (random effect) full group GLM. It is worth emphasizing that identical results were obtained when implicit baseline (zero) was replaced by the weight of the regressor modeling the fixation periods (see Results section). With respect to group-level analyses, multiple comparisons correction of statistical maps was conducted using a

[1]http://www.fil.ion.ucl.ac.uk/spm/software/spm8

cluster-based extent thresholding for $p < 0.05$ (FWER-corrected) calculated based on the Gaussian random field method and following previous recommendations (Woo et al., 2014).

RESULTS

All participants were successful in performing the categorization task inside the scanner, with a good and similar categorization accuracy for both the normogravity and the microgravity displays as assessed using independent two-sample *t*-test (mean ± SD: 75.85 ± 7.55% and 72.98 ± 8.22%, respectively; $t = 1.15$; $p = 0.25$; $d = 0.36$).

As previously mentioned, a first analysis of the fMRI data consisted in identifying brain regions that were similarly activated during the observation of point light displays independently of whether the movements were performed under normogravity or microgravity. For this purpose, we identified voxels that were common to the contrasts "normogravity > baseline" and "microgravity > baseline," by masking inclusively ($p = 0.05$) the latter contrast with the former contrast (**Figure 2** and **Table 1**). Results indicated that the observation of point light displays moving either under normogravity or microgravity led to a widespread pattern of activity, with significant clusters of activation located in frontal, parietal and occipito-temporal regions. In particular, regions subtending the pattern of activity included the middle occipital gyrus, the lingual gyrus, the fusiform gyrus, the superior, middle and inferior temporal gyrus, the cuneus, the inferior and superior parietal lobules, motor-related areas (primary motor cortex, primary somatosensory cortex, pre-motor cortex, and pre-supplementary motor area) and the inferior frontal gyrus. On the other hand, neither the "normogravity > baseline" contrast nor the "microgravity > baseline" contrast revealed exclusive clusters of significant activation, as examined by looking for activated voxels in either contrasts while using an exclusive masking approach (i.e., "normogravity > baseline" masked exclusively by "microgravity > baseline," and inversely). Therefore, the networks subtending the perception of human movement under either microgravity or normogravity perfectly

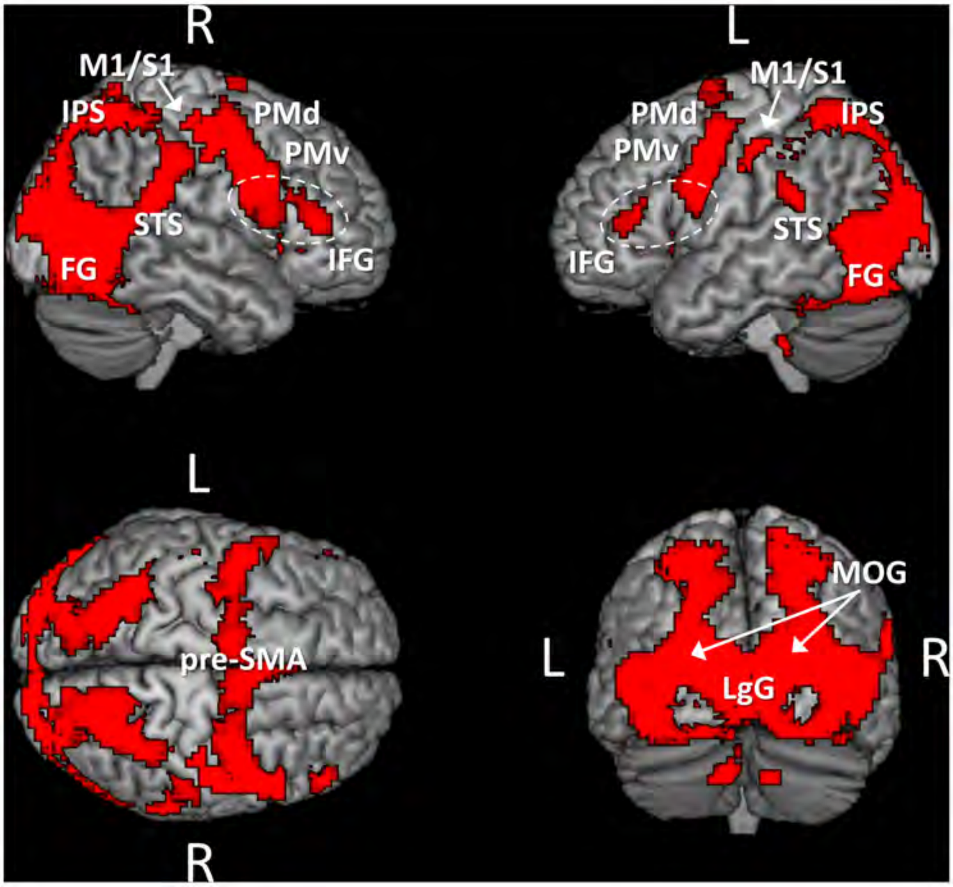

FIGURE 2 | Brain areas activated by the point light displays independently of the gravity condition. The activation pattern was obtained by masking inclusively ($p = 0.05$) the contrast "microgravity > baseline" with the contrast "normogravity > baseline." Activations are thresholded at $p < 0.001$ (uncorrected) at the voxel level and at $p < 0.05$ (FWE-corrected) at the cluster level. Abbreviations: M1/S1, primary motor and somatosensory cortices; PMd, dorsal premotor cortex; PMv, ventral premotor cortex; pre-SMA, pre-supplementary motor area; IFG, inferior frontal gyrus; IPS, intraparietal sulcus; STS, superior temporal sulcus; FG, fusiform gyrus; LgG, lingual gyrus; MOG, middle occipital gyrus.

TABLE 1 | Activated brain regions during the observation of point light displays, as obtained by masking inclusively ($p = 0.05$) the contrast "microgravity > baseline" with the contrast "normogravity > baseline."

Region	BA	Side	*X, Y, Z*	*Z*
Cluster #1: 6082 voxels				
Middle occipital gyrus	18/19	R	30, −87, 12	>8
			30, −84, 21	>8
		L	−30, −93, 12	>8
			−30, −87, 12	>8
			−27, −90, 15	>8
Fusiform gyrus	19	R	27, −75, −12	7.79
		L	−21, −81, −12	>8
Lingual gyrus	18	R	9, −87, −6	6.28
		L	−6, −87, −9	7.33
Superior temporal gyrus	22	R	60, −36, 21	7.69
		L	−62, −37, 20	6.06
Middle temporal gyrus	37	R	48, −63, 3	>8
		L	−42, −66, 6	>8
Inferior temporal gyrus	19	R	48, −62, −4	>8
		L	−45, −72, 0	>8
Cuneus	17	R	12, −96, 3	7.82
		L	−15, −96, 6	>8
Inferior parietal lobule	40	R	60, −35, 21	7.69
		L	−57, −38, 24	5.93
Superior parietal lobule	7	R	33, −60, 54	7.65
		L	−27, −57, 54	6.78
Cluster #2: 2069 voxels				
M1/S1	4	R	51, −12, 39	6.58
		L	−46, −14, 49	6.32
	3	R	54, −9, 48	5.72
		L	−50, −18, 40	5.05
PMd	6	R	39, −3, 51	6.40
		L	−27, −6, 48	7.25
PMv	6	L	−48, 3, 33	6.57
			−42, −3, 42	6.20
			−48, 0, 48	5.69
		R	33, −9, 48	6.33
			48, 0, 45	5.94
Pre-SMA	6	R	9, 12, 51	6.57
			6, 9, 54	6.28
			9, 0, 66	5.12
		L	−3, 9, 51	6.34
			−6, 6, 54	6.32
			−12, −6, 69	4.78
Insula	13	R	30, 24, 0	5.55
		L	−30, 21, 3	4.91
Inferior frontal gyrus	9	R	45, 3, 33	7.37
		L	−48, 6, 27	7.37
	46	R	51, 36, 12	4.79
		L	−45, 33, 15	4.81

For each region, MNI coordinates at the center of gravity are specified along with the corresponding Brodmann area (BA). Z-values refer to significant activation peaks at $p < 0.001$ (uncorrected for multiple comparisons). In addition, all reported regions were significantly active at $p < 0.05$ (FWE correction). Abbreviations: L, left hemisphere; R, Right hemisphere; M1, primary motor cortex; S1, primary somatosensory cortex. PMd, dorsal premotor cortex; PMv, ventral premotor cortex; pre-SMA, pre-supplementary motor area.

overlapped. Furthermore, the same network was identified when baseline was modeled as the fixation period. Significant clusters of activation were located along the regions previously identified, although the spatial extent of activation was reduced (Supplementary Figure S1).

In the second analysis, we identified regions where activation was modulated by the gravity condition of the displays (**Figure 3** and **Table 2**). Three significant clusters of activation were revealed by the contrast normogravity > microgravity. The first cluster was located in the primary motor cortex (BA 4). Activation also extended into the primary somatosensory cortex (BA 3), as shown in greater detail in Supplementary Figure S2. The other clusters belonged to the kinetic occipital region (BA 18) in both the right and left hemispheres. The reverse contrast, namely microgravity > normogravity, revealed increased activation of a cluster that included regions of the right and left lingual gyrus and cuneus.

DISCUSSION

The present study investigated whether gravity-related changes in movement kinematics is reflected through activation gradients in regions of the MNS, which would support the premise that coding of gravitational content in BM displays relies on motor resonance. For this purpose, we examined BOLD signal when participants were observing point-light displays of human movements performed under either normogravity or microgravity. The result is twofold: first, independently of the gravity conditions, the perception of human movement relied on a large-scale network that encompassed frontal (inferior frontal gyrus, motor-related areas), parietal (inferior and superior parietal lobules), and occipito-temporal (superior temporal sulcus region, inferior temporal gyrus, fusiform gyrus, lingual gyrus, middle occipital gyrus) regions, which is in keeping with previous findings on the perception of point-light BM (Vaina et al., 2001; Saygin et al., 2004); and second, gravity information modulated the activation of a restricted set of regions of the network including visual (kinetic occipital region, lingual gyrus, cuneus) and motor-related (primary motor and somatosensory cortices) areas. Notably, the portions of the primary motor cortex along with those of the primary somatosensory cortex were significantly more active when acceleration in the point-light displays was consistent with natural (Earth) gravity. Previous studies on the neuronal encoding of the kinematic laws of motion during both abstract (cloud of dots) motion observation (Dayan et al., 2007) and human action observation (Casile et al., 2010) already demonstrated a larger involvement of the motor-related regions in processing normal kinematics compared to perturbed kinematics. The authors proposed that cortical representations of motion are optimally tuned to the kinematic invariants characterizing biological actions, with discrimination of normal vs. abnormal kinematics being carried out via a motor-matching process of the observed movements onto the observer's motor system. The present finding adds to this view by providing evidence that compliance of gravity cues embedded in the kinematics of human motion with normal gravity is encoded

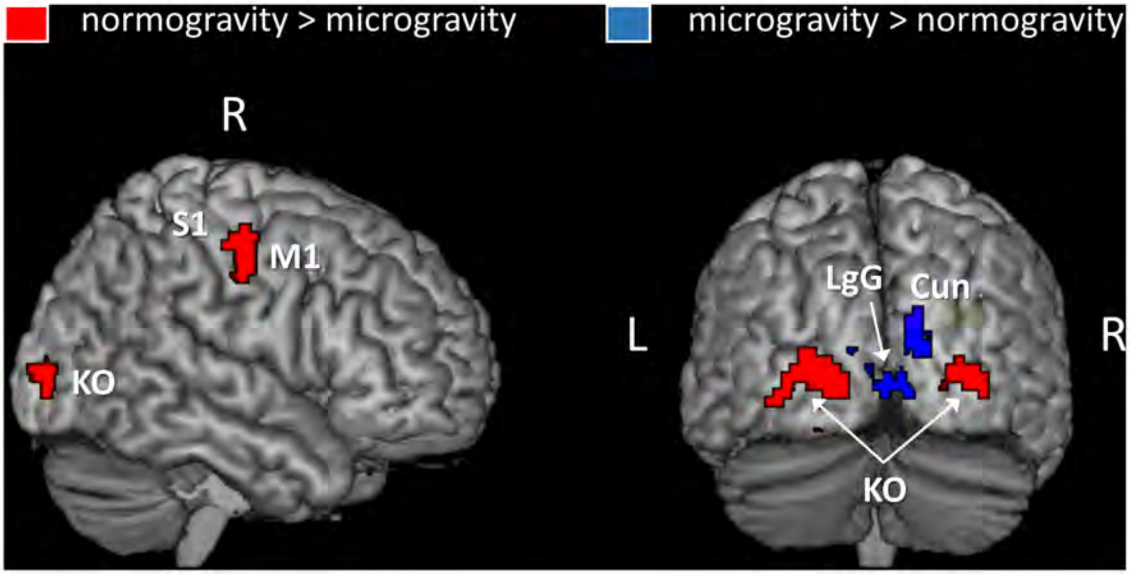

FIGURE 3 | Brain areas showing an activation gradient as a function of the gravity information embedded in the point light displays. Activations are thresholded at $p < 0.001$ (uncorrected) at the voxel level and at $p < 0.05$ (FWE-corrected) at the cluster level. An inclusive mask ($p = 0.05$, "normogravity > baseline" contrast) was applied. Abbreviations: M1, primary motor cortex; S1, primary somatosensory cortex; Cun, Cuneus; LgG, lingual gyrus; KO, kinetic occipital region.

TABLE 2 | Activated brain regions during the observation of normogravity displays vs. microgravity displays and microgravity displays vs. normogravity displays.

Region	BA	Side	*X, Y, Z*	*Z*
Normogravity > Microgravity				
Middle occipital gyrus	18/19	R	27, −90, 0	4.11
k: 101 voxels		L	−24, −93, 3	4.23
			−27, −83, −3	4.15
			−21, −99, 6	3.46
Motor-related areas (M1/S1)	4	R	51, −12, 39	4.18
k: 45 voxels			56, −8, 48	3.99
	3	R	54, −9, 48	3.98
Microgravity > Normogravity				
Lingual gyrus	18	R	9, −84, 3	4.01
k: 107 voxels		L	−9, −78, 0	4.29
Cuneus	17	R	12, −90, 18	3.77
			15, −93, 24	3.63
			18, −84, 18	3.54
		L	−3, −90, 6	3.98

For each region, MNI coordinates at the center of gravity are specified along with the corresponding Brodmann area (BA). Z-values refer to significant activation peaks at $p < 0.001$ (uncorrected for multiple comparisons). In addition, all reported regions were significantly active at $p < 0.05$ (FWE correction). Abbreviations: L, left hemisphere; R, Right hemisphere; M1, primary motor cortex; S1, primary somatosensory cortex.

in motor-related areas, possibly by transforming the visual inputs into the specific motor capabilities of the observer and thus coding the plausibility of actually performing the same movements.

Although the above result favors our hypothesis that the interpretation of gravitational cues embedded in BM relies on a mechanism of motor resonance, the primary motor and sensorimotor cortices are not classically considered to be part of the human MNS subtending motor resonance whose core regions are the inferior frontal/ventral premotor and posterior parietal areas (Rizzolatti and Craighero, 2004; Iacoboni and Dapretto, 2006). In particular, several studies that manipulated indirectly the kinematic characteristics of the movement, by contrasting the observation of natural as opposed to unnatural movements (Stevens et al., 2000; Tai et al., 2004; Costantini et al., 2005; Gazzola et al., 2007; Lestou et al., 2008), revealed a further involvement of these core regions in processing movement displays that conform with normal kinematics. However, there is growing evidence that mirror activity extends beyond brain regions identified as being part of the classical MNS, including the primary somatosensory cortex (Keysers and Gazzola, 2009; Molenberghs et al., 2012) and the primary motor cortex (Fadiga et al., 2005; Tkach et al., 2007; Dushanova and Donoghue, 2010). Thus, it is reasonable to assume that the individuals discriminated between normogravity and microgravity displays via the mirror property of these two regions, by simulating the motor commands and their sensory consequences for observed movements.

Such an implicit coding of gravity effects through motor resonance contrasts with a recent study by Maffei et al. (2015) where judgment on graviceptive information embedded in point-light BM is proposed to result from a predictive code generated by an internal model of gravity effects (whose primary sites are in temporo-parietal junction and insula) that is conveyed to the occipito-temporal cortex where it is compared to the incoming stimuli to produce a prediction error, and thereby an activation. Specifically, BM stimuli under a condition of abnormal gravity evoked stronger activation in occipito-temporal regions than BM stimuli under normal gravity. Studies on the recognition of BM when stimuli are displayed upside down (i.e., violation of physical gravity) reconcile this result with our own. Indeed, it was reported a sensitivity to the inversion effect in the occipito-temporal cortex (Grezes et al., 2001; Grossman and Blake, 2001; Pavlova et al., 2004; Peuskens et al., 2005) as well as in parietal (i.e., intraparietal sulcus) and frontal (i.e., caudal part of the middle/inferior frontal gyrus) areas (Grezes et al., 2001; Pavlova et al., 2004) that belong to the MNS. The discrepancy between results from Maffei et al. (2015) and ours may have to do with differences in the complexity of the portrayed movements. They used gait movements that are likely to be of lower complexity than the movements used in our experiment. Exploring gait movements and complex movements close to those used in our experiment, Jastorff and Orban (2009) showed enhanced activations by complex biological kinematics in the occipito-temporal cortex and in frontal regions belonging to the MNS, therefore suggesting that one destination of the BM signals is the occipito-temporal cortex and another destination is the MNS. Accordingly, gravity cues are likely coded in these two main loci of BM processing, with a hierarchy from the occipito-temporal cortex to the MNS as BM becomes more complex.

Another intriguing result was that gravity discrimination between displays also relied on visual regions known to be involved in form-from-motion perception, defined as the ability to extract the form of a stimuli entirely from motion cues. The kinetic occipital region, which was found to be more active for movements performed under normogravity, is selective to kinetic boundaries (Dupont et al., 1997; Van Oostende et al., 1997). In the case of point-light BM, Vaina et al. (2001) showed that this region integrates local motion of the light points with the goal of determining whether they altogether constitute the outline of a human silhouette. The lingual gyrus at the cuneus border, which was inversely found to be more active for movements performed under microgravity, is also involved in processing motion and deriving global form information in the perception of BM (Servos et al., 2002). Accordingly, variations of gravity information in point-light displays of human movement was likely also coded based on the familiarity of the human form reconstructed from the moving dots. Furthermore, the opposite pattern of activation found between the kinetic occipital region (i.e., more active in normogravity) and the lingual gyrus/cuneus complex (i.e., more active in microgravity) may indicate different functions in form-from-motion perception, the former region coding visual familiarity with the observed form-from-motion and the latter region visual unfamiliarity.

In sum, findings of the present experiment suggest that discrimination of point-light movements whose kinematic characteristics either did or did not comply with natural gravity was carried out by (i) mapping the movements onto the observer's motor system, and (ii) extracting the overall form of the movements from local motion of the moving light points. Such a dual-mechanism plausibly coded both the possibility of actually performing the same movements and the visual familiarity of the observer with the form defined by the movements. Therefore, judgment on graviceptive information embedded in point-light BM may not be restricted to accessing the internal model of gravity effects (Maffei et al., 2015), also relying on motor representations and visual knowledge of what is observed.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiment: P-YC, CS, MV, and CA. Subject recruitment and screening: P-YC, MV, and CA. Acquisition of data: FC, P-YC, CS, MV, and CA. Analysis and interpretation of data: FC, P-YC, JM, J-LA, MV, and CA. Drafting the work: FC, MV, and CA. Final approval of the work: FC, P-YC, J-LA, JM, CS, MV, and CA. Being accountable for the accuracy and integrity of the work: FC, P-YC, J-LA, JM, CS, MV, and CA.

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Sensation Seeking and Adaptation in Parabonauts

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Evidence from extreme environments suggests that there are relationships between difficulties of adaptation and psychological factors such as personality. In the framework of microgravity research on humans, the aim of this exploratory study was to investigate inter-individual differences of parabonauts on the basis of quality of adaptation to the physical demands of parabolic flights. The personality characteristics of two groups of parabonauts with a different quality of adaptation (an Adaptive group, $N = 7$, and a Maladaptive group, $N = 15$) were assessed using the Sensation Seeking Scale, Brief COPE, and MSSQ-Short. Compared to the Maladaptive group, the individuals of the Adaptive group scored higher on Boredom Susceptibility (i.e., a subscale of the Sensation Seeking Scale), lower on scales of susceptibility to motion sickness (MSSQ-Short) and tended to score lower on Instrumental Support Seeking (i.e., a subscale of the Brief COPE). These results suggest that individuals of the Adaptive group are more intolerant to monotony, present an aversion to repetitive and routine activities, are less susceptible to motion sickness and less dependent on problem-focused strategies. These characteristics may have contributed to developing a certain degree of flexibility in these subjects when faced with the parabolic flight situation and thus, may have favored them. The identification of differences of personality characteristics between individuals who have expressed difficulties of adaptation from those who have adapted successfully could help to prevent the risk of maladaptation and improve the well-being of (future) commercial or occupational aerospace passengers. More generally, these results could be extended to extreme environments and professional and/or sports domains likely to involve risk taking and unusual situations.

Keywords: adaptation, parabolic flights, microgravity, sensation seeking, coping strategies, motion sickness susceptibility, parabonauts' characteristics, Zero-G fliers

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INTRODUCTION

Described as mimicking spaceflight-associated conditions (e.g., Strewe et al., 2012), parabolic flights constitute the best ecological model on earth to investigate the effects of microgravity and/or different gravity transitions and, thus, to study human adaptation to these physical demands presented by the space environment. In fact, microgravity and gravitational changes that involve unique physical demands lead to perceptual mismatches between various information from the vestibular system on the one hand (i.e., canal-otolith conflict) and between information from the visual system and from the vestibular system on the other hand (i.e., visuo-vestibular conflict) (Reason, 1978; Benson, 2002; Baker et al., 2008; Schmäl, 2013). These sensory conflicts can induce maladaptation (i.e., motion sickness symptoms) and seem to affect people differently (Reason and Brand, 1975; Davis et al., 1988; Reschke, 1990; Lackner and DiZio, 2006; Golding et al., 2017). In fact, studies carried out in a microgravity environment have reported differences not only in the frequency of appearance of maladaptation but also in their severity (e.g., Davis et al., 1988; Benson, 2002; Golding et al., 2017). Consequently, these studies suggest the existence of individual differences faced with the physical demands of the unusual environment (i.e., microgravity and gravitational changes). Interestingly, it should be noted that ground-based studies in the context of a broader field of research have suggested an influence of psychological factors such as dispositional characteristics in adaptation to the physical demands of the environment (e.g., Collins and Lentz, 1977; Bick, 1983; Fox and Arnon, 1988; Gordon et al., 1994; Paillard et al., 2013). Among dispositional characteristics, personality has been studied in relation to the difficulties of adapting to a physical environment on earth (i.e., susceptibility to air sickness, seasickness, etc.). On the basis of several studies, evidence suggests a relationship between the characteristics of personality and difficulties of adaptation to a physical environment (Collins and Lentz, 1977; Bick, 1983; Gordon et al., 1994; Paillard et al., 2013). Moreover, studies on other extreme environments highlight the fact that personality could influence the extent to which an individual adapts effectively. Although each extreme environment contains unique physical (and social) demands, some characteristics such as emotional instability, high neuroticism, sensation seeking or anxiety seem to be unfavorable overall to adapting (Gunderson, 1974; Steel et al., 1997; Abraini et al., 1998; Palinkas et al., 2000; Palinkas and Suedfeld, 2008; Lafère et al., 2017). Consequently, personality plays a crucial role in the adaptation process. It can therefore compromise this adaptation process (e.g., Sandal et al., 1996; Palinkas et al., 2000; Bolmont et al., 2001; Bolmont and Collado, 2014).

In parabolic flight situations, recent studies have investigated the psychological factors affecting people participating in parabolic flights (i.e., parabonauts) in order to try to identify possible predictors of maladaptation on one hand (e.g., Choukèr et al., 2010; Strewe et al., 2012; Van Ombergen et al., 2016; Collado et al., 2017; Golding et al., 2017) and to better describe this specific population on the other hand (e.g., Collado et al., 2014; Montag et al., 2016). Most studies carried out on possible predictors of maladaptation signs have mainly focused on the situational characteristics of the voluntary participants (Choukèr et al., 2010; Strewe et al., 2012; Van Ombergen et al., 2016; Collado et al., 2017; Golding et al., 2017). It should be noted that few studies have investigated the dispositional characteristics of people participating in parabolic flight and have highlighted a specific personality profile characterizing parabonauts (Collado et al., 2014; Montag et al., 2016). Parabonauts appear to be stimulation seekers who are conscientious, emotionally stable, less anxious and who tolerate stress better than the general population (Collado et al., 2014) or than a control group (Montag et al., 2016). In their study, Collado et al. (2014) revealed that people attracted by parabolic flights scored higher on Extraversion and differed in four out of six NEO-PI-R facets of this domain (e.g., Activity, Excitement-Seeking, Positive Emotions). These distinctive facets suggest that voluntary participants have a more rapid pace of living, and need to be more stimulated by the environment than the general population. Given that parabonauts need to be permanently stimulated by their environment, and as suggested previously (Collado et al., 2014), it would be interesting to focus on sensation seeking in parabonauts in order to examine whether this dispositional characteristic can provide information on quality of adaptation to the physical demands of parabolic flights. Interestingly, a study on major affective disorders has shown that a sensation seeking pattern could predict hyperthymic temperament (Engel-Yeger et al., 2016), a personal disposition with "positive" traits such as being optimistic, fun-loving, confident, outgoing, jocular, on the go but also being a risk-taker (Akiskal et al., 2005). It should be noted that sensation seeking has been investigated in a recent parabolic flight study (Montag et al., 2016). However, the authors only assessed this dispositional characteristic in a control group without the possibility of considering parabonauts.

Considering the unique physical demands of parabolic flights that are likely to hinder adaptation and the involvement of the personality in the adaptation process on the one hand (Sandal et al., 1996; Palinkas et al., 2000; Bolmont and Collado, 2014), and given the involvement of personality domains in dispositional coping on the other (e.g., Costa et al., 1996; Watson and Hubbard, 1996; Ferguson, 2001), the main objective of this exploratory study is to identify differences in dispositional characteristics such as sensation seeking or trait coping strategies on the basis of the quality of adaptation (successfully adapted or not) to the physical demands of parabolic flights. In the present study, our hypothesis was that parabonauts who have expressed difficulties adapting could present differences in trait-coping strategies or subscales of sensation seeking that are likely to hinder their adaptation compared to parabonauts who have adapted successfully.

MATERIALS AND METHODS

Participants

The data presented in this study were drawn from a larger *ETAP-0g Project* study, which investigated behavioral, psychological, and physiological parameters during parabolic flights. Data was collected over 2 parabolic flight campaigns, scheduled between 2010 and 2011. The study was approved in advance by the CNES and the local institutional ethics committee (CPP OUEST II-Angers; approval no. 2007/18). Participants were informed about the experimental procedure and the parabola profile. They were also notified that they were voluntary, anonymous and that their data were protected by the applicable legislation. Each subject was then asked to fill out an informed consent form in accordance with the Declaration of Helsinki, and all participants provided this written consent before participating. The selection criteria for the *ETAP-0g Project* study were as follows: subjects had to be healthy men, with at least a second-year university level education, have no previous experience in parabolic flight, no history of severe motion sickness, no history of psychiatric, neurological or vestibular disorders, and comply with the medical requirements for parabolic flights. During the parabolic flight, all participants were only "subjects of the experiment" to the exclusion of any other role, and were assigned to perform the same tasks with relatively simple reaction times under the same conditions, i.e., to press a response-button as quickly as possible as soon as they perceived a stimulus (results presented below). Caffeine and alcohol were strictly prohibited 24 h before the beginning of the flight. No anti-emetics were used before or during the flight.

A total of 24 participants were involved in this study (mean age: 24.71 ± 4.88 year). In order to recruit a large group for this exploratory study, the sex variable was excluded. Because women respond to stress differently from men (e.g., intra-individual hormonal variability) and present more limitations for participation in parabolic flights (i.e., risk of pregnancy), only men were recruited to participate in this study during parabolic flights.

Assessment

Personality characteristics and susceptibility to motion sickness were assessed on the basis of forms filled out after the parabolic flights during the laboratory session (second phase) of the ETAP-0g Project. The objective was to limit response biases (i.e., to eliminate individuals who could respond in an overly desirable manner in order to be selected for this experiment).

Sensation Seeking Scale

Sensation seeking was assessed by the Zuckerman's Sensation Seeking Scale-V (Zuckerman et al., 1978; French version by Carton et al., 1992) which consists of 40 items in which participants have to choose between two statements per item. The Sensation Seeking Scale has four item subscales, each of them ranging from 0 to 10: (1) Disinhibition (i.e., adoption of socially "uninhibited" and extraverted behaviors, seeking stimulation through various sexual experiences or psychoactive substances), (2) Thrill and Adventure Seeking (i.e., a set of sports and activities that include a risk-taking dimension), (3) Experience Seeking (i.e., seeking an unconventional lifestyle and new sensory or intellectual experiences), and (4) Boredom Susceptibility (i.e., intolerance to monotony manifested by an aversion to repetitive and routine activities). In the present sample, the Sensation Seeking Scale-V was characterized by a Cronbach's alpha of 0.81. The subscales ranged from 0.51 to 0.71.

Brief COPE

Trait coping was assessed by the Brief COPE (Carver, 1997; French version by Muller and Spitz, 2003) which consists in a self-evaluation questionnaire about the usual way individuals deal with the stressors of everyday life. The Brief COPE consists of 28 items divided into 14 scales allowing assessment of 14 distinct dimensions of coping: (1) active coping, (2) planning, (3) using instrumental support, (4) using emotional support, (5) venting, (6) behavioral disengagement, (7) self-distraction, (8) self-blame, (9) positive reframing, (10) humor, (11) denial, (12) acceptance, (13) religion, and (14) substance use. Each items scored on a four-point scale and scores for each dimension have a range from 2 to 8. The Brief COPE was characterized by a Cronbach's alpha of 0.72. Internal consistencies of the questionnaire were satisfactory ranging from 0.58 to 1.00. Self-blame and Acceptance were excluded from analysis, because of low internal consistencies (i.e., <0.5).

MSSQ-Short

Susceptibility to motion sickness was assessed by the MSSQ-Short questionnaire (i.e., Motion Sickness Susceptibility Questionnaire Short-form, Golding, 1998; French version by Paillard et al., 2013). This short self-assessment questionnaire consists of two parts evaluating the general experience of motion sickness symptoms in childhood (before the age of 12 years, Child section A, i.e., MSA score) and in the last 10 years (section Adult section B, i.e., MSB score). The subject must indicate how often he has felt sick or nauseated in different situations (e.g., cars, trains, ships, swings, and roundabouts in playgrounds, fairground rides...) by varying their intensity on the following scale: "Not Applicable – Never Travelled," "Never Felt Sick," "Rarely Felt Sick," "Sometimes Felt Sick," and "Frequently Felt Sick." MSA and MSB raw scores range from 0 to 27 and add up to give a total MSS score that ranges from 0 to 54. Cronbach's alpha was 0.83.

Maladaptation/Adaptation

Maladaptation during parabolic flights was assessed after the flight by questionnaires listing potential symptoms of motion sickness (sweating, drowsiness, headache, stomach discomfort, nausea, vomiting), their frequency and severity. Self-reported symptoms in the form of observations by subjects were collected and compared to the experimenters' observations in order to check data consistency. Because this study was exploratory, frequency, and severity were only noted in order to remove any doubt about identification of the occurrence of adaptation difficulties. Data from two participants were removed from the analyses due to their inability to perform required reaction time tasks during parabolic flights. From this cohort (n = 22), two groups were constituted. The subjects who presented signs of maladaptation with at least one symptom of motion sickness constituted the Maladaptive group (25.33 ± 5.63 year; n = 15). The others formed the Adaptive group (23.00 ± 3.46 year; n = 7).

Procedure

Experiments were performed during parabolic flight campaigns (three flights per campaign) aboard the A300 ZeroG (Bordeaux International Airport, France). These flights are funded by the CNES (*Centre National d'Etudes Spatiales*: French national space research center) and organized by Novespace. They are run under the authority of the *Centre d'Essais en Vol*. The parabolic flights have a standard profile defined by Novespace. They each last about two and half hours (between 9:30 and 12:00) and consist in 30 experimental parabolas preceded by a preliminary test parabola. The parabolas are executed in sets of five with 90 s intervals between parabolas and with 4–8 min intervals between sets of parabolas. A parabolic flight maneuver is characterized by gravitational changes from 1 to 1.8G to 0G to 1.8G to 1G. Each change lasts approximately 20–30 s. Consequently, each parabola lasts approximately 70 s. The complete parabola is followed by 90 s of flight at 1G level.

Statistical Analyses

Shapiro–Wilk and Levene tests were used to check the normality of distribution and the homogeneity of variance respectively. Because the assumption of normality of distribution and/or homogeneity of variance was contradicted (except for demographic characteristics for which a Student test were applied), non-parametric analyses were conducted in order to determine whether differences existed between the groups. Comparisons between these groups were carried out using a Mann–Whitney U-test in order to determine whether differences existed in sensation seeking, coping strategies, and motion sickness susceptibility. Logistic regression was carried out to test the existence of possible predictors for the binary variable "successfully adapted" versus "not successfully adapted." Because this study was exploratory, and given the sample for all subscales tested that was too small, only the most relevant characteristics for which the inter-group difference was significant were considered. For all statistical analysis, we considered p-values less than 0.05 to be statistically significant.

RESULTS

Demographic Characteristics

The demographic characteristics of the Adaptive group and the Maladaptive group are presented in **Table 1**. No inter-group differences were found for age ($t = -0.63$; *ns,* $\eta^2 = 0.05$) and Trait-Anxiety ($t = -1.00$; *ns,* η2 = 0.02) assessed by the YA form of the Spielberger State-Trait Anxiety Inventory (i.e., STAI; Spielberger et al., 1983).

Sensation Seeking

The differences between the Adaptive group and the Maladaptive group on the Sensation Seeking Scale are presented in **Figure 1**. The results showed a significant difference in one out of four subscales. The Adaptive group scored higher than the Maladaptive group on Boredom Susceptibility ($U = 24.5$, $z = 2.01$, $p < 0.05$, $\eta^2 = 0.21$). No significant differences were found for Disinhibition ($U = 42.5$, $z = 0.71$, *ns*, $\eta^2 = 0.02$), Thrill and Adventure Seeking ($U = 43.5$, $z = -0.69$, *ns*, $\eta^2 = 0.05$), and Experience Seeking ($U = 37.5$, $z = -1.07$, *ns*, $\eta^2 = 0.04$).

Trait Coping

The differences between the Adaptive group and the Maladaptive group in the framework of the dimensions of the Brief COPE are presented in **Table 2**. A trend was observed for coping Using instrumental support. Individuals in the Maladaptive group scored higher than those in the Adaptive group ($U = 27$, $z = -1.94$, $p = 0.078$, $\eta^2 = 0.17$). No significant differences were found for Active coping ($U = 42.5$, $z = -0.75$, *ns*, $\eta^2 = 0.03$), Planning ($U = 47$, $z = -0.41$, *ns*, $\eta^2 = 0.02$), Using emotional support ($U = 43$, $z = -0.69$, *ns*, $\eta^2 = 0.04$), Venting ($U = 52$, $z = 0.04$, *ns*, $\eta^2 < 0.01$), Behavioral disengagement ($U = 34$, $z = -1.43$, *ns*, $\eta^2 = 0.07$), Self-distraction ($U = 45$, $z = 1.46$, *ns*,

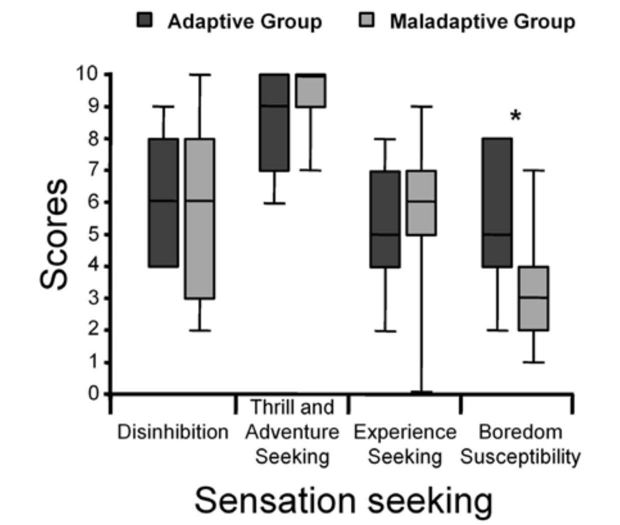

FIGURE 1 | Medians and interquartile ranges of Sensation Seeking subscales in the Adaptive group and the Maladaptive group. Significant differences between the scores of the Adaptive group and the Maladaptive group have been marked as follows: $^*p < 0.05$.

TABLE 1 | Demographic characteristics by group.

	Adaptive group (*n* = 7)	Maladaptive group (*n*= 15)
	Mean ± *SD*	Mean ± *SD*
Age (years)	23.00 ± 3.46	25.33 ± 5.63
Trait-Anxiety	34.71 ± 5.06	36.53 ± 6.84

Means and standard deviations for the Age and the Trait-Anxiety (STAI) scores of the Adaptive group and the Maladaptive group.

TABLE 2 | Comparison of Brief COPE between Adaptive group and Maladaptive group.

	Adaptive group (*n* = 7)	Maladaptive group (*n* = 15)	*p*-Value
	Median (IQR)	Median (IQR)	
Active coping	6.00 (6.00–6.50)	6.00 (6.00–8.00)	*ns*
Planning	6.00 (6.00–7.00)	6.00 (6.00–7.50)	*ns*
Using instrumental support	4.00 (4.00–4.00)	5.00 (4.00–6.00)	*ns*
Using emotional support	6.00 (4.00–7.50)	7.00 (6.00–7.00)	*ns*
Venting	6.00 (5.00–7.50)	6.00 (5.50–7.00)	*ns*
Behavioral disengagement	4.00 (3.50–4.00)	4.00 (4.00–5.00)	*ns*
Self-distraction	2.00 (2.00–2.00)	2.00 (2.00–2.00)	*ns*
Positive reframing	4.00 (3.00–4.50)	4.00 (3.00–5.50)	*ns*
Humor	5.00 (4.50–6.00)	5.00 (4.00–6.00)	*ns*
Denial	2.00 (2.00–2.00)	2.00 (2.00–2.00)	*ns*
Religion	2.00 (2.00–3.50)	2.00 (2.00–3.00)	*ns*
Substance use	2.00 (2.00–2.00)	2.00 (2.00–2.00)	*ns*

Medians and interquartile ranges (IQR) for the Brief COPE scores of the Adaptive group and the Maladaptive group.

$\eta^2 = 0.10$), Positive reframing ($U = 47.5$, $z = -0.37$, *ns*, $\eta^2 = 0.01$), Humor ($U = 46.5$, $z = 0.44$, *ns*, $\eta^2 = 0.02$), Denial ($U = 42$, $z = -1.24$, *ns*, $\eta^2 = 0.06$), Religion ($U = 48$, $z = 0.37$, *ns*, $\eta^2 < 0.01$), and Substance use ($U = 49$, $z = -0.68$, *ns*, $\eta^2 = 0.02$).

Motion Sickness Susceptibility

Differences between the Adaptive group and the Maladaptive group for the MSSQ-Short are presented in **Table 3**. The results showed significant differences in the MSA score (Childhood), the MSB score (Adulthood) and Total MSS score. Compared with the Adaptive group, individuals in the Maladaptive group scored higher on the MSA score ($U = 21$, $z = -2.26$, $p < 0.05$, $\eta^2 = 0.22$), the MSB score ($U = 16.5$, $z = -2.67$, $p < 0.01$, $\eta^2 = 0.16$) and the Total MSS score ($U = 17$, $z = -2.52$, $p < 0.05$, $\eta^2 = 0.22$).

Predictors of Maladaptation Signs

The total MSS score and the Boredom Susceptibility score were included in the logistic regression model. The logistic regression model ($\chi^2 = 13.26$, df = 2, $p < 0.001$; Nagelkerke $R^2 = 0.63$) gave 93.33% correct classification for "not successfully adapted" and showed that "not successfully adapted" was predicted by the Total MSS score ($p < 0.01$) and the Boredom Susceptibility ($p < 0.05$).

DISCUSSION

The objective of this exploratory study was to identify differences in dispositional characteristics such as sensation seeking or trait coping strategies according to the quality of adaptation (successfully adapted or not) to the physical demands of parabolic flights. Compared to the individuals in the Maladaptive group, those in the Adaptive group scored higher on Boredom Susceptibility (i.e., a subscale of the Sensation Seeking Scale) and lower on scales of susceptibility to motion sickness. A low level of Boredom Susceptibility and a high Total MSS score were found to predict Maladaptive group membership. No significant differences were found in the subscale of the Brief COPE, except for a trend in Instrumental Support Seeking (i.e., $p = 0.078$)—a problem-focused strategy that corresponds to seeking information, assistance and/or advice (Muller and Spitz, 2003) with a higher score for the Maladaptive group compared to the Adaptive group.

TABLE 3 | Comparison of Motion Sickness Susceptibility between the Adaptive group and the Maladaptive group.

	Adaptive group (*n* = 7)	Maladaptive group (*n* = 15)
	Median (IQR)	Median (IQR)
MSA score (Child section)	0.00 (0.00–2.25)	5.63 (1.75–7.71)*
MSB score (Adult section)	0.00 (0.00–0.00)	1.29 (0.50–2.50)**
Total MSS score	1.00 (0.00–2.25)	6.75 (2.50–8.92)*

Medians and interquartile ranges (IQR) for the Motion Sickness Susceptibility Questionnaire Short-form (MSSQ-Short) scores of the Adaptive group and the Maladaptive group. **Significantly different from the Adaptive group (p < 0.05).* ***Significantly different from the Adaptive group (p < 0.01).*

With respect to the scales of susceptibility to motion sickness (i.e., MSSQ), the Adaptive group showed a lower score in the raw score, the childhood and the adulthood section compared to the Maladaptive group. These results seem to be consistent with the distinction criteria applied in this study in order to separate individuals with adaptation difficulties from those who have successfully adapted to parabolic flight conditions. This distinction criterion was also confirmed by our logistic regression model which identifies the motion sickness raw score as a "not successfully adapted" predictor. Interestingly, a recent study conducted on the predictors of motion sickness in parabolic flights has shown that participants who vomited had significantly higher MSSQ scores, but concluded that the MSSQ failed as a vomiting predictor (Golding et al., 2017). In their study, Golding et al. (2017) used the binary variable "vomiting versus no vomiting" in their predictor model. In our study, as well as in a previous study (i.e., Collado et al., 2017), individuals who successfully adapted showed significant differences with individuals who manifested at least one symptom of motion sickness (e.g., sweating, drowsiness, headache, stomach discomfort, nausea, vomiting) and a not exclusively vomiting symptom. Thus, the binary variable "vomiting versus no vomiting" used by Golding et al. (2017) may not be sufficiently discriminating, and this distinction criterion could have been broader (presence or absence of motion sickness symptoms), given that our results supported this. Moreover, on the basis of Golding's average and conversions table (Golding, 1998), both groups of our subjects appear to be less sensitive than the general population average. This result agrees with previous studies conducted on motion sickness, which have shown low motion sickness susceptibility in participants in parabolic flights compared with the general population, and which have carried out a self-selection of the volunteers (Gaudeau et al., 2002; Golding, 2006; Golding et al., 2017). Nevertheless, although both groups are below Golding's average, there appear to be two levels of adaptation. Individuals with very low MSSQ-Short scores have adapted successfully to this particular situation, while the environment may have been too novel and disruptive for the others. Thus, it seems that individuals in the lowest 10th percentile of Golding's conversions table will have no trouble adapting to the demanding situation of parabolic flights.

As far as the Sensation Seeking Scale is concerned, the Adaptive group scored significantly higher in Boredom Susceptibility than the Maladaptive group. As far as the other subscales are concerned, it should be noted that the lack of any difference for Experience Seeking and Thrill and Adventure Seeking does not seem surprising for a population that had been described previously as sensation seekers (Collado et al., 2014; Montag et al., 2016). However, Boredom Susceptibility could be a more subtle behavioral characteristic that would have made individuals in the Adaptive group more dynamic and proactive in their sensation seeking. In fact, Boredom Susceptibility is described as an intolerance to monotony with an aversion to repetitive and routine activities (Zuckerman et al., 1978; Carton et al., 1992). Individuals with a high Boredom Susceptibility score would therefore be regularly looking for new activities.

According to Zuckerman (1971), this subscale "incorporates the need for change and variety more than any of the other factors." In our study, individuals of the Adaptive group may be more accustomed to seeking and experimenting with all sorts of new activities that are related to sensation seeking. This habit, which regularly exposes members of the Adaptive group to new situations, could have led them to develop a certain degree of flexibility when faced with the parabolic flights situation. Given that previous studies from a larger area highlighted the resilient characteristic of sensation seeking behavior (e.g., Engel-Yeger et al., 2016), our results may suggest a 'protective' effect on a particular subscale of Sensation Seeking (i.e., the Boredom Susceptibility). Thus, and as corroborated by the logistic regression result, such novelty seeking behavior could, in the context of parabolic flights, have favored the subjects in the Adaptive group to adapt compared to the individuals in the Maladaptive group.

Overall, the individuals who successfully adapted in parabolic flights appear to be more susceptible to boredom with an aversion to routine activities and less susceptible to motion sickness than individuals with difficulties adapting. These dispositional characteristics could have preserved individuals of the Adaptive group faced with the challenging and unusual parabolic flights situation. Although the difference was not significant, coping strategies also seem to distinguish both groups, which have clearly shown differences of symptoms manifested during parabolic flights. As parabolic flights constitute a particular situation in which it is difficult to have a direct action on the "problem" (i.e., different gravity transitions), use of a problem-focused strategy may not have been advantageous in this context. Nevertheless, caution is needed given the small samples of our study but also the use of an abbreviated version of a dispositional coping questionnaire. This result must be completed and refined in future studies with a larger sample and/or another trait coping questionnaire. Moreover, because the quality of adaptation in parabolic flights could be multifactorial, further data are required in order to investigate the involvement of other psychological parameters such as state coping strategies or motivation but also other characteristics such as degree of adaptation (parabolic) flight experience or gender. As part of the development and use of a potential tool to prevent adaptation difficulties in parabolic flights, the results of this exploratory study suggest that it is necessary to consider (1) out of all the motion sickness symptoms (not just vomiting), the presence of at least one characteristic symptom of motion sickness that may reveal the beginnings of difficulties and, (2) the dispositional characteristics of the candidate for parabolic flights. In addition, in future studies, it would be interesting to develop a more suitable tool in order to subtly detect the degree of adaptation difficulties under parabolic flight conditions. A tool like this could refine the detection of adaptation difficulties in parabolic flights and be used for future research. Moreover, in order to achieve a powerful statistical model of predictability, future investigations need to recruit a very large number of subjects. This would lead to substantial, homogeneous groups with different degrees of adaptation difficulties. Nevertheless, because very few studies have been conducted on the psychological aspects of parabonauts, this study enhances the database on the dispositional characteristics of parabonauts and could help to improve the selection of participants for experimental research and/or to adapt the design of future research but could also help prevent the risk of maladaptation and improve the well-being of (future) commercial or occupational aerospace passengers. Finally, the results of this present study primarily confirm the need to consider the quality of adaptation, which is likely to influence the behavior of individuals involved in parabolic flight studies, or more broadly, in extreme environments with high physical demands such as deep-sea diving or very high altitude expeditions.

AUTHOR CONTRIBUTIONS

AC, J-PH, VM, and BB: conceived, designed, and performed the research. AC: analyzed the data. AC and BB: contributed to the writing of the manuscript.

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Anxiety and Psycho-Physiological Stress Response to Competitive Sport Exercise

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Introduction: Sport is recognized as beneficial for health. In certain situation of practice, it nevertheless appears likely to induce a stress response. Anxiety is a stress response-modulating factor. Our objective is to characterize the role of anxiety in the stress response induced by a selective physical exercise.

Method: Sixty-three young male military conducted a selective sporting running event (a 8-km commando-walk) and were recorded the day before, the day of the race, and the day after. The variables were psychometric [personality questionnaires, coping and anxious/stress state, and physiological (nocturnal heart rate variability and actigraphy)]. The subjects were classified, using scores on anxiety questionnaires at baseline, into two groups according to their anxious (G ANX) or non-anxious (G N-ANX).

Results: Before the race, the G ANX was characterized by a lower level of self-esteem, higher scores in dysfunctional coping and a greater perceived stress compared to the G N-ANX. Compared to G N-ANX, the stress response to the exercise was higher in G ANX: G ANX exhibited (Selye, 1950) in immediate post-exercise, greater level in activation markers, and mental fatigue associated with a same level of physical fatigue and (Kim et al., 2018) in nocturnal post-exercise, an increase in sympathetic activation associated with a higher sleep fragmentation.

Conclusion: A competition selection sport exercise causes a stress response, particularly for anxious subjects. Anxious status could be involved in the risk of emergence of overtraining in sport practice. These results must be taken into account when sport practice is used for anxiety management.

Keywords: anxiety management, stress, selective physical exercise, special forces, overtraining

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INTRODUCTION

Stress is a non-specific and complex response of a human body submitted to a stressor, which responds to an adaptive function. It is described as the general adaptation syndrome (GAS) (Selye, 1950) and is divided into three stages: an initial alarm stage, followed by a resistance stage to the stressor, which lasts and to which the human body has to adapt to, and lastly a recovery stage.

The stressor characterizes any situation activating the stress pathway, regardless its nature, its depth and its duration (Selye, 1950). It can be external to the subject, imposed by an environmental change, or auto-generated by affects or negative-valence thoughts, especially anxious thoughts.

From a physiological point of view, if we can artificially consider all the stress players separately in regard to their very nature, it is important to consider them within their dynamics and their interactions. Strictly speaking, stress corresponds to the activation of the catabolic mechanisms: the activation of the corticotropic axis and of the sympathetic autonomic nervous system (ANS) and withdrawal of the parasympathetic ANS. The sympathetic ANS prepares the body to action when facing a stressor thanks to an increased mobilization of the energy resources of the body in order to support the alert reaction (flight or fight) and attention to the world. The corticotropic axis facilitates the availability of the body energy resources over time. Recovery is possible through anabolic pathways entailing in particular sleep and activation of the parasympathetic ANS. These pathways represent the link between the central nervous system and the periphery, allowing the body to act in a coordinated and adjusted manner. They allow the rating of the level of stress response of a body by peripheral physiological measurements.

The heart rate variability dynamically informs about the regulation of the balance between the sympathetic and the parasympathetic ANS (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Kim et al., 2018). When it is well-regulated (eustress), the stress expresses a physiological mechanism managing acute and chronic biological costs. A burnout situation will result if the stressor is too intensive and/or too long, or else if the stressed individual's response capacities are not adapted (distress). There is a strong inter-individual variability in the psychobiological reaction to a stressor, and it is identical within the same individual for the physical or psychic stressors (McEwen, 2007). The stress response, whatever the stressor, forms part of an earthly body whose limits are influenced by the genome and the history of the subject. These factors inherent to each subject constitute an endogenous limitation, which expresses the more or less big efficiency of the biological systems to cope with the demands imposed by the stressors. This biological efficiency is modulated by the *psyche* that is going to deal with an event as a stressor as soon as it is perceived as new, unpredictable, or else uncontrollable. As soon as an individual assigns one or several of these characteristics to an event, he perceives his/her resources as inappropriate to the perception he has of the coercion (Lazarus, 1993). The psychological factors participating in this inter-individual variability belong to two main categories: the moderators, which determine whether or not the response to stress will emerge (the most studied are the personality traits) (Baron and Kenny, 1986), and the mediators, which are going to modulate this response once it is implemented (we also speak about adjusting factors). Among these psychological factors, anxiety plays a major part. As for any psychic dimension, the trait (personality, moderator factor) must be distinguished from the state and the mood (mediator factor; Bolmont and Abraini, 2001). The anxiety trait is considered as a relatively stable emotional disorder that characterizes a personality manifesting through a sense of insecurity (Spielberger and Smith, 1966). The anxiety-state is a fear characterized by behavioral, physiological, and cognitive responses, which encompass those of stress but do not stop at them. The individuals with an anxious personality are particularly sensitive to emotional stimuli with a negative valence, which contribute to the risk of developing anxious pathologies. This sensitivity contributes to reducing the adaptation of anxious individuals to stressors. The anxiety trait is therefore considered as a moderator fostering the emergence of important and repeated stress reactions. The anxiety-state reflects a time period focused to anxious feeling relatively to a present or future meaningful context (Hainaut and Bolmont, 2006). So, if the anxious trait empirically allows to prejudge an anxious state, the opposite is less systematic as a result of the anxious state depending on its context. The anxiety context would express a dynamic emotional process that could include an anxious emotion or an anxious mood depending on its intensity and duration. The anxious state is considered as a stress reaction mediator positioning the perception of context as a *per se* actor of the dynamic to stress response. Numerous managements of anxiety do exist in terms of prevention and/or treatment. Practicing a physical activity, aerobic or anaerobic, has been put forward for a long time as an ecological method to reduce the anxiety-state whatever the level of anxiety-trait (Petruzzello et al., 1991; Anderson and Shivakuvar, 2013). Furthermore, the physical activity is of undeniable interest for the prevention and treatment of mental diseases in relation to anxiety (Anderson and Shivakuvar, 2013; Kumar, 2017). Beyond this, somatic benefits do exist, especially cardiovascular effects are benefits (Paffenbarger et al., 1986). It also plays a beneficial role on the stress-related diseases. These benefits are all the more important when the practice is regular (Petruzzello et al., 1991). The WHO recommendations suggest to practice a moderate activity (at least 3 h a week) or an intense activity (at least 20 min three times a week). However, some sport activities generate deleterious stress responses. In the context of a compulsory practice, the physical activity turns up to act as a stressor for the individual. Preclinical studies on rats undergoing rehabilitation show a stress response, which delays recovery when the rehabilitation exercises are compulsorily done (Ke et al., 2011). As far as humans are concerned, a compulsory sport practice also triggers a stress reaction (Nicolas, 2009; Ke et al., 2011). In the context of a regular practice, 13% of the sportsmen develop a state ranging from exhaustion to overtraining, and 7.8% of the military are addicted to sport (Tello et al., 2011). A need for performing a more-and-more frequent and more-and-more intense physical activity is observed, particularly among sportsmen characterized by a high anxiety-trait level (Cook and Hausenblas, 2008). In the context of competition, an anxiety is often present following a sports contest (Shabnam Hamidia, 2010). It is fostered by the existence of an anxiety-trait, -state and/or -mood and of a strong social desirability (Craft et al., 2003; Shabnam Hamidia, 2010). Finally, after a competitive effort, compared to a similar effort in a training context, a longer physiological recovery is observed, resulting in the persistence of a high sympathetic tone (Foster, 1998). Thus, practicing a physical activity in the context of an external and/or internal compulsory practice induces a stress response likely to be potentiated among the anxious sportsmen. This response could have consequences in terms of recovery among these patients. These data suggest that certain sport practices conditions might not be beneficial to the health, especially among anxious patients.

The military environment provides a model to explore these issues. As part of the army forces retention, the military institution imposes a regular physical activity practice to its staff for a minimum of 8 h a week. In this sense, soldiers are professional sportsmen. The purpose of this training is twofold: toughening the soldiers for the future struggles, but also preparing them to sports events selection, among which the commando march is the most common selection procedure used. This paradigm characterizes a selective physical exercise, intense in duration and in "intensity," which forms part of well-codified regular practice. It is legitimate under these elements to consider that the military sports selection is similar to competitions) and that it constitutes a practice setting likely to generate a stress response, especially among anxious staff members. We wish to study the psycho-physiological stress response in the military individual compelled to a selective commando march. The purpose of this study is to assess the impact of the anxious status on the dynamic of stress response following the commando march.

MATERIALS AND METHODS

Participants

Sixty-three voluntary French male soldiers (age: 20.3 ± 2 years; BMI 23.4 ± 2.3) were included in a prospective study. All were in initial training for the fusilier commando specialty, a French navy special force unit. The data collection was carried out on four commando training-courses that took place between May 2012 and January 2013. Three individuals were excluded from the analysis due to missing data; the individuals had not completed the submitted questionnaires. Chart 1 sums up the characteristics of these training-courses. This study was agreed by the Ouest six persons protection comity under the reference 2011-A01660-41. All the prospective subjects were informed about the conduct of this study and gave their written consent prior to their participation.

Physical Test

The experimental paradigm is an 8-km commando march in the minimum amount of time. This physical test specific to the military environment consists in a race dressed in combat gear with "rangers" type of shoes carrying an 11-kg backpack. As regards a qualifying event, the subjects were to perform the best possible time, the time for this event interfering in their ranking and in their final selection for the fusilier commando specialty. None of the subjects had already the experience of this event.

Physiological Variables

Night Heart Rate Variability

The heart rate was measured, thanks to a heart rate monitor (ActiHeart®, CE). It is a small (35 mm × 35 mm × 15 mm), light (16 g) self-adhesive device placed on the chest and connected to two self-adhesive ECG electrodes causing no discomfort. To isolate specifically the responsiveness of the sympathovagal balance, the calculation of the LF/HF (Low Frequency/High Frequency) ratio, was chosen as variable of interest (Kubios® software). The higher the value is, the more important the sympathetic activation is.

The Quality of Sleep

The quality of sleep is assessed thanks to a wrist actimeter (Wellness Wireless Watch VIVAGO EU644534000). The devices are light watches causing no inconvenience for the activities. The processed variables are the time asleep, the sleep efficiency, the sleep onset latency, and the sleep fragmentation (Wellness Wireless VIVAGO® software).

Psychological Variables

Three questionnaires have assessed the psychological dispositions (trait). The anxious personality was assessed by the anxiety-trait Spielberger questionnaire (State-Trait-Anxiety Inventory; STAI Y-B) (Spielberger, 1983). This self-assessment questionnaire consists of 20 items. For each item, the subject must indicate if he characterizes his usual anxious feeling from a Likert scale ranging from 1 (no) to 4 (yes). The average value is 41.9 ± 9.5 in a French population. A score greater than or equal to 47 is considered as being pathological (Bruchon-Schweitzer and Paulhan, 1993).

The self-esteem has been measured, thanks to the Rosenberg Self Esteem Scale (SES) (Rosenberg, 1979). This self-assessment scale measures, thanks to 10 items, the opinion one has about oneself, in a non-specific way. For each of the items, the subject must indicate if he characterizes his feeling from a Likert scale ranging from 1 (totally disagree) to 4 (totally agree). A score of less than 15 is considered as assessing a low self-esteem; a score between 15 and 25 is considered as assessing a normal self-esteem.

The stress adjustment, or "coping," has been measured by the Coping Inventory of Stressful Situations (CISS) (Endler and Parker, 1999). This questionnaire assesses via 48 items the way the subject usually copes with stresses. It allows to assess the subject in the three types of coping: the task focused coping, the emotion focused coping, and the passive coping (avoidance, social distraction, and diversion). The average value in the population is of 58.6 ± 10 for the task focused coping, 39.2 ± 11.5 for the emotion focused coping, 38.1 ± 9 for the avoidance focused coping, 17.5 ± 5.5 for the distraction focused coping, and 13.3 ± 4.1 for the social diversion type coping (Trousselard et al., 2010).

Three Questionnaires Assess the Psychological State

The anxious state was assessed through the Spielberger's state-anxiety questionnaire (STAI Y-A). This self-questionnaire includes 20 items. For each of these items, the subject must indicate if he characterizes his anxious feeling while filling in the questionnaire from a Likert's scale ranging from 1 (no) to 4 (yes). The average value is of 35.7 ± 10.3 in a French population. An equal or higher score than 41 is considered as being high (Bruchon-Schweitzer and Paulhan, 1993).

The subject's mood was measured by the *Profile of Mood State* (POMS) (Shacham, 1983). This self-questionnaire assesses, by the means of 37 adjectives, the different states of mood of a subject and their fluctuation (repetition of questionnaires). The subject must indicate how this adjective reflects his state from a Likert's scale ranging from 1 (not at all) to 4 (extremely). The adjectives gather in six factors defining six states of mood, which are anxiety, depression, confusion, anger, fatigue, and vigor. This questionnaire, assessing the state of mood of a subject at the moment when he answers the different items, has a transitional value.

The perceived stress was assessed through Cohen's perceived stress scale [*Perceived Stress Scale* (PSS)] (Cohen et al., 1983). This self-questionnaire includes 14 items. For each of the items, the subject must indicate his perception of stress while filling it in from a Likert's scale ranging from 1 (never) to 4 (often).

Experimental Procedure

Each of the trainings followed an identical protocol including four sessions and was organized around the commando march challenge, which systematically started at 10 a.m. **Figure 1** sums up the experimental protocol with the distribution of the four sessions over time: at 5 p.m. on the day before the challenge (inclusion session; D-1), in the morning just before the challenge (pre-challenge session; prechallenge D1), at the end of the challenge (post-challenge session; post-challenge D1), and at 9 a.m. on the day after the challenge (recovery session; D+1).

All fusilier commando training, participants benefited from a medical examination during the week before the beginning of the training assessing their medical fitness to do it. On D-1, the subjects filled in the following questionnaires: socio-biographic and sports practice, self-esteem (EES), anxiety-trait and -state (STAI Y-A and-B), mood (POMS), coping (CISS), perceived stress (PSS). During the D1 pre-challenge and post-challenge sessions and D+1, the subjects filled in the anxiety-state and mood questionnaires.

The heart rate variability and actimetrics data were collected in $N - 1$ and $N + 1$ on a recording time of 6 h for all subjects. This period was established within an 11 p.m. to 5 a.m. timetable corresponding to the moments when the subjects were all in bed.

Statistical Analysis

The data were registered under Excel 2010 (Microsoft®, Redmond, WA, United States) and analyzed with Statistica V7.1 (Statsoft®, France). The discrete variables were compared through the Chi^2 test or through the Fisher's exact test when the conditions for the Chi^2 use were not completed. The continuous variables, presented by their average ± standard deviation, were compared by variance analyses (ANOVAs). A principal components analysis pre-treatment was conducted in order to assess the relevance of the anxiety psychometric data reduction collected in the inclusion (anxiety-trait, -state, -mood baselines) with less descriptors. The results of the analysis isolate one single factor of own value above 1 (λ = 2.22) and which explains 73.9% of the variance. The three variables used have a positive force on this factor, meaning that they have the same meaning: the most anxious subjects express high scores in the three anxiety questionnaires taken into account. The pre-treatment

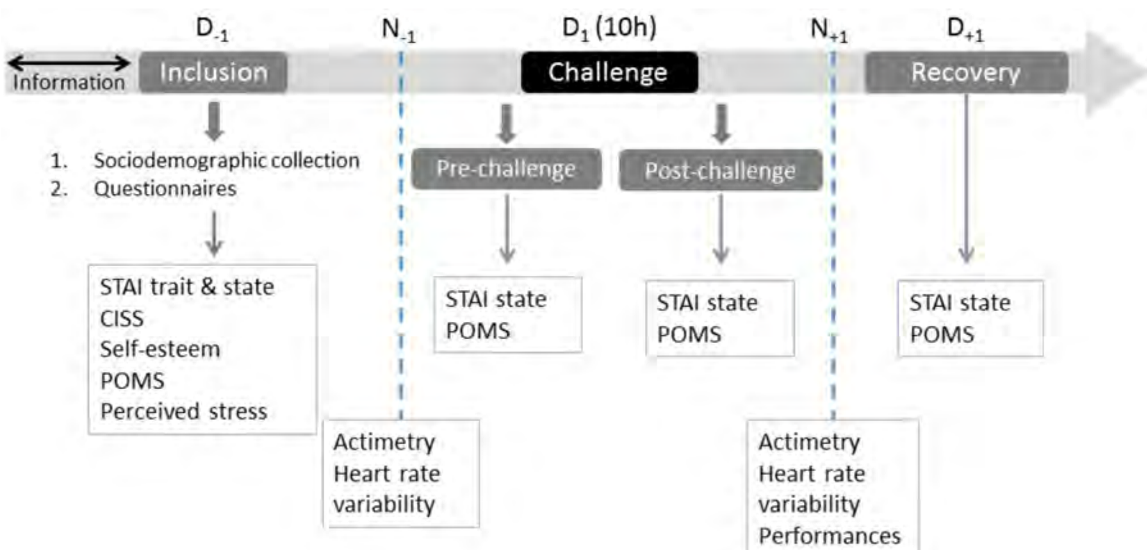

FIGURE 1 | Experimental protocol with the distribution of the four sessions: day before the challenge (inclusion session; D_{-1}), morning just before the challenge (pre-challenge session; pre-challenge D_1), end of the challenge (post-challenge session; post-challenge D_1), and day after the challenge (recovery session; D_{+1}).

performed was complemented by a distribution of subjects relatively to the anxiety-trait, -state, -mood variables, collected in the inclusion, through the clustering technic (K-mean) in two groups allowing to characterize an anxious group (G ANX) composed of 17 subjects and a non-anxious group (G N-ANX) composed of 47 subjects. In all cases, we considered that a difference was significant as soon as $p < 0.05$.

RESULTS

Study Population

Table 1 sums up the bio-demographic characteristics of the subjects. The night-time physiological data collected in $N - 1$ are summed up in **Table 2**. No difference was found between the trainings on each of the physiological variables. The psychological profile in the inclusion is summed up in **Table 3**. No difference is found between the trainings on the psychological variables collected.

Effect of the Commando March on the Population

All the trainees completed the commando march. The average performance was of 54.71 (±8.85) minutes. The psycho-physiological responses to the commando march show a post-challenge stress reaction. Physiologically, a post-challenge night-time sympathic activation is noticed: comparatively to $N - 1$, the LF/HF index in $N + 1$ is higher ($F = 6.77$; $p = 0.01$), and the objective sleep is of less good quality in terms of sleep quality ($F = 11.03$; $p = 0.002$), and of sleep onset latency ($F = 6.02$; $p = 0.02$). Psychologically, we notice that the anxiety-state is the highest in the D1 post-challenge ($F = 11.54$; $p < 0.001$) and that the negative moods scores are the highest [fatigue ($F = 7.32$; $p < 0.001$); depression ($F = 7.59$; $p < 0.001$); and anger ($F = 11.48$; $p < 0.001$)]. In D + 1, these scores are not different from the values collected in D − 1 and D1 pre-challenge.

TABLE 1 | Sociodemographic characteristics of the subjects.

Mean age in year (standard deviation)	20(±2)
Military seniority (months)	1 [1 − 2]*
Educational level (%)	
- Undergraduate studies	32
- Graduate studies	60
- Post-graduate studies	5
Reported stressfull event (%)	10
Levels of sport (%):	
- Recreational	49
- Regional	14
- National	10
Monthly hours of sport practice	9 [1, 75–20]*

**For non-Gaussian distribution values, the description is presented by the median (first–second interquartile).*

TABLE 2 | Nocturnal physiological data collected in N − 1 session.

Variables		Inclusion
Nocturnal Actimetry	Mean (±SD) sleep duration (min)	407(±42)
	Micro-awakening duration (min)	45
	Sleep efficiency index (%)	89
	Sleep fragmentation index (%)	30
	Mean (±SD) easiness to go to sleep (min)	2
Mean (±SD) nocturnal heart rate variability (LF/HF)		1.32(±0.77)

SD, standard deviation; Min, minutes; LF/HF, low frequency/high frequency.

TABLE 3 | Psychological scores in the inclusion session (D_{-1}).

Variables	*M* (±SD)
Self-esteem	32.8 (±5.5)
Coping:	
– Task	57.4 (±9.3)
– Avoidance	37.2 (±10.7)
– Emotion	43.7 (±12.3)
– Distraction	17.9 (±6.8)
– Social diversion	16.6 (±5.4)
Anxiété:	
– Trait	33.8 (±8.6)
– Etat	30.5 (±8.1)
Perceived stress	31.5 (±5.9)
Mood:	
– Tension/anxiety	3.1 (±3.6)
– Activity/vigor	12.3 (±5.2)
– Fatigue	2.4 (±2)
– Depression	0.7 (±1.3)
– Angor	1.3 (±2.2)
– Confusion	1.2 (±1.8)

M(±SD), mean (±standard deviation).

Anxious Cluster Effect in the Inclusion (G-ANX vs. G N-ANX) Onto the Psycho-Physiological Responses to the Commando March

Anxious Cluster Effect Onto the Inclusion Psycho-Physiological Variables

No difference is found between both groups neither in terms of average age, marital status, school level, nor in terms of hours of sports training during the previous month ($p > 0.05$). The G-ANX trainees revealed more stress events throughout their life than the G N-ANX trainees ($X^2 = 5.45$; $p = 0.02$). No difference is found in the inclusion ($N - 1$) neither on the nocturnal actimetrics variables, nor on the nocturnal heart rate variability. No difference is found between both groups in terms of performance ($p > 0.05$). **Table 4** sums up the differences observed in psychological terms between both groups.

Anxious Cluster Effects Onto the Physiological Responses of the Commando March

Regarding the LF/HF heart rate variability index, there is a session effect [LF:HF is higher at $N + 1$ (recovery) comparatively to

TABLE 4 | Differences observed in mean psychological scores between both groups in the inclusion session (D_{-1}).

Variables		G ANX *M* (±SD)	G N-ANX *M* (±SD)	*p*
Population size		**17**	**43**	
Self-esteem		30.3 (±6.1)	33.8 (±5)	0.03
Coping	CISS task	54.2 (±10.9)	58.4 (±8.7)	0.15
	CISS emotion	48.2 (±9.6)	33.6 (±8.5)	0.00
	CISS avoidance	49.5 (±12)	41.5 (±11.9)	0.03
	CISS distraction	20.2 (±6.5)	17 (±6.9)	0.13
	CISS social diversion	18.2 (±6.8)	15.9 (±4.7)	0.16
Perceived stress		36.7 (±4.2)	29.4 (±5.2)	0.00
Mood	Vigor	10.9 (±6)	12.7 (±4.9)	0.24
	Fatigue	2.9 (±1.6)	2.2 (±2.1)	0.21
	Depression	1.8 (±1.8)	0.3 (±0.7)	0.00
	Anger	2.6 (±2.5)	0.8 (±1.9)	0.00
	Confusion	2.4 (±2.4)	0.6 (±1)	0.00

G-ANX, anxious group; G N-ANX, non-anxious; M, mean; SD, standard deviation; CISS, Coping Inventory Stress Scale.

$N - 1$; $F = 25.68$; $p < 0.001$] without any effect on the anxious status ($F = 0.73$; $p = 0.39$]. The significant interaction between the session factor and the anxiety status shows that the LF/HF index is higher at $N + 1$ for the G ANX comparatively to the G N-ANX ($F = 13.45$; $p < 0.001$). Regarding the quality of sleep, it is noticed that the fragmentation index is higher at $N + 1$ (recovery) comparatively to $N - 1$ ($F = 8.5$; $p = 0.006$). The significant interaction between the session factor and the anxiety status shows that the fragmentation index is higher at $N + 1$ for the G ANX comparatively to the G N-ANX ($F = 5.64$; $p = 0.02$).

Anxious Cluster Effects Onto the Psychological Responses Following the Commando March

Regarding the anxiety-state, there is an effect of the session ($F = 5.97$; $p < 0.001$) with an anxiety-state score higher at D1 post-challenge comparatively to the other sessions. There is an anxious status effect (G ANX et G N-ANX; $F = 41.95$; $p = 0.39$) with an anxiety-state score higher for the G ANX comparatively to the G N-ANX. The significant interaction between the session factor and the anxious status shows that the anxiety-state score is higher in D1 post-challenge comparatively to the other sessions for the G N-ANX ($F = 4.17$; $p = 0.006$).

Regarding the tension-anxiety mood, there is an effect of the session ($F = 6.79$; $p = 0.002$) with a tension-anxiety score higher in D+1 (recovery) comparatively to the other sessions. There is an effect of the anxious status (G ANX et G N-ANX; $F = 18.67$; $p < 0.001$) with a tension-anxiety score higher for the G ANX comparatively to the G N-ANX. No interaction between the factors is noticed ($F = 1.75$; $p = 0.15$).

Regarding the activity-vigor mood, there is an effect of the session with a lower activity-vigor score at D1 post-challenge comparatively to the other sessions ($F = 10.09$; $p < 0.001$) and an anxious status effect with an activity-vigor score lower for the G ANX comparatively to the G N-ANX ($F = 4.02$; $p = 0.04$). A trend toward an anxious session–status interaction is noticed ($F = 2.1$; $p = 0.1$). For the G ANX, the activity-vigor score tends to be stable between the sessions whereas for the G N-ANX, it is lower at D1 post-challenge comparatively to the other sessions.

Regarding the fatigue mood, there is a session effect with a higher score in D1 post-challenge comparatively to the other sessions ($F = 10.09$; $p < 0.001$), without any effect of the anxious status ($F = 2.17$; $p = 0.14$), and without any interaction between the session factor and the anxious status ($F = 0.25$; $p = 0.85$).

Regarding the depression mood, we notice that the score is higher in D1 post-challenge comparatively to the other sessions ($F = 8.09$; $p < 0.001$), and that it is higher for the G ANX comparatively to the G N-ANX ($F = 13.06$; $p < 0.001$).

Regarding the anger mood, we notice that the score is higher in D1 post-challenge comparatively to the other sessions ($F = 9.87$; $p < 0.001$) and that it is higher for the G ANX comparatively to the G N-ANX ($F = 8.67$; $p = 0.004$).

Regarding the confusion mood, the score is higher in D1 post-challenge comparatively to the other sessions ($F = 11.03$; $p < 0.001$). The score is higher for the G ANX ($F = 28.75$; $p < 0.001$). A trend toward an interaction is observed between the session factors and the anxious status ($F = 2.1$; $p = 0.1$). For the G N-ANX, the confusion score tends to be stable between the sessions whereas for the G ANX, it is higher in D1 post-challenge comparatively to the other sessions. **Figure 2** sums up the differences between the groups over the mood variables during the different sessions (D-1, D1 pre-challenge, D1 post-challenge, and D+1).

DISCUSSION

The purpose of this study was to assess the reaction to stress induced by a sports challenge with a strong commitment and the recovery the day after the challenge according to the subjects' anxious status. First, it confirmed the stressor role of the selecting challenge played by the commando march. The increase of the activation psychological markers (anxiety, anger moods) was associated with physical fatigue (fatigue mood) and mental fatigue (depression mood). The psychological recovery was observed the very next day after the exercise. The night that followed the exercise allowed to assess the

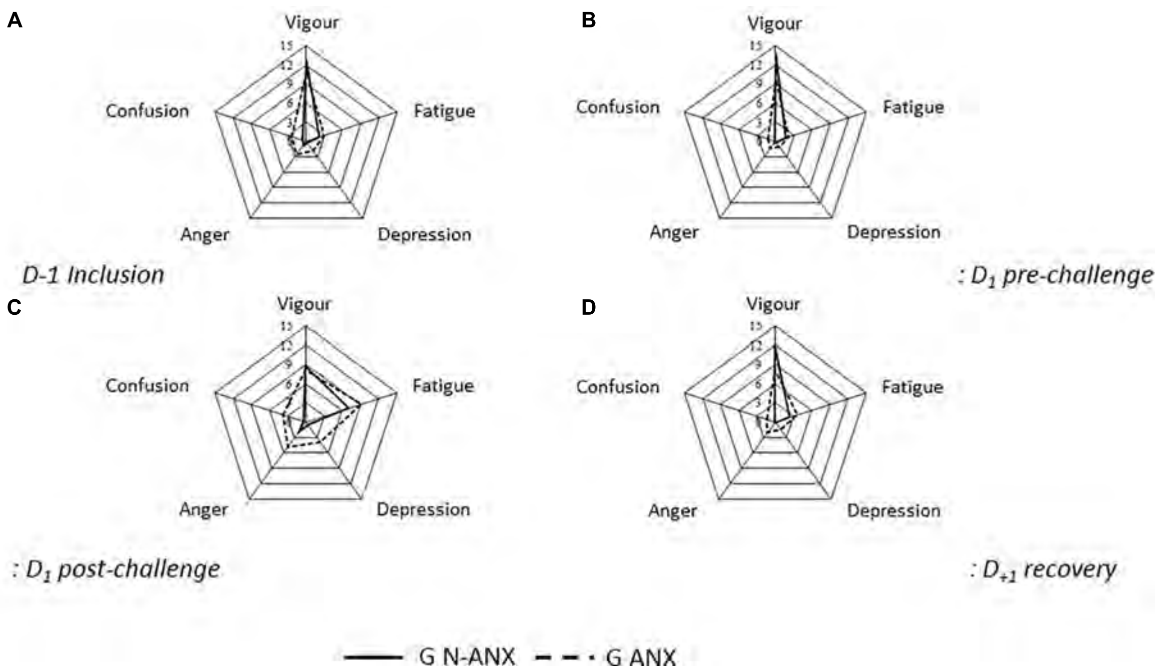

FIGURE 2 | Differences between the groups over the mood variables during the different sessions. **(A)** D_{-1} inclusion, **(B)** D_1 pre-challenge, **(C)** D_1 post-challenge, **(D)** D_{+1} recovery. G-ANX, anxious group; GN-ANX, non-anxious group.

recovery. A night-time parasympathetic rebound (Myllymaki et al., 2012) and a quality of sleep with few awakenings reflect the quality of the anabolic response of the sleep in the post-exercise recovery (Schall et al., 2013). The results show that the sleep was not restorative as shown by the night-time heart rate variability and the deterioration of the sleep quality. These observations contrast with the data showing that the isolated or regular physical exercise practice is an important factor to promote the sleep (Youngstedt et al., 1999). The disruption of the post-exercise night-time recovery is all the more important to consider that the subjects enrolled in the study are characterized by a protective psychological profile: high level of self-esteem and low level of anxiety-trait. The adjustments strategies show an adaptive pattern relatively to the general population, the subjects being characterized by a high level of on-task coping, and a moderate level of emotional coping.

Second, in our study, the population was segregated into two subgroups according to the anxious status and by taking into account both the anxious personality and the dynamic process induced by the commando march (anxiety-state and -mood). This segregation showed that the subjects of the anxious group were characterized by a lower level of self-esteem and a higher level of coping focused on emotion and avoidance. It also had implications on the stress response at different steps of the exercise. Before the challenge, the anxious subjects expressed a higher level of perceived stress as well as an increase of the activation psychological markers (anxiety, anger moods) and of mental fatigue (depression and confusion moods) for a same level of physical fatigue (fatigue mood). If this state reflects an anticipatory anxiety in relation to previous experiences, this reactivity before the exercise raises questions the memorization of previous physical exercises (Stranahan et al., 2008) and the way he anticipates the level of constraint (Dishman et al., 2000). A neurobiological profile with an increase of reactivity to a stressor was put forward (Pruessner et al., 2005). It combines a reduction of the hippocampus volume in a functional MRI, an increase of the reactivity of the corticotropic axis, and a reduction of the self-esteem. The pertinence of this profile requests to be assessed within the frame of an excessive reaction to stress when facing a sport event with a strong commitment. Immediately after the exercise, the anxious status was characterized by the maintenance of the psychological markers of activation (anxiety, anger) and of mental fatigue (depressive and confused mood) at a higher level than the non-anxious status. However, the anxious status did not have any impact neither onto the physical performance nor onto the fatigue.

Lastly, the night-time recovery is of less good quality in the anxious subjects than in the subjects with a non-anxious status. Hughes et al. (2006) have shown that the existence of depressive symptoms were slowing down the recovery after an exercise. Thus, the stress activation excess in peri-exercise was associated with an anomaly of its extinction in the recovery phase. In the end, these data suggest that in subjects with an anxious status, practicing an implying activity generates an inadequate stress reaction. This excessive reactivity concerns on the one hand the modalities of sports practice in the treatment of anxiety. The physical activity modalities in these treatments must focus on promoting a practice without generating neither an internal nor an external obligation in the anxious subjects. On the other hand, the excessive reactivity questions in the longer term about the consequences of a repeated and constraint physical activity onto these subjects' psychological and physical health. In particular, it questions the role that the anxious status could have in the risk of emergence of an overtraining syndrome. This syndrome, which is characterized by a performance level decrement without any modification of the investment in the sports practice, is clinically expressed in very different ways: anxiety, depression, fatigue, anger, lack of confidence, insomnia (Kentta and Hassmen, 1998). The absence of any clinical sign pathognomonic for an overtraining state, is associated with a difficulty to extract specific biological signs from it (Lee et al., 2017). However, the repetition of excessive stress reactions was put forward as contributing to a risky sports practice (Angeli et al., 2004). The repeated stress would induce a functional problem likely to lead to a dysfunction. The overtraining could be considered as a stress pathology expressing the impossibility for an individual to manage his stress response to the minimum required by the demand. These costs fall within the framework of the allostasis theory, which characterizes the recovery process or not of the homeostasis in the presence of constraint (Charney, 2004). The evaluation of the anxious status would allow to improve the detection of subjects at highest risk complementary to the overtraining questionnaire. The latter does not allow the systematic detection of the most motivated subjects who are the most vulnerable (Maso et al., 2005): these subjects, who barely listen to the body alarm signals, will meet any decrease of performance by an increase of training. This description fits in with the absence of impact of the anxious status onto the physical fatigue experienced after the commando march. Taking this status into account could be necessary to determinate the training programs for regular sportsmen. These data are all the more important that the consequences of overtraining can be dramatic for a sports career.

This study has shown two main limitations. Even though no difference in the main variables was observed over the four training sessions organized in different seasons, an impact of the seasonality onto the mood cannot be excluded (Kurlansik and Ibay, 2012). Then, the study has only included male subjects, which implies reproducing these results in a female population.

CONCLUSION

The commando march constitutes a military paradigm of sport under constraint that can be transposed to the civilian environment within the frame of a selective or competitive sports practice. The results obtained have applications for the health of both civilian and military sportsmen. They clearly raise the question of taking into account the anxiety in the sports practice programming. When the anxiety is taken into account, the modalities must detect any practice under constraint. For regular sportspeople, the modalities must integrate the anxious status in the programs of the sessions' repetition. These results highlight the interest for coupling the physical activity to stress and anxiety management techniques whether on the occasional sportsman, the regular sportsman or the competitor.

AUTHOR CONTRIBUTIONS

ES, ZF, FC, and MT were involved in the conception and trial design. GT, ES, CM-K, and MT wrote the draft of the article. ES, ZF, FC, CM-K, and MT contributed to the refinement of the study protocol and provided expert insight. ES and FC were responsible for the ethics committee. All the authors were involved in final approval of the manuscript.

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Psychological and Physiological Biomarkers of Neuromuscular Fatigue after Two Bouts of Sprint Interval Exercise

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The main aim of our study was to determine whether a repeated bout (RB) (vs. first bout [FB]) of sprint interval cycling exercise (SIE) is sufficient to mitigate SIE-induced psychological and physiological biomarker kinetics within 48 h after the exercise. Ten physically active men (age, 22.6 ± 5.2 years; VO_2max, 44.3 ± 5.7 ml/kg/min) performed the FB of SIE (12 repeats of 5 s each) on one day and the RB 2 weeks later. The following parameters were measured: motor performance (voluntary, electrically induced and isokinetic skeletal muscle contraction torque, and central activation ratio [CAR]); stress markers [brain-derived neurotrophic factor (BDNF), cortisol, norepinephrine, and epinephrine]; inflammatory markers (IL-6, IL-10, and TNF-α); metabolic markers (glucose and lactate); muscle and rectal temperature; cycling power output; and psychological perceptions. The average cycling power output and neuromuscular fatigue after exercise did not differ between the FB and RB. There were significant decreases in cortisol and BDNF concentration at 12 h ($P < 0.05$) and 24 h ($P < 0.001$) after the FB, respectively. The decrease in cortisol concentration observed 12 h after exercise was significantly greater after the RB ($P < 0.05$) than after the FB. The immune-metabolic response to the RB (vs. FB) SIE was suppressed and accompanied by lower psychological exertion. Most of the changes in psychological and physiological biomarkers in the FB and RB were closely related to the response kinetics of changes in BDNF concentration.

Keywords: high-intensity exercise, brain derived neurotrophic factor, stress hormones, immune-metabolic response, perception

INTRODUCTION

Sprint interval exercise (SIE) is being used increasingly as a form of exercise training. A typical session comprises a series of brief bursts of vigorous exercise separated by periods of rest or low-intensity exercise (Buchheit and Laursen, 2013; Biddle and Batterham, 2015). Compared with traditional endurance training, the major advantage of SIE is that beneficial adaptations can be obtained with a shorter exercise duration (Bacon et al., 2013). Recent research shows that regular and optimum physical exercise plays a key role in general well-being, mental and physical health, disease prevention, and longevity in humans, and that some of these effects are mediated by the production and release of the brain-derived neurotrophic factor (BDNF) and other myokines, such as interleukin 6 (IL-6), IL-10, and the tumor necrosis factor-alpha (TNF-α) (Kramer et al., 2006; Cotman et al., 2007; Pedersen et al., 2009). High-intensity exercise improves cardiovascular health (Lowensteyn et al., 2000; Lee et al., 2011) and increases BDNF levels (Oliff et al., 1998); therefore, it may help promote synaptic plasticity and growth and the survival neurons in the brain (Neeper et al., 1995; Cotman and Berchtold, 2002), which is of great importance for overall brain heath (Marquez et al., 2015).

Sprint interval exercise is a type of exercise that should not cause skeletal muscle damage, but should cause fatigue because of metabolic stress to muscle fibers (Place et al., 2015). Regarding metabolic response, lactate has been proposed as an important glycolytically produced metabolite that is most likely released because of increased or accelerated anaerobic glycolysis and stress response. When the rate of glucose metabolism exceeds the oxidative capacity of the mitochondria (Dienel, 2013), lactate assists as a critical buffer, allowing glycolysis to produce significant amounts of energy rapidly. The acute immune-metabolic response to exercise is connected to glucose homeostasis in muscle cells (Steinacker et al., 2004; Rosa Neto et al., 2011). Acute exercise enhances IL-6 concentration by increasing its production in skeletal muscle, which acts in the regulation of the muscle energetic status (Pedersen and Febbraio, 2008). Moreover, IL-6 upregulates anti-inflammatory cytokines (such as IL-10) that prevent the exacerbation of the proinflammatory milieu, thus blocking a possible persistent inflammatory status, and downregulates TNF-α. Alterations of cortisol and IL-6 levels can regulate the availability of substrate during the exercise, and these alterations are modified by the type, intensity, and duration of the exercise (Petersen and Pedersen, 2005). In addition, possible BDNF modulation factors, such as lactate, cortisol, and intensity, have been proposed. It has been speculated that skeletal muscle cells might be involved in mediating changes in BDNF, and that skeletal muscle contractions during high-intensity exercise might be a possible trigger of a biochemical pathway linking an exercise-induced secreted factor from skeletal muscle to *BDNF* gene expression in the brain (Wrann et al., 2013). Cortisol is commonly known as a stress hormone (Sapolsky et al., 2000), and chronically elevated levels of cortisol inhibit neurogenesis and neural plasticity. More specifically, exposure to corticosterone decreases BDNF expression in the brain; this suggests a negative relationship between cortisol and BDNF (Smith et al., 1995), which alters mood and may cause depression (Brunoni et al., 2008; Stein et al., 2008; Hashimoto, 2010; Polyakova et al., 2015).

The repeated bout (RB) effect refers to the adaptation via which a single bout of eccentric exercise protects against muscle damage from subsequent eccentric bouts (Kamandulis et al., 2010; Gorianovas et al., 2013). The functional effects of applying repeated SIE on physiological and psychological biomarkers with respect to neuromuscular fatigue in healthy young adult men have not been investigated. A maximal sprint effort that lasts >30 s is associated with unpleasant outcomes, such as nausea, vomiting, and dizziness (Inbar et al., 1996). By contrast, a 5-s maximal sprint effort has been shown to be well tolerated (Verbickas et al., 2017). Considering this, in the present study, participants performed the same exercise protocol—12 repeats of 5 s—twice, once at the start of the exercise and again 2 weeks later, on the assumption that the first bout (FB) would reduce the metabolic stress on muscle fibers and the brain in the subsequent SIE bout (i.e., RB). If so, one would expect that a lower increase in lactate, glucose, cortisol, norepinephrine (NEp), and epinephrine (Ep) levels, in line with a lower increase in body temperature after RB, would promote a decreased cytokine response and a greater increase in BDNF level compared with the FB. Therefore, the participants would be more motivated to engage in exercise, would feel less exertion during exercise, and would be better oriented regarding time perception during exercise and recovery. In addition, we hypothesized that reduced metabolic stress on muscle fibers would induce a decreased release of reactive oxygen/nitrogen species-dependent ryanodine receptor 1 (RyR1) fragmentation (Place et al., 2015), thereby leading to reduced sarcoplasmic reticulum (SR) Ca^{2+} leakage and depression in muscle contractility properties and overall force production.

Therefore, the primary purpose of our study was to determine whether RB (vs. FB) is sufficient to mitigate SIE-induced psychological and physiological biomarker kinetics within 48 h after exercise.

MATERIALS AND METHODS

Participants

Ten physically active men (mean ± SD; age: 22.6 ± 5.2 year; height: 181.1 ± 4.4 cm; body mass: 76.5 ± 9.1 kg, and VO_2max: 44.3 ± 5.7 ml/kg/min) participated in the current study. All volunteers were non-smokers and had no indications of cardiovascular, pulmonary, mental, or neuromuscular disease or trauma. The experimental protocol was approved by the Kaunas Regional Biomedical Research Ethics Committee and conformed to the Declaration of Helsinki.

Experimental Design

Familiarization

On the familiarization day, participants completed a maximum graded exercise test to determine maximum oxygen uptake (VO_2max) (Cernych et al., 2017) on a cycle ergometer (Ergoline, Ergoselect 100, Bitz, Germany) using a mobile spirometry system (Oxycon Mobile, Jaeger/VIASYS Healthcare, Hoechberg, Germany). Subjects with a VO_2max < 42.2 ml/kg/min were excluded from the study based on the American College of Sports Medicine recommendation for individuals performing high-intensity exercise (Thompson et al., 2010). Each subject was familiarized with all laboratory testing procedures, equipment, psychological perception scales, and protocols to ensure that they would be able to maintain MVC of the knee extensors, to perform isokinetic knee extension, and to tolerate electrical muscle stimulation. The familiarization day was at least 7 days before the first investigation day. All participants avoided strenuous exercise for 72 h, abstained from alcohol and caffeine intake for at least for 24 h, and were well hydrated before each session.

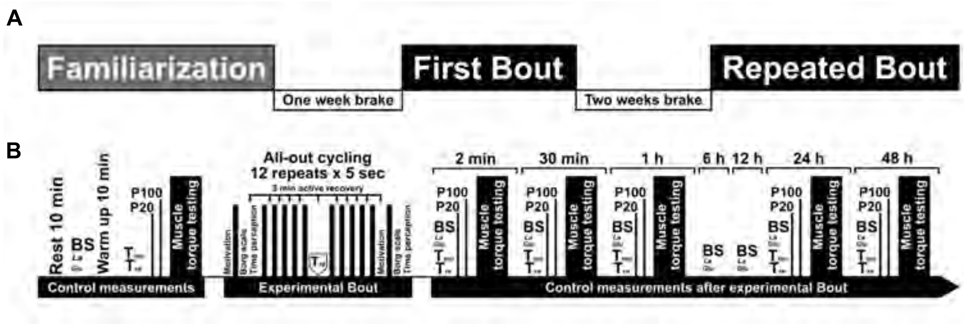

FIGURE 1 | Graphical overview of the experimental protocol. **(A)** Experimental design; **(B)** protocol and control measurements. BS, blood sample; Muscle torque testing (electrical muscle stimulation; maximum voluntary contraction torque; central activation ratio (CAR); isokinetic knee extension torque at 180°/s; handgrip torque); T_{mu}, muscle temperature; T_{re}, rectal temperature; La, lactate; Glu, glucose.

Experimental Protocol

The experimental protocol was performed at the same time of day (8:00 a.m.) for all subjects. At the beginning of the first visit, body mass was measured on a scale (Tanita, TBF-300, Arlington Heights, IL, United States), and the subject rested for 10 min, after which blood samples were taken (**Figure 1**). All blood samples were collected at the following time points: before exercise, 2 and 30 min, and 1, 6, 12, 24, and 48 h after completion of the exercise. The subject performed a warm-up on a cycle ergometer (Ergoline GmbH, Ergoselect 100, Bitz, Germany) for 10 min at a power output (W) approximately equal to the subject body's mass (kg) and at a rate of 70 rpm. After the warm-up, body temperatures, voluntarily and electrically induced muscle torque, and central activation ratio (CAR) were measured. Muscle temperature (T_{mu}) and rectal temperature (T_{re}) were measured at the following time points: before exercise (exception: T_{re} was measured after the sixth series and 1 h after exercise), 2 and 30 min completion of the exercise. Knee extensor muscle torque was measured at the same times plus 24 and 48 h after exercise. Handgrip strength was measured at the same time as the measurement of knee extensor muscle torque before and 2 min after exercise; the best of three attempts was recorded.

After all control measurements were made before exercise, the subject performed a standardized warm up comprising 5 min of unloaded cycling at 60 rpm. The SIE protocol comprised 12 repeats of 5 s each interspersed with 3 min of active recovery (unloaded cycling at 60 rpm). The subject was instructed to perform a maximum effort from the beginning of the test until instructed to stop. The subject was asked to indicate his motivation level before the first and 12th interval, perceived exertion and subjective perception of work time after first and 12th interval, and subjective perception of rest time before the second and 12th interval.

Each subject performed the FB of the SIE on the first experimental day and repeated the same exercise (i.e., RB) under the same conditions 2 weeks later. This study involved several complex measurements performed at various times after SIE. After the FB and RB, the immediate priority was to obtain the blood samples and then to measure T_{mu} and T_{re}, followed by voluntarily and electrically induced muscle force, and CAR testing. The time required for the blood sample collection through the muscle force testing was about 2 min.

Experimental Measurements

Sprint Interval Cycling Exercise (SIE)

Cycling power output was measured during the first and the last trials of the FB and RB. Each bout of exercise comprised 12 repeats of 5 s (Verbickas et al., 2017) on a cycle ergometer (Monark, Ergomedic 894 EA, Vansbro, Sweden) interfaced with a laptop computer. Foot straps were used to secure the feet to the pedals. The pedal right arm crank starting position was 45° forward to the vertical axis. Upon the start command, the subject began pedaling and continued until the stop command. During cycling, when the subject had reached 100 rpm pedaling frequency, the computer automatically added the weight (7.5% of body mass). During the entire SIE, the subject received strong verbal encouragement.

Body Temperatures Measurements

T_{mu} was measured by needle thermocouple (model DM-852; Ellab, Roedovre, Denmark) inserted to a depth of 3 cm below the skin surface into the vastus lateralis muscle mid-thigh and slightly lateral to the femur. The temperature recorded at a depth of 3 cm was assumed to represent the average temperature of the active muscle mass (Brazaitis et al., 2015). T_{re} was measured using a rectal thermocouple (Rectal probe, Ellab, Hvidovre, Denmark) inserted to a depth of 12 cm past the anal sphincter. The thermocouples remained in place for 1 h after the exercise bouts. The room temperature was 21.2 ± 0.3°C.

Maximal Voluntary Contraction and Torque Measurements

Isokinetic dynamometer (Biodex System 3, Shirley, NY, United States) was used to measure knee extensor muscle torque. The participant sat upright in the dynamometer chair with vertical back support, and a strap secured the hips and thighs to minimize uncontrolled movement. The knee joint was positioned at a 120° angle (180° is full knee extension). MVC was reached and maintained for ~5 s before relaxation and was measured twice, each separated by a 1 min rest, and the larger value was used in the analyses (Bernecke et al., 2017).

Isokinetic knee extension torque (IT) was measured at a speed of 180°/s (Skurvydas et al., 2010). The subject was required to extend the knee from an 80° angle. The best of three attempts was used in the calculations.

Handgrip strength was measured at the same time points as knee extensor torque using a Jamar Handgrip Dynamometer (Bolingbrook, IL, United States). The subject sat in a chair with his elbow bent at 90°. Three attempts were performed with 1 min rest between each, and the best attempt was used in the calculations.

Direct Electrical Stimulation and Torque Measurements

The equipment and procedure for electrical stimulation were essentially the same as previously described (Kyguoliene et al., 2017). Direct muscle stimulation was applied using two carbonized rubber electrodes covered with a thin layer of electrode gel (ECG-EEG Gel; Medigel, Modi'in, Israel). One of the electrodes (6 cm × 20 cm) was placed transversely across the width of the proximal portion of the quadriceps femoris muscle. Another electrode (6 cm × 11 cm) covered the distal portion of the muscle above the patella. A standard electrical stimulator (Digitimer DS7, Hertfordshire, England) was used. The electrical stimulation was delivered in square-wave pulses of 0.5 ms duration. Peak torques induced by a 1-s electrical stimulation at 20 Hz (P20; representing the steep section of the force–frequency relationship curve) and at 100 Hz (P100; which is close to maximum force) were measured with a ∼4-s rest interval between electrical stimulation.

Central Activation Ratio

The CAR was obtained during the 5 s MVC (Brazaitis et al., 2016). At ∼3 s of the MVC, a 250 ms test train of stimuli at 100 Hz (TT-100 Hz) was superimposed on the voluntary contraction. The CAR was calculated as the ratio of the maximum voluntary torque to the peak torque generated with an additional TT-100 Hz superimposed on the MVC. The 5 s MVC with the superimposed stimuli was performed twice; the larger CAR value was used in the analyses.

Blood Samples

Fasting blood samples were collected from median antecubital vein. Blood serum was obtained by venipuncture into vacuum tubes with a gel separator (5 ml; BD Vacutainer, Franklin Lakes, NJ, United States). The samples were allowed to clot, and the serum was separated by centrifugation (1200 × *g*, 15 min) at room temperature. Blood samples for measurement of NEp and Ep in plasma were collected into vacuum tubes with an anticoagulant (3 ml; BD Vacutainer). The serum and plasma samples were aliquoted and stored at −70°C until analysis. The concentrations of human BDNF, cortisol, IL-6, IL-10, and TNF-α were measured in serum. The concentrations of NEp, Ep, BDNF, IL-6, IL-10, and TNF-α were measured using a Gemini immunoassay ELISA analyzer (Stratec Biomedical GMBH, Birkenfeld, Germany). Cortisol concentration was measured using an AIA-2000 automated enzyme immunoassay analyzer (Tosoh Corp., Tokyo, Japan).

Glucose concentration was measured immediately in venous blood (Glucocard X-mini Plus, Japan) at the following time points: before exercise, 2 and 30 min, and 1 h after completion of the exercise. Blood lactate concentration was measured at the same times. Blood samples (0.3 μL) were taken from the fingertip and analyzed immediately for blood lactate concentration using reagent strips (Lactate Pro, Arkray, Inc., Kyoto, Japan).

Measurement of Perception

Motivation was assessed using a visual analog scale that ranged from 1 (not motivated at all) to 10 (extremely motivated) on a 10-cm horizontal line (Kleih and Kübler, 2013). Participants indicated their motivation with regard to the load by marking one position on the line that best represented their subjective motivation.

Participants were also asked about perceived exertion using a Borg rating scale (Borg, 1970) ranging from 6 (no exertion at all) to 20 (maximal exertion). The participants were told that they were going to exercise for 12 repeats of sprints on a cycle ergometer and that they must rate how hard and strenuous the exercise felt in the legs. They were instructed that a score of 20 on the Rating of Perceived Exertion (RPE) scale should correspond to the highest exercise load they thought they could maintain for exact sprint. RPE was recorded immediately after the first and 12th repeats.

The dimension of time is essential for everyday behavior and survival (Wittmann, 2013). The subject was not informed of the amount of work (sprint) and rest time periods during the SIE. The subject was asked to rate his subjective perceptions of work and rest time using simple visual time scales. He was asked to estimate his perception of work time from 2 to 10 s (graded in 1 s increments) immediately after each repeat. The subject's perception of rest time was evaluated before the start of the next repeat using a scale graded in 30 s increments from 1 to 5 min.

Statistical Analyses

The data were tested for normal distribution using the Kolmogorov–Smirnov test, and all data were found to be normally distributed. Descriptive data are presented as the mean ± standard deviation (SD). A multivariate one-way analysis of variance (MANOVA) for repeated measures was used to determine the effects of each repeated trial (FB vs. RB) on the before-exercise values of MVC, CAR, IT, P20, P100, P20/P100 ratio, body temperature, and blood markers. A two-way MANOVA for repeated measures was used to determine the effects of each repeated trial (FB vs. RB) and time on the mean cycling power output, blood markers (IL-6, IL-10, TNF-α, NEp, Ep, BDNF, cortisol, lactate, and glucose levels), body temperature (T_{re} and T_{mu}), MVC, CAR, IT, handgrip strength, and electrically induced muscle properties (P20 and P100). If significant effects were found, Sidak's *post hoc* adjustment was used for multiple comparisons across a set of conditions within each repeated-measure MANOVA. Single time-point comparisons between conditions were analyzed using independent-sample *t*-tests. In addition, an exploratory data evaluation of relationships between changes in BDNF and physiological and psychological variables was performed. Statistical significance was set at $P < 0.05$. Calculations of statistical observed power (OP, in percent) were performed, and the partial eta squared (η_p^2) was estimated as a measure of the RB task effect size. The OP for a significant effect was considered to be >80%. The non-parametric Wilcoxon signed-rank test was used to compare changes in subjective ratings of perceptions (motivation, perceived exertion, and perceptions of work time and rest time) between trials. Statistical analyses were performed using IBM SPSS Statistics software (v. 22; IBM Corp., Armonk, NY, United States).

RESULTS

Effects of RB on Average Cycling Power

There were no significant changes in average cycling power between the FB and RB; the first and 12th repeats of FB were 767.7 ± 116.5 W and 782.8 ± 120.1 W (102.2 ± 9.1% of the first repeat); and RB were 786.8 ± 116.1 W and 796.9 ± 123.7 W (101.3 ± 6.1% of the first repeat), respectively.

Effects of RB on Body Temperature

The T_{mu} before and 2 and 30 min after exercise was 37.0 ± 0.45°C, 38.0 ± 0.5°C ($P < 0.001$, $\eta_p^2 > 0.9$, OP > 99%), and 36.9 ± 0.4°C, respectively, for the FB and 37.1 ± 0.52°C, 37.9 ± 0.38°C ($P < 0.001$, $\eta_p^2 > 0.9$, OP > 99%), and 36.9 ± 0.47°C, respectively, for the RB. There were no significant differences in the changes in T_{mu} between the FB and RB. By contrast, the increase in T_{re} was significantly greater ($P < 0.05$, $\eta_p^2 > 0.7$, OP > 80%) after the FB compared with after the RB (**Figure 2**).

There was a significant inverse relationship between the Δ% from before to after SIE in T_{re} and BDNF ($r = -0.74$ and −0.89, respectively, in the FB and RB sessions; $P < 0.05$). The Δ% in T_{re} from before to after SIE was correlated positively with basal BDNF levels (r = 0.83 and 0.86, respectively, in the FB and RB sessions; $P < 0.05$).

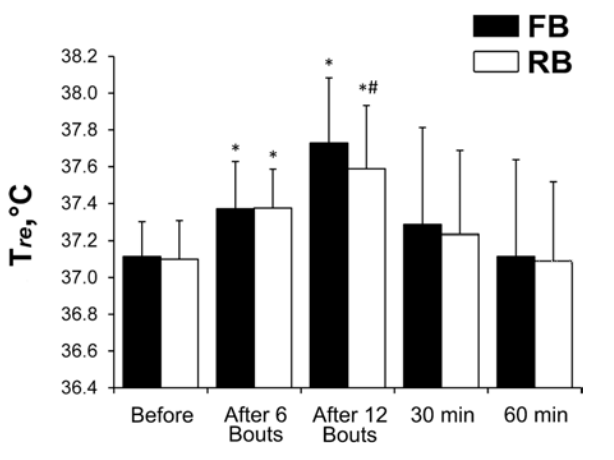

FIGURE 2 | Rectal temperature changes. FB, first bout; RB, repeated bout; *$P < 0.05$ compared with preexercise values; #$P < 0.05$ between FB and RB.

Effects of RB on Blood Markers

NEp, Ep, cortisol, and BDNF concentrations increased significantly 2 min after the FB and RB (**Figure 3**). After the FB, there were significant decreases in cortisol at 12 h ($P < 0.05$, $\eta_p^2 > 0.65$, OP > 80%) and in BDNF concentration at 12 and 24 h ($P < 0.01$, $\eta_p^2 > 0.8$, OP > 95%) (**Figures 3A,B**). The decrease in cortisol concentration observed 12 h after exercise was significantly ($P < 0.05$, $\eta_p^2 > 0.7$, OP > 85%) smaller after the RB compared with after the FB. The RB caused less stress, as shown by a smaller increase ($P < 0.01$, $\eta_p^2 > 0.8$, OP > 90%) in cortisol concentration after exercise. By contrast, BDNF concentration did not decrease 24 h after the RB.

IL-6 concentration increased significantly within 30 min to 12 h after both the FB and RB ($P < 0.001$, $\eta_p^2 > 0.9$, OP > 99%). IL-10 concentration increased significantly within 2 min to 1 h after the FB and at 1 h after the RB ($P < 0.05$, $\eta_p^2 > 0.7$, OP > 80%). The concentration of TNF-α did not change after either bout (**Figure 4**). The increase in IL-6 concentration was significantly smaller ($P < 0.05$, $\eta_p^2 > 0.75$, OP > 90%) at 12 h after the RB compared with after the FB. IL-10 concentration from 2 to 30 min was significantly lower after the RB ($P < 0.05$, $\eta_p^2 > 0.65$, OP > 80%) compared with the same time after the FB.

Glucose concentration increased significantly 2 min after the FB ($P < 0.05$, $\eta_p^2 > 0.65$, OP > 80%) and 30 min after both the FB and RB ($P < 0.001$, $\eta_p^2 > 0.9$, OP > 99% and $P < 0.05$, $\eta_p^2 > 0.75$, OP > 80%, respectively) (**Figure 5A**). Glucose concentration at 30 min was significantly lower after the RB ($P < 0.05$, $\eta_p^2 > 0.65$, OP > 80%) compared with the same time after the FB.

Lactate concentration increased significantly 2 min after ($P < 0.001$, $\eta_p^2 > 0.9$, OP > 99%), and remained significantly elevated 30 min after the FB and RB ($P < 0.001$, $\eta_p^2 > 0.9$, OP > 99% and $P < 0.05$, $\eta_p^2 > 0.8$, OP > 95%, respectively) (**Figure 5B**). The increase in lactate concentration was significantly smaller ($P < 0.01$, $\eta_p^2 > 0.75$, OP > 90%) after the RB compared with after the FB.

The Δ% in BDNF concentration at 24 h was correlated significantly with the Δ% in cortisol concentration 24 h after the FB ($r = 0.85$, $P < 0.05$). We found an inverse relationship between the Δ% from before to after SIE in La and BDNF ($r = -0.73$ and −0.79, respectively, in the FB and RB sessions; $P < 0.05$).

Effects of RB on Neuromuscular Performance

There were no significant differences in any of the muscle torque measurements at rest before the FB and RB (**Table 1**).

After both the FB and RB, MVC, CAR, and IT decreased significantly ($P < 0.05$, $\eta_p^2 > 0.8$, OP > 90%); however, there was no difference between the FB and RB (**Figure 6**). Recovery of IT within 24–48 h was significantly faster for the RB than it was for the FB ($P < 0.05$, $\eta_p^2 > 0.65$, OP > 80%) (**Figure 6C**). Unexpectedly, MVC and CAR recovered more slowly after the RB compared with after the FB. Twenty-four hours after the RB, the

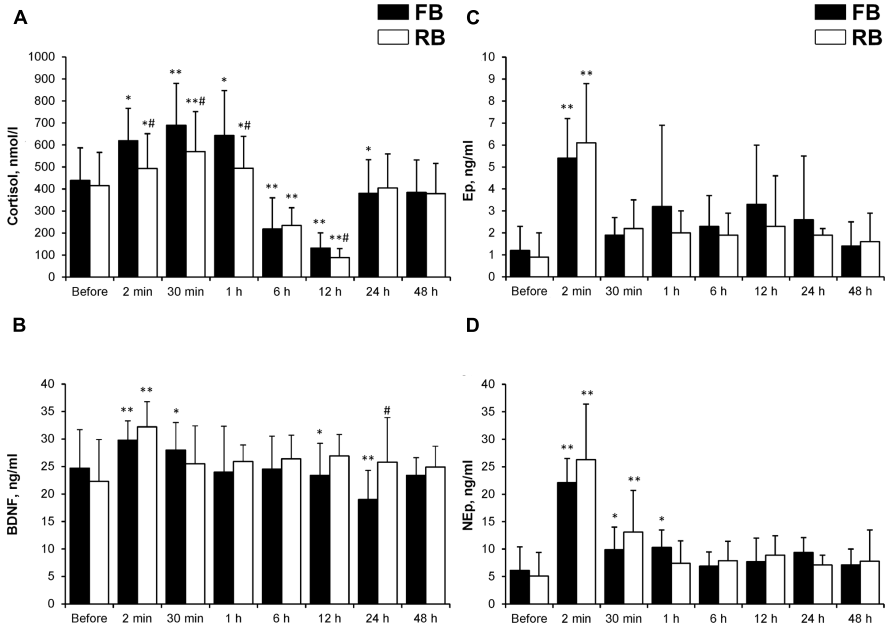

FIGURE 3 | Blood indicators. **(A)** Cortisol; **(B)** BDNF; **(C)** Ep; **(D)** Nep. FB, first bout; RB, repeated bout; BDNF, brain-derived neurotrophic factor; Ep, epinephrine; NEp, norepinephrine. *$P < 0.05$, **$P < 0.001$ compared with preexercise values; #$P < 0.05$ between FB and RB.

MVC and CAR values did not differ from those recorded before exercise.

Handgrip strength did not change significantly from before to after exercise: in the FB, it varied from 56.7 ± 6.1 to 57.5 ± 8.8 kg, and in the RB, from 56.0 ± 7.1 to 58.5 ± 7.5 kg. There were no significant differences between the FB and RB.

There was no significant difference in any of the electrical muscle stimulation variables between the FB and RB (**Figure 7**). The low-frequency stimulation-induced torque decreased significantly more than did the high-frequency stimulation-induced torque in both the FB and RB ($P < 0.001$, $\eta_p^2 > 0.9$, OP > 99%). The high-frequency stimulation-induced torque recovered within 24 h, whereas the low-frequency stimulation-induced torque did not recover fully within 48 h after either the FB or RB.

The percentage change (Δ%) in BDNF concentration at 24 h was correlated significantly with the Δ% in MVC and CAR 24 h after the FB ($r = 0.80$ and 0.76, respectively). The Δ% in MVC and CAR from before to 24 h after SIE were correlated inversely with basal BDNF levels ($r = -0.75$ and -0.92, respectively, in the FB session; and $r = -0.66$ and -0.67, respectively, in the RB session; $P < 0.05$). The Δ% in BDNF concentration from before to after exercise correlated positively with the Δ% in MVC from before to 24 h after exercise in the RB session ($r = 0.76$; $P < 0.05$), and with the Δ% in CAR from before to 24 h after exercise in the FB and RB sessions ($r = 0.81$ and 0.68, respectively; $P < 0.05$).

Effects of RB on Psychological Variables

The average motivation scores were 8.8 ± 1.2 and 9.0 ± 1.1 points ($P > 0.05$) during the FB and RB, respectively. Perceived exertion increased significantly during both the FB and RB ($P < 0.05$). The average scores for perceived exertion (Borg scale) were 11.9 ± 2.8 and 11.4 ± 3.1 points for the FB and RB, respectively ($P > 0.05$ between the FB and RB) after the first repeat, and 16.3 ± 2.4 and 15.3 ± 2.1 points after the 12th repeat ($P < 0.05$ between the FB and RB).

The subjective perception of work time in the first and last repeats was 5.3 ± 0.9 and 6.4 ± 1.1 s, respectively ($P < 0.05$) during the FB, and 4.9 ± 1.1 and 5.9 ± 1.1 s ($P < 0.05$) during the RB. There was no significant difference between the FB and RB.

The subjective perception of rest time after the first and the 11th repeats was 150 ± 27 and 236 ± 43 s, respectively, for the FB ($P < 0.05$), and 176 ± 25 and 210 ± 39 s, respectively, for the RB ($P < 0.05$). There were no significant differences between the FB and RB.

The perception of work time in the 12th repeat was correlated inversely with the Δ% in BDNF concentration from before to after exercise ($r = -0.74$ and -0.72, respectively, in the FB and RB sessions; $P < 0.05$). The psychological sensations of perceived exertion during the 12th repeat and the subjective perception of work time during the FB were correlated inversely with the Δ% in BDNF concentration from before to 24 h after exercise ($r = -0.79$ and -0.82, respectively; $P < 0.05$).

DISCUSSION

To our knowledge, this is the first study to investigate the effects of repeated SIE on body temperature, neuromuscular functions, and markers of the immune, metabolic, and stress system in healthy young men.

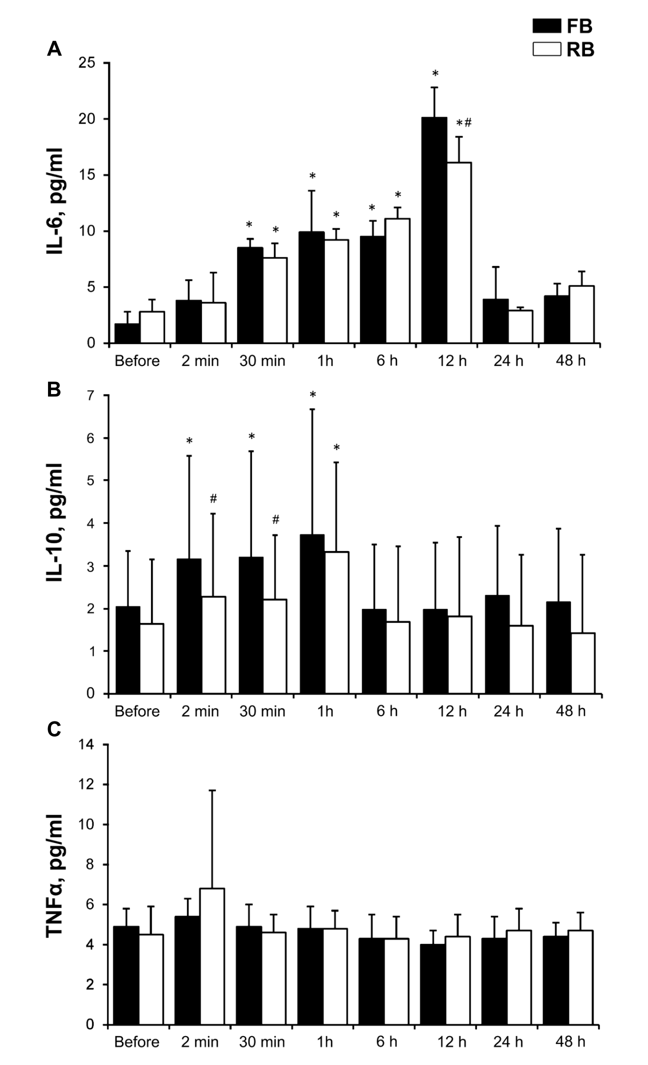

FIGURE 4 | Blood indicators. **(A)** IL-6; **(B)** TNFα; **(C)** IL-10. FB, first bout; RB, repeated bout; IL-6, interleukin 6; IL-10, interleukin 10; TNFα, tumor necrosis factor-alpha. **P* < 0.05 compared with preexercise values. #*P* < 0.05 between FB and RB.

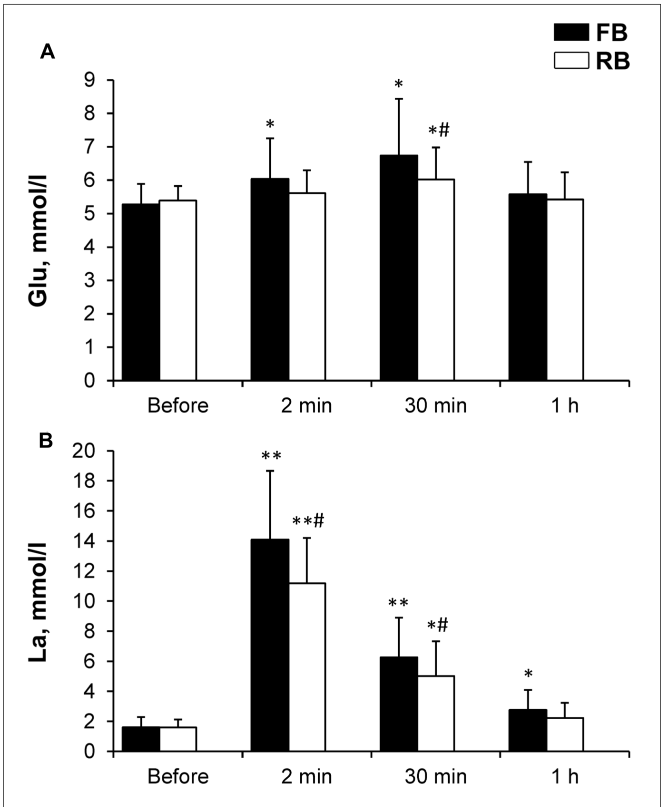

FIGURE 5 | Glucose and lactate concentrations. **(A)** Glucose; **(B)** Lactate. FB, first bout; RB, repeated bout. **P* < 0.05, ***P* < 0.001 compared with preexercise values; #*P* < 0.05 between FB and RB.

In the present study, CAR decreased by about 5%, MVC and P100 by about 15%, and P20 by about 50% after both the FB and RB. We believe that the main reason for fatigue is undoubtedly associated with exhaustion of energy substrates and increased metabolite concentrations (e.g., inorganic phosphate, ADP, H^+), which can affect the attachment of myosin cross-bridges to actin filaments, attachment strength (Allen et al., 2008), and muscle fiber activation by reducing the Ca^{2+} released from the SR (Place et al., 2015). After SIE, we found a slight but significant decrease in voluntary muscle CAR of about 5%, which returned to the initial level within 24 h. The long-lasting decrease in force observed after various types of physical exercise is more marked at low than at high stimulation frequencies and is referred to as "prolonged low-frequency force depression" (PLFFD) (Allen et al., 2008). In our study, PLFFD was apparent after both the FB and RB. The decreased force production in muscle fibers can be caused, in principle, by reduced free myoplasmic concentration Ca^{2+} ($[Ca^{2+}]i$) during contraction, decreased myofibrillar Ca^{2+} sensitivity, and reduced ability of the contractile machinery to produce force (Allen et al., 2008). On a simplified level, the first two factors should increase the magnitude of the force depression at low compared with high stimulation frequencies because of the sigmoidal shape of the force – $[Ca^{2+}]i$ relationship, whereas the third factor should cause a similar decrease in force at all stimulation frequencies (Allen et al., 2008). In a recent study (Place et al., 2015), we showed marked PLFFD and RyR1 fragmentation in muscles of recreationally active subjects after six cycling bouts of 30 s each (Wingate bouts). Intriguingly, the same exercise caused a similar PLFFD in elite endurance athletes, but the RyR1 remained intact, and the difference could be explained by a greater superoxide dismutase expression in the elite athletes (Place et al., 2015). Thus, depending on the training status, the mechanism responsible for the PLFFD induced by repeated Wingate cycling bouts may be either impaired Ca^{2+} release from SR or reduced myofibrillar Ca^{2+} sensitivity. In this study, PLFFD did not decrease after the RB.

Acute exercise bouts have been shown to promote an acute phase response, resulting in post-exercise cytokine levels that are similar to those observed during sepsis or inflammatory disease (Nunes et al., 2015). Skeletal muscle is a major source of several cytokines, and the response is dependent on the duration,

TABLE 1 | Mean (±SD) values of voluntary and electrically induced torque before first bout (FB) and repeated bout (RB).

	FB	RB
MVC, Nm	311.8 ± 41.5	307.2 ± 44.3
CAR, %	94.7 ± 7.3	94.1 ± 7.2
IT, Nm	171.9 ± 17.7	167.4 ± 18.9
P20, Nm	181.5 ± 27.2	189.0 ± 31.9
P100, Nm	272.3 ± 30.1	270.1 ± 31.0
P20/P100	0.67 ± 0.06	0.70 ± 0.07

MVC, maximal voluntary contraction torque; CAR, central activation ratio; IT, isokinetic knee extension torque at 180°/s; P20, torque of electrical muscle stimulation at 20 Hz; P100, torque of electrical muscle stimulation at 100 Hz.

intensity, and session volume of the exercise. The cytokines have several functions and play a crucial role in energy metabolism; for instance, IL-6 and TNF-α are important in the anti-inflammatory response and exert effects on glucose and lipid metabolism, by stimulating an increase in the processes of lipolysis and glycogenolysis, to provide an energy supply for skeletal muscle and other tissues after exercise (Pedersen and Febbraio, 2008). The transient increase in the circulating levels of IL-6 during exercise appears to be responsible for a further increase in the circulating levels of the anti-inflammatory cytokine IL-10 by stimulating the release of cortisol and decreasing the levels of TNF-α. Consistently, we showed here that after SIE, the IL-10 and cortisol response initially increased progressively and reached its peak at 30 min, followed by a decrease and return to the baseline values. By contrast, IL-6 concentration increased progressively and reached its peak at 12 h. The level of TNF-α was unaffected. Such an interaction in cytokine kinetics may indicate an effect of both pro- and anti-inflammatory activity (Peake et al., 2015). The increase in cortisol concentration was a clear indicator that the SIE load caused a marked response of the HPA axis (Frx et al., 2000). As expected, in this study, the RB blunted the release of these cytokines and cortisol. Moreover, glucose concentration was significantly lower after the RB compared with after the FB; therefore, we can only speculate that the decrease in glucose concentration in the blood observed in our study reflects an increase in insulin sensitivity (Cartee, 2015).

The increase in BDNF concentration after the FB and RB in our study coincides with the findings of other researchers who reported that brief intense exercise increases BDNF concentration (Marquez et al., 2015). Shorter bouts of high-intensity exercise (1 min work and 1 min rest) are slightly more effective than continuous high-intensity exercise for elevating serum BDNF concentration (Marquez et al., 2015). The authors concluded that the SIE protocol might represent an effective and preferred intervention for elevating BDNF level and promoting brain health (Marquez et al., 2015). However, in our study, the increase in BDNF concentration was much smaller than that reported by Mang et al. (2014). Those authors showed that systemic BDNF concentration increased on average by 3.4-fold following aerobic exercise, but the changes did not relate to neurophysiological or behavioral measures. By contrast, in our study, the changes in BDNF concentration correlated strongly with prolonged changes in motor performance. Our most interesting finding is that BDNF concentration decreased gradually 24 h after exercise and that this decline was significantly greater after the FB (**Figure 3B**). We speculate that the BDNF

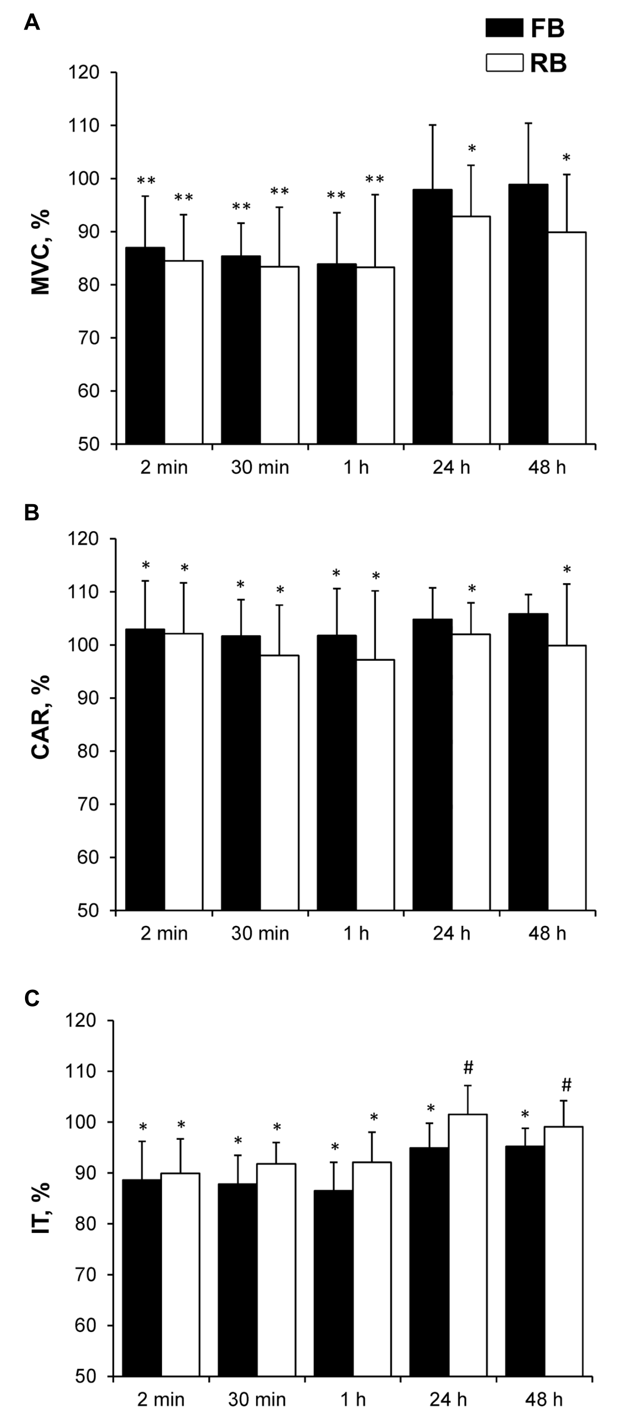

FIGURE 6 | Changes in muscle force after FB and RB. **(A)** MVC; **(B)** CAR; **(C)** IT. FB, first bout; RB, repeated bout; MVC, maximum voluntary contraction; CAR, central activation ratio; IT, isokinetic knee extension torque at 180°/s; *$P < 0.05$, **$P < 0.001$ compared with preexercise value; #$P < 0.05$ between FB and RB.

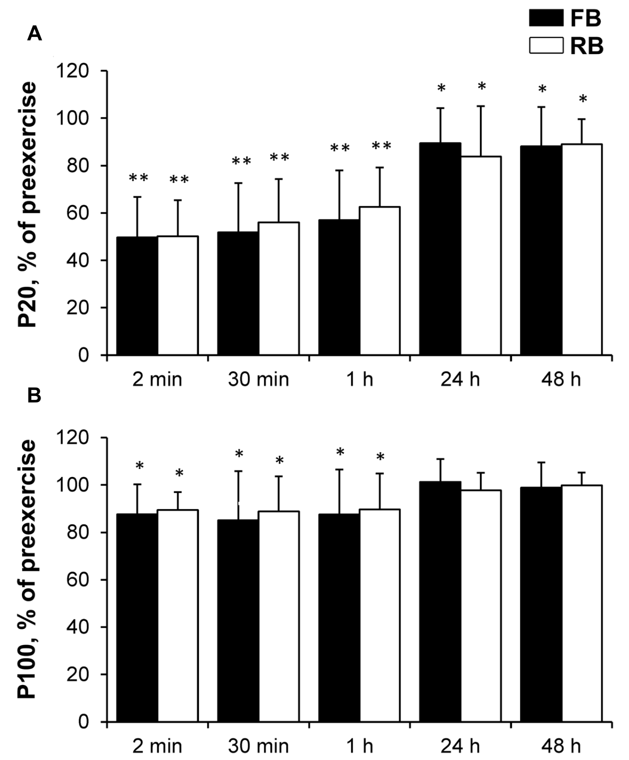

FIGURE 7 | Percentage changes in electrically induced muscle torque compared with preexercise values. **(A)** P20; **(B)** P100. P20, electrical muscle stimulation at 20 Hz; P100, electrical muscle stimulation at 100 Hz; FB, first bout; RB, repeated bout; *$P < 0.05$, **$P < 0.001$ compared with preexercise values.

"pit" is an indicator of a slower motor system work rate; i.e., FB was psychologically and metabolically more demanding, as shown by the greater increases in T_{re} and lactate concentration. This idea is supported by the significant correlation between the psychological feeling of perceived exertion and decrease in BDNF concentration 24 h after the FB. In our study, BDNF concentration did not decrease 12–24 h after exercise. By contrast, Ieraci et al. (2015) found that BDNF level returned to the initial value within 24 h after exercise. Rojas Vega et al. (2006) and Marquez et al. (2015) showed that BDNF level is restored to the initial level within 15–20 min after exercise. Contrary to our findings, research has shown that long-term regular aerobic exercise has a positive effect on the increase in serum BDNF level (Saligan et al., 2016). BDNF signaling mediates adaptive responses of the central, autonomic, and peripheral nervous systems to exercise (Weinstein et al., 2014), and BDNF helps to regulate energy homeostasis and energy metabolism (Pedersen et al., 2009). Our data are consistent with this concept and suggest that changes in BDNF level are closely related to changes in T_{re} and lactate concentration, although the mechanisms responsible for this association are unclear. We found that the preexercise BDNF concentration and kinetics of the change in BDNF concentration after both the FB and RB correlated significantly with indicators of prolonged neuromuscular fatigue, including central motor fatigue. That is, BDNF was a good biomarker of prolonged motor fatigue and especially central motor fatigue.

Stress is hypothesized to cause a reduction in BDNF (protein) level in the brain, which alters mood and may cause depression (Stein et al., 2008). Low BDNF level leads to a greater subjective feeling of fatigue (Saligan et al., 2016). Higher serum BDNF level may protect against the future occurrence of dementia (Weinstein et al., 2014). BDNF level may be a biomarker of mood disorders (Hashimoto, 2010) and may be a central factor in the network of multimorbidity in old populations (Krabbe et al., 2009). Low BDNF levels are found in patients with neurodegenerative diseases, including Alzheimer's disease, and major depression (Pedersen et al., 2009). The peripheral BDNF level at rest is lower in exercise-trained people than in untrained people (Nofuji et al., 2008; Huang et al., 2014) and BDNF level increases more after acute exercise in trained than in untrained people (Knaepen et al., 2010). Taken together, these studies show that there is no consensus on the effects of exercise on the basal BDNF level and the functional significance of changes in BDNF level after exercise. The relationship between the decrease in BDNF concentration and central fatigue after 24 h has also been reported by other researchers; that is, brain performance and human well-being are associated with BDNF level (Krabbe et al., 2009; Hashimoto, 2010; Weinstein et al., 2014). It is interesting that the decrease in BDNF concentration was closely associated with the decrease in cortisol concentration after both exercise bouts. However, one may expect the opposite effect; that is, the higher the cortisol concentration, the lower the BDNF concentration because most studies show an inverse relationship between cortisol and BDNF concentrations (Issa et al., 2010; Rothman and Mattson, 2013). However, Marquez et al. (2015) did not find a negative correlation between cortisol and BDNF concentrations in humans.

BDNF inhibits the proinflammatory process (Cotman et al., 2007; Tong et al., 2008; Calabrese et al., 2014; Medina et al., 2015). However, we found no significant correlation between the changes in IL-6 and BDNF concentrations within 24 h after exercise. Physical exercise and stress can differentially modulate the expression of BDNF transcripts possibly through different epigenetic mechanisms, and this may explain why BDNF level is increased by exercise but decreased by acute stress (Rojas Vega et al., 2006). It seems that the lower initial BDNF level and the greater change after SIE should have been related to better exercise performance in our study. This idea was confirmed by the correlational analysis, which showed that the greater the change in BDNF concentration, the smaller the change in lactate concentration after exercise. Smaller changes in lactate concentration after exercise indicate higher aerobic capacity. Therefore, we speculate that a larger change in BDNF concentration after SIE may be a better indicator of aerobic capacity. BDNF is an essential neurotrophin that is also intimately connected to the central and peripheral molecular processes of energy metabolism and homeostasis (Knaepen et al., 2010). Thus, it is not surprising that the BDNF kinetics after exercise was closely related to the metabolic responses to an intensive load, as shown by the change in lactate concentration and T_{re} kinetics. The decrease in BDNF level associated with various diseases and depression (Brunoni et al., 2008; Stein et al., 2008; Hashimoto, 2010; Polyakova et al., 2015) suggest then that the decrease in BDNF concentration 24 h after exercise may be associated with increased central motor fatigue.

The psychological indicators of the prediction of BDNF kinetics after bouts seem surprising remembering the conclusions of other researchers that firstly the brain experiences the size of stress (McEwen et al., 2015). The brain perceives load size and pleasure/enjoyment very quickly and uses this information to modulate motor performance (Frazão et al., 2016). The brain is a central organ for perceiving stressors via multiple interacting mediators, including the HPA axis, the autonomic nervous system, and their non-linear interactions with the metabolic system and the pro- and anti-inflammatory components of the immune defense system (McEwen et al., 2015). Although motor performance and immune responses did not differ between FB and RB, the psychological and metabolic responses, and the stress level were lower after RB. These changes were closely related to the kinetics of the changes in BDNF.

It was surprising that the initial BDNF level predicted prolonged central motor fatigue after SIE. Other researchers have reported that BDNF level is lower at rest in exercise-trained people than in untrained people (Nofuji et al., 2008; Huang et al., 2014). We speculate that the higher training level of our subjects may have dampened the change in the CAR after the exercise bouts. Considering that the change in BDNF concentration is greater in trained people than in untrained people after an exercise load (Knaepen et al., 2010), we suggest that the greater the change in BDNF concentration, the smaller the long-term decline in the CAR after exercise.

CONCLUSION

Even though the FB and RB generated similar average cycling power and caused similar neuromuscular fatigue, the stress and immune-metabolic responses to repeated (vs. FB) SIE were suppressed and accompanied by a smaller increase in T_{re} and lower psychological exertion. Most of the changes in the psychological and physiological biomarkers observed in the FB and RB were closely related to the response kinetics of changes in BDNF concentration.

AUTHOR CONTRIBUTIONS

The authors MB and AS contributed to the design of the work. The authors MB, VV, NB, NE, MC, ES, and LD performed the experiments. The authors MB, VV, NB, NE, MC, ES, LD, and AS contributed to the analysis and interpretation of data for the work. The authors MB and AS drafted the work for important intellectual content. The authors MB, VV, NB, NE, MC, ES, LD, and AS finally approved the version to be submitted. The author MB contributed to the revision of this work. All the authors agreed 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.

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Behavioral Repertoire Influences the Rate and Nature of Learning in Climbing: Implications for Individualized Learning Design in Preparation for Extreme Sports Participation

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Extreme climbing where participants perform while knowing that a simple mistake could result in death requires a skill set normally acquired in non-extreme environments. In the ecological dynamics approach to perception and action, skill acquisition involves a process where the existing repertoire of behavioral capabilities (or coordination repertoire) of a learner are destabilized and re-organized through practice—this process can expand the individuals affordance boundaries allowing the individual to explore new environments. Change in coordination repertoire has been observed in bi-manual coordination and postural regulation tasks, where individuals begin practice using one mode of coordination before transitioning to another, more effective, coordination mode during practice. However, individuals may also improve through practice without qualitatively reorganizing movement system components—they do not find a new mode of coordination. To explain these individual differences during learning (i.e., whether or not a new action is discovered), a key candidate is the existing coordination repertoire present prior to practice. In this study, the learning dynamics of body configuration patterns organized with respect to an indoor climbing surface were observed and the existing repertoire of coordination evaluated prior to and after practice. Specifically, performance outcomes and movement patterns of eight beginners were observed across 42 trials of practice over a 7-week period. A pre- and post-test scanning procedure was used to determine existing patterns of movement coordination and the emergence of new movement patterns after the practice period. Data suggested the presence of different learning dynamics by examining trial-to-trial performance in terms of jerk (an indicator of climbing fluency), at the individual level of analysis. The different learning dynamics (identified qualitatively) included: continuous improvement, sudden improvement, and no improvement. Individuals showing sudden improvement appeared to develop a new movement pattern of coordination in terms of their capability to climb using new body-wall orientations, whereas those showing continuous improvement did not, they simply improved performance. The individual who did not improve in terms of jerk, improved in terms of distance climbed. We discuss implications for determining and predicting how individual differences can shape learning dynamics and interact with metastable learning design.

Keywords: learning dynamics, scanning procedure, intrinsic dynamics, rock climbing, motor learning, system degeneracy

INTRODUCTION

In recent years participation rates in extreme sports such as free solo climbing, where climbers perform in extreme environments without the use of safety aides such as ropes, have outstripped many traditional sports (Brymer and Schweitzer, 2013; Seifert et al., 2017). Performance in extreme climbing environments, where a fall would most likely result in death, places considerable physiological and psychological demands on the climber (Llewellyn et al., 2008). Deaths in climbing are most often attributed to climbing in extreme environments and when climbing without ropes (Lack et al., 2012). While the emotional and psychological requirements for climbing in extreme environments are often different from those required to climb in non-extreme environments, such as indoor climbing walls, many of the underlying skills required to complete a particular move when climbing a 3,000 m wall without ropes are the same as those required to undertake the same move in an indoor context (Brymer and Schweitzer, 2017).

Climbing in extreme environments not only requires a profound environmental knowledge and the ability to effectively assess environmental constraints such as weather conditions and rock or ice quality but also requires effective adaptability and highly tuned skills (Seifert et al., 2017). Understanding how individual climbers effectively acquire the skills required to perform at this level is important for the development of the individual climber and ultimately supports safer and more sustainable participation (Immonen et al., 2017).

How Environmental Design Can Support Adaptability in Climbing

This study adopts the ecological dynamics approach to perception and action. Ecological dynamics integrates ideas from dynamical systems theory and ecological psychology toward understand learning and behavioral change by the individual toward becoming adapted to a particular environment (Rietveld and Kiverstein, 2014; Davids et al., 2015; Immonen et al., 2017). In this framework, behavioral change is underpinned by principles of self-organization which overtime, enhance the individual-environment fit (Schöner et al., 1992; Edelman and Gally, 2001; Sumpter, 2006). Self-organizing systems can be described as systems which are initially disordered and where global order can emerge under the influence of the system's own dynamics (Bruineberg and Rietveld, 2014, p. 4). The effort to satisfy current constraints (interacting environmental, task, and individual factors) gives rise to perceptual-motor couplings that function to support the individual's perception of affordances (opportunities) for action (Davids et al., 2008). By learning new ways of acting adaptive, or acting adaptive in new situations, the individual enhances their movement system degeneracy (i.e., the capability to use structurally different elements for the same functions: Kelso, 2012) which can extend the boundaries of what their environment affords for action (Orth et al., 2017c).

The idea of affordances, first introduced by Gibson (1979), suggests that successful behavior is predicated on the information-based relationship between the individual and their environment. The generation and pick-up of information supports the perception of affordances (or invitations for action) (Withagen et al., 2012). During practice, instability facilitates exploration of alternative motor solutions, and, hence their adaptability (Hristovski et al., 2011; Van Orden et al., 2011; Bril et al., 2012). Practically speaking, a coach or experimentalist might develop knowledge for affordances of other individuals (i.e., affordances the coach can provide by establishing a certain set of constraints). In doing so, the coach or researcher can interact with constraints so as to shape learning without prescribing or presupposing a solution in advance (Silva et al., 2013). Assuming that the individual behaves in such a way that takes into account the limits on their action capabilities (an hypothesis associated with affordance-based control: Fajen, 2007; Croft et al., 2018), when the individual is positioned at the limits of their affordance boundaries this can lead to an increase in movement variability during practice (Prieske et al., 2015; Orth et al., 2018), which may drive learning (Schöllhorn et al., 2009; Chow et al., 2011). For example, in climbing, the individual will control their actions in some respects based on how long they perceive they can continue to remain in contact with the wall, which can be influenced fatigue (Fryer et al., 2012). In order to manage fatigue, participants need to learn to use holds in different ways, such as with different grasping actions or with body positions that are more mechanically efficient. Through practice, as the individual becomes capable of using more efficient actions they will climb increasingly difficult routes or in more complex environments—reflecting their action boundaries, separating their ability to climb possible and impossible routes, have been expanded (Fajen, 2007). **Figure 1** exemplifies these ideas, suggesting that constraints impinge on affordances at both the individual and the sociocultural frame of reference. Functional movement variability, including exploration, enhanced degeneracy, and discovery of new and

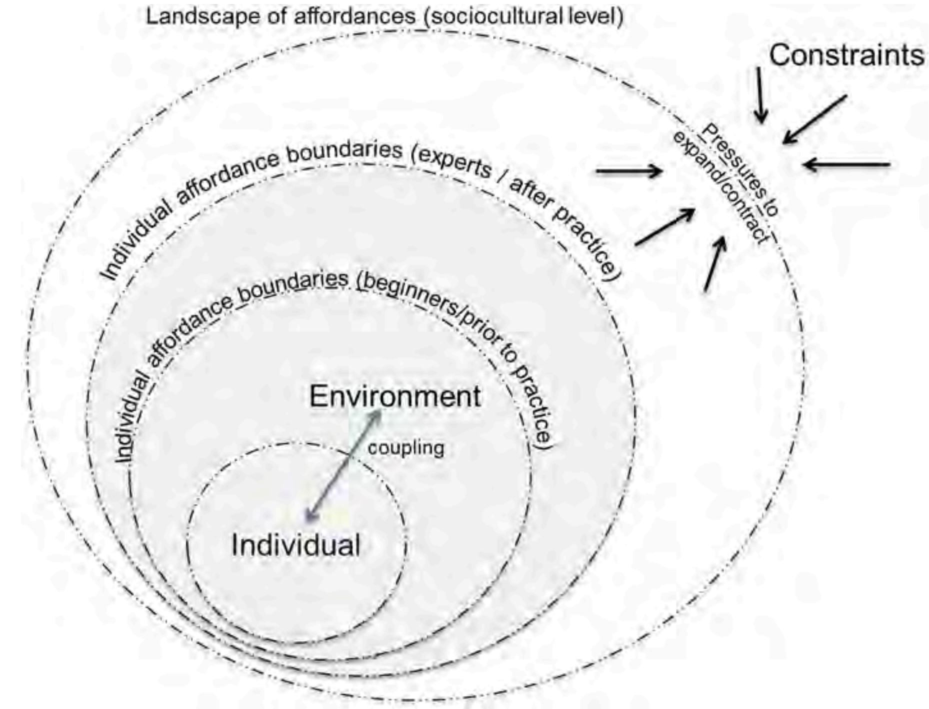


FIGURE 1 | The individuals practice is nested within their learning and development. Constraints at both the individual and the sociocultural frame of reference, interact to impinge on perceptual-motor couplings, supporting an expansion or contraction of affordance boundaries. Through functional movement variability, including exploration, enhanced degeneracy, and discovery of new and functional actions the individual and social landscape of affordances can be enriched.

functional actions are a behavioral mechanisms that support the expansion of affordance boundaries.

Operationalising these ideas, Orth et al. (2018) showed how a route designed with holds that could be grasped either using an overhand grip (like a ladder) or a side-grip (like grasping a cup handle) invited participants to carry out more exploratory actions during climbing (actions where individuals would touch a hold only to subsequently withdraw their hand to reposition it). Due to the route's design, beginners learned to explore holds whilst at the same time maintain climbing fluency when constraints were modified in a transfer test. In doing so, the learners extended their affordance boundaries (i.e., their ability to climb on new routes), by learning how to explore whilst maintaining fluency. According to Orth et al. (2018), the enhanced exploration in the dual-grasping route was because it allowed a fall-back option to an already stabilized movement pattern (grasping using the over hand grip and with body orientated face-on to the wall) thus, making exploration less risky. The study by Orth et al. (2018) also suggests that beginners need to learn how to use side-on body-wall orientations. Indeed, as shown by Seifert et al. (2015) intermediate skilled climbers tend to increase the amount of rolling motion at the hips when climbing holds were designed to promote these actions (specifically, edges running perpendicular to the ground plane were made available). Thus, the individual's current behavioral repertoire may have a strong influence on the nature and rate of learning in climbing tasks.

The Current Study

The purpose of the current study was to determine to what extent the individual's current behavioral repertoire (i.e., the extant perceptual-motor landscape of performance solutions that can be adapted by the individual with respect to a particular environment: Davids et al., 2015) affect the learning dynamics and subsequent emergence of new skills. In order to determine if a solution is new relative to the individual, a scanning procedure can be used to assess an individual's current behavioral repertoire, allowing determination of how it changes during and after practice (Zanone and Kelso, 1992). For example, prior to learning a new skill, a scanning procedure can uncover pre-existing stable and unstable coordination solutions when performing under a given set of constraints. In doing so, this can be used to determine how the perceptual-motor landscape is altered by practice (Zanone and Kelso, 1992). The main aim of this study was to evaluate, in beginner climbers, possible relationships between learning dynamics and the emergence of new patterns of movement coordination during skills practice in a route climbing task. We also aimed to evaluate, using a scanning procedure, any mediating relationship between the learning dynamics and the learners behavioral repertoire as assessed prior to and after practice.

In order to assess learning, we used variables that quantify climbing fluency (Orth et al., 2017b). Fluency in climbing is globally captured as jerk (a measure sensitive to the number of sub-movements made while climbing: Seifert et al., 2014), and can further be assessed separately along temporal and spatial dimensions (Orth et al., 2017b). To assess temporal performance, mobility is used and reflects the time spent moving relative to remaining stationary (Billat et al., 1995). To assess spatial performance, the entropy of the hip trajectory is used, where more straight forward trajectories are associated with behavioral certainty (Cordier et al., 1994b).

Using these variables, performance was observed over an extended time period (7 weeks of practice, 2 sessions per week). During practice we encouraged exploration by designing a climbing route where each hold had four good edges (top, bottom, and sides). The route also encouraged exploration of different pathways though the route. Following previous results (Seifert et al., 2015; Orth et al., 2018), we anticipated beginner climbers would be able to immediately climb the route with a face-on orientation to the wall. However, in order for beginners to substantially improve their climbing fluency, they would need to also use side-on body wall configurations. We predicted that the discovery of these new movement patterns would support a sudden improvement in performance. Additionally, we expected these movement patterns to absent prior to practice and be present after practice.

METHODS

Participants

Eight participants without prior experience of outdoor rock climbing were recruited to be involved in the learning study, noting that one dropped out during the experiment (see **Table 1**). Noting however, that participants had received a minimum of 10h of practice in indoor climbing, because this amount of practice was the minimum required to guarantee that participants can correctly know to set harness, rope and know belaying. Moreover, participants had roughly a 16–18 Ewbank skill level and so were not completely inexperienced. Further inclusion criteria required that participants be within the healthy BMI range (<25) and have an arm span of no <140 cm. This was done to ensure that climbers were able to reach holds as intended by two professional route setters. Notably one participant (P14) had a BMI of 26.8. However, because this result was because the individual had a larger proportion of muscle mass, he was permitted to participate. All participants were right handed.

Finally, testing occurred as part of a physical activity course for students enrolled at local university (Rouen Normandy University). In participating, they received a grade for their participation for the climb course unit. The local ethics committee of the Rouen Normandy University approved the protocol, who verified that wearing data acquisition equipment was compatible with climbing, which was validated. This study was carried out in accordance with the recommendations of the guidelines of the International Committee of Medical Journal Editors. The protocol was explained to all participants who gave written informed consent in accordance with the Declaration of Helsinki.

TABLE 1 | Participant details.

Learners	P12	P13	P14	P15	P17**	P18	P19	P21
Age (years)	18.0	19.0	21.0	20.0	21.0	22.0	24.0	18.0
Gender	F	M	F	M	F	F	M	F
On-sight ability (Ewbank*)	17	17	17	18	17	17	18	16
Standing height (cm)	162	182	186	171	176	156	165	163
Arm span (cm)	162	185	173	178	174	152	166	166
Body weight (kg)	54.6	68.4	83.0	58.5	64.2	53.0	72.5	59
Grip strength (kg)	26.2	54.6	52.0	26.0	28.3	23.8	58.0	22.5
Grip strength to weight ratio	0.48	0.80	0.63	0.44	0.44	0.45	0.88	0.38

** For conversions from Ewbank across other systems see Draper et al. (2011a).*
*** Dropped out during intervention.*

Experimental Design and Climbing Route Design

The study involved two pre-test and two post-test sessions, and 14 learning sessions in total. The learning sessions were distributed such that two learning sessions per week (e.g., Tuesday, Friday) were carried out over a 7-week period. Within each session participants were required to climb the same route three times, equating to 42 trials of practice overall. The volume of practice corresponded roughly to the typical length of a beginner level climbing course and matched values in existing studies reporting the acquisition dynamics of multi-articular skill (Delignières et al., 1998; Chow et al., 2008a). Pre-testing was carried out 1 week prior to commencing the learning sessions and post-testing was carried out 1 week after the final learning session (see **Figure 2**).

Across all testing sessions, participants upon arrival were fitted with climbing shoes and afforded a 10-min period to warm up their hands, feet, and body. Aside from stretching and mobility, during this time a very easy traverse was also carried out to allow participants to safely use their fingers and were prepared to support their body weight. They were then fitted with a harness and instrumentation (detailed below). Prior to undertaking each climb, the following global task instructions were given: "climb the route as fluently as possible, minimizing jerky movement, taking an efficient path through the route and minimizing prolonged pauses." Participants were then afforded a 3-min period to view the route from the ground, following which the trial was commenced. Between each climb, a seated 5-min rest was enforced to minimize effects of fatigue on performance. Globally, all routes were designed at 5b F-RSD by agreement of two qualified route-setters (Draper et al., 2011b). This difficulty was chosen as it corresponds to a beginner level of difficulty (Draper et al., 2011a).

Pre- and Post-test Scanning Procedure

Session 1 and Session 16

The pre- and post-test sessions required participants to undergo a scanning procedure modified to the climbing task and required three performance trials carried out on the same day. In theory

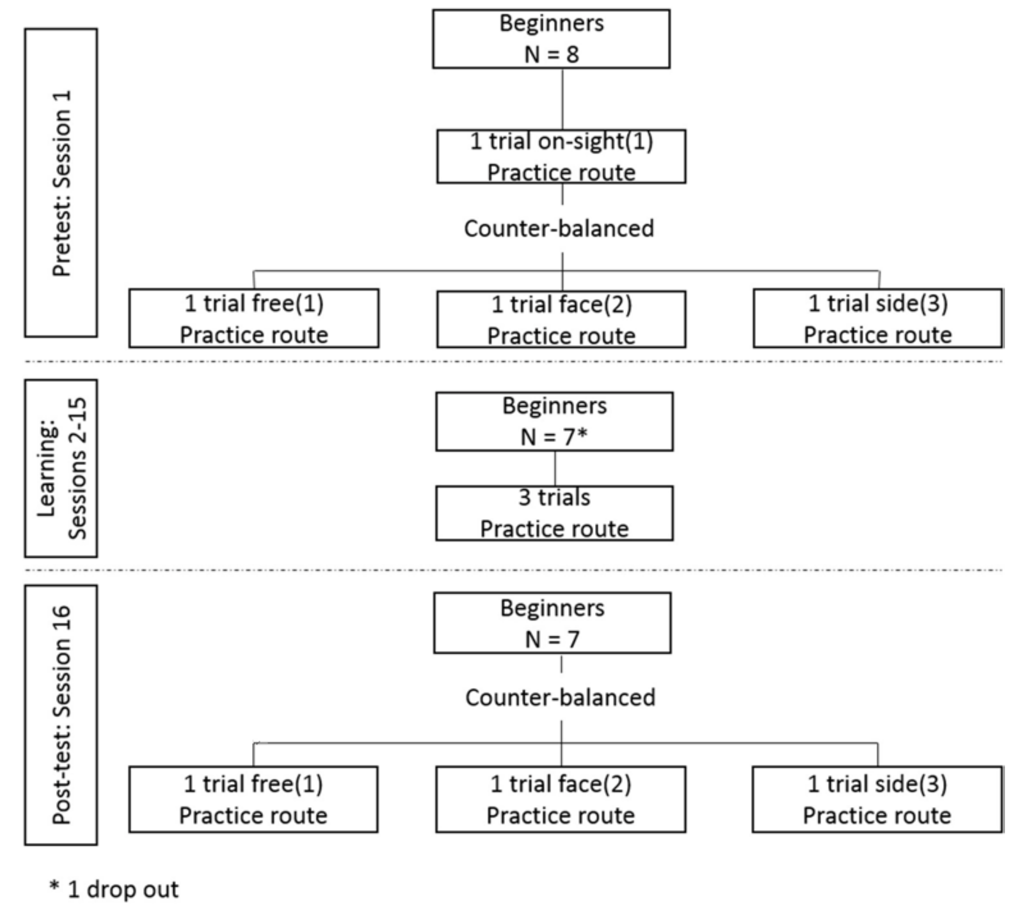


FIGURE 2 | Experimental design.

the approach involves scaling a parameter, in this case required body position, and observing effects on overall movement coordination and climbing fluency (including jerk, mobility, and entropy).

In order to assess how beginners organized their body with respect to the wall while climbing the orientation of the pelvis with respect to the wall was gathered while mobile. Obtaining the pelvis orientation during mobility was important as we were interested in body-wall coordination modes during route progression and not during resting (see the example given in **Figure 3**). Thus, the scanning procedure was designed to assess participants' capacity to coordinate different body-wall positions while mobile (when displacement at the hips was occurring).

The first climbing condition of the scanning procedure, acted as a reference and required individuals to climb under global task instructions (i.e., to get to the end of the route). The second condition required participants to climb the same route under instructions to maintain as much as possible the front of their body facing the wall (i.e., the "face-on" condition). The third condition required climbing with the side of the body facing the wall for as much as possible (i.e., the "side-on" condition). It was anticipated that the beginners would show better performance under the face-on condition, but, be unable to remain mobile when in the side-on condition. The order for each condition was counterbalanced to control for possible order of treatment effects. Participants were not given time to preview the route for the scanning procedure. Note also that the scanning procedure was carried out on the same route as the learning route. **Figure 3A** shows the position of each hold of the scanning and learning route with respect to the climbing wall plane.

Learning Sessions: Route and Procedures

Session 2 to Session 15

Learning sessions were carried out twice weekly with at least 2 days in between (e.g., Tuesday, Friday) and over a 7-week period. Within each learning session participants carried out three trials of practice per session on the same route. Between trials, participants were required to sit for no less than 5 min between trials without viewing the route. Before each trial they were allowed to preview the route for a maximum of 3 min if desired. Before participants climbed for the first time they were informed that they would be given feedback about their performance in terms of jerk, entropy and immobility. These data were also explained to

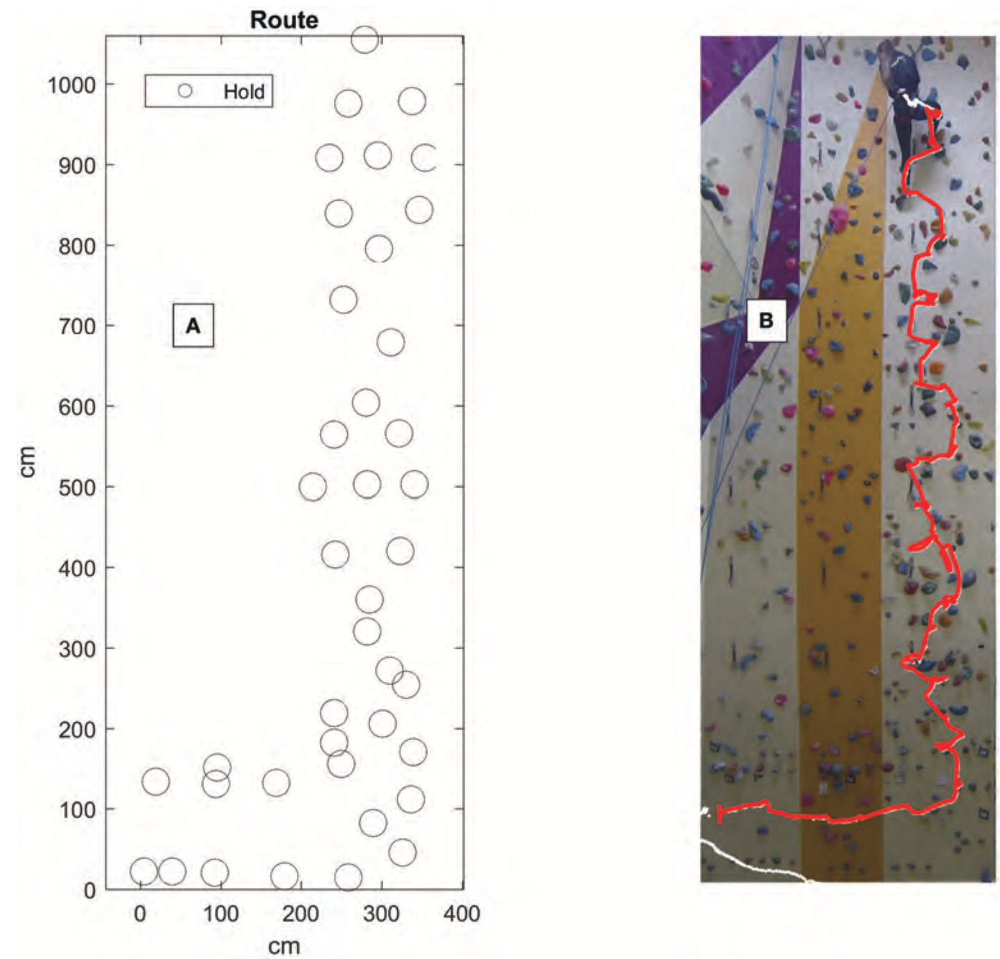


FIGURE 3 | Route design. **(A)** Circles indicate the location of the holds of the route used for the scanning and learning procedure. **(B)** Shows an example of the hip tracked and mapped over a photo of the route. The redline indicates the hip tracked from the beginning the end of the climb.

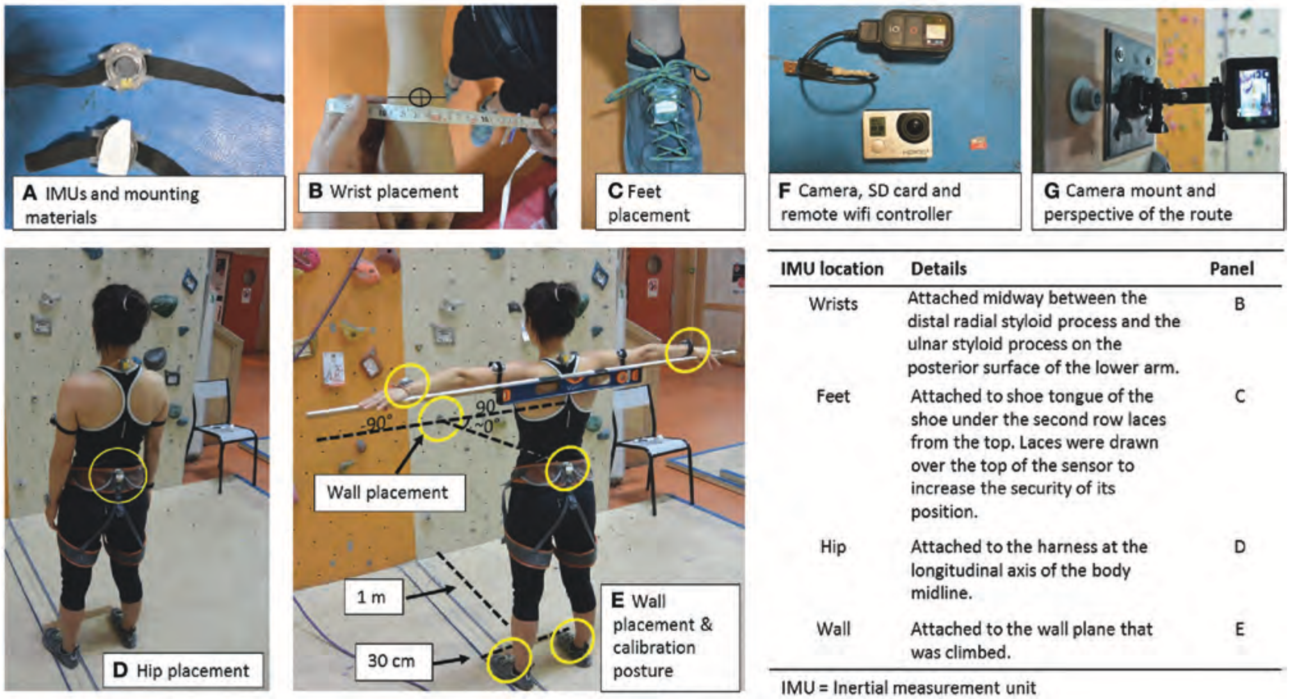
participants in terms of how movements at the hips affects these values. Jerk was explained to increase the more they fluctuated between increasing and decreasing their speed while climbing. Entropy was explained to increase with the more movements they used to get to the top. Immobility was explained to increase the longer they stayed still. Finally, participants were informed lower values of jerk, entropy, and immobility were indications of better performance. All instructions, belaying, and feedback were given by the same researcher across all sessions.

At the beginning of each learning session (not including the first session) feedback of climbing fluency was provided regarding the previous learning session. In addition to this, participants were emailed their feedback 48 h after each learning session. Specifically, the feedback given included three values, jerk, entropy, and immobility and also the adopted trajectory through the route for each trial. The climbed trajectory was conveyed in the form of photo overlay of their climbed trajectory onto a photo of the climbing wall. **Figure 3B** is an example image given to participants that exemplifies the data given.

Instrumentation

Data on directions of the trunk (3D unit vectors in Earth reference) were collected from small, wearable, inertial measurement units (IMU: **Figure 4A**). These IMUs contain three sensor components: a tri-axial accelerometer (±8G); tri-axial gyroscope (1,600°/s); and a tri-axial magnetometer (MotionPod, Movea©, Grenoble, France). Data collected from the IMUs were recorded with North magnetic reference at 100 Hz and transmitted by wireless connection with a control unit run off a desktop operating system. IMUs were attached to the hip to estimate the movement at the climber's center of mass without interfering with movement (**Figures 4B–D**). Finally, in order to orientate the sensor with respect to the vertical and wall references, participants were required to adopt a calibration position prior to each climb. This position was recorded for 10 s prior to each attempt (**Figure 4E**) and the average value was used to zero the angular positions relative to the wall reference. The same sensor and relative placement location, orientation, and procedure were used throughout the entire experiment.

Raw sensor recordings were then prepared. Specifically, gyroscope, accelerometer, and magnetometer information were



IMU location	Details	Panel
Wrists	Attached midway between the distal radial styloid process and the ulnar styloid process on the posterior surface of the lower arm.	B
Feet	Attached to shoe tongue of the shoe under the second row laces from the top. Laces were drawn over the top of the sensor to increase the security of its position.	C
Hip	Attached to the harness at the longitudinal axis of the body midline.	D
Wall	Attached to the wall plane that was climbed.	E

IMU = Inertial measurement unit

FIGURE 4 | Instrumentation. **(A–E)** show the inertial measurement unit placement and initial calibration posture (see embedded table, bottom right, for details). **(F,G)** show the instrumentation and positioning of the camera used to record each climb.

converted into a 3 × 3 rotation matrix that describes each sensor in an Earth frame (North, West and vertical). Specifically, as described in, Boulanger et al. (2016) two of the sensor components (accelerometer and gyroscope) in a rotation signal provides a better signal/noise ratio and the third sensor, the magnetometer, is used to obtain the Earth reference. A transformation is then performed through a complementary filter based algorithm (as described in, Madgwick et al., 2011).

Each trial was also captured with a frontal camera (GoPro© Hero 3) fixed 9.5 m away from the climbing wall and at a distance of 5.4 m from the ground and operated via remote wi-fi, with recordings directly captured to a SD card (**Figures 4F,G**). A red light was equipped to the back of the harness midpoint. Post processing involved automatic tracking of the red light position on a frame by frame basis (Boulanger et al., 2016). In order to synchronize the signals, we used the obtained trajectory data from the video to compute a time series estimating the acceleration of the pelvis. Then, using a maximum correlation measure between the sensor-recorded accelerations of the pelvis (the norm of the lateral and vertical components), the delay between each signal, video and sensor, was estimated (Boulanger et al., 2016). Finally, the start (first discernible reaching action made from quadrupedal support) and end (first discernible contact made with the final hold with both hands) were then manually determined from the video and the two signals extracted over the same time period accounting for the delay obtained in the previous step.

Computations

From the raw signals recorded during each climb, three forms of data were computed to assess performance and learning dynamics:

- jerk coefficient of translation–jerk reflects the spatial-temporal indicator of performance;
- geometric index of entropy–entropy reflects the spatial indicator of performance, and;
- the threshold based immobility to mobility ratio–immobility is the temporal indicator of performance.

Finally, in order to assess the body state dynamics, we took the orientation of the hip-wall angle when the individual was mobile.

Jerk

Jerk (the derivative of acceleration) is correlated with the number of sub-movements that compose gross actions (Seifert et al., 2014). The fewer sub movements made, the lower the jerk value.

When the trajectory is known, such as when the hip position is tracked relative to the wall surface, allowing that for a given trajectory $x:[O,T] \rightarrow R^3$, the dimensionless jerk coefficient of translation is defined as:

$$Jerk = \frac{T^5}{(\Delta x)^2} \int_0^T \left\| \frac{d^3x}{dt^3}(s) \right\|^2 ds \tag{1}$$

Noting that Δx is the length of the climbed trajectory.

Geometric Index of Entropy

The geometric index of entropy is a ratio of the path length of a trajectory to the perimeter of its convex hull and is a uniquely spatial indicator of performance (Cordier et al., 1994b). For a given trajectory $x:[O,T] \rightarrow R^3$, letting Δx as the distance of the path covered by the hips, and Δc the perimeter of the convex hull, we find:

$$Entropy_x = \frac{\log\left(2*\Delta x\right) - \log(\Delta c(x))}{\log(2)} \quad (2)$$

Note that the division by log(2) places the geometric index of entropy in dimensionless terms (bits). Thus, the greater amount of displacement that occurs within a given convex hull, the higher this value and more complex (or chaotic) the movement trajectory.

Threshold Based Immobility to Mobility Ratio

The relationship between periods of mobility to immobility is estimated by determining how long, with respect to the total climb time, an individual's COM remains in a stationary state, relative to a moving state. It is a uniquely temporal indicator of performance (Orth et al., 2017b). Time spent "immobile" reflects time under isometric contraction, incurring an energy cost (Billat et al., 1995). Since hip mobility is determined as a given level of displacement over time, a solution to remove potential operator bias is to directly use hip velocity, and apply a threshold. Thus, for this study a threshold value was applied to the velocity of the climber's trajectory.

Specifically, for a trajectory $x : [O, T] \rightarrow R^3$, we find the threshold based immobility to mobility ratio as:

$$Ratio\ of\ immobility\ to\ mobilty_x = \frac{\sum_{i=1}^{N} P_i}{N} \quad (3)$$

$$P_i = \begin{cases} 1, & if\ v_i < threshold \\ 0, & if\ v_i \geq threshold \end{cases} \quad (4)$$

$$v_i = f\sqrt{x_i^2 + y_i^2} \quad (5)$$

Hence the larger the threshold based immobility to mobility ratio, the longer the individual's respective COM is considered to be in a more immobile state.

Orientation of the Trunk When Mobile

The hip-wall orientation was taken as the angle formed between the hip sensor and a sensor positioned on the climbing surface (recall **Figure 4E**). Following Seifert et al. (2015), the time series of rotation around the axis perpendicular to the transverse plane was extracted using the wall reference such that 0°Corresponded to a face-wall position (the sagittal plane perpendicular to the climbing surface; and following the right hand rule, 90° rotation right side of the body is parallel to the wall plane, and; −90° left side of the body is parallel to the wall plane (**Figure 5A**). It was anticipated that when participants were requested to climb side-on to the wall, the body-wall angle distributions during mobility would be concentrated around 0° in the pre-test, and, more toward ±90° in the post-test. Thus, the probability distributions of the hip-wall angle segmented above threshold were used to assess the initial coordination and changes after practice revealed when undergoing the scanning procedure.

Indeed, it was found in pilot work that adopting a side-wall orientation could be achieved by experts while using holds for route progression. This is shown in **Figures 5D–F**. In contrast, beginners were less capable of using the side-on position when mobile. However, during periods of immobility it was anticipated that beginners might rest in the side-on position (as shown in **Figures 5D**, **4E**). As described in Fuss et al. (2013), skill effects related to more advanced movement patterns can require that the climber achieve a threshold of mobility in conjunction to the relative positioning of the COM with respect to wall. Thus, it was important to segment the hip-wall orientation according to states of more or less mobility. To separate between states of mobility and immobility, we chose to set a threshold of movement at the hip of 20 cm/s which allowed a feasible and objective quantification of mobility. Whilst previous work has classified an individual as immobile using frame-by-frame analysis of an operator, this is extremely time consuming and open to operator bias. For example, criteria for mobility have included statements like: "progress of the hips was observed" (Billat et al., 1995) whereas, criteria for static climbing have included: "no discernible movement in pelvic girdle" (White and Olsen, 2010). **Figure 5**, provides face validity of using 20 cm/s, shown in the normalized density histograms comparing the hip-wall angles segmented above and below the threshold used to determine mobility (for additional discussion see: Orth et al., 2017b).

Statistical Procedures

The experimental design for addressing pre- and post-test findings required a repeated measures ANOVA with three levels of instruction (free, face, and side) and two levels of time (pre and post). In cases where main or interaction effects were significant, planned contrasts were carried out assuming that variables indicating skilfulness (i.e., jerk, entropy, and immobility) would improve due to practice. For effects related to condition, it was anticipated that performance in the side-on condition would be worse relative to the face-on condition (i.e., revealed in higher levels of jerk, entropy and degree of immobility relative to the face-on condition).

To examine learning dynamics, a one-way repeated measures ANOVA was used with planned contrasts to assess, at the group level, at what point a plateau in performance was evident by contrasting each trial with the final trial. It was anticipated, that the level of mobility would plateau after entropy (Cordier et al., 1994b, 1996; Orth et al., 2017a).

When the sphericity assumption was violated in the repeated measures variables, Greenhouse-Geisser adjustments are made. Finally, effect sizes, were reported in cases where a focused effect is addressed (i.e., comparisons involving two groups) by converting F-ratios to r-values following Field (2009, p. 501). Noting that: $r = 0.10$ reflects small effect; $r = 0.30$ is a

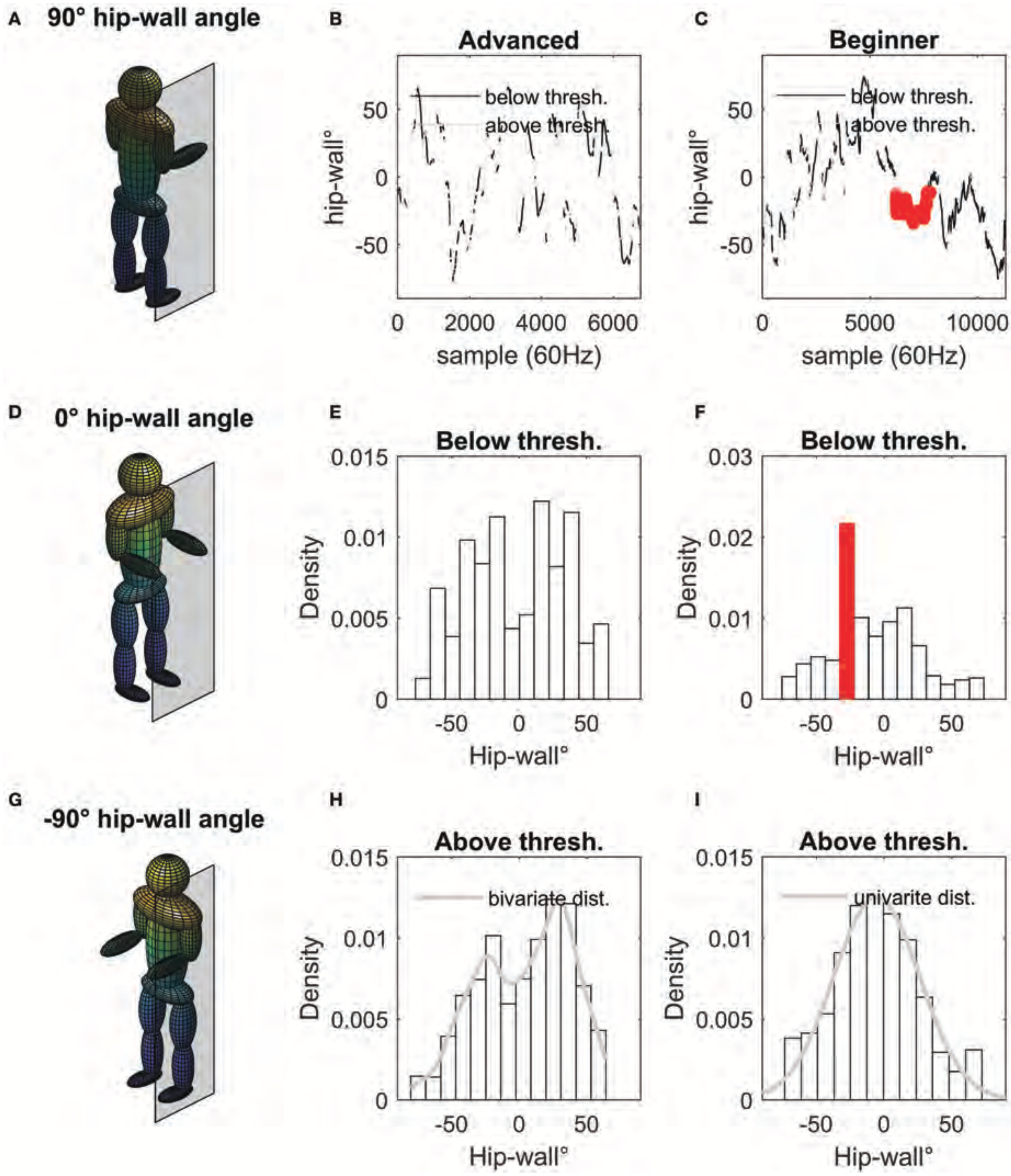


FIGURE 5 | The hip-to-wall angle data reduction. The mannequins **(A,D,G)** on the left show two general coordination patterns: face-on (middle mannequin), and; side-on (top and bottom mannequins). The middle column of graphs **(B,E,H)** shows the angular position of the hip of an expert climber. The time series data of the hip **(B)** shows the hip-wall angle when moving below 20 cm/s (black line) and above (gray line). When moving below 20 cm/s the climber is considered "immobile" (**E** shows these data as a frequency histogram). When moving above 20 cm/s the climber is considered "mobile" **(H)**. For contrast, a beginners data is shown **(C,F,I)**. When the beginner is immobile they may rest in a side-on position **(F)**. However, beginners find it difficult to use a side-on body-wall position when mobile **(I)**. Note that density (y-axis) of the histograms is used in place of frequency as these data have been normalized so that the area under the curve is equal to one. dist. = distribution. thresh. = threshold.

medium effect, and; $r = 0.50$ is a large effect. Additional follow-up tests beyond planned contrasts were done using pair-wise (dependent) t-tests with Bonferroni corrections. All statistics were run using IBM® SPSS® Statistics version 21. All effects are reported at a statistical significance level of $p < 0.05$.

RESULTS

In the following sections, group outcomes of the pre- and post-test on the scanning procedure are given. We then address the learning dynamics, finally moving to individual analyses. Note that since participant 17 did not complete the practice intervention, she was removed from any statistical analysis.

Results at the Group Level

Grouped Pre- and Post-test Scanning Procedure

The outcomes, jerk, entropy, and degree of mobility of the scanning procedure were assessed across three levels of instruction (free, face, and side) and two levels of time (pre and post). There was a significant main effect of practice on: jerk, $F_{(1, 6)} = 11.56$, $p < 0.01$, $r = 0.81$; entropy, $F_{(1, 6)} = 72.96$, $p < 0.001$, $r = 0.96$, and; level of mobility, $F_{(1, 6)} = 59.53$, $p < 0.001$, $r = 0.95$. The decreases in jerk, entropy, and immobility after practice were all large effects. There were no significant effects for instruction, nor was there a significant interaction between practice and instruction. Also shown in **Figure 6** are the hip-wall angle distributions (bottom row of histograms). These findings suggest that, at the group level, participants were capable of being mobile and oriented face-on and side-on to the wall both before and after practice.

Grouped Learning Dynamics

The grouped outcomes showed that across each outcome (jerk, entropy, and immobility), an improvement in performance was observed through practice (see **Figure 7** which summarizes the session average for each variable). The main effect of trial was significant for all outcomes: jerk, $F_{(1, 13)} = 5.15$, $p < 0.001$; entropy, $F_{(1, 13)} = 4.68$, $p < 0.001$, and; level of mobility, $F_{(1, 13)} = 11.62$, $p < 0.001$.

Repeated contrasts were then performed to evaluate whether performance on each outcome variable improved at the same or different rates. Contrasts were, therefore, set up to compare each session relative to the penultimate session of practice (session 14). When contrasts were not statistically significant to the final trial of practice, performance can be considered plateaued (Cordier et al., 1994b). These outcomes are summarized in **Table 2**, which shows that both jerk and entropy values plateaued at session 7 whilst participants continued to improve their level of mobility until session 9.

We then performed a final follow-up test to address whether any sudden improvements in performance could be identified at the group level. That is, the purpose of the follow-up tests were to examine whether from one session to the next if, at the group level, performance could be shown to improve gradually or abruptly. Pairwise (dependent) comparisons were performed comparing each session of practice to the next session (e.g., session 1 vs. session 2, session 2 vs. session 3, and so on until session 13 vs. session 14). These were performed (with Bonferroni corrections) on jerk, entropy, and immobility values. No statistically significant differences between sessions were uncovered.

DISCUSSION

Grouped Outcomes

At the group level, findings are generally in support of previous literature in climbing. Jerk, entropy, and immobility have all been implicated as indicators of skill in climbing (Cordier et al., 1994b; Billat et al., 1995; Seifert et al., 2014), and is well corroborated in these data, each showing clear tendencies to improve through practice. This is the first study that examined jerk, entropy, and immobility in combination and we anticipated that participants would learn to co-vary movement complexity (entropy) with climbing mobility (Orth et al., 2017b), but that, initially, these two outcomes would improve at different rates through practice (Cordier et al., 1994a, 1996; Orth et al., 2017a). The latter expectation is supported, the former is not.

In this first instance, we expected that the learners would increase movement complexity and level of mobility in the side-on condition. In doing so, this should help to maintain a stable level of jerk (Orth et al., 2017b). The outcomes, when compared across the pre- and post-test, did not support this prediction–in so far that they did not reveal a significant interaction of route and time (pre- vs. post-test) for any of the outcome variables. A reason for this may be that the post-test scanning procedure was carried out on the same route as was practiced. This probably led to a tendency to climb faster compared to unfamiliar routes, and without needing to adapt movement complexity alongside mobility when using either the face-on or side-on body positions.

Additionally, at the group level, we did not find a clear indication that prior to practice, the beginners needed to learn how to climb, whilst mobile, in a side-on position. As shown in the probability density plots in **Figure 6**, these findings suggest that, as a group, the individuals had the capability to immediately adapt this position–and that further practice was beneficial at a level of general refinement or optimization of movement parameters supporting fluent climbing (Chow et al., 2008b; Hristovski et al., 2011).

These ideas are generally supported in the data on learning dynamics at the group level (see **Figure 7**), where over practice, improvement in terms of jerk, entropy, and immobility followed a fairly linear progression (also generally corroborated with the lack of significant session to session differences tested in the follow-up). However, given the large standard deviations present at the group level, additional individualized analyses were carried out. Indeed, examining the grouped hip tracings in **Figure 7** shows that a large range of climbed trajectories were used and prompted us to carry out an exploratory analysis to examine any important differences at the individual level (Liu et al., 2006).

Individual Analysis: Qualitative Assessment of the Learning Curves

Individual Learning Dynamics and Their Relationship to the Scanning Procedure

In the first step of the exploratory analysis, we examined each of the individual's learning curves. Here we present the values on jerk for each trial of practice (jerk is presented since is a spatial-temporal indicator of fluency and provides a more global indication of change than immobility and entropy; Orth et al.,

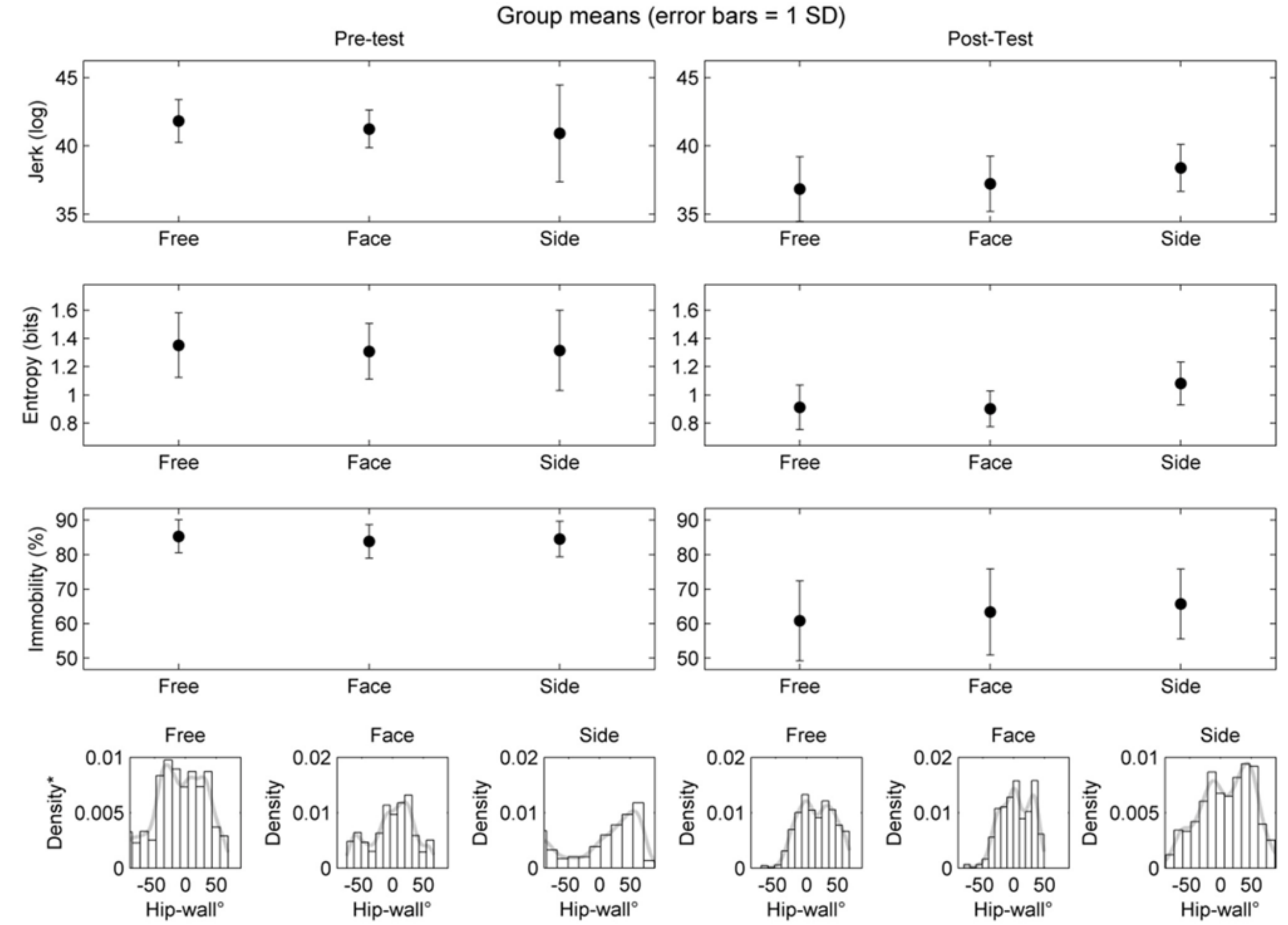


FIGURE 6 | Grouped outcomes from the scanning procedure for the pre-test **(left)** and post-test **(right)**. Only the main effect of practice was found to be statistically significant. Note that free refers to the condition where climbers climbed as they liked. Face refers to the condition where climbers were asked to try to climb the route while maintaining as much as possible facing the wall with the front of their body. Side refers to when climbers were asked to climb the route as much as possible with the side of their body facing the wall. * = y-axis of histograms represent the normalized density of the hip-wall angle during mobility.

2017b). In doing so, three types of curves were qualitatively identified (see **Figure 8**):

- progressive (continuous) improvement (participants 12, 13, 15, and 19);
- sudden improvement (participants 14 and 18 and possibly 17);
- no improvement (participant 21).

After identifying these differences using the individual learning curves, we re-examined the pre- and post-test hip-wall angle data grouped as progressive; sudden improvement, and; no improvement. These data, presented in **Figure 9**, suggest that the initial capability to climb side-on to the wall while mobile affected the learning dynamics. Specifically, the most compelling findings as shown in **Figure 9**, which suggests that for participants where the hip wall angle was well spread from −90 to 90 degrees in the pre-test showed a progressive improvement during learning. For participants who showed a concentration of the hip-wall angle around 0 degrees in the pre-test showed a sudden improvement during learning.

Progressive Improvement

Participants who appeared to improve progressively during practice, in the pre-test when asked to climb as much as possible with the side of their body relative to the wall, this hip-wall angle reflects a flatter, more spread distribution (see also the pre-test post-test histograms for each individual in **Figure 10**). By examining the time normalized raw data of the hip roll (see the primary axis of the line plots for each individual in **Figure 10**), it appears these individuals were able to transition multiple times from around 0° (indicating a face-on position) to more oblique angles toward positive or negative 90°.

Sudden Improvement

In **Figure 11** the individual results for participants 14 and 18 (along with participant 17) are presented. In contrast to the progressive improvement group, the nature of the histograms for this group are qualitatively different. For these individuals, in the pre-test, the histograms are less spread out and they are more concentrated toward a 0° value suggesting that while climbing,

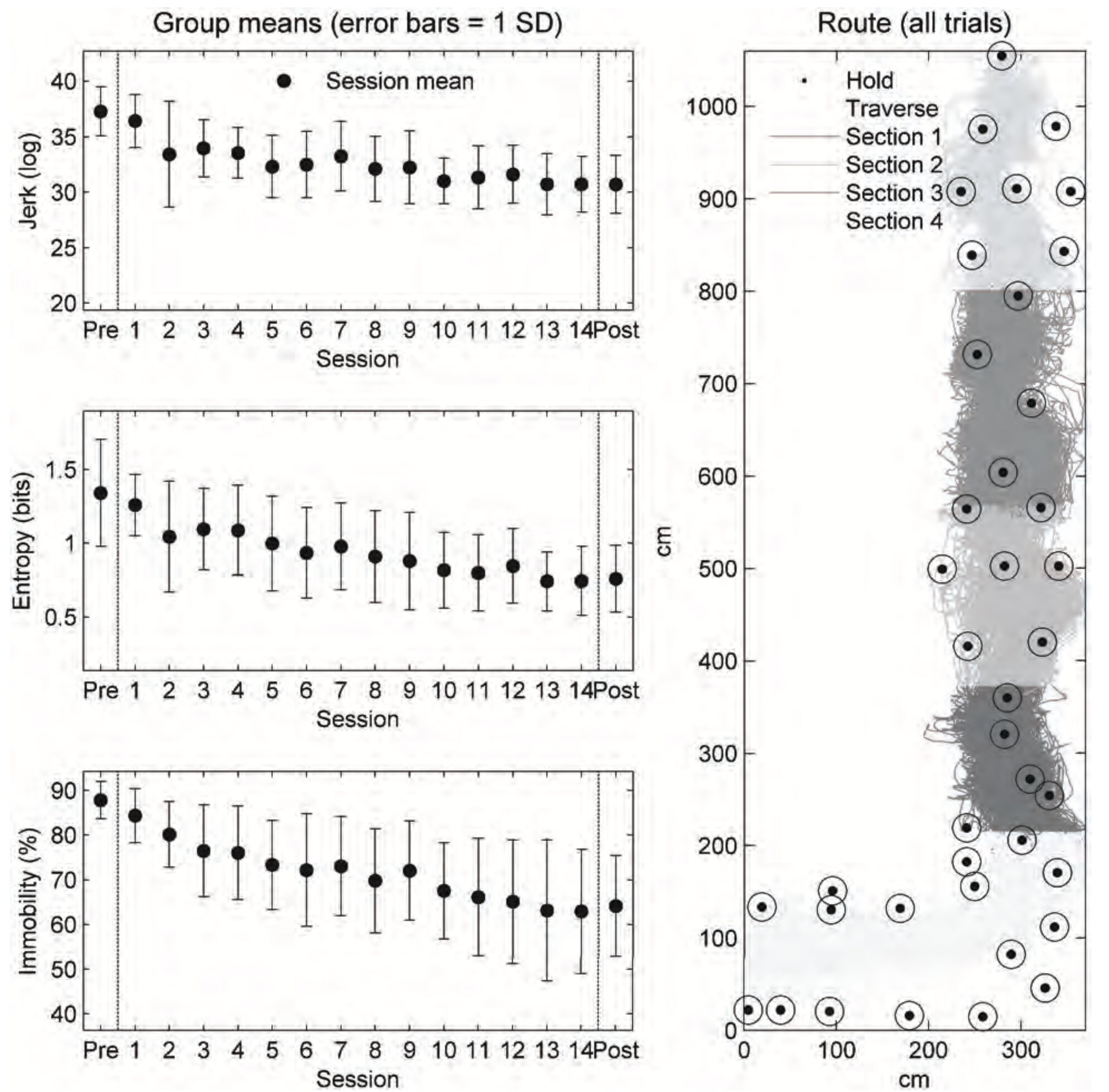


FIGURE 7 | Grouped outcomes through practice. The graphs on the left show the session average and standard deviations of jerk **(top)**, entropy **(middle)**, and immobility **(bottom)** for the seven climbers followed through practice. The graph on the right indicates the design characteristics of the route. Dotted circles indicate the position of the holds. The hip position of all trials of practice across all participants onto the wall plane. The different shades of the hip data are a function of the section of the route (see the legend).

they were more face-on to the wall. The time-normalized data of the hip (primary axis of the line plots) provide support for this interpretation. Additionally, in clear contrast to the progressive improvement subgroup, the time series data of the hip reflect a general inability of these individuals to switch from a facing (~0°) and remain for any extended period of time in an oblique (around ±50°) or side-on (around ±90°) position relative to the wall. Also notable is that in the pre-test, participant 14 fell about halfway up the route (see the secondary red axis of the time-series data) and, that participant 18 took much longer to finish the route (355 s) than the participants in the progressive improvement subgroup (where the longest time for the progressive improvement group was 190 s). Finally, participant 17 was also included with this subgroup, because after examining her pre-test data, her hip-wall orientation was also concentrated around 0°. This leads us to speculate that if participant 17 had continued to practice, a sudden transition would have occurred in her performance dynamics.

TABLE 2 | Group level ($n = 7$) contrasts of each trial against the final trial for each outcome variable.

Contrast	Jerk (plateau at Session 7)	Entropy (plateau at Session 7)	Immobility (plateau at Session 9)
Session 1 vs. Session 14	$F_{(1, 6)} = 148.26$, $p < 0.001$, $r = 0.98$	$F_{(1, 6)} = 48.43$, $p < 0.001$, $r = 0.94$	$F_{(1, 6)} = 34.23$, $p = 0.001$, $r = 0.92$
Session 2 vs. Session 14	$F_{(1, 6)} = 2.10$, $p > 0.05$, $r = 0.51$	$F_{(1, 6)} = 4.47$, $p > 0.05$, $r = 0.65$	$F_{(1, 6)} = 18.66$, $p < 0.05$, $r = 0.87$
Session 3 vs. Session 14	$F_{(1, 6)} = 9.28$, $p < 0.05$, $r = 0.78$	$F_{(1, 6)} = 9.91$, $p < 0.05$, $r = 0.79$	$F_{(1, 6)} = 16.90$, $p < 0.05$, $r = 0.86$
Session 4 vs. Session 14	$F_{(1, 6)} = 9.29$, $p < 0.05$, $r = 0.78$	$F_{(1, 6)} = 21.21$, $p < 0.05$, $r = 0.88$	$F_{(1, 6)} = 12.81$, $p < 0.05$, $r = 0.83$
Session 5 vs. Session 14	$F_{(1, 6)} = 6.28$, $p < 0.05$, $r = 0.72$	$F_{(1, 6)} = 7.88$, $p < 0.05$, $r = 0.75$	$F_{(1, 6)} = 39.14$, $p < 0.05$, $r = 0.93$
Session 6 vs. Session 14	$F_{(1, 6)} = 10.60$, $p < 0.05$, $r = 0.80$	$F_{(1, 6)} = 5.16$, $p > 0.05$, $r = 0.68$	$F_{(1, 6)} = 11.77$, $p < 0.05$, $r = 0.81$
Session 7 vs. Session 14	$F_{(1, 6)} = 9.35$, $p < 0.05$, $r = 0.78$	$F_{(1, 6)} = 6.98$, $p < 0.05$, $r = 0.73$	$F_{(1, 6)} = 7.72$, $p < 0.05$, $r = 0.75$
Session 8 vs. Session 14	$F_{(1, 6)} = 5.59$, $p > 0.05$, $r = 0.69$	$F_{(1, 6)} = 3.41$, $p > 0.05$, $r = 0.60$	$F_{(1, 6)} = 7.21$, $p < 0.05$, $r = 0.74$
Session 9 vs. Session 14	$F_{(1, 6)} = 4.90$, $p > 0.05$, $r = 0.67$	$F_{(1, 6)} = 2.43$, $p > 0.05$, $r = 0.54$	$F_{(1, 6)} = 7.14$, $p < 0.05$, $r = 0.74$
Session 10 vs. Session 14	$F_{(1, 6)} = 0.41$, $p > 0.05$, $r = 0.25$	$F_{(1, 6)} = 1.47$, $p > 0.05$, $r = 0.44$	$F_{(1, 6)} = 4.69$, $p > 0.05$, $r = 0.66$
Session 11 vs. Session 14	$F_{(1, 6)} = 3.16$, $p > 0.05$, $r = 0.59$	$F_{(1, 6)} = 0.66$, $p > 0.05$, $r = 0.31$	$F_{(1, 6)} = 3.36$, $p > 0.05$, $r = 0.60$
Session 12 vs. Session 14	$F_{(1, 6)} = 43.67$, $p < 0.05$, $r = 0.94$	$F_{(1, 6)} = 8.73$ $p < 0.05$, $r = 0.77$	$F_{(1, 6)} = 1.14$, $p > 0.05$, $r = 0.38$
Session 13 vs. Session 14	$F_{(1, 6)} = 0.00$, $p > 0.05$, $r = 0$	$F_{(1, 6)} = 0.06$, $p > 0.05$, $r = 0.10$	$F_{(1, 6)} = 0.03$, $p > 0.05$, $r = 0.07$

*For mean differences and directions of effects see **Figure 7**.*
Note that diagonal lines indicate that the differences in the outcome variable during climbing between the final session of practice and the first session of practice were not statistically significant (alpha set at p < 0.05).

No Improvement

Figure 12 shows the data for participant 21 (the individual showing "no improvement" in terms of jerk). **Figure 12** shows all data from the pre- and post-test scanning procedure (free, face-on, and side-on). In the pre-test, across all conditions, participant 21 fell very early in the route. In the free and side-on condition she fell during the traverse (the first horizontal portion of the route). In the face-on condition, she was able to climb with a total vertical displacement of roughly 300 m (falling at about 400 m up the route). to be in a These findings indicate that the key difference between this individual in the pre-test and those in the progressive improvement and sudden improvement groups, was the ability to move vertically. Participant 21 appeared in a stage of learning where only postural stability was possible (i.e., traversing left and right, or standing stationary). Further examination her hip position data through practice also indicated that a significant amount of practice was required for her to successfully ascend the route. Interestingly, her learning curve in terms of jerk (**Figure 8**) shows a tendency to increase before finally, at around trial 27, it started to improve.

Discussion of the Individual Analysis

In sum, the idea that some participants exhibited a specific learning dynamic as a function of their initial capabilities to ascend the route in a side-on position and move vertically, is a possible interpretation of these data (summarized in **Figures 7**, **8**).

That is, it might be predicted that:

1. Individuals with a flat distribution of body wall orientation when required to be mobile whilst side-on will exhibit a progressive improvement in learning dynamics.

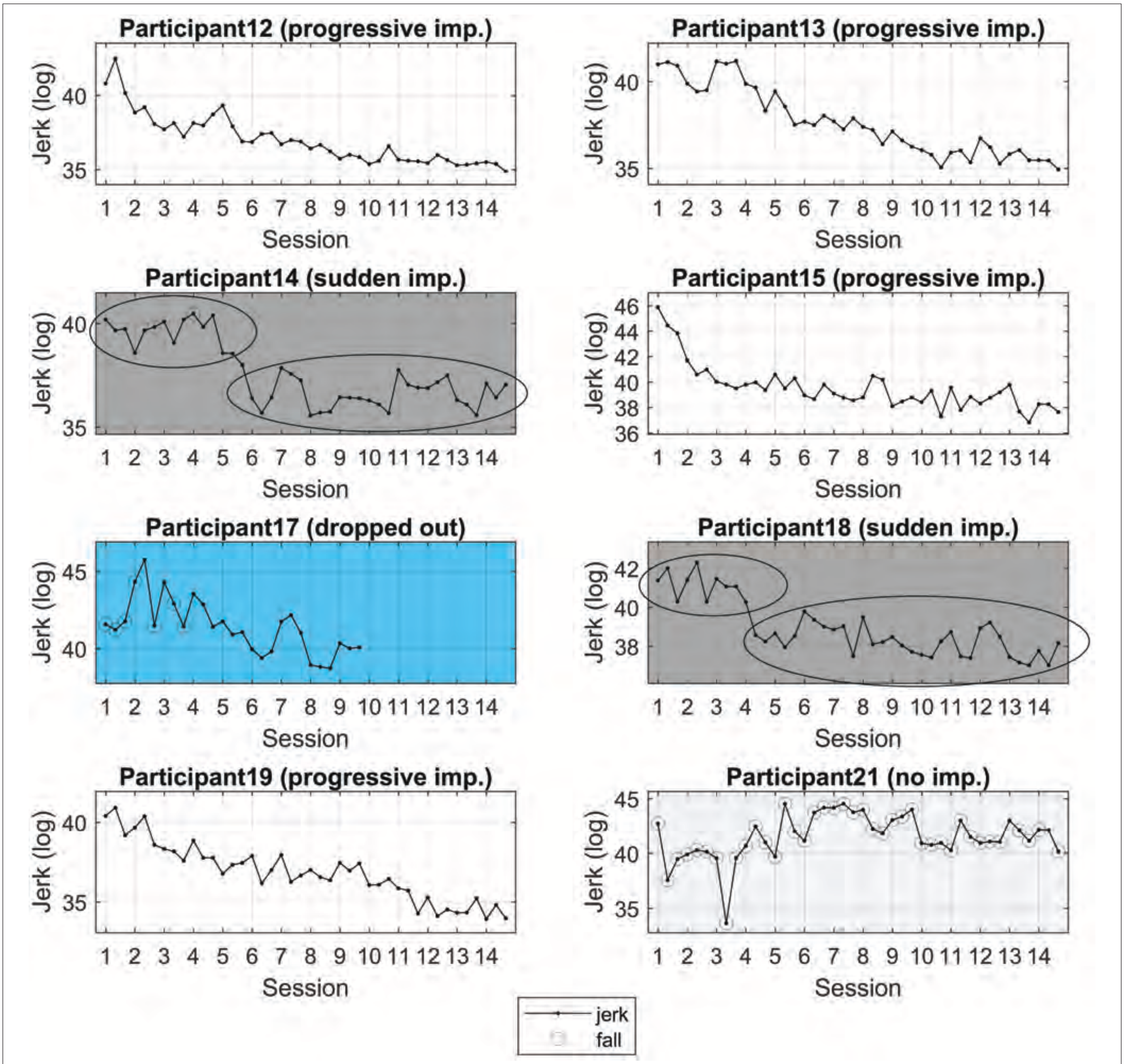


FIGURE 8 | Individual learning curves. Three types of learning curves are apparent. Continuous improvement (participants 12, 13, 15, and 19); sudden improvement (participants 14 and 18), and; no improvement (participants 21). imp. = improvement. Note that here different scales are used in the y-axis in order to accentuate the nature of the learning curve for each individual. Different color schemes are used to highlight subgroupings.

2. Individuals who exhibit a concentration toward a face-on position and show a limited capability to use a side-on coordination pattern will exhibit learning dynamics where an initial period of little improvement gives way to a sudden jump in performance.
3. Individuals unable to move vertically whilst face-on to the wall will exhibit very slow learning dynamics.

GENERAL DISCUSSION

The aim of this study was to determine if through practice, individuals would acquire a new, more advanced pattern of coordination. However, this seemed only to occur for some participants and not others. Our exploratory findings revealed the nature of each individual's learning is not dependent on whether he/she can be classified prior to practice as a beginner

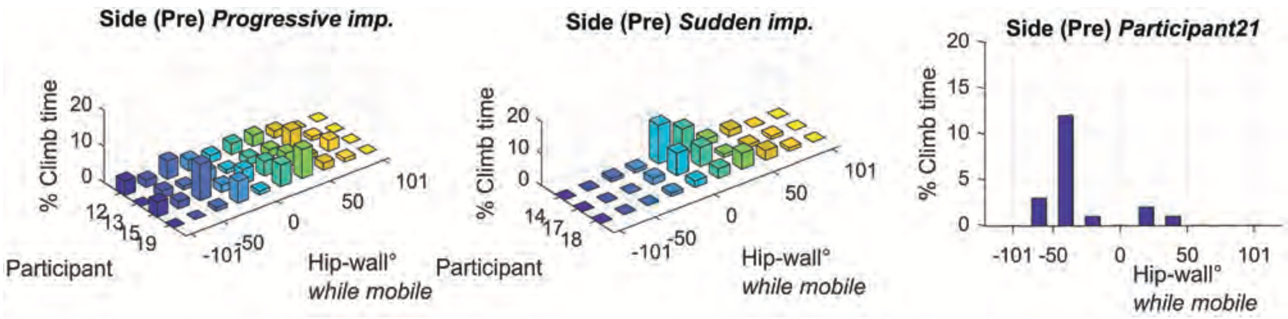


FIGURE 9 | Histograms of each individual's hip to wall angle, expressed as a percentage of time spent climbing under the side-on condition during the pre-test. The left graph shows the data for the participants 12, 13, 15, and 19. Note the spread out nature of the hip-wall angle data. The middle graph shows the data for the participants 14, 17, and 18. Note the concentrated nature of the hip-wall angle. The right graph shows participant 21. The sub groups were formed according to the nature of the learning curves discussed and shown in **Figure 8**. Note that, based on the histogram data here, participant 17 was included with the sudden improvement group as her data is highly concentrated around 0 degrees.

(e.g., such as based on their amount of prior specific task experience or categorical ability level; Draper et al., 2011b). Rather, the nature of learning dynamics was likely dependent on each individual's behavioral repertoire prior to practice.

These findings lend support to previous work showing that individuals display different responses during learning to a given set of constraints, in terms of the nature and/or rate of learning (Liu et al., 2006, 2012). In Liu et al. (2006) participants were required to practice a hand held ball roller task over 7 days, where the aim was to achieve and maintain a certain rotation speed of the ball. In their study 3 out of 11 participants were not able to succeed in achieving a set criterion level of performance over the time given to practice. Additionally the successful participants had two subgroups of response: one subgroup improved in terms of both a qualitative and quantitative change and; the other subgroup did not undergo a transition (qualitative change) but still improved performance with practice. Other studies have also shown similar results, such that some individuals do not improve, others improve suddenly, and others improve gradually if at all (Vereijken et al., 1992, 1997; Delignières et al., 1998, 1999; Nourrit et al., 2000, 2003; Teulier et al., 2006; Teulier and Delignières, 2007). Although these studies have successfully identified different coordination regimes, they have largely failed to provide an understanding for why individuals differ in terms of their learning dynamics.

By identifying prior to practice current coordinative capabilities, our findings provide support for the idea that individual differences present prior to practice manifest themselves during practice in the rate and nature of learning. In doing so, differences in learning dynamics are explained as a function of the individuals prior repertoire of coordination (Kostrubiec et al., 2006, 2012). Indeed, in bi-manual coordination tasks (learning to finger waggle at specific frequencies and relative phase) where scanning procedures were initially operationalized (Zanone and Kelso, 1992), it has been proposed that there are two basic mechanisms or routes for learning a new required movement pattern–smooth shift or abrupt qualitative change (Kostrubiec et al., 2006, 2012). In cases where prior to learning, individuals who were initially bi-stable (able to produce in-phase and antiphase regimes), the tendency is for abrupt qualitative change in overall movement behavior. In individuals with initially multi-stable [able to produce in-phase, antiphase and some other regime(s)] solutions, the tendency is for a smooth shift in overall behavior.

Seifert et al. (2015) showed that experienced climbers tend to use more oblique positions of the hip relative to the wall when using climbing holds that encourage a side-on pattern of coordination. Similarly, in Orth et al. (2018), it was found that inexperienced climbers used more complex climbing trajectories on routes where holds encourage the use of side-on body positions. Furthermore, the beginners used less complex climbing trajectories when holds encouraged a face-on position. In contrast, a group of experienced climbers showed no significant differences in movement complexity across routes encouraging either face on and side-on climbing actions (Orth et al., 2018). In explaining the results of these studies (Seifert et al., 2015; Orth et al., 2018), the experienced climbers were deemed to have a larger repertoire of movement patterns that they could adapt as constraints (climbing hold orientation) changed. Beginners on the other hand, still needed to "find" these stable regimes of coordination. To explain the emergence of new movement patterns, it has been previously argued that exploration of different ways of grasping or using holds is a key mechanism (Seifert et al., 2013).

This study uncovered a more nuanced explanation, that the individuals behavioral repertoire is a key candidate for determining how exploration is functional, such as for either finding an efficient pathway through the route or finding new movement patterns of coordination (Orth et al., 2017a). This is an important distinction, since previous research in climbing has quantified exploration where the hand or foot comes into contact with a hold and is subsequently withdrawn without using that hold for progression or support (Pijpers et al., 2006; Orth et al., 2018). A key question in understanding the role of movement variability that accompanies the learning process is to determine the specific intentions that underlies exploratory behaviors (Orth

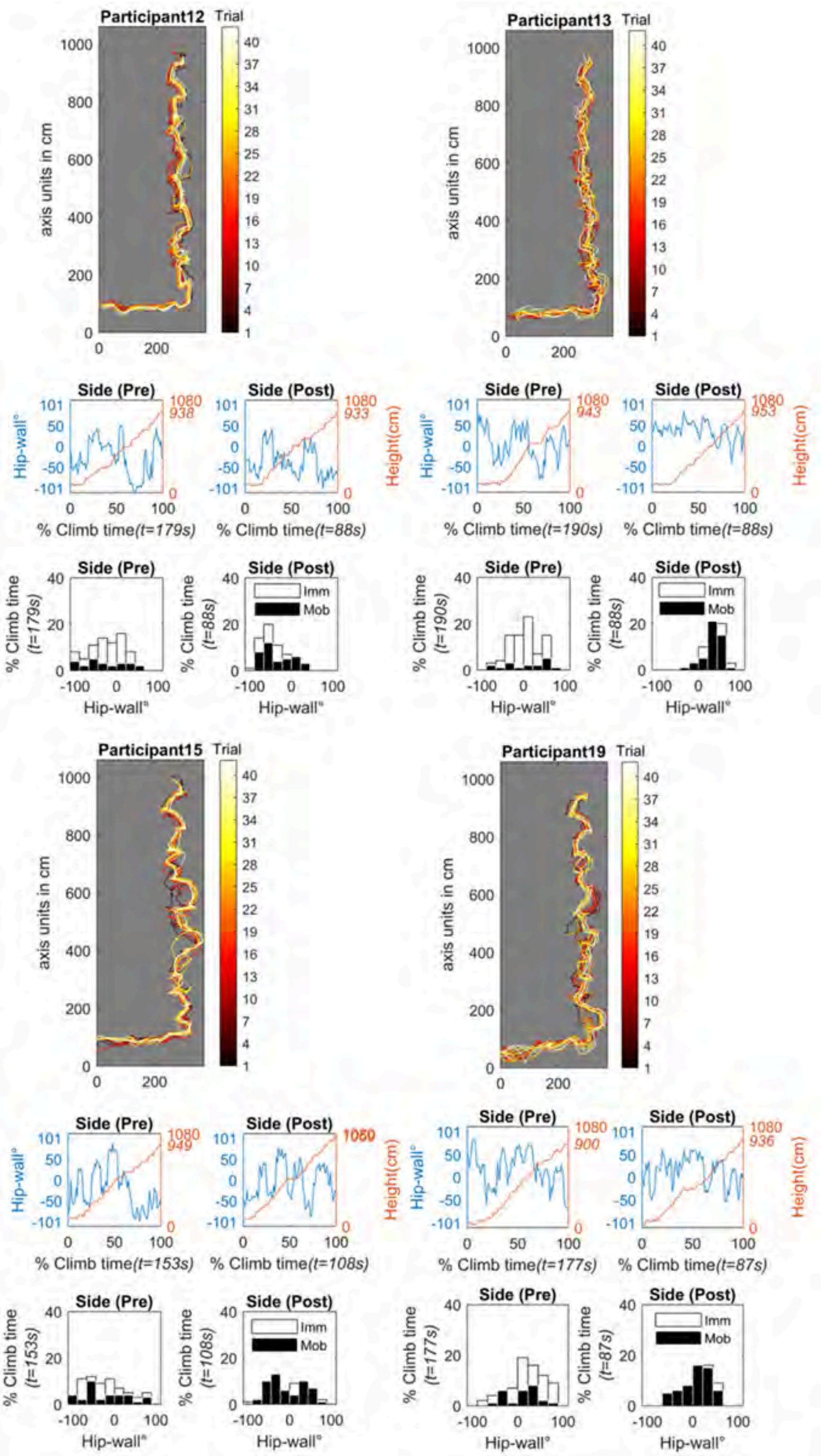


FIGURE 10 | Progressive improvement subgroup made up of participants 12 **(top–left)**, 13 **(top–right)**, 15 **(bottom left)**, and 19 **(bottom right)**. For each participant, all practice trials are projected onto the wall plane (the black trace represents trail 1, the white trace is trial 42). The time-series graphs show the pre-test and post-test of the side-on condition. Shown are the time-normalized hip-wall angle (primary y-axis, blue line) and the time-series of the height of the hip position (secondary y-axis, red line). The histogram graphs show the pre- and post-test of the side-on condition. Shown is the relative time spent in different body-wall angles while mobile (black bins) or immobile (white bins) angle in degrees. Imm, Immobile; Mob, Mobile; t, total time.

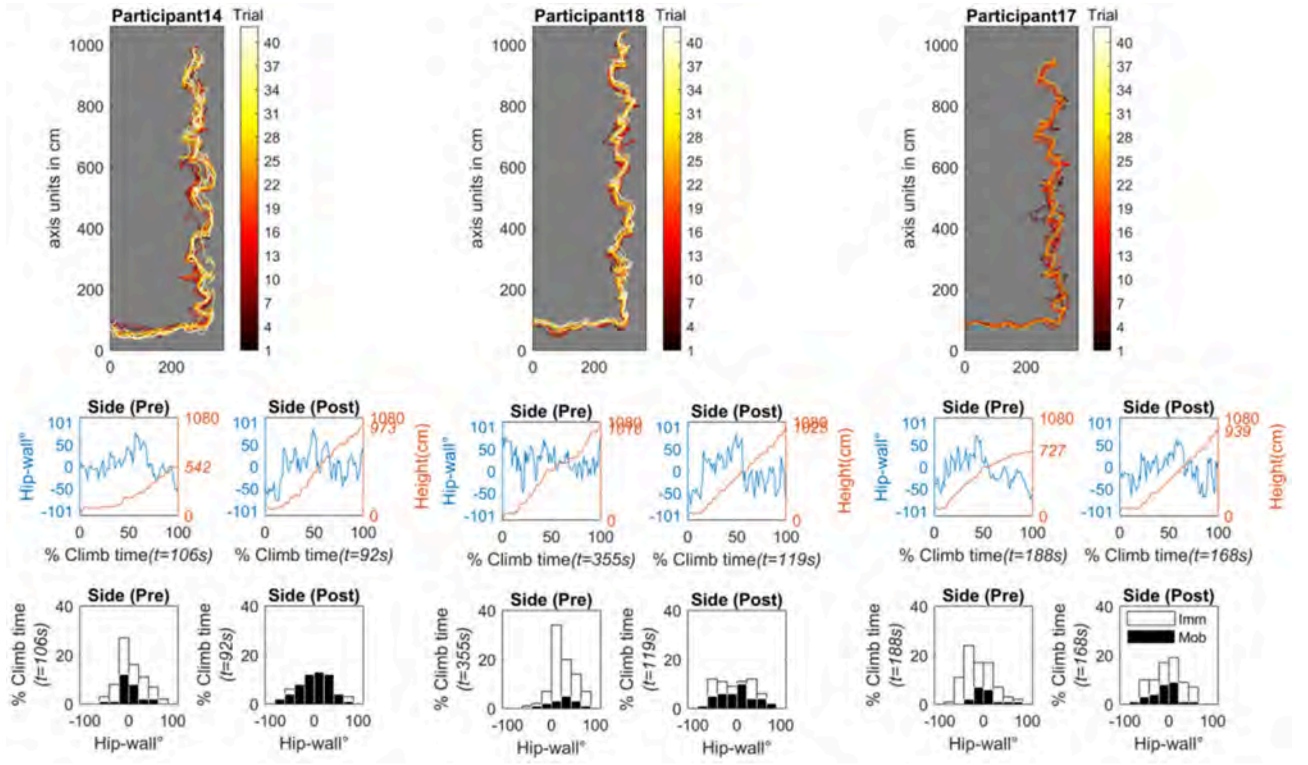


FIGURE 11 | Sudden improvement subgroup made up of participants 14 **(left)**, 18 **(middle)**, and 17 **(right)**. Note participant 17 (the participant who dropped out after practicing 27 trials) was also added to this subgroup after examining the participant's histogram results relative to participants 14 and 18 (recall **Figure 9**). For each participant, all practice trials are projected onto the wall plane (the black trace represents trail 1, the white trace is trial 42). The time-series graphs show the pre- and post-test of the side-on condition. Shown are the time-normalized hip-wall angle (primary y-axis, blue line) and the time-series of the height of the hip position (secondary y-axis, red line). The histogram graphs show the pre- and post-test of the side-on condition. Shown is the relative time spent in different body-wall angles while mobile (black bins) or immobile (white bins) angle in degrees. Imm, Immobile; Mob, Mobile; t, total time.

et al., 2017b). According to the results in this study, we predict that a learners intentions will be in some way determined by their pre-existing repertoire of coordination. The way an individual will explore and learn in a given task is influenced by the number of movement solutions that they can already exhibit under that set of constraints (Kostrubiec et al., 2012). The implications for climbing in extreme environments is that climbing walls can be used as effective learning contexts for the development of movement skills in extreme environments, if accompanied by opportunities that enhance environmental knowledge and personal judgment skills.

More broadly, these findings suggest that, if the individual is able to safely explore (e.g., explore without failing the task), new movement patterns of coordination may be learned more rapidly (Seifert et al., 2015; Orth et al., 2018). Alternatively, in cases where a continuous improvement in performance occurs through practice, it is possible that these individuals do not need to qualitatively reorganize their overall movement patterning (Newell, 1985). Continuous improvement could reflect a refinement of a stable performance solution to achieve an outcome (Chow et al., 2007, 2008a). In this case, individuals may be able to effectively improve performance through making minor movement adaptations. In sum, these findings suggest when preparing individuals to learn in new contexts (for example, using the climbing wall environment to learn skills potentially required for extreme climbing environments), by understanding an individual's capabilities prior to practice, practitioners can more effectively plan the design of learning problems so that the individual is invited to seek out and potentially discover new motor solutions.

CONCLUSION

In sum, personal (behavioral repertoire) and task/environmental (equipment, surfaces, edges, etc.) constraints that can influence effective exploration of a learning environment are an important consideration since they may influence whether or when a transition to a new movement solution eventuates (Delignières et al., 1998; Pacheco et al., 2017). In experimental designs where the search for new and functional solutions are allowed to emerge spontaneously (a solution is not proscribed), learners can display different trajectory dynamics (or learning curves). For example, different learning curves can include continuous improvement, sudden improvement; and, no improvement (Liu et al., 2006; Pacheco et al., 2017). One explanation for different routes to learning is the level

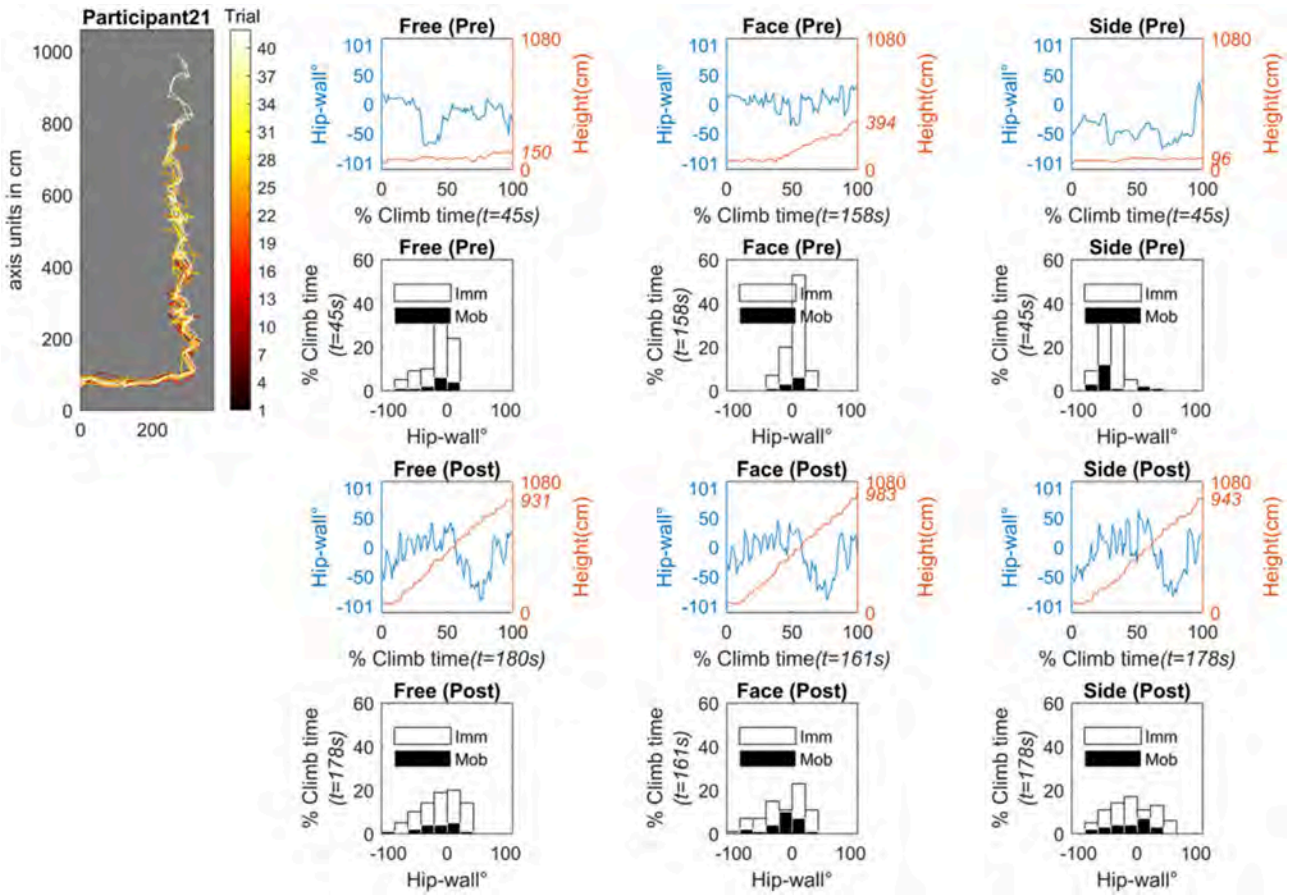


FIGURE 12 | The individual (participant 21) who showed no improvement in terms of jerk, however, it should be clear that she did improve in other ways with practice. The graph on the top-left shows all practice trials projected onto the wall plane (the black trace represents trail 1, the white trace is trial 42). The pre-test data for the scanning procedure is shown in the top two rows of graphs and the bottom two rows show the post test data. The free condition (where the climber climbed as they liked) is given in the left column. The face condition (where the climber is asked to climb as much as possible while facing the wall) is given in the middle column. The side condition (where the climber is asked to climb as much as possible with the side of their body facing the wall) is given in the right column. Time series data are the time-normalized hip-wall angle (primary y-axis, blue line) and the time-series of the height of the hip position (secondary y-axis, red line). Histogram graphs show the pre- and post-test of the side-on condition. Shown is the relative time spent in different body-wall angles while mobile (black bins) or immobile (white bins) angle in degrees. Imm, Immobile; Mob, Mobile; t, total time.

of competition of a to-be-learned pattern with an already established behavioral repertoire (Nourrit et al., 2003; Kostrubiec et al., 2012).

A continuous-improvement in performance through practice may be more likely in individuals who do not need to dramatically modify their overall movement patterning and could reflect a refining of the current movement pattern to achieve the outcome. These individuals, may be able to effectively improve performance through making minor adjustments in control processes because their current behavioral repertoire is sufficient (Newell, 1985; Chow et al., 2008a). Alternately, individuals who exhibit sudden improvement can show higher levels of behavioral variability surrounding transitional periods which suggests the to-be-learned behavior is initially unstable (Teulier et al., 2006; Delignières et al., 2011). Finally, in situations where an individual does not improve through practice, the task dynamics may be too complex relative to the individual's current performance capabilities. A transitional (new) behavior may not surface, possibly preventing the individual from achieving the task goal even after extensive practice (Delignières et al., 1998; Liu et al., 2006; Pacheco et al., 2017). One reason individuals may show no improvement is that they do not have sufficient capability to explore effectively. The ability to explore, or exploration itself, has been identified as a candidate cause of sudden improvement as it may uncover "transitional information" needed to support a new mode of coordination (Newell, 1991; Teulier et al., 2006; Pacheco et al., 2017). In climbing, because of the added element of height from the ground and risk of injury due to falling, facilitating safe exploration is particularly relevant (Seifert et al., 2015). Indeed, if an individual feels unsafe to climb they can become more restricted in their movements (Pijpers et al., 2006), perhaps leading to ineffective exploration of the task dynamics.

One of the key challenges to the practitioner is to appropriately scale task difficulty relative to the learner over time. The data presented in this study suggests that task difficulty can be understood in terms of the extent to which the individual's current capabilities will compete or cooperate with the task. The level of competition may be better understood through operationalizing scanning procedures as exemplified in this study. Subsequently, the learner or coach can identify constraints that influence the individual's stability in the search of ways for achieving a fluent and successful climb on new routes. Performing in extreme environments requires effective decision making skills as well as climbing skills paying attention to individual differences in skill acquisition is important to ensure that learners undertake climbing in extreme environments at an appropriate time. Recognizing that individuals develop at different rates and in different ways depending on prior capacities not only supports effective skill acquisition in climbing but also broader preparation for climbing in extreme environments.

AUTHOR CONTRIBUTIONS

DO, KD, LS: Planning experiments, developing rationale; DO: Performing data acquisition and analysis, and writing the manuscript; DO, KD, J-YC, EB, and LS: Revising follow up versions of the manuscript.

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frontiers in Integrative Neuroscience

ORIGINAL RESEARCH
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Hyperbaric Oxygen Environment Can Enhance Brain Activity and Multitasking Performance

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Background: The Brain uses 20% of the total oxygen supply consumed by the entire body. Even though, <10% of the brain is active at any given time, it utilizes almost all the oxygen delivered. In order to perform complex tasks or more than one task (multitasking), the oxygen supply is shifted from one brain region to another, via blood perfusion modulation. The aim of the present study was to evaluate whether a hyperbaric oxygen (HBO) environment, with increased oxygen supply to the brain, will enhance the performance of complex and/or multiple activities.

Methods: A prospective, double-blind randomized control, crossover trial including 22 healthy volunteers. Participants were asked to perform a cognitive task, a motor task and a simultaneous cognitive-motor task (multitasking). Participants were randomized to perform the tasks in two environments: (a) normobaric air (1 ATA 21% oxygen) (b) HBO (2 ATA 100% oxygen). Two weeks later participants were crossed to the alternative environment. Blinding of the normobaric environment was achieved in the same chamber with masks on while hyperbaric sensation was simulated by increasing pressure in the first minute and gradually decreasing to normobaric environment prior to tasks performance.

Results: Compared to the performance at normobaric conditions, both cognitive and motor single tasks scores were significantly enhanced by HBO environment ($p < 0.001$ for both). Multitasking performance was also significantly enhanced in HBO environment ($p = 0.006$ for the cognitive part and $p = 0.02$ for the motor part).

Conclusions: The improvement in performance of both single and multi-tasking while in an HBO environment supports the hypothesis which according to, oxygen is indeed a rate limiting factor for brain activity. Hyperbaric oxygenation can serve as an environment for brain performance. Further studies are needed to evaluate the optimal oxygen levels for maximal brain performance.

Keywords: HBOT, hyperbaric oxygenation, dual tasking, oxygen limitation, enhancing brain activity
ClinicalTrials.gov ID: NCT03126669, Unique Protocol ID: 213/13

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INTRODUCTION

The brain is the body's largest consumer of oxygen, utilizing roughly 20% of the total oxygen and consuming 25–30% of the total glucose (Lennie, 2003). Even though <10% of the brain's maximal capacity is active at every given time, the brain utilizes almost all delivered oxygen (Sokoloff et al., 1955). In order to perform different tasks or more than one task at a time (multitasking), the oxygen supply is shifted from one region of the brain to another via blood perfusion modulation (Lennie, 2003). These perfusion changes can be easily visualized by functional magnetic resonance tomography (fMRI) technology (Tombu et al., 2011). Multiple studies have demonstrated that our ability to perform complex activities decreases under oxygen depleted environments (Shukitt-Hale et al., 1998; Lieberman et al., 2005; Malle et al., 2013). However, the possible effect on brain performance by a single hyperbaric oxygen (HBO) environmental exposure has not been studied in humans.

Brain performance is highly sensitive to any decrease in oxygen supply. A reduction of the plasma oxygen pressure to 65 mmHg will impair the brain's ability to perform complex tasks, at 55 mmHg short-term memory will be impaired, while at <35 mmHg consciousness will be lost (Zauner, 2002). The effects of a hypobaric environment (decreased oxygen level) in individual motoric and cognitive performances were studied at high altitudes. At high altitudes or other oxygen depleted environments, cognitive and motor performances are impaired while performing relatively simple tasks (Shukitt-Hale et al., 1998; West, 2002, 2003; Mortazavi et al., 2003; Lieberman et al., 2005; Malle et al., 2013). On the other hand, elevation of oxygen levels, even at normobaric conditions, was found to facilitate cognition by decreasing the response time in the elderly (Choi et al., 2013). In a randomized control trial where memory consolidation (as a measure for cognition) was evaluated, increased oxygen supply at normobaric conditions (sea level) to healthy young participants, word memorization was more efficient compared to control group (Moss and Scholey, 1996).

While multitasking at a normal environment (normal air at sea level), oxygen is required in multiple brain regions simultaneously. The relative deficiency of oxygen may explain the decrease in processing speed, accuracy and other neuro-cognitive performances (Spelke et al., 1976; Han and Marois, 2013). It is assumed that conscious attention to two different actions performed at the same time is possible only if the tasks are coordinated into a single, higher-order activity (Spelke et al., 1976) or that at least one of the activities is being done "automatically" without conscious awareness (Spelke et al., 1976; Han and Marois, 2013). Donohue et al. have found that the ability to perform more than one task is limited even in individuals who are very experienced in one of the given tasks (Donohue et al., 2012).

The aim of the present study was to evaluate whether an HBO environment, with increased oxygen supply to the brain, will enable better performance of complex and/or multiple activities in healthy individuals.

MATERIALS AND METHODS

A prospective, double blind randomized control, crossover trial was performed at the Sagol Center for Hyperbaric Medicine and Research, Assaf-Harofeh Medical Center, Israel. This study was carried out in accordance with the recommendations of Assaf-Harofeh medical center institutional review board with written informed consent from all subjects. All subjects gave written informed consent in accordance with the declaration of Helsinki. The protocol was approved by the Center's institutional review board.

Participants

The study included 22 healthy volunteers, aged 20 years or older with a minimum of 12 years of formal education. Patients were excluded if they had any inner ear pathologies, lung disease, or any mental or physical limitations of being exposed to a hyperbaric chamber environment.

Protocol and End-Points

Participants were randomly assigned to be evaluated in one of the two environments: normal room air in normal pressure (control) and HBO (intervention). After a 2-week wash-out period, participants were crossed to the alternative environment (**Figure 1**).

During each session, participants were seated in the chamber with masks on. In the intervention session, the pressure in the chamber was increased to 2 ATA, and the participants breathed

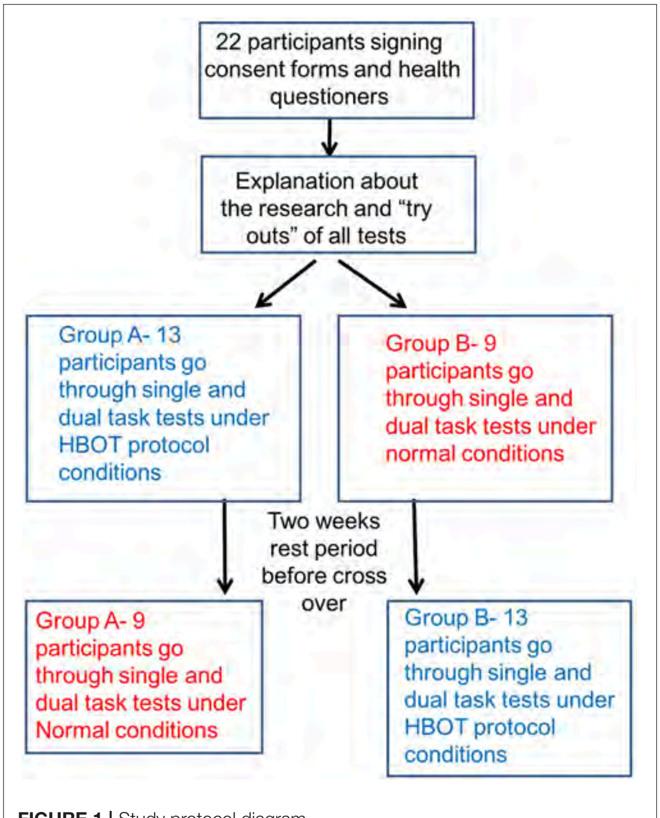

FIGURE 1 | Study protocol diagram.

100% oxygen for 45 min. In the control session, in order to manipulate the participants, the chamber pressure was increased for less than a minute so they felt some pressure in the ears, and a flow of 21% oxygen was used for the masks. The tests/tasks were initiated 30 min after the masks were on within the chamber.

Cognitive performance was evaluated by a symbol-digit test (example in Appendix 1), and the motor performance was evaluated by the units of beans that were transferred from a plate to a cup (One of the tasks in the motor assessment scale test-MASS, Appendix 2). The dual tasking was a combination of the cognitive and the motor tests which were done simultaneously.

Cognitive/Attention Symbol-Digit Modality Test (SDMT)

The symbol digit modalities test (SDMT, Appendix 1) is a test used to assess divided attention, visual scanning, tracking, and motor speed (Strauss et al., 2006). In this test, participants are presented with series of symbols, each indicates a different number. While they are repeatedly presented with those different symbols they are asked to write the adequate number next to each symbol (the Appendix includes an example of the task recoding). The test was found to be reliable in four different forms in healthy and cognitive impaired conditions (McCaffrey, 1988; Hinton Bayre and Geffen, 2005; Dickinson et al., 2007; Paramenter, 2007). Three different forms of the SDMT were used in each evaluation session. The first was a try-out for the test, ensuring that the task was understood. The second tested the ability to perform an individual task. The third form was used in combination of the motor task for examining dual task performance. Standard administration procedures were followed as indicated in the test manual (Smith, 1973). The score of the test is the number of correct substitutions during a 90-s interval.

Motor Task: Transferring of Beans

Motor performance was evaluated by the motor assessment scale test (MAS, Appendix 2)—transferring individual beans between two tea cups an arm's length away. The MAS test was found to be reliable for assessing advance hand-motor functioning in post stroke patients (Carr, 1985; Pool and Whiteny, 1988). One of the advantages of this task is that during its performance the participant is not required to have full vision focus on the hands, allowing the participant to do other tasks. In addition, the test can be easily performed under the conditions inside a hyperbaric chamber. The MAS score is based on the number of beans that were successfully transferred in the given time (90 s).

Statistical Analysis

Statistical analysis was done using SPSS software (version 22.0). Continuous data are expressed by mean ± STD (standard deviation) and compared by an independent t-test for inter-group comparison and by a paired t-test for intra-group comparison. P-values < 0.05 were considered statistically significant. All randomly allocated patients were included in the safety analysis and those that went through all the assessments were included in the efficacy analyses.

RESULTS

Twenty-four healthy participants signed an informed consent and were included in the study. Two patients were excluded: One did not complete the crossed evaluation as required by study protocol and the other could not adjust to the hyperbaric conditions. Accordingly, a total of 22 healthy volunteers were included in the final analysis (11 females and 11 males, age 22–68 years (mean 42 ± 13 years) with 12–30 education years at the time of the study (mean 16 ± 4 years). Baseline participant characteristics are summarized in **Table 1**. The results of the cognitive and the motor tests are summarized in **Table 2**.

A significant performance decline was observed in all tests scores when performed dual tasks compared to single tasks in both normobaric and HBO environments ($p < 0.001$ for both).

The SDMT cognitive task was considered the primary task and the motor task was considered the distraction when we analyzed the dual task performance at the two different environments. SDMT scores, the number of correct answers minus the incorrect answers in the test, were significantly increased when performed under the HBO environment compared to the normobaric environment (**Table 2**, **Figures 2–4**). The improvement in SDMT in the HBO environment was significant when tested either as a single (43.9 ± 11.6 vs. 40.2 ± 9.8, $p < 0.001$) (**Figure 2**) or as a dual task (38.7 ± 11.7 vs. 35.2 ± 10.8, $p = 0.006$) (**Figure 3**).

Motor task scores were also significantly increased when performed under the HBO environment compared to the normobaric environment. The improvement in the motor task results, or the number of bean units that were transferred from the plate to the cup, under the HBO environment was significantly higher either as a single (83.8 ± 9.3 vs. 89.3 ± 11.5 $p < 0.001$) or as a dual task (51.4 ± 14.7 vs. 56 ± 14.2 $p = 0.029$).

TABLE 1 | Patient baseline characteristics.

		All participants
Number of participants		22
Gender	Female	11
	Male	11
Age		42 ± 13
Years of education		16.3 ± 4

TABLE 2 | Cognitive and motor task scores.

	General		
	Room air	Hyperbaric oxygen	P-value
Single task Cognitive	40.2 ± 9.8	43.9 ± 11.6	$P < 0.001$
Dual task Cognitive	35.2 ± 10.8	38.7 ± 11.7	$P = 0.006$
Single task Motor	83.8 ± 9.3	89.3 ± 11.5	$P < 0.001$
Dual task Motor	51.4 ± 14.7	56 ± 14.2	$P = 0.029$

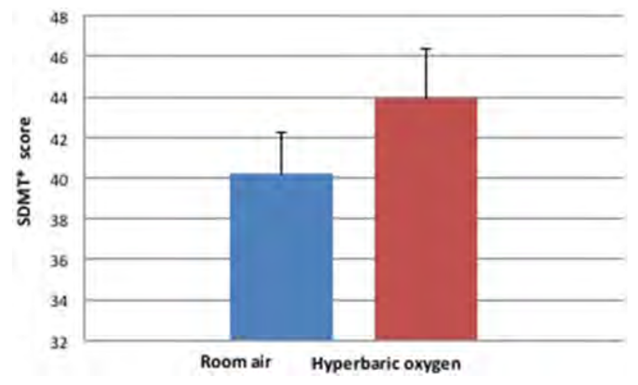

FIGURE 2 | Cognitive single task during hyperbaric oxygen compared to normal environments. *SDMT—Symbol Digit Modality Test.

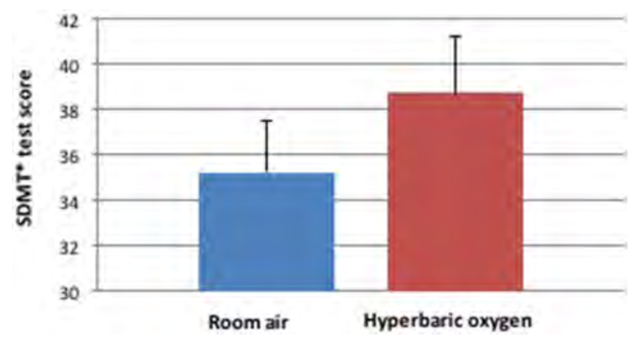

FIGURE 3 | Cognitive score during dual task during hyperbaric oxygen compared to normal environments. *SDMT—Symbol Digit Modality Test.

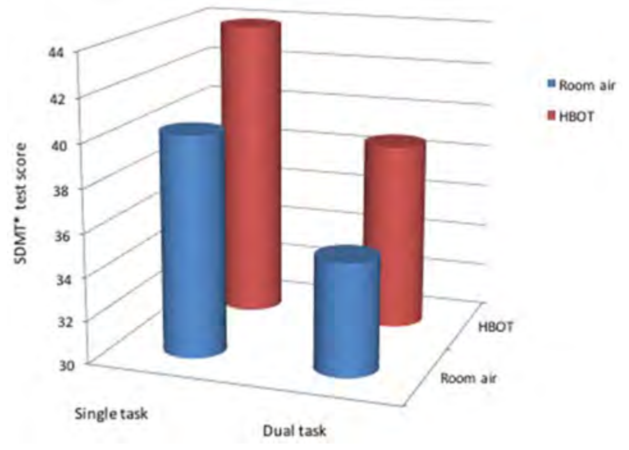

FIGURE 4 | General cognitive score during hyperbaric oxygen compared to normal environment. *SDMT—Symbol Digit Modality Test.

DISCUSSION

The study's findings indicate that even for healthy individuals, oxygen at normal conditions is a limiting factor for brain activity. The ability to perform cognitive and/or motor tasks as a single or a combined task (multitask) was evaluated at a normal air/oxygen environment and at HBO, a hyperbaric oxygen enriched environment. Increasing oxygenation using a HBO environment significantly enhanced both cognitive and motor performance. Significant improvements were found for both single tasks as well as simultaneous multiple tasks.

Many studies have confirmed that environments low in oxygen levels have a negative effect on performing single and multiple tasks (Shukitt-Hale et al., 1998; Lieberman et al., 2005). A study performed by Yu et al. evaluated the effect of repeated exposure to HBO treatment for healthy individuals (Yu et al., 2015). Their study demonstrates that repeated daily HBO sessions, 5 days per week, 80 min with 100% at 2 ATA, can enhance memory performance. The improved memory correlated with enhanced functional connectivity in the left hippocampus, right inferior frontal and lingual gyri as demonstrated by fMRI analysis (Yu et al., 2015). In the current study, the immediate effect of a single HBO environment exposure was evaluated in healthy volunteers. Since it is a single exposure, the immediate effect on neuro-cognitive performance cannot be related to the neuroplasticity effects of HBO but rather to a limiting factor preventing the brain to function at a higher capacity. This finding means that oxygen is indeed a limiting factor for brain performance at standard conditions in healthy human beings.

The minimal effective dosages of pressure and oxygen concentration are still unknown, and future studies are needed to test this issue by evaluating the optimal, case-specific dose response curves (Efrati and Ben-Jacob, 2014). There is a huge variability in HBO dosages (1.3–2.4 ATA) used in previous studies aimed to induce neuroplasticity in post stroke and TBI patients (Boussi-Gross et al., 2013; Efrati and Ben-Jacob, 2014; Tal et al., 2015; Hadanny and Efrati, 2016). Oxygen is not a drug and because it is mainly metabolized in the mitochondria, its pharmacodynamics varies greatly from patient to patient. Thus, no simple dose-response curve has been defined so far. Emphasizing the latter, there are many case reports illustrating the significant effect of relatively low increases in air pressure (Golding et al., 1960; Austin, 1998). For example, the Dead Sea (altitude 402 m below sea level, 1.05 ATM) can serve as a good model for a relatively "low" hyperbaric environment. The beneficial effect of this slight increase in air partial pressure is well-known and was studied and evaluated in different populations (Kramer et al., 1998; Falk et al., 2006; Goldbart et al., 2007). With respect to the current study, the aim was to confirm that oxygen is indeed a rate limiting factor for enhancing the activity and not to investigate the minimal effective dosage oxygen. For that purpose, we chose to use 100% oxygen at 2 ATA as the challenge dose. Now that we have demonstrated that indeed oxygen is a limiting factor, further studies are needed to evaluate the dose-response curve related to enhancing brain/cognitive performance.

In today's modern life, there is an increased need for multitasking, which is unfortunately limited (Carrier et al., 2009). The inability to perform well while multitasking could have severe and even life threatening consequences, as was found in emergency room care physicians (Chisholm et al., 2000) and in military drone pilots (Shanker and Richtel, 2011). Considering that an oxygen enriched environment could enhance performance, improve multitasking and decision making, the use of this environment could have a significant impact for those who needs it. However, before being used for large scale populations, the minimal and maximal effective dosage should be evaluated.

In addition to immediately enhancing brain/cognitive functions, there is growing convincing evidence that HBO therapy can revitalize chronically damage brain tissue in patients suffering from chronic neuro-cognitive impairment due to TBI, stroke or anoxic brain damage even years after the acute insult (Boussi-Gross et al., 2013; Efrati et al., 2013; Efrati and Ben-Jacob, 2014; Hadanny et al., 2015; Tal et al., 2015; Hadanny and Efrati, 2016). As detailed above, brain metabolism reaches its upper limit of oxygen consumption even at normal healthy conditions, which makes it dependent on cerebral blood flow (CBF) for its oxygen supply. After brain insults, when the CBF is compromised, there is a further decrease in oxygen delivery to the injured brain tissue and oxygen becomes a limiting factor for brain recovery (Hadanny and Efrati, 2015). Consequently, achieving higher tissue oxygen delivery by using higher paO_2 is crucial for maintaining the sufficient oxygenation needed for the damaged brain tissue (Hadanny and Efrati, 2015). Clinical studies published in recent years present convincing evidences that HBO therapy (HBOT) can assist in brain repair (Boussi-Gross et al., 2013, 2015; Efrati and Ben-Jacob, 2014). In addition to delivering sufficient oxygen to the brain for tissue repair, HBOT might initiate cellular and vascular repair mechanisms and improve cerebral vascular flow (Efrati and Ben-Jacob, 2014). At the cellular level, HBOT can improve mitochondrial function (in both neurons and glial cells), improve blood-brain barrier and inflammatory reactions, reduce apoptosis, alleviate oxidative stress, increase levels of neutrophils and nitric oxide, and upregulate axon guidance agents (Efrati and Ben-Jacob, 2014). Moreover, the effects of HBOT on neurons can be mediated indirectly by glial cells, including astrocytes (Efrati and Ben-Jacob, 2014). HBOT may also promote neurogenesis of the endogenous neural stem cells (Efrati and Ben-Jacob, 2014). At the vascular level, HBOT was found to have a role in initiating and/or facilitating angiogenesis and cell proliferation needed for axonal regeneration (Efrati and Ben-Jacob, 2014).

Another potential effect of HBOT may be its possible contribution to perception. Perception provides meaning for sensation. Mendez-Balbuena et al, has shown that by providing audio tactile stimulation, the sensory experience (perception) of vision, could be expended (Mendez-Balbuena et al., 2015). It might be possible that enhanced brain activity by HBOT may also increase sensory perception. However, it was not directly evaluated in the current study and it could be an interesting goal for additional studies.

The current study has several challenges and potential limitations. One important limitation relates to the test re-test learning effect due to the crossover design. Every participant has performed the tests twice under both conditions in separate sessions. To overcome this limitation, participants were randomly divided into two groups in a way that part of the participants started under the HBO environment and the other part started with the sham environment (normobaric with room air). Accordingly, the two groups are almost matched for their learning effect. The other challenge is related to generating the control intervention that would mimic hyperbaric environment where participants can sense the increased pressure in their ears. To overcome this challenge, the chamber pressure was increased and then gradually decreased during the control session so that the participants felt some pressure in their ears, and a flow of 21% oxygen was used for the masks. The tests/tasking were initiated 30 min after the masks were on within the chamber and the pressure at that time was already reduced back to sea level during the placebo session.

CONCLUSION

The improvement in performance of both single and multitasking while in a HBO environment supports the hypothesis that oxygen is indeed a rate limiting factor for brain activity. Hyperbaric oxygenation can serve as an environment for enhancing brain performance. Such a brain enhancing environment can be of significant importance when many skills are becoming more and more dependent on enhanced cognitive functions and multitasking. Further studies are needed to evaluate the optimal oxygen-performance relation for maximal brain performance.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnint.2017.00025/full#supplementary-material

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Recovery of Repressed Memories in Fibromyalgia Patients Treated With Hyperbaric Oxygen – Case Series Presentation and Suggested Bio-Psycho-Social Mechanism

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Fibromyalgia Syndrome (FMS) is a condition considered to represent a prototype of central sensitization syndrome, characterized by chronic widespread pain and along with symptoms of fatigue, non-restorative sleep and cognitive difficulties. FMS can be induced by trauma, infection or emotional stress with cumulative evidence that dissociation is relatively frequent in FMS patients. Two randomized controlled trials have shown that hyperbaric oxygen therapy (HBOT) can induce neuroplasticity and be effective in patients suffering from FMS. In this paper we present, for the first time, case series of female fibromyalgia patients who, in the course of HBOT, suddenly recalled repressed traumatic memories of childhood sexual abuse (CSA). The surfacing of the repressed (dissociative) memories decades after the sexual abuse events was sudden and utterly surprising. No psychological intervention was involved. As the memories surfaced, the physical pain related to FMS subsided. In one patient who had brain single photon emission CT (SPECT) before and after HBOT, the prefrontal cortex appeared suppressed before and reactivated after. The 3 cases reported in this article are representative of a total of nine fibromyalgia patients who experienced a retrieval of repressed memory during HBOT. These cases provide insights on dissociative amnesia and suggested mechanism hypothesis that is further discussed in the article. Obviously, prospective studies cannot be planned since patients are not aware of their repressed memories. However, it is very important to keep in mind the possibility of surfacing memories when treating fibromyalgia patients with HBOT or other interventions capable of awakening dormant brain regions.

Keywords: hyperbaric oxygen, repressed memories, fibromyalgia, biopsychophysical mechanism, childhood sexual abuse

INTRODUCTION

Fibromyalgia Syndrome (FMS) is a condition characterized by chronic widespread pain and diffuse tenderness, along with symptoms of fatigue, non-restorative sleep and cognitive difficulties. It affects 2–5% of the general population worldwide, with 9:1 female-to-male incidence ratio (Buskila, 2009; Branco et al., 2010; Clauw et al., 2011; Schmidt-Wilcke and Clauw, 2011; Jones et al., 2015). FMS is considered to represent a prototype of central sensitization, i.e., a condition characterized by an increase in the transmission and processing of pain within the central nervous system (Yunus, 2007a,b; Efrati et al., 2015; Ablin et al., 2016). Like other functional somatic syndromes, despite suffering from pain affecting soft tissues (muscles, ligaments), and tendons, patients seem well without obvious abnormalities on neither physical examination nor evidence of tissue inflammation on laboratory and radiologic studies (Clauw, 2014).

In recent years, there are cumulative data regarding the complex biology of chronic pain within the CNS (Ablin et al., 2016; Sancassiani et al., 2017; Yavne et al., 2018). Chronic pain syndromes in general, and FMS in particular, are not congenital; these conditions rather evolve over lifetime, often in response to various external factors such as physical trauma, infection or emotional stress, which emphasizes the capability of the CNS to morph and re-wire during life, even at a fully developed stage (Ablin et al., 2016; Yavne et al., 2018). Hence, the general concept of neuro-plasticity is an essential pattern of chronic pain evolution.

In this study, our main focus is on FMS induced by childhood sexual abuse (CSA). CSA has negative long term physical and psychological effects and women with a history of CSA may develop depression, post-traumatic stress disorder (PTSD), dissociative disorders, and chronic pain syndromes such as FMS (Spiegel et al., 2015). Dissociation in CSA is particularly relevant to amnesia of all or part of the traumatic events (Bohn et al., 2013). Studies have shown that dissociation is relatively frequent in FMS patients (Naring et al., 2007; Bohn et al., 2013; Kilic et al., 2014; Berkol et al., 2017). Unfortunately, despite the fact that chronic pain syndromes are common in adult survivors of CSA, patients rarely reveal their abuse history to their care providers due to amnesia, dissociation, or merely shame, embarrassment or trust issues.

Hyperbaric oxygen therapy (HBOT), the application of hyperbaric pressure in conjunction with increased oxygen content, has been shown in several clinical studies to have the capacity to induce neuroplasticity in injured brains even years after an acute insult (Boussi-Gross et al., 2013, 2015; Efrati et al., 2013; Efrati and Ben-Jacob, 2014; Hadanny et al., 2015; Tal et al., 2015; Hadanny and Efrati, 2016). As demonstrated in different animal models, HBOT induces neuroplasticity by stimulating cell proliferation (Mu et al., 2013), promotes neurogenesis of endogenous neural stem cells (Yang et al., 2008), regenerates axonal white matter (Chang et al., 2009), improves maturation and myelination of injured peripheral and cranial neural fibers (Haapaniemi et al., 1998; Vilela et al., 2008), induces brain angiogenesis (Tal et al., 2015), and stimulates axonal growth (Mukoyama et al., 1975; Bradshaw et al., 1996).

Despite the fact that HBOT for neurological disorders is somewhat still controversial there is recent evidence of its effectiveness in treating FMS (Ablin et al., 2016). To date, two prospective randomized controlled trials have demonstrated the efficacy of HBOT in fibromyalgia (Yildiz et al., 2004; Efrati et al., 2015). The improvement was demonstrated in all aspects of FMS including pain threshold, fatigue, distress and quality of life (Yildiz et al., 2004; Hadanny et al., 2015).

In this paper we present three cases of women suffering from fibromyalgia, who recalled suppressed traumatic memories of CSA during HBOT. These cases provide additional important insights on memories retrieval and dissociative amnesia and the opportunity to suggest a possible mechanism hypothesis that is further discussed in the article. The participants in the current paper were not subject to any psychological intervention during the time of HBOT. As the memories surfaced, around the 20th HBOT session (with more memories surfacing as the treatment continued) the physical pain related to the FMS subsided. Following participants' disclosure to a nurse, they were assigned to a physician for further assistance and investigation.

It should be noted that these events of self-disclosure were unfamiliar and unexpected for both patients and medical staff.

MATERIALS AND METHODS

A retrospective case presentation of FMS patients who reported retrieval of repressed memories during HBOT at the Sagol Center for Hyperbaric Medicine and Research at Assaf Harofeh Medical Center, Israel. The three cases reported in this article represent nine FMS patients who experienced the same during HBOT, out of a larger cohort of ∼200 FMS patients treated by HBOT in our center between 2013 and 2016. All reported patients, included in this article, signed an informed consent for their case publication. After patients signed consent, they reviewed and proofed their presented case. There were six additional patients with repressed memories recovery who did not agree for case publication. Thus, their cases are not presented. Vulnerable populations (for example minors, persons with disabilities) were not involved in this study. The publication process of those cases was approved by the head of Assaf Harofeh Medical Center Institutional Review Board.

The following HBOT protocol was practiced: 60 daily sessions, 5 days a week, 90 min each session, breathing 100% oxygen at 2.0 absolute atmospheres (ATA) with 5 min air breaks every 30 min. Sessions were performed in "multiplace" chambers, which may treat up to 12 patients simultaneously.

The diagnosis of FMS was based on the following criteria: (1) Symptoms of widespread pain, both above and below the waist and bilaterally. (2) Physical findings of at least 11 out of a set of 18 known tender points.

RESULTS

Case Study 1

N, a 21 year-old female, was diagnosed at the age of seventeen with severe FMS, because of which she had to quit studying. N was a popular, good student, a third-born of three children to her highly educated parents. At the age of fourteen she insisted on moving to another school, rationalizing it as her simply wishing to study at a "better school." Her parents agreed. She then invested all her energy in studying and in dancing lessons, in which she excelled until the onset of her fibromyalgia. N was referred to the Sagol hyperbaric institute by the family physician to receive HBOT for her fibromyalgia. Prior to her referral to HBOT she was treated with several different medications and with medical Cannabis for her severe pain. Between the 17 and the 34 HBOT sessions, N complained on low energy and tiredness. In additional, N told the medical staff that she feels sadder and more anxious than prior to HBOT. During that time, she started to experience flashbacks of herself being sexually molested. The flashbacks occurred both during HBOT sessions (inside the chamber) and in the hours following a HBOT session (at her normal environment). The flashbacks started with unbearable sexual arousal, accompanied with shortness of breath, burst into tears and other panic attack related symptoms such as feeling of loss of control and overwhelming fear. Fragments of memories appeared, in which she was being raped by several peers which added up to a full recovery of her repressed memory of being repeatedly molested and raped by classmates at the age of fourteen.

Looking back, N said: "I can't understand how I, or anyone else for that matter, could forget such experiences. . . I continued to function as an excellent student; I focused on my dancing. . . I avoided peer gatherings, rationalizing this avoidance to myself and to my parents as "lack of interest" and "will to excel."

N reported that, after the repressed memories emerged, she felt much stronger, slept better, had more energy and had almost no pain. According to N, she was fully recovered from her FMS.

Case Study 2

A, a 56 year-old businesswoman, married with four children, the eldest daughter in her family of origin. As a child she had urolithiasis, for which she underwent bladder surgery three times. The relationships between her parents were complicated. Her mother used to have love affairs and brought her sexual partners home in the father's absence. The mother also used to share her sexual experiences with A, who was a "parental child." At the age of seven, A has been visiting an 18 year-old neighbor in his apartment three times a week and was receiving little presents from him. After the first visit, A cut her long, beautiful, blond hair without informing her parents, telling them later that she "preferred to be ugly." About a year later, during the 6 days War, the family moved for a week to her grandmother's. A describes this week as "the best, quietest, calmest week of my life, despite the fear of being at war." Upon returning home, she refused her neighbor's invitations and forgot his name. Shortly after graduation she got married. In 2011, she had a car accident and referred to treatment at the Sagol HBOT institute. Around that time she was also diagnosed with FMS. Between the 37th and 44th HBOT sessions, A experienced flashbacks of sexual molestation. The flashbacks were accompanied with anxiety and feelings of losing control in addition to sympathetic signs such as sweating. The flashbacks began with unbearable sexual arousal and concurrent panic attacks. Flashbacks initially occurred during the hyperbaric sessions, and later on happened during her daily routines between sessions. Visually vivid memories surfaced including her being ordered to have oral intercourse. These flashbacks gradually joined into detailed, coherent memories of sexual molestation she had experienced at the age of seven by her 18 year-old neighbor, who threatened to kill her if she ever revealed their "secret." In an attempt to validate these memories, A returned to her childhood neighborhood, found the perpetrator, and confronted him. He confirmed that this had indeed occurred as remembered.

Case Study 3

B, a 36 year-old female, was the youngest of three siblings in her family of origin. When admitted to HBOT for fibromyalgia, she reported having been sexually abused by her uncle (her mother's brother) between the ages of six and sixteen. She said that her parents didn't know about the abuse. "Actually," she said "I think that, back then, I myself did not remember it most of the day, kind of detached." Her mother died of cancer a year after the abuse ceased. B described her mother as "an innocent, good mother who knew nothing of what was going on." In adulthood, B had psychotherapy for her CSA. During her Social Work studies, B began to suffer from fibromyalgia. The disease prevented her from committing to a full-time work as a social worker. At the age of thirty-six, she started HBOT for her FMS. During the first twenty sessions, she reported sexual arousal and unbearable pain in her abdomen, "felt as if someone was stabbing me with a knife." These symptoms were followed by surfacing of new memories of the abuse revealing another abuser – her mother. "My mother took me to the shower after he [the uncle] abused me. She undressed me, ordered me to open my legs and then began to abuse my genitals." These new, vivid, coherent, and detailed memories exposed her to different narratives on her mother and her childhood. Those memories of abuse by B's mother did not surface prior to the HBOT, even during psychotherapy. After those new memories surfaced she returned to psychotherapy.

B's baseline and post HBOT brain single photon emission CT (SPECT) scans are presented in **Figure 1**. The highest relative increases in activity (>15%) are seen in the visual cortex (BA 19), prefrontal cortex (BA 9), orbito-frontal gyrus (BA 11) and paratemporal cortex (BA 37) (**Figure 1**).

DISCUSSION

This article is the first report of retrieval of repressed memories of CSA in FMS patients during HBOT. In the three cases presented, HBOT was initiated for FMS, decades after the sexual abuse events occurred. The retrieval of the repressed memory through physiological intervention that

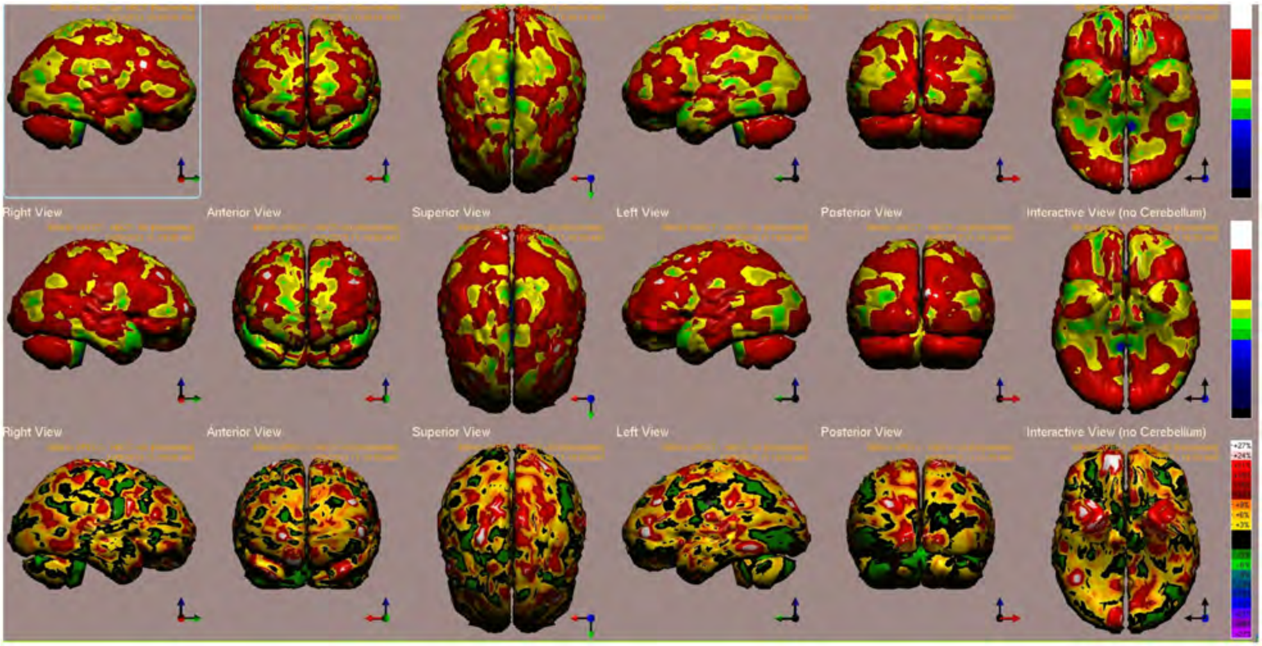

FIGURE 1 | Case 1 single photon emission CT (SPECT) calculated change after hyperbaric oxygen therapy (HBOT) compared to baseline. In the **Top** two rows colors, represent maximal functional brain activity relative to the entire brain activity; in the **Bottom** row, colors represent regional changes in functional brain activity. White and red areas show the biggest relative changes in cerebral blood flow/perfusion. Significant changes can be seen at the right prefrontal and temporal areas (white). Abbreviations: Analysis of brain SPECT performed pre- and post HBOT showed the highest relative increase in activity (>15%) in the visual cortex (BA 19), prefrontal cortex (BA 9), orbito-frontal gyrus (BA 11), and paratemporal cortex (BA 37).

induces neuroplasticity was unexpected. This phenomenon may augment our knowledge on the pathophysiological process related to dissociation and dissociative amnesia and suggest new physiological interventions to enhance the current psychological interventions.

Dissociation, Dissociative Amnesia and Biopsychophyisical Mechanism

Trauma is a biopsychophysical experience, even when the traumatic event doesn't cause direct mechanical harm to the body. According to this, the organism is defined by a collection of multiple biological and psychological interactions as well as social factors throughout its life (Bowman, 2011; Nijenhuis and van der Hart, 2011). The term "Dissociation" refers to the failure of integrating aspects of memory, identity, perception, and consciousness. Dissociative amnesia, is a failure of the ability to remember autobiographical information in the absence of brain damage (Staniloiu and Markowitsch, 2014). Thus "Dissociation" means that a memory is neither lost nor forgotten but is unavailable for retrieval for a period of time, which could last even decades (Chu et al., 1999). Dissociative amnesia or the suppression of traumatic memories, is well-documented in the literature and is coded as a dissociative disorder in Diagnostic and Statistical Manual of Mental Disorders (DSM-5®), Fifth Edition (American Psychiatric Association, 2013 and American Psychiatric Association. DSM-5 Task Force).

Individuals with dissociative disorders usually report a history of exposure to traumatic stress, development trauma, or other events that trigger stress known as stressors (Andrews et al., 1999; Bohn et al., 2013; Fergusson et al., 2013; Lev-Wiesel and Zohar, 2014). In psychological terms, dissociation may be considered as a protective mechanism to shield the traumatized individual from the unbearable pain of the memory and its consequences. CSA is an exceptionally severe form of emotional and physical trauma that may lead to disorders of memory and dissociation (Andrews et al., 1999; McNally, 2003; van der Kolk et al., 2005; Fergusson et al., 2013; Lev-Wiesel and Zohar, 2014). Several clinical studies demonstrate that CSA is associated with FMS severity and may shape the biological development of interoception in ways that predispose to pain and polysymptomatic distress (Romans et al., 2002; Ciccone et al., 2005; Jiao et al., 2015; Ortiz et al., 2016).

The classic fight-or-flight response to a perceived threat is a reflexive neural phenomenon which has obvious survival advantages in evolutionary terms (Sherin and Nemeroff, 2011). However, under certain circumstances, such as the CSA cases described above, exposure to a perceived threat can cause dysregulated response (Scaer, 2001; Sherin and Nemeroff, 2011). Chronic dysregulation can lead to functional impairment in certain individuals who become "psychologically traumatized" with long standing neurobiological abnormalities, which overlap features found in patients with post-concussion syndrome due to traumatic brain injury (TBI) (Scaer, 2001; Sherin and Nemeroff, 2011). Certain brain areas become disconnected from the normal trophic stimulations of cerebral perception and subjected to the extremes of vasomotor instability of trauma that may lead to pathologic vasoconstriction and ischemia (Scaer, 2001). This model explains the somatoform dissociative symptomatology that may arise in TBI or CSA, followed by FMS. Thus, FMS can be induced by either TBI or CSA.

Suggested Physiological Mechanism for HBOT Effect in Dissociation

From the neurobiological/physiological perspective, the authors suggest a possible mechanism for the recovery of repressed memories induced by HBOT.

Astrup et al. (1977), demonstrated in a paper argued to be one of the most important in applied neurology, that after the onset of focal ischemia (such as blood vessel occlusion), measurements of electrical activity reveal brain regions that are dysfunctional but still viable (Astrup et al., 1977). The neurons in this area retained enough energy to maintain ion pumps and sustain the −70-mV resting membrane potentials required for their existence. However, those neurons did not have enough energy to generate the action potentials needed for their purpose functioning. In the last decade, functional imaging of the brain by positron emission tomography (PET) and SPECT scanning (Heiss, 2000; Baron, 2001; Siddique et al., 2002; Ebinger et al., 2009; Meerwaldt et al., 2010), afforded us images of such areas, and the accumulating data indicate that apparently dead areas may persist for years after an acute event (Boussi-Gross et al., 2013; Efrati et al., 2013). The stunned/hibernating areas, unable to fire action potentials, are characterized by metabolic dysfunction, namely anaerobic metabolism and ATP depletion (**Figure 2**). Loss of ATP and the resultant intracellular acidosis would bring about energetic breakdown of ion pumps and, consequently, additional damage to the mitochondria and endoplasmic reticulum (Hossmann, 2006; Culmsee and Krieglstein, 2007). Furthermore, anaerobic metabolism would bring about elevation of free radical levels (Hossmann, 2006; Culmsee and Krieglstein, 2007), persistent inhibition of protein synthesis (DeGracia, 2004), selective neuronal damage demonstrable by decreased density of benzodiazepine and/or 5-HT2 receptors (Vera et al., 1996; Yamauchi et al., 2005), augmented intracellular calcium (Hossmann, 2006), blood-brain barrier damage and augmented inflammation evidenced by higher levels of cytokines and cyclooxygenase-2 (COX-2) (Yin et al., 2002).

The brain regions with continuous hypoperfusion, with their very low, anaerobic metabolic rate, settle into a quasi-steady energy well from which they cannot climb up on their own. The hibernation state typical of some mammalian species seems to be the most proper model created by nature, simulating the post injury and post ischemia states of brain (Drew et al., 2007). The energy consumption in hibernation is less than 0.1 WT/kg, i.e., less than 1% of the metabolism in a resting state. The corresponding outcomes at the cellular level include a dramatic (~2500-fold) decrease in protein synthesis (Frerichs et al., 1998), complete interruption of neuronal spike activity (Carey et al., 2003), and a switch from carbohydrate-based to fat-based metabolism (Carey et al., 2003). In turn, metabolic changes induced by minimized levels of energy availability have an impact on brain structure and functioning at the cellular level, such as reorganization of cell membranes, formation of protein-free domains that displace membrane proteins, and altered cell membrane permeability due to alterations in cytoplasmic matrix (Azzam et al., 2000; Adibhatla and Hatcher, 2008).

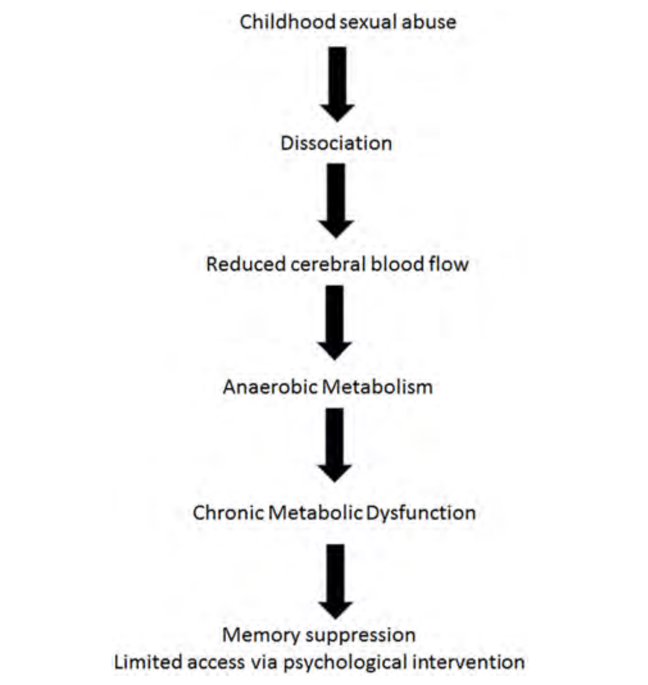

FIGURE 2 | Dissociation and repressed memory – suggested model for the neuropathology cascade.

The neurobiological mechanism of dissociation, as mentioned above involves disconnection of certain brain areas, may be explained by hibernation, a hypometabolic state at the cellular level (minimal energy generation by the mitochondria in order to maintain membrane potential for cell survival). Hibernated areas may correlate with apathy and low responsiveness (Schore, 2009). This model supports the assumption that dissociation could be interpreted from the biological perspective as a hypometabolic state and from the biopsychological perspective as a deficiency of psychological energy. In the cases presented, the metabolic dysfunction of the dissociated brain regions serves as a physiological boundary for psychological intervention (**Figure 2**).

Energy wise, climbing back up from the "metabolic well" mandates energy input. As discussed above, HBOT has been shown to induce neuroplasticity and reactivation of cells in chronic metabolic dysfunction in different type of brain injuries, and in FMS specifically (Mukoyama et al., 1975; Bradshaw et al., 1996; Haapaniemi et al., 1998; Yildiz et al., 2004; Vilela et al., 2008; Yang et al., 2008; Chang et al., 2009; Boussi-Gross et al., 2013, 2015; Efrati et al., 2013; Mu et al., 2013; Efrati and Ben-Jacob, 2014; Hadanny and Efrati, 2015, 2016; Hadanny et al., 2015; Tal et al., 2015). HBOT supplies extra oxygen to the anaerobic brain regions, providing them with the energy needed to exit metabolic well. Once these regions of metabolic dysfunction are re-activated by HBOT, cerebral blood flow is increased to these regions and they return to normal metabolism. Hibernating brain regions which may have been responsible to memory repression, can be reactivated by HBOT, regain their normal function and resurface suppressed memories (**Figure 3**).

Potential Role of Prefrontal Cortex Role in Suppressed Memories

One of the patients (Case 1) presented in this article had brain SPECT before and after HBOT (**Figure 1**). In this case, the prefrontal cortex that was suppressed at baseline was reactivated by the use of HBOT.

Repression of memories, or dissociative amnesia, has been correlated with decreased activity of the dorsomedial prefrontal cortex, while recovery is correlated with increased prefrontal cortex activity as shown by functional magnetic resonance imaging (fMRI) (Anderson et al., 2004; Kuhl et al., 2008; Che et al., 2015). PET studies in patients suffering from PTSD demonstrated increased cerebral blood blow in the prefontral cortex when re-experiencing emotionally charged episodic memories (Masaki et al., 2006; Im et al., 2016).

Study Limitations

This article has several obvious limitations. Most limitations are related to the small number of patients and the lack of brain SPECT imaging in the other two patients. Another limitation relates to the fact that there is no known way to fully and objectively validate the retrieved memories. Still, two of the women confronted their past perpetrators, who begged for their forgiveness. The three cases presented in this article are representative of 9 women who had retrieved suppressed memories during HBOT at our center. In order to gain better understanding of dissociation/dissociative amnesia and its suggested biopsychophyisical mechanism and long term biological consequences, direct evaluation of brain metabolism/activity in animal models should be investigated.

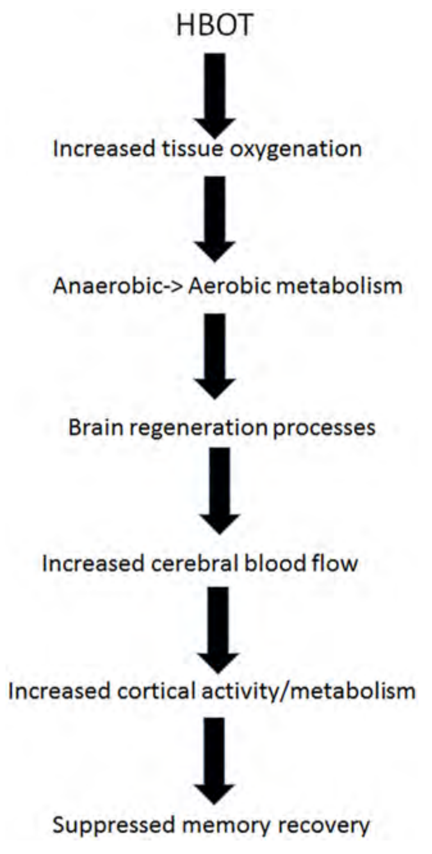

FIGURE 3 | Recovery of repressed memory by HBOT – suggested mechanism.

CONCLUSION

This is the first clinical report of recovery of suppressed memories with HBOT alone as physiological intervention. Even though further studies are needed, it would not be easy to carry out a study in patients that are unaware of their difficult childhood history. Obviously, prospective studies cannot be planned since patients are not aware of their repressed memories. However, it is very important to keep in mind the possibility of surfacing memories when treating FMS patients with HBOT or other interventions capable of awakening dormant brain regions. We recommend that HBOT practitioners treating FMS patients should add the potential risk for unexpected memory of traumatic events to occur to their informed consent. All medical professionals who are dealing with FMS patients should be aware and prepared to handle such outcome. It would be highly beneficial if anyone who encounters such a case documents and reports it. Through the cumulative data we may get important insight into the neurobiology of suppressed memories.

AUTHOR CONTRIBUTIONS

SE: study initiator, interpretation of study results, and wrote the first and final drafts of the manuscript. AH: interpretation of study results, brain Image analysis, and co-wrote with SE first and final drafts of the manuscript. SD-T: interpretation of study results, data collection, patients follow up, and revision of the manuscript. YB: data collection and patients follow up. KT: interpretation of study results and revision of the manuscript. NP: patients follow up and interpretation of study results. GS: interpretation of study results and revision of the manuscript. RL-W: patients follow-up, interpretation of study results, and revision of the manuscript. All authors read and approved the final manuscript.

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frontiers in Psychology

REVIEW
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Environmental Physiology and Diving Medicine

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Man's experience and exploration of the underwater environment has been recorded from ancient times and today encompasses large sections of the population for sport enjoyment, recreational and commercial purpose, as well as military strategic goals. Knowledge, respect and maintenance of the underwater world is an essential development for our future and the knowledge acquired over the last few dozen years will change rapidly in the near future with plans to establish secure habitats with specific long-term goals of exploration, maintenance and survival. This summary will illustrate briefly the physiological changes induced by immersion, swimming, breath-hold diving and exploring while using special equipment in the water. Cardiac, circulatory and pulmonary vascular adaptation and the pathophysiology of novel syndromes have been demonstrated, which will allow selection of individual characteristics in order to succeed in various environments. Training and treatment for these new microenvironments will be suggested with description of successful pioneers in this field. This is a summary of the physiology and the present status of pathology and therapy for the field.

Keywords: diving, oxygen, decompression sickness, pulmonary edema, gas exchange

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INTRODUCTION

Exploration of underwater environments predates documentation for possibly 1000s of years, but written evidence dates back to Ancient Greece in the 4th century BCE; Aristotle recounts the use of a primitive diving bell, an overturned cauldron that trapped air from the atmosphere. Today diving is incorporated into many aspects of modern society including: sports, recreation, industry, and widespread strategic military goals. Subaquatic environments are an essential piece of modern day life, and will only play a bigger role in the future. Within the last century, there has been growing interest in diving for both recreational and professional purposes. The worldwide number of annual diving certifications has tripled during the past 20 years, and in the United States alone there are an estimated 4 million sport divers (Lynch and Bove, 2009). Diving will change rapidly again in the coming years, with plans to establish permanent underwater installations that have specific research goals of exploration, health, and survival.

Profound pulmonary, circulatory, and cardiac changes are induced by immersion in water; divers must tolerate and compensate for changes in pressure and temperature. In this review, we will discuss suggested treatments and preventive training for these new microenvironments as gleaned from successful pioneers; we will summarize the physiology and the present status of pathology and therapy in the field; and we will illustrate the physiological changes induced by immersion, swimming, breath-hold diving, and exploring while using special equipment in the water.

Only in the last 100 years has technology allowed people to reach extreme depths using compressed gas delivery devices and diving suits containing air at atmospheric pressure. Free-diving has also been achieved at considerable depths (Balestra and Germonpré, 2014). We will review the major environmental challenges at these depths: density, pressure, temperature. Then we will briefly explain the physiological changes induced by immersion and discuss the clinical implications of diving. When underwater the human body is challenged by forces and physiological concerns not faced in its every-day environment. Familiarity with the risks involved with diving and mindfulness are critical to maintain one's health during diving exposure (Lanphier, 1957).

THE WATER ENVIRONMENT

Understanding the physical properties of the underwater environment remains the best approach to minimize risks while diving when breathing support systems are needed (Pendergast and Lundgren, 2009; Pendergast et al., 2015). The hazardous characteristics of submersion are: density, pressure, temperature, and optical phenomena.

Density

Flotation in any fluid is ruled by the density of water, 1 $\frac{g}{cm^3}$, (Baronti et al., 2012; Pendergast et al., 2015). According to Archimedes' law objects denser than water will sink. As seawater has a high salinity the density is closer to 1.029 $\frac{g}{cm^3}$ at the surface. Hence, it is easier to float in saltwater as it is denser than fresh water. Net buoyancy (equal to the magnitude of the weight of fluid displaced by the body) of breath-hold divers (humans or animals) affects their swimming behavior and energetics (Williams et al., 2000). However, the high density of water implies an enhanced energy cost of locomotion, reducing the human efficiency in the water environment (Bosco et al., 2014; Pendergast et al., 2015). A diver's overall density depends upon incompressible non-gas tissues (tissue density), and the volume and density of highly compressible gas stores carried within the body (i.e., natural cavities such as lungs, stomach and other viscera) (Baronti et al., 2012). Another important parameter, strictly related to density is dynamic viscosity. It determines how easily bodies move through water and divided by density represents kinematic viscosity (Pendergast et al., 2015).

Moreover, density of the inspired gas determines breathing resistance. During immersion using underwater breathing apparatus, air (or any other respiratory mixture) is delivered at the diver's ambient pressure. Breathing gas density increases in direct proportion to ambient pressure, with a corresponding increase in work of breathing. For this reason, helium-oxygen composite mixtures used during deeper dives to eliminate the narcotic effects of nitrogen also reduce breathing resistance (Brubakk et al., 2003; Balestra and Germonpré, 2014).

Pressure

Small pressure effects on biological systems can be observed at 40–60 atmospheres, where there are small reductions in the sedative properties of propofol (Tonner et al., 1992) and changes in the affinity of hemoglobin for oxygen (Reeves and Morin, 1986). The major effect of pressure changes within the depths usually encountered by man is therefore on gas properties. Several simple gas laws were derived from the ideal gas law (PV = nRT) and are currently used under conditions associated with diving (i.e., Boyle's law, Dalton's law, and Henry's law). A column of liquid exerts a pressure that is proportional to its height, density, and the acceleration of gravity. At sea level the ambient pressure is 1 atmosphere absolute (ATA). For each 10 m depth in water a diver is exposed to an additional pressure of 1 ATA Because gases are compressible the lung may therefore experience significant changes in volume, especially during hasty ascents (Pendergast et al., 2015).

Temperature

Thermal conductivity is the property of a material to transfer heat from one molecule to another through a liquid, solid, or gas. Typically, heat loss in the human body is mainly related to radiative and convective factors, so temperatures within 18–24°C are comfortable for a body surrounded by air. Conversely when in water, a short period of time exposed to that temperature range can cause great discomfort or worse. Thermoneutrality in complete submersion, defined as the temperature at which there is no heat transfer between the water and the body, is 28–30°C. When the water is colder, suitable protection is needed to reduce heat loss. Similarly, active cooling may be required for diving in warmer waters. For respiratory heat loss, the important parameter is thermal or heat capacity. *Heat capacity* is higher for diatomic molecules like N_2 and O_2 than for monoatomic gases such as helium or argon. Because of the high thermal capacity of gases under pressure, breathing mixtures during deep diving in cold water are sometimes preheated (Brubakk et al., 2003).

Optical Phenomena

When underwater, it is extremely difficult to focus images on the retina due to the similar refractive index between the cornea and water. Therefore, an air interface by means of a mask or special glasses is required, creating two interfaces with different refractive powers (water vs. glass and glass vs. air). Kent (1966) found, however, due to this layering of interfaces, that except at short ranges, both size and distance were overestimated underwater compared to normal viewing under above water conditions. Since the refractive index of the water is four-thirds that of air, the optical (apparent) distance of a target under water is three-fourths of that in air (Ono et al., 1970). Immersed objects appear about 30% larger and closer, due to the distorted perception.

This distortion derives from the passage of the rays of light from the water into the gas environment within the mask, due to the refraction at the interface where the light speed increases, as described by Snell's law:

$$\left(\frac{Sin\theta_1}{Sin\theta_2} = \frac{v_1}{v_2} = \frac{\lambda_1}{\lambda_2}\right),$$

θ as the angle measured from the normal of the boundary, v as the velocity of light in the respective medium (SI units are meters per second, or m/s), λ as the wavelength of light in the respective medium.

Moreover, refraction alters the form and location of the image resulting in variations in the perceived lateral position as the angle of incidence of the light rays increases. These physiological alterations may disturb the divers' hand-eye coordination and motor skills. However, individuals own notable abilities to adjust to changes in different conditions, such as light, color and optical distortions (Luria and Kinney, 1970). Light traveling in the air does not considerably modify its spectral composition, but light transmission through water can limit color recognition (Kinney et al., 1967). Furthermore, both the quantity and quality of the change depend on the particular body of water involved. Pure water has its greatest transmittance at 480 mμ in the blue-green region of the spectrum. Kinney et al. (1967) determined empirically the underwater visibility of colored paints, both fluorescent and non-fluorescent, in different waters. The easiest color to see when the water clarity increases ranges from yellow-green to green to blue-green. Conversely, gray and black represent the two colors hardest to see in a water environment with a natural illumination. Moreover, other colors whose spectral components are absorbed by the water are orange and red in clear water and blue and green in murky water, implying poor visibility (Kinney et al., 1967).

MAN AND WATER

We can summarize the human body immersion in the water in three different aspects:

(a) partial immersion of the human body, such as face, lower limbs, body without head; (b) full immersion (submersion) breath-hold diving at various lung volumes; (c) submersion with auxiliary breathing apparatus to provide an appropriate gas mixture (oxygen, air, helium-oxygen, helium-oxygen-nitrogen, oxygen-hydrogen etc.) (Pendergast et al., 2015).

Partial Body Diving

Immersion results in a substantial modification on the dynamics of venous return toward the intrathoracic vessels and the right part of the heart. The increased intrathoracic blood flow is due to the reduction of blood flow in the peripheral veins. This phenomenon is induced by the increase in surrounding pressure of the submerged limb and, assuming average ocean surface water temperature 17°C, the active vasoconstriction of veins triggered by the loss of heat due to the high thermal conductivity of water. Swimming with head above water induces an estimated 500 ml increase in intrathoracic blood volume. Blood-shift due to immersion is responsible for the expansion of the right heart cavities and the neuroendocrine modifications shown after immersion. Specifically, immersion activates stretch receptors located in the atrial wall, stimulating the secretion of atrial natriuretic peptide (ANP). This explains the strong natriuresis and diuresis that occurs during immersion (Kollias et al., 1976; Lundgren and Miller, 1999).

Facial immersion in cold water results in the *diving reflex*, consisting of: apnoea, bradycardia, vasoconstriction, increased mean arterial pressure and splenic contraction (Schagatay et al., 2001, 2005; Lemaitre et al., 2015). The diving response in humans can be influenced by several factors, and water temperature is one of the most important. Gooden (Gooden, 1994) summarized a series of findings focusing on face immersion with breath-holding and showed a well-defined inverse relationship between the water temperature and the degree of the diving-induced bradycardia. Recently Lemaitre et al. (2015) elucidated the many similarities and the functional purposes shared by trigeminocardiac reflex (TCR) and diving reflex (DR). Decreasing water temperature proportionally increases the response of the TCR; this is a factor in the intensity of stimulation of the DR (Lemaitre et al., 2015).

Breath-Hold Diving

Immersion with voluntary breath holding requires no equipment but induces extreme changes in cardiovascular physiology. In breath-hold diving, two main factors affect the physiological changes of the human body: the *time* of breath suspension and the *depth* of submersion (Lundgren and Miller, 1999). Individuals have reached depths greater than 200 m using weight-assisted descent and ascent facilitated by balloon. Basing on the concept of the thoracic squeeze these dives reach greater depth than predicted by Boyle-Mariotte's law. Until a few years ago, it was hypothesized that the maximum depth reached after a forced inspiration was determined by the ratio between the total lung capacity (TLC) and residual volume (RV). During progressively deeper submersion lung volume is compressed from TLC toward RV and it was hypothesized that overstepping this predicted depth, the hydrostatic pressure should induce pulmonary vascular rupture. Based on this concept, divers could not have reached more than 30–35 m depths, while in fact in 1903 a Greek sponge fisherman, Georgios Stattis, descended to 71 m holding his breath to recover the anchor of an Italian ship, the "Regina Margherita" (Bosco et al., 2007). However, hemoptysis can occur during breath-hold diving, and fatal pulmonary hemorrhage has been described (Mijacika and Dujic, 2016). Furthermore, rupture of the pulmonary capillaries can allow air to enter the vasculature and cause cerebral arterial gas embolism (Kohshi et al., 2000, 2005).

The possibility of reaching greater depths is due to a physiological compensation of physical laws; blood from peripheral circulation is shifted to the chest and being an incompressible fluid, it modulates the expected reduction of the pulmonary volume below the residual volume, and can further compress alveolar gases. In this way, a simpler application of physical laws in breath-hold deep diving does not apply. The intrathoracic blood volume increase, investigated with different methods (plethysmography, bioelectrical impedance analysis, scintigraphy, radiography), and is nowadays hypothesized to exceed 2 l (Arborelius et al., 1972; Robertson et al., 1978; Data et al., 1979; Lanphier, 1987). Additionally, the classic diving reflex, induced by face immersion leads to bradycardia. This physiological response appears in many different species both mammals and birds, although varying in terms of time and intensity. Breath-holding at rest, out of water, induces non-significant changes in heart rate; as well as immersion itself do not cause bradycardia while swimming and snorkeling (breathing through an aerator tube). Breath-hold swimming, even on the surface, instead causes pronounced bradycardia (Ferrigno et al., 1986; Andersson et al., 2002; Ferretti and Costa, 2003; Lindholm and Lundgren, 2008). Severe hypertension has also been observed in championship breath hold divers during dives in a hyperbaric chamber (Ferrigno et al., 1997).

Submersion with Self-Contained Breathing Apparatus

Diving with self-contained breathing apparatus induces physiological changes in the subject, with possible pathological events. Major adjustments are those concerning respiratory mechanics (Camporesi and Bosco, 2003). Simple body immersion in water results in a significant reduction of vital capacity, residual volume, lung distensibility, and functional residual capacity due to the increased venous return and resulting pulmonary blood content. These changes reduce respiratory performance and increase tidal volume, while maintaining the respiratory minute volume constant (Moon et al., 2009).

In addition, the pressure of the air provided by the breathing apparatus is influenced by the position of the diver's body while diving. In particular, when the diving regulator is positioned higher in the water column than the lung, it delivers air at a lower pressure and thus increasing the inspiratory work. This phenomenon is not specific only for scuba diving, but it also exists in partial body immersion in a vertical position.

When breathing gas with a fixed oxygen concentration, alveolar and arterial oxygen partial pressures increase with depth. This tends to attenuate ventilatory chemosensitivity (Dunworth et al., 2017) and facilitate CO_2 retention. Seasoned divers indeed become less sensitive to high CO_2 partial pressures.

The nitrogen distribution between the various compartments of the organism are characterized by latency that at the end of the submersion leads to a nitrogen excess in the body with respect to pre-diving levels. Tissue supersaturation with inert gas such as nitrogen or helium during decompression is the mechanism for decompression sickness (DCS) (Vann et al., 2011). If the rate of ascent is too fast in relation to the quantities of nitrogen absorbed then the solubilized nitrogen will be released into gaseous phase with bubble formation in the circulating blood and tissues (Bosco et al., 2010; Arieli and Marmur, 2013). For this reason, decompression procedures have been developed, in which decompression is staged, in order to allow inert gas to be eliminated via the lung at a rate consistent with a minimal risk of bubble formation.

DIVING DISORDERS

Subaquatic environments expose divers to a variety of pathologies, varying from *drowning*, probably the most frequent complication induced by inhalation of water and resulting in asphyxia, to *transient syncope*, i.e., loss of consciousness usually resulting from systemic or district hypoxia during breath-hold diving. Sometimes it is due to or associated with hypercapnia. There might be multiple causes for drowning and not all causes are related to the submerged environment: the most notable causes are immersion barotrauma and immersion pulmonary edema (IPE). More complex and unique pathologies derive from *decompression*. Decompression disorders are defined as pathological events that result from a reduced environmental pressure caused by the development or penetration of gaseous bubbles within the blood and tissues.

Barotrauma

Barotrauma is a common disorder among professional divers. It is caused by volume changes (increase or reduction) of gas placed in spaces within, or adjacent to, the body. This results in tissue damage (Strauss, 1979). The aforementioned Boyle's Law describes the changes of gas volume in relation to the pressure applied to it. The greater the pressure applied, the bigger the gas compression. Conversely, as the pressure decreases the gas volume is allowed to expand. Hence, the gas compression/expansion derived from this mechanism, widely enhances the possibility of damage to tissues (Brubakk et al., 2003).

While the diver is submerging, the pressure applied to his body increases; the diminished volume of the surrounded gases will result in barotrauma of descent. Differently, the related drop in pressure applied to the emerging diver, will result in barotrauma of ascent due to gas expansion. Though descent and ascent barotrauma affect the same sites, the clinical symptoms are different, considering the different pathophysiological changes. Gas within the body can be enclosed in distensible or in bony tissues. However, the possibility that barotrauma occurs when gases are surrounded by solid organs (i.e., sinus and middle ear) is likely. Similarly, when the gas is trapped in artificial sites (i.e., face mask) the changes of pressure equally result in potential barotrauma (Strauss, 1979).

Scientific literature divides barotrauma considering the affected organs. *Ear barotrauma* is the most common type of barotrauma and can cause a range of issues from mild hyperemia of the tympanic membrane to its actual rupture (Sadri and Cooper, 2017). It is due to the discrepancy in pressure between the outside environment and the middle ear. Insufficient pressure equalization can result in ear pain during descent, and subsequent Valsalva maneuvers may cause acute, spinning vertigo, disorientation, nausea, and vomiting (Brubakk et al., 2003). Similarly, nasal functionality and equalization maneuvers are primary to avoid *sinus barotrauma*. Rosińska et al. (2015) identified inflammation of the mucous membrane, nasal or sinus polyps, as well as nasal septum curvature and nasal concha hypertrophy as risk factors for sinus barotrauma. Indeed, since the gas is blocked in the sinus cavities the increase of pressure brings to absorption of the air hosted in the sinus with void development (Strauss, 1979). During descent or ascent, obstruction of the sinus ostia or eustachian tube generates a pressure differential that can result in pathological manifestation such as hemorrhage (Strauss, 1979), headache and paresthesia in the area innervated by the infraorbital nerve (Rosińska et al., 2015).

Pulmonary barotrauma is the most severe of the barotraumas and causes the most concern in all types of diving operations (Strauss, 1979). It represents one of the commonest cause of arterial gas embolism (AGE). It may be considered as barotrauma of descent and barotrauma of ascent. As the depth increases the total lung volume decreases up to approach the residual lung volume. Since the lung compressibility reaches its limit deeper submersion will cause lung collapse, preceded by pulmonary congestion, edema and hemorrhage. Although rare, pulmonary barotrauma during decompression (Wolf et al., 1990) may occur due to breath holding or focal gas trapping due to airway obstruction. Significant air trapping and history of spontaneous pneumothorax are also causes for concern during hyperbaric oxygen therapy and mandate a careful analysis of potential benefit from hyperbaric medicine oxygen therapy vs. the associated risk (UHMS, 2014).

Mask barotrauma, also known as "mask squeeze" or facial barotrauma, is caused when the diver inadvertently closes his nasopharynx, causing a pressure differential between the gas trapped within the mask worn by the diver and his face. Hence, when the diver descends in the water, periorbital soft tissues are forced into the mask, causing edema and bleeding (Lynch and Deaton, 2014). A case report defined the subconjunctival hemorrhage among the symptoms of the ocular barotrauma of descent (Ergözen, 2017). It is possible to prevent this type of barotrauma wearing mask rather than goggles, also during free-diving, and to apply correct techniques of mask equalization. *Gastrointestinal barotrauma* is caused by the augmented volume of the gas stored in the gastrointestinal tract. Gas expansion during the ascent phase can result in symptoms such as cramping, pain, distention, and bloating. A therapeutic approach may modulate food and liquid ingestion before diving (Lynch and Deaton, 2014). *Dental barotrauma* happens when artificial gas-filled spaces (i.e. caps, crowns, root canals) are damaged due to gas expansion during ascent. As reported by Zadik and Drucker (2011) dental barotrauma occurs while the diver is ascending. At the surface, after completing the dive, dental barotrauma may result in tooth fracture, restoration fracture, and reduced retention of dental restoration (Zadik, 2009).

Immersion Pulmonary Edema (IPE)

This condition, also referred to as swimming induced pulmonary edema (SIPE), was first described in healthy swimmers and divers by Wilmshurst et al. (1989). SIPE presents during a swim or dive with acute onset of dyspnea and cough, often productive of bloody sputum. Other manifestations include hypoxemia and bilateral pulmonary infiltrates. Many patients with SIPE require hospitalization. In Wilmshurst's series these individuals, many of whom subsequently developed hypertension, had an exaggerated hemodynamic and vasoconstrictive response to cold applied to the skin (packing of head and neck in towels soaked in ice-cold water), and it was hypothesized that during immersion in cold water the increase in afterload precipitated heart failure. This syndrome was subsequently reported in naval recruits (Weiler-Ravell et al., 1995; Shupak et al., 2000; Mahon et al., 2002; Lund et al., 2003), triathletes (Miller et al., 2010) and many non-divers and non-elite swimmers (Peacher et al., 2015).

Uncertainty existed as to how the mechanism initially proposed could apply to exceptionally fit individuals such as triathletes and special naval forces trainees. Water aspiration was implausible in most cases, and increased permeability as in acute respiratory distress syndrome (ARDS) did not seem likely. Recent invasive hemodynamic investigations have been performed in volunteers with and without a SIPE history during submersed exercise in cold (20°C) water. SIPE-susceptible individuals have higher pulmonary artery and pulmonary artery wedge pressures than controls (Moon et al., 2016b), which solidified the notion of a hemodynamic mechanism for the capillary leak. However, the reason for the higher vascular pressures is still unknown. Medical predisposing factors in some individuals include hypertension, cardiomyopathy left ventricular hypertrophy (LVH) and valve disease (Peacher et al., 2015). It is now hypothesized that a common mechanism for SIPE is the combination of heavy exercise, mild to moderately stiff left ventricle (mildly abnormal LV diastolic properties) and central blood redistribution due to immersion/submersion, causing excessively high filling pressures.

Deaths have been reported due to SIPE (Cochard et al., 2005). Indeed, a significant portion of deaths during triathlon events occur in individuals with LVH, suggesting that fatalities are often precipitated by SIPE (Moon et al., 2016a).

Preventive measures include avoidance of fluid loading before a competitive swimming event, which presumably attenuates the central blood redistribution. Sildenafil administered before a swim in cold water lowers pulmonary vascular pressures (Moon et al., 2016b) and a case report supports the notion that sildenafil administered before a triathlon swim may prevent SIPE in predisposed individuals (Martina et al., 2017). It should be noted that sildenafil may not be safe in divers who breathe oxygen tensions greater than 1 ATA, as the drug causes cerebral vasodilatation and is likely to predispose to convulsions caused by CNS oxygen toxicity (Demchenko et al., 2009). Data from Blatteau et al. (2013) also reported that sildenafil increase the risk of DCS.

Decompression Illness

Bubble formation in blood stream or tissue during or after a decrease in environmental pressure (decompression) is defined decompression illness (Vann et al., 2011). There are two fundamental decompression pathologies in the underwater environment, characterized by different pathophysiological genesis: *decompression sickness* (DCS) and *arterial gas embolism* (AGE). Each pathophysiology involves different mechanisms that can result in damages in the particular tissue affected via: direct blockage of blood flow; tissue damage or compression by direct mechanical effect; endothelial damage resulting in impaired regulation of the endothelium-mediated microcirculation and capillary leak; secondary effects mediated by immune or inflammatory pathways.

In decompression sickness (DCS) bubbles form, due to insufficient time to achieve decompression and gas elimination from the lungs. Bubbles form in tissues or in the blood-stream when local inert gas pressure exceeds a threshold value and inert gas comes out of solution. The exact biophysical mechanism is not clear, but there is agreement that in the diving environment supersaturation occurs when the rate of ambient pressure reduction exceeds the rate of inert (usually nitrogen) gas washout from tissues. The different manners of diving (i.e., breath-hold or scuba) may also induce different physiological or pathophysiological effects, depending on the altitude at which the immersion is performed: sea level immersion, or at high altitude, with or without previous changes induced by acclimation (Egi and Brubakk, 1995; Bosco et al., 2001; Clarke et al., 2015).

Traditional classification of DCS comprises type 1, including pain and cutaneous manifestations, plus subjective symptomatology; and type 2, where tingling, paresthesia and numbness will appear, at times with muscle weakness and mental and motor abnormalities.

Treatment begins at sea level with 100% oxygen breathing and recompression therapy as soon as possible. While around the world various recompression procedures are implemented, since their introduction in the 1960's the US Navy oxygen treatment tables with initial recompression to 18 m (2.8 atmospheres absolute) and administration of 100% O_2 (US Navy Table 6, **Figure 1**) have been the most widely used. They are highly effective if administered without excessive delay after development of symptoms (Vann et al., 2011). Other procedures used in Europe and elsewhere follow the same general principles of pressure and oxygen breathing.

Arterial gas embolism might cause a more overt and extensive symptomatology but the treatment will converge to recompression and oxygen breathing. Embolism results from rupture of alveoli during ascent from a dive resulting in entry of air into the bloodstream. The most common cause of embolism in underwater activities is a rapid ascent with breath holding or regional gas trapping in the lung due to lung pathology. Therapeutic remedies are similar both for DCS and air embolism, both utilizing O_2 breathing and recompression. It is recommended to use only Table 6.

Recently, certain interventions such as exercise are viewed as protective form of preconditioning reducing the venous gas emboli quantity (Dujic et al., 2008). Moreover, oxygen pre-breathing has shown protective effect on bubble production and platelets activation; deeply, it has been shown correlation among oxygen partial pressure breathing and modulation of reactive oxygen species and related antioxidant enzyme (Landolfi et al., 2006; Bosco et al., 2010; Morabito et al., 2011).

Thom et al. (2015) offered a novel opportunity to explore associations of circulating microparticles and neutrophil activation that may contribute to development of DCS. Further work will be needed to explain and clarify DCS pathophysiology.

Nitrogen Narcosis

Nitrogen does not participate in any metabolic process and therefore is defined as an inert gas; hence, nitrogen levels in the body are determined solely by the atmospheric pressure (Freiberger et al., 2016). During air breathing at sea level, the body is therefore "saturated" with nitrogen. During immersion with underwater air breathing, nitrogen follows its alveolar-arterial pressure gradient passing from the alveoli to the lung capillaries, and hence to peripheral tissues. Reversal occurs in the ascending phase and after the dive.

Narcosis while diving (also known as nitrogen narcosis, inert gas narcosis, raptures of the deep, Martini effect) is a pathology that arises following a considerable increase in nitrogen partial pressure and consists of alteration in consciousness and neuro-sensory state. Manifestations consist of distorted perceptions, hallucinations, difficulty in concentrating and performing elementary tasks, confusion and loss of consciousness. There is a wide inter-individual susceptibility to the effects of hyperbaric nitrogen, which can be augmented by hypercapnia (Freiberger et al., 2016). Reducing the nitrogen partial pressure (basically reducing the depth) causes rapid recession of the symptoms, but recent experimental data on a controlled group of healthy divers (Balestra et al., 2012) supports the view that nitrogen narcosis can interact with the production and release of a

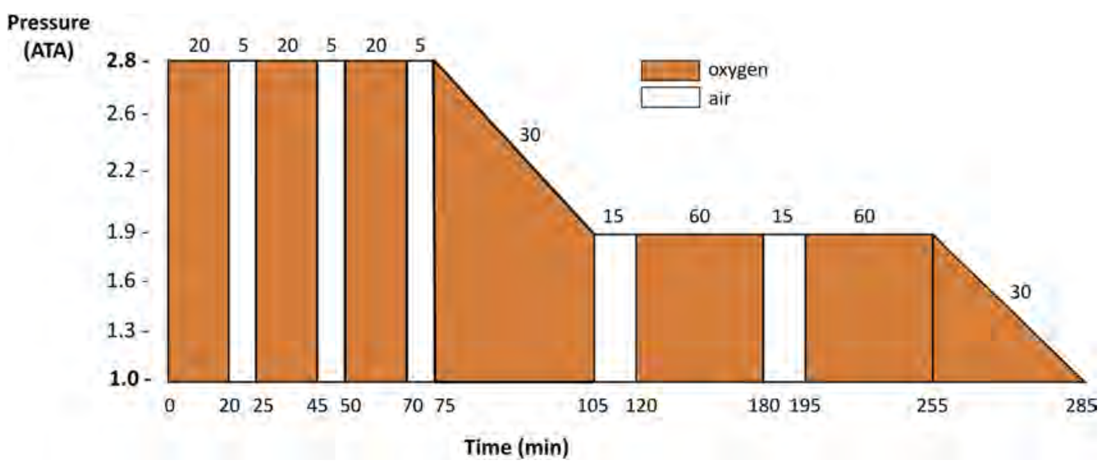

FIGURE 1 | US Navy Table 6 profile commonly used in clinical routine for DCS recompression procedure for recompression of divers with DCS or arterial gas embolism. Redrawn from Navy Department (2016).

variety of neurotransmitters and might require substantial time delay to recover after exposure. In this last study, the authors utilized Critical Flicker Fusion Frequency (CFFF) measurements to time the onset and resolution of N_2 narcosis after a dive. The impairment of CFFF persisted 30 min after surfacing and the narcotic effect dissipated only after this time delay.

Oxygen Toxicity

The use of enriched air or closed-circuit rebreather during diving, can potentially result in oxidative injury affecting the brain, lungs and eyes. However, lung and brain injuries develop mainly because of the toxic effects of oxygen free radicals on lung parenchyma, airway and brain (Demchenko et al., 2007, 2008; Buzzacott and Denoble, 2017). Central nervous system oxygen toxicity (CNS-OT) is one of the most harmful effects of Enriched Air Nitrox (EAN) diving and it is related to oxidative stress and inflammation (Fock et al., 2013; Arieli et al., 2014). CNS-OT may cause convulsions similar to epileptic seizures, with sudden loss of consciousness, and other symptoms such as nausea, vomiting, palpitations, visual field constriction, tinnitus and auditory hallucinations. Recent studies have shown that seizures due to CNS O_2 toxicity are probably preventable by conventional anticonvulsant drugs (Demchenko et al., 2017).

A breathing mixture with sufficiently high PO_2 can also induce pulmonary oxygen toxicity (Arieli, 2006; Balestra and Germonpre, 2006). Indeed, exposure to HBO_2 may lead to temporary reductions in pulmonary function (Van Ooij et al., 2013). While pulmonary mechanics are altered during hyperbaric exposure due to gas density effects (Hrnčíř, 1996), there are also data on possible long-term effects of diving on respiratory mechanics. A spirometric investigation revealed a long-lasting impairment of the conducting function of the small airways in humans accustomed to perform deep dives. Interestingly, these results were observed in subjects using oxygen but also in subjects using air as breathing gas (Tetzlaff et al., 1998). In addition, a small but significant reduction in FVC was observed 24 h after a single dive to a depth of 50 m while breathing air (Tetzlaff et al., 2001), suggesting that an increment in airway resistance occurred and persisted long after the dive.

Recent experimental work has been performed on animals to investigate the possible effects of hyperoxic hyperbaric exposure on the mechanics of respiratory parameters. Previously, it has been demonstrated that the exposure to hyperbaric hyperoxia increased respiratory system elastance and both the "ohmic" and viscoelastic components of inspiratory resistances, probably due to increased nitrogen reactive species production and iNOS activity (Rubini et al., 2013, 2014). Conversely, a single exposure to hyperbaric air at 2.5 atmospheres for 90 minutes does not affect either the elastic or the resistive respiratory system properties (Rubini et al., 2014). More researches using new experimental technologies are requested to continue the predictive studies on work capability and physiological effects under pressure (Lambertsen, 1976).

High Pressure Nervous Syndrome

High Pressure Nervous Syndrome (HPNS) is a set of motor and sensory symptoms that appear during deep helium diving, typically at depths deeper than 150–180 m. Subjects affected by HPNS complain of general illness, intentional and postural tremor, asthenia, drowsiness (or otherwise insomnia), nightmares, fasciculation or muscle twitches and myoclonic contractions (Bennett et al., 1981). Electroencephalographic anomalies are also observed, which include a generalized increase in slow activity and a decrease in frequency at higher frequencies. This pathology appears to be produced by the direct effect of high environmental pressure on cells, through modifications in neurotransmitter function (Rostain and Balon, 2006).

CONCLUSION

Underwater physiology research today, considering its tradition, focuses on the prevention of underwater accidents, with pharmacological and physical approach as preconditioning (Bosco et al., 2010; Morabito et al., 2011) to reduce the bubble formation, inflammation and the risk of DCS (Castagna et al., 2009; Germonpré and Balestra, 2017). Long sojourning and efficient maneuverability and working capabilities can only be achieved with underwater habitats capable of being maintained for weeks and months with human safety factors, appropriate rotation of selected crews and safe decompression protocols. It is conceivable that fast evacuation from such environments might require rapid "at depth" extraction of the crew with capability of surface decompression in appropriate support vessels. Such specialized and costly stations at depth could only be justified for surveillance of mineral extraction resources, oil pumping stations or military purposes at significant depths, where breathing gases might be tailored to the specialized needs.

NOTES

Archimedes' law: An object partially or wholly immersed in a fluid is buoyed up by a force equal to the weight of the fluid displaced by the object.

Boyle's law: the absolute pressure exerted by a given mass of an ideal gas is inversely proportional to the volume it occupies if the temperature and amount of gas remain unchanged within a closed system.

Dalton's law: in a mixture of non-reacting gases, the total pressure exerted is equal to the sum of the partial pressures of the individual gases.

Henry's law: the amount of dissolved gas is proportional to its partial pressure in the gas phase.

AUTHOR CONTRIBUTIONS

GB substantial contributions to the design of the work and drafting the manuscript. AR reviewed the literature and wrote the manuscript. RM wrote the paper and revised it critically for important intellectual content. EC contributed to review and write the manuscript, editing of the language. All authors critical reviewed the final draft of the manuscript and approved the version submitted.

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Dive Risk Factors, Gas Bubble Formation, and Decompression Illness in Recreational SCUBA Diving: Analysis of DAN Europe DSL Data Base

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Introduction: The popularity of SCUBA diving is steadily increasing together with the number of dives and correlated diseases per year. The rules that govern correct decompression procedures are considered well known even if the majority of Decompression Sickness (DCS) cases are considered unexpected confirming a bias in the "mathematical ability" to predict DCS by the current algorithms. Furthermore, little is still known about diving risk factors and any individual predisposition to DCS. This study provides an in-depth epidemiological analysis of the diving community, to include additional risk factors correlated with the development of circulating bubbles and DCS.

Materials and Methods: An originally developed database (DAN DB) including specific questionnaires for data collection allowed the statistical analysis of 39,099 electronically recorded open circuit dives made by 2,629 European divers (2,189 males 83.3%, 440 females 16.7%) over 5 years. The same dive parameters and risk factors were investigated also in 970 out of the 39,099 collected dives investigated for bubble formation, by 1-min precordial Doppler, and in 320 sea-level dives followed by DCS symptoms.

Results: Mean depth and GF high of all the recorded dives were 27.1 m, and 0.66, respectively; the average ascent speed was lower than the currently recommended "safe" one (9–10 m/min). We found statistically significant relationships between higher bubble grades and BMI, fat mass, age, and diving exposure. Regarding incidence of DCS, we identified additional non-bubble related risk factors, which appear significantly related to a higher DCS incidence, namely: gender, strong current, heavy exercise, and workload during diving. We found that the majority of the recorded DCS cases were not predicted by the adopted decompression algorithm and would have therefore been defined as "undeserved."

Conclusion: The DAN DB analysis shows that most dives were made in a "safe zone," even if data show an evident "gray area" in the "mathematical" ability to predict DCS by the current algorithms. Some other risk factors seem to influence the possibility to develop DCS, irrespective of their effect on bubble formation, thus suggesting the existence of some factors influencing or enhancing the effects of bubbles.

Keywords: SCUBA diving, decompression sickness, decompression illness, vascular gas emboli, decompression algorithms

INTRODUCTION

The popularity of SCUBA diving is steadily increasing together with the number of dives and correlated diseases per year, even if the total number of exposed individuals (i.e., commercial divers, hyperbaric attendants, and recreational divers) and the exact incidence of decompression illness DCI is unknown (Trout et al., 2012).

This pathology is affecting divers, astronauts, pilots, and compressed air workers, and although its occurrence is relatively rare, with rates of 0.01–0.1% per dive (the higher end of the spectrum reflecting rates for commercial diving and the lower rates for scientific and recreational diving), the consequences can be dramatic (Balestra et al., 2016).

The increase in ambient pressure, the different breathing gases used for diving (with different fractions of inert, saturating gases), the rules that govern their behavior and the correct decompression procedures are considered well known (Bennett and Elliott, 1982). Commonly, decompression tables or diving computers are used to control the risk of decompression sickness (DCS) using the "leading tissue" concept to calculate decompression stop depth and time (Buhlmann, 1982).

In Haldane-Bulhmann decompression models, for instance, the decompression algorithm is calculated not to exceed a given maximum inert gas level, for each "compartment," the so called: *M*-value (Buhlmann, 1982).

Even if the ultimate pathogenic mechanism of DCS is still debated, the link between circulating inert gas emboli (Vascular Gas Emboli: VGE) and DCS is well accepted, as well as the presence of "silent" VGE in many divers without any DCS symptom (Weathersby et al., 1987; Eftedal et al., 2007).

New recently developed hypotheses indicating that inert gas embolism can trigger cell-mediated mechanisms assimilating DCS to an inflammatory disease (Thom et al., 2015) make the presence of even "silent bubbles" worth considering and investigating to identify further risk factors that may correlate with an increase in the incidence of bubble formation and DCS.

Our hypothesis may also encompass that some predisposing factors and/or peripheral humoral variables can contribute to develop DCS at the same level of bubble degree.

This study aims at three goals:

1. An in-depth epidemiological analysis focusing on habits and risks of the diving community.
2. Investigating additional risk factors correlated with the development of circulating bubbles other than pressure differentials.
3. Analyzing 320 DCS cases from the DAN Europe Diving Safety Laboratory (DSL) database (DAN DB) to identify related risk factors and to improve the current decompression guidelines.

MATERIALS AND METHODS

An original database (DAN DB) including specific questionnaires for data collection was developed allowing retrospective statistical analysis from 2,629 European divers (2,189 men 83.3%, 440 women 16.7%) who made 39,099 open circuit dives over 5 years. All dives were considered started upon reaching 1 m depth and finished when reaching the same depth without any return to deeper depth within 5 min.

All dives shallower than 5 m and shorter than 10 min were excluded.

Description of the SCUBA Community and Dives

Information about gender, age, and anthropometric data (height, weight) were requested, BMI was calculated. Starting from age, gender and BMI we also extrapolated the percentage of fat mass and lean body weight, using the Deurenberg (Deurenberg et al., 1991a,b) and the James formula, respectively (James and Waterlow, 1976).

All the 39,099 dives have been digitally recorded including depth, diving time, relative gradient factor (GF), and real water temperature.

The maximum gradient factor (GF) was calculated according to the Buhlmann ZHL16 C model, taking into account any previous dive if the surface interval was less than 48 hours (repetitive dives). Dives made after a surface interval longer than 48 hours were considered as non-repetitive.

GF is a way to measure nitrogen supersaturation in the "leading tissue" (the compartment with the highest supersaturation level) at any given time and depth during the ascent to the surface, represented as a fraction of the maximum inert gas supersaturation (*M*-value) allowed for the 16 tissues considered by the Buhlmann ZH-16 Model C, from 4 to 635 min half saturation/desaturation times (HT). Calculations of GF were performed for all the 16 tissues, and the maximum GF-value in the leading tissue was recorded (Baker, 1998).

The frequency of leading tissue involvement was also investigated. Aqueous tissues, with low gas solubility, were usually considered "fast tissues" (in terms of saturation time) as compared to "slow tissues" with high gas solubility (Bennett and

Elliott, 1982; Marroni et al., 2004); starting from this the various "tissues" were grouped into three Leading Tissue Groups (LTG) in the DAN DB, to better manage the 16 investigated tissues, data recording and the related statistical analysis:

✓ Fast tissues (from 4 to 18.5 HT)
✓ Medium tissues (from 27 to 38.3 HT)
✓ Slow tissues (from 54.3 to 635 HT)

The dive profiles were collected from different models of diving computers and converted into the original DAN DB format known as DAN DL7 allowing us to recalculate the dive profile in an extended way that permits a detailed analysis with different calculations based on "real time" depth and time data points.

Trimix dives (open circuit, semi closed and closed circuit) were excluded.

A specific questionnaire was used to also record other dive characteristics such as:

✓ Environment (sea, lake, other)
✓ Purpose of diving (sightseeing, research, learning, photography, other)
✓ Gas used (air or nitrox)
✓ Current (absent or present)
✓ Visibility (low < 7/10 m; high >7/10 m)
✓ Perceived individual thermal comfort
✓ Type of diving suit (dry or wet)
✓ Physical condition before the dive (rested or tired)
✓ Level of exercise during the 24 h preceding diving (no-exercise, moderate or heavy exercise)
✓ Workload during the dive (no-workload, light or intense)
✓ Diver related problems during diving (difficult ear equalization, out of air, buoyancy control, shared air, rapid ascent, omitted deco, vertigo, seasickness, other)
✓ Equipment problems (jacket, regulator, dive computer, mask, fins, suit, weight belt, other)
✓ Use of alcohol
✓ Use of "habitual" drugs (medically prescribed) during the 24 h before diving
✓ Use of "occasional" drugs (non-medically prescribed) during the 24 h before diving

Additional information about general medical history (allergy, asthma, heart, and vascular disease, pulmonary problems, diabetes, recurrent back pain, sinus problems, previous DCS, and use of tobacco were also requested.

Bubble Formation Risk

Nine hundred and seventy out of the 39099 collected dives were also investigated for bubble formation by 1-min precordial Doppler recording at 30 min after surfacing, 448 out of 970 were also recorded every 15 min and for 90 min after surfacing.

Doppler recordings were evaluated according to a modified Spencer Scale (Spencer and Johanson, 1974) named Expanded Spencer Scale (ESS) (Marroni et al., 2004) as follows:

Grade 0	No Bubble Signals
Grade 0.5	1–2 sporadic Bubble signals
Grade 1	up to 5 Bubble signals
Grade 1.5	up to 15 Bubble signals
Grade 2	up to 30 Bubble signals
Grade 2.5	more than 30 Bubble signals
Grade 3	virtually continuous Bubble signals
Grade 3.5	continuous Bubble signals, with numerous bubble showers
Grade 4	continuous Bubble signals, with continuous bubble showers.

However a simplified bubble grading system was used for our statistical evaluation, as follows: (Marroni et al., 2004)

Zero	No Bubble signal
LBG	Low Bubble Grade: occasional bubble signals, lower than 2 in the ESS
HBG	HBG High Bubble Grade: Frequent to continuous bubble signals, 2 and 2.5 in the ESS
HBG+	High Bubble Grade plus: Bubble signals reaching grade 3, 3.5, and 4 in the ESS.

Bubble grade was compared with several parameters and possible risk factors such:

✓ Gender and age
✓ Height
✓ Weight
✓ BMI
✓ Fat mass and lean body weight
✓ Diving profile (depth, time, GF, and LTG)
✓ Minimum water temperature
✓ Environment
✓ Purpose of diving
✓ Gas used
✓ Current
✓ Visibility
✓ Perceived individual thermal-comfort
✓ Type of diving suit
✓ Physical conditions before dives
✓ Exercise during the 24 h preceding diving
✓ Workload during the dive
✓ Any diver and equipment related problem during diving
✓ Use of alcohol

DCS Associated Risk Factors

To better understand the mechanisms of DCS, we also comparatively analyzed 320 sea-level dives followed by DCS symptoms.

These DCS cases were evaluated according to the same dive parameters and risk factors investigated for bubble formation and compared to the no-DCS dives in the DAN DB.

We also made an in-depth analysis of GF and LTG distribution in the DCS cases.

STATISTICAL ANALYSIS

Data are presented as the mean ± standard deviation (SD) for parametric data and median and range for non-parametric data (e.g., bubble grades).

The risk factors related effect in bubbles formations were investigated by non-parametric analysis of variance (Kruskal–Wallis test), after normality testing (Kolmogorov–Smirnov test) for anthropometric and diving data and by the chi-square test for gender and environmental data, leading tissue, and for the other risk factors.

The influences of the risk factors in the development of DCS were compared with the no-symptomatic dives in the DB by the Mann-Whitney test for non- parametric data, after the normality test (Kolmogorov-Smirnov) for anthropometric, diving and other risk factors data. Some data such as: gender, visibility, current, environmental, exercise before diving, state before, thermal comfort, use of alcohol, diver, and equipment problem were investigated using the chi-square test.

RESULTS

Description of the SCUBA Community and Dives (Table 1)

Two thousand six hundred and twenty-nine divers (2,189 male, 440 female; mean age 37.36 ± 9.17 years) completed 39,099 open circuit dives (32,311 – 82.6% performed by males and 6,788 – 17.4% performed by females); mean age (mean ± SD) was 37.4 ± 9.2 years (38 for males and 34 for females).

Anthropometric and diving profile data were as follows:

- ✓ Mean height 175.3 cm ± 6.22 (177 males–164 females)
- ✓ Weight 77.6 Kg ± 9.27 (81 males–61 females)
- ✓ BMI 25.16 (Kg/m^2) ±1.83 (26 males–23 females)
- ✓ Fat mass calculated 23.7% range 6.5–43.6 (23.2 males–29.4 females)
- ✓ Lean body weight 62.6% range 34.3–87.3 (62.6 males–44.8 females)
- ✓ Depth 27.1 m (range 5–104); Dive time 46.4 min (range 10–130); GF 0.66 (range 0.05–1.25)
- ✓ Half Time grouping: medium tissue 70.0%; fast tissue 24.5%; slow tissue 5.5% of cases
- ✓ Mean water temperature 17.28°C (SD ± 6.53)
- ✓ All dives were performed with open circuit SCUBA (95.3% Air; 4.7% Nitrox)

The other characteristics recorded by the questionnaire were:

- ✓ Dives in seawater 86.1%, in lake 6.2% and in different conditions 7.7%
- ✓ 66.2% were made for sightseeing, 11.2% for research, 6.1% for learning, 2.6% for photography, and 13.9% for other scope
- ✓ Current was reported as absent in 77.3% and present in 22.7%
- ✓ Visibility was reported as low in 33.3%, and high in 66.7% of cases (Data about current and visibility were filled only for 14,361 dives)
- ✓ 5.3% declared to have "felt cold" while 94.7% reported thermal comfort during the dive
- ✓ Wet suits were used in 60.5% of the dives, dry suits in 19.0% (in 20.6% of dives this field has not been filled)
- ✓ 90.9% of the divers declared being rested, while 9.1% declared being tired before the dive
- ✓ 30.3% declared no exercise, 68.8% moderate exercise and 0.9% heavy exercise during the pre-dive 24 h period
- ✓ Reported Workload during the dive: No or light-workload 92.1%, intense 7.9%
- ✓ Diver related problem during diving were reported by 3.6% while 96.4% declared no problem (details in **Table 1**)
- ✓ Equipment malfunction during diving occurred in 2.7% of the dives (details in **Table 1**)
- ✓ 42.4% of the divers declared moderate alcohol use and 57.6% no alcohol use in the 24 h before diving
- ✓ In 1,010 dives out of 39,099 (2.6%), divers declared use of "habitual" drugs (medically prescribed) and 490 (1.2%) use of occasional (non-medically prescribed) drugs; a total of 1,500 dives (3.8%) were performed after some drug use
- ✓ 316 subjects (12%) declared at the least one chronic disease; 386 were smokers (14.7%) and 28 had suffered previous DCS (1.1%)

Bubble Formation Risk (Table 2)

Nine hundred and seventy (892 male and 78 female) precordial Doppler files were evaluated according to the ESS scale and converted into the simplified Doppler grading system: 369 dives (38%) showed no bubble, 446 dives (46%) Low Grade Bubbles (LBG), 106 dives (11%) High Grade Bubbles (HBG), and 49 dives (5%) showed High Grade Bubbles Plus (HBG+).

We found a statistically significant difference when comparing grade Zero vs. other grades in:

- ✓ BMI lower in grade Zero vs. HBG ($p = 0.02$)
- ✓ Fat mass was significantly lower in subjects with grade Zero vs. LBG (0.012), HBG (0.0005) and HBG+(<0.0001)
- ✓ Age related difference was found lower in grade Zero vs. LBG ($p = 0.01$) than for HBG ($p < 0.001$) and HBG+ ($p < 0.001$)
- ✓ Diving Exposure (see **Table 2** for details)

The bubble grade showed no difference between fast tissues and medium tissue groups ($p = 0.51$); while slow tissues showed an increase in bubble grade when compared with both fast ($p = 0.014$) and medium ($p = 0.01$). However the slow tissues involvement regarded only 15 dives (one zero; 8 LBG; 5HBG; 1 HBG+).

Other risk factors seem linked with bubble formation but an in depth investigation showed that the effect was associated with an influence of diving exposure and consequently GF:

- ✓ Low visibility decreases High Bubble Grades (HBG and HBG+) ($p = 0.002$) but through significantly lower GF-values as compared to high visibility dives $P < 0.001$
- ✓ Intense workload during the dives seems to reduce High Bubble Grades ($p = 0.001$) most probably through a reduction of diving exposure

The other investigated risk factors did not show any significant relation with High Bubble Grades.

Finally in the 448 dives for which Precordial Doppler recorded every 15 min we found that bubbles peaked between 30 and

TABLE 1 | Description of the SCUBA community.

Anthropometric data	**Male**	**Female**	**Total**
Gender	***N* = 2,189** **83.3%**	***N* = 440** **16.7%**	***N* = 2,629**
Age (years)	38	34	37.4 ± 9.2
Height (cm)	177	164	175.3 cm ± 6.22
Weight (km)	81	61	77.6 Kg ± 9.27
BMI (kg/m^2)	26	23	25.16 ± 1.83
Fat mass (%)	23.2	29.4	23.7% (6.5–43.6)
Lean body weight (%)	62.6	44.8	62.6 (34.3–87.3)
CHARACTERISTIC OF DIVES			
Diving profile	27.1 (5–104) Depth (m)	46.4 (10–130) Diving time (min)	0.66 (0.05–1.25) GF
Leading tissues	70% Medium	24.5 Fast	5.5% Slow
Real temperature recorded	17.28°C (±6.53)		
RISK FACTORS			
Environment (Lake/Sea)	86.1% Seawater	6.2% Lake	7.7% Other
Scope	66.2% Sightseeing	11.2% Research	22.6% Other
Gas used	95.3 Air	4.7 Nitrox	
Current	77.3 Absent	22.7 Present	–
Visibility	33.3 < of 7/10 m	66.7 > of 7/10 m	–
Perceived temperature-comfort	94.7% Thermal comfort	5.3% “Felt cold”	
Suit	60.5% Wet	19.0% Dry	20.6% Not filled
Feeling before the dive	90.9% Rested	9.1% Tired	–
Physical exercise during the 24 h before dive	30.3% No exercise	68.8% Moderate	0.9% Heavy
Workload during the dives	92.1% No-workload or light	7.9% Intense	–
Diver related problems	96.4 No problem	1.25 Equalization 0.45 Out of air 0.42 Buoyancy	0.05 Omiss. Deco 0.28 Rapid ascent 1.19 Other
Equipment problems	97.2 No problem	0.29 Jacket 0.26 Octopus 0.10 Computer 0.83 Mask	0.08 Fins 0.40 Suit 0.32 Weight belt 0.47 Others
Moderate use of alcohol before	57.6 No use	42.4 Moderate use	–
Use of drugs	2.6% Habitual drugs (Prescribed)	1.2% Occasional drugs (Not prescribed)	3.8% Total
Medical history	12% At least one chronic diseases	14.7% Smoker	1.1% Previous DCS

45 min after the dive, irrespective of the bubble grade level (**Figure 1**).

DCS Risk Factors (Table 3)

The 320 cases of DCS recorded in a specific section of the DAN Data base (all occurred in open circuit diving) were related to the analyzed factors such as:

- ✓ Higher percentage of female divers $P < 0.0001$
- ✓ Age significantly higher $p < 0.001$
- ✓ Less height, weight, and BMI $p < 0.0001$
- ✓ Higher percentage of fat mass $P < 0.0001$
- ✓ Lower lean body weight $P < 0.0001$
- ✓ Depth, dive time, and GF were statistically higher $p < 0.0001$, $p = 0.001$, $p < 0.0001$
- ✓ Lake diving $p = 0.004$
- ✓ Strong current and low visibility $p < 0.0001$ and $p = 0.026$
- ✓ Heavy exercise before diving $p < 0.0001$
- ✓ Heavy workload during diving $p \leq 0.0001$

Other risk factors also appeared related to DCS but an in depth investigation showed that the effect was associated with increasing diving exposure and consequently GF:

- ✓ Higher water temperature $p < 0.0001$
- ✓ Dry Suit diving $p < 0.0001$

We did not find any other significant difference for all the other investigated risk factors (**Table 3**).

In-Depth Analysis of GF-Value in the 320 DCS Cases (Table 4)

- ✓ Only eight cases (2.5%) showed a GF > 1
- ✓ 14 cases had a GF > 0.9 (4.4%)
- ✓ The majority of cases (236–73.7%) showed GF-values between 0.70 and 0.90

TABLE 2 | Bubble formation Risk: Only Age and BMI influence bubble formation, all the other investigated risk factors did not show any effect on bubble formation or had an influence BUT trough modification of diving exposure.

Sample description				
Gender	*N* = 892 Male	*N* = 78 Female	*N* = 970 Total	
Grade of bubbles %	38 Zero	46 LBG	11 HBG	5 HBG+
	Zero vs. LBG (38)	**Zero vs. HBG**	**Zero vs. HBG+**	**Note**
ANTHROPOMETRIC RISK FACTORS				
Gender	–	–	–	Analysis of contingency did not show any gender related difference $P = 0.40$
Age (years)	$P = 0.002$	$P < 0.0001$	$P < 0.0001$	Age related bubble increase
Height (cm)	Ns	Ns	Ns	**NO** influence on bubbles
Weight (km)	Ns	Ns	Ns	**NO** influence on bubbles
BMI (kg/m^2)	Ns	$P = 0.01$	$P = 0.04$	Increased BMI seems to increase bubbles
Fat mass (%)	0.012	0.0005	<0.0001	Increased of fat mass seems to increase bubbles
Lean body weight (%)	Ns	Ns	Ns	**NO** influence on bubbles
DIVING RISK FACTORS				
Depth (m)	$P = 0.048$	$P < 0.02$	$P < 0.001$	Depth related Bubble increase
Diving time (min)	$P = 0.02$	Ns	Ns	**NO** relevant influence
GF	$P = 0.0001$	$P = 0.0002$	$P < 0.0001$	Increasing GF Increase bubbles
Leading tissues	–	–	–	**NO** influence on bubbles $P = 0.48$ (Analysis of contingency)
Minimum temperature	Ns	Ns	Ns	**NO** influence on bubbles

Other risk factors	***P*-values**	**Analysis of contingency**
Low visibility	$P = 0.0001$	Low visibility reduces bubbles **BUT** by reduction of diving exposure $P = 0.001$
Workload	$P = 0.0003$	Workload reduces high bubbles grade **BUT** by reduction of diving exposure $P = 0.001$
Environment	$P = 0.001$	Diving in lake reduces high bubbles grade **BUT** by reduction of diving exposure $P = 0.001$
Gas used (Nitrox/Air)	$P = 0.90$	**NO** influence on bubbles
Current	$P = 0.06$	**NO** influence on bubbles
Perceived temperature	$P = 0.35$	**NO** influence on bubbles
Suit	$P = 0.38$	**NO** influence on bubbles
Feeling before diving rested/tired	$P = 0.13$	**NO** influence on bubbles
Exercise before diving	$P = 0.06$	**NO** influence on bubbles
Divers related problem	$P = 0.55$	**NO** influence on bubbles
Equipment malfunction	$P = 0.38$	**NO** influence on bubbles
Use of alcohol before diving	$P = 0.43$	**NO** influence on bubbles

  * 37.5% between 0.8 and 0.9
  * 36.2% between 0.7 and 0.9

✓ 46 cases (14.4%) had a GF lower than 0.70
✓ 10 cases (3.4%) lower than 0.60
✓ Only 3 cases had a GF lower than 0.50

It is intriguing to note that all the eight cases that exceeded GF-value 1 involved the fast or the slow tissues, while no case of GF > 1 involved the medium tissues, indicating an apparent inability to correctly calculate and predict DCS by the current decompression models when medium HT compartments are involved as the leading tissue.

The distribution of DCS cases divided by single tissue is shown in **Table 5**.

Grouping the tissues into LTG we found:

✓ 75.9% of the DCS cases involved the Medium Tissues
✓ 15.9% the Fast tissues
✓ 8.1% the Slow tissues

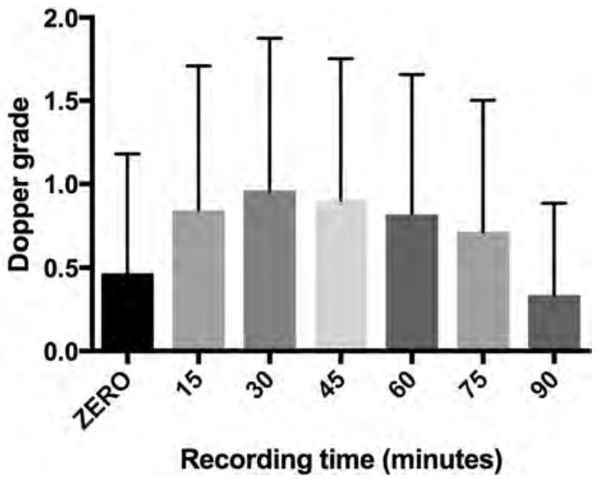

FIGURE 1 | Trend of bubbles after dives. The peak of bubbles was localized around 30 and 45 min after dives: 90 min after dive the bubbles were significantly reduced.

This finding indicates that the prevalence of the different LTG was statistically different in the DCS group than in the total DB ($p = 0.0005$), in particular the DCS cases involved a lower percentage of "Fast Tissues" than expected.

A more in-depth statistical analysis considering the prevalence of the three groups separately showed:

✓ Prevalence of Fast tissues was statistically lower as compared to Medium tissues $p = 0.0008$
✓ Prevalence of Fast tissues was statistically lower as compared to Slow tissues $p = 0.0005$
✓ No difference in prevalence was found between medium and slow tissues $p = 0.13$ (**Table 5**)

DISCUSSION

The data collected by the DAN Europe Database have two important characteristics, in fact if on the one hand data recorded come from real-life dives, allowing for a "real picture" of the recreational diving community, on the other hand more than 11% of dives were performed during field research trips with an *ad hoc* research protocol allowing for accurate collection and in depth analysis of important variables, providing a large base of comparison to investigate Bubble and DCS related risk factors.

The DAN DB analysis shows that most dives were made in a "safe zone," with an average depth of 27.1 m, average GF 0.66, and an average ascent speed lower than the currently recommended "safe" one. Even more importantly, very few deco omissions occurred; this indicates that divers tend to dive very conservatively.

Another interesting information is about the incidence of diver and equipment related problems which is reported to occur in only 6.3% of dives and that serious problems, fortunately, occurred only in a very limited fraction of these dives; for instance problems with breathing apparatus occurred only in 103 cases out of 39,099 dives, deco omission in just 20 dives and rapid ascent in only 109 dives. All together summing up to less than 0.6% of all recorded dives.

Our data confirm that the bubble peak occurs between 30 and 45 min after surfacing. This aspect is very important and indicates the importance to avoid efforts during this post-dive time interval, also considering that conditions increasing intrathoracic pressure, such as Valsalva maneuvers and physical efforts, can have negative implications for divers with Patent Foramen Ovale (Balestra et al., 1998).

But the main focus of this analysis was to investigate how certain risk factors may influence bubble formation (in particular high bubble grades) and DCS and the capacity to predict DCS trough the current decompression models, considering that in recent years diving medicine experts began to suspect that bubble formation and DCS occurrence could be linked not "only" to the dive profile but also to certain pre-dive conditions (Theunissen et al., 2013, 2015) and possibly to specific individual predisposition as already confirmed in a other diving related illnesses (Cialoni et al., 2015). The relation between bubble formation and DCS also seems to be more complex than previously believed and DCS in the presence of high bubble grades to be possibly influenced by other peripheral variables (Thom et al., 2015).

Our analysis showed little or no relation between bubble formation and many investigated "risk factors," in fact only increased age and BMI appear to be related to increased bubble formation. It is interesting to note that height and weight separately did not appear to increase bubble formation, while their combined value (BMI) appeared to have a certain relation with higher bubble grades.

Because of this we included the analysis of fat mass, confirming a link with bubble formation, and apparently even more so when considering the DCS cases.

Although we could not find any really significant relation between the non-dive-profile related risk factors and bubbles it is intriguing to note that such risk factors, although not increasing bubble formation, appear to be related to DCS, allowing to infer that these risk factors may cause effects that, at similar bubble formation levels, can influence the diver's defense mechanism.

Such risk factors (current, low visibility, lake diving—usually cold and with very low visibility-, high workload during the dive) are all likely to cause a condition of stress. Therefore it is possible to hypothesize that humoral factors (including hormones) released in a stress condition can influence the effect of bubbles, and we have already started a more in-depth study about these possible variables.

A similar explanation could be used to understand why women are more subject to DCS even without marked (St. Leger Dowse et al., 2002; Lee et al., 2003) difference in bubble formation as compared to similar dives in men. As already claimed in the literature, different moments of the menstrual cycle can be considered as increasing the risk of DCS (Lee et al., 2003) in fact the DCS incidents were unevenly distributed throughout the cycle with the greatest percentage of incidents occurring in the first week of the menstrual cycle.

TABLE 3 | Investigation on DCS risk factors.

Sample description	Male $N = 188$ (59%)	Female $N = 132$ (41%)	Total $N = 320$	
	DCS	**Data base**	**Results**	**Note**
ANTHROPOMETRIC RISK FACTORS				
Gender	41.2% Female	17% Female	$P < 0.0001$	Females have higher possibility to develop DCS Notwithstanding similar bubble formation as compared to males
Age (years)	42 (23–67)	37 (10–82)	$P < 0.0001$	DCS increases when AGE increases
Height (cm)	173 (155–191)	178 (150–203)	$P < 0.0001$	
Weight (km)	75 (49–120)	81 (40–125)	$P < 0.0001$	
BMI (kg/m^2)	24.5 (17–44)	25.6 (17–37)	$P < 0.0001$	Decrease of BMI seems to increase DCS
Fat mass (%)	34.05	23.7	$P < 0.0001$	Increased of fat mass seems to increase DCS
Lean body weight (%)	53.05	62.6	$P < 0.0001$	In DCS case we found a lower lean mass
Depth (m)	32.4 (13–82)	27.1 (5–104)	$P < 0.0001$	Increase of depth Increases DCS
Diving time (min)	48.4 (17–104)	46 (10–130)	$P = 0.001$	Increase of time Increases DCS
GF	0.79 (0.4–1.1)	0.66 (0.05–1.2)	$P < 0.0001$	Increase of GF Increases DCS
Real minimum recorded temperature	23 (0.0–36)	17 (0.0–32)	$P < 0.0001$	Incidence of DCS increases when water temperature increases **BUT** In these cases warmer temperature increases diving exposure $P < 0.0001$
OTHER RISK FACTORS				
Environmental (Lake/Sea)	10.9%	6.7%	$P = 0.005$	Higher DCS incidence increases for lake dives **NOT** diving related $P = 0.71$
Presence of current	35.6%	24.8%	$P < 0.0001$	Incidence of DCS increases in presence of current **NOT** diving related $P = 0.72$
Low visibility	39.1%	33.3%	$P = 0.026$	Incidence of DCS increases in low visibility **NOT** diving related $P = 0.09$
Physical exercise into 24 h before	90.3%	69.7%	$P < 0.0001$	Exercise before diving increases DCS **NOT** diving related $P = 0.56$
Workload (Intense)	86.6%	7.93%	$P < 0.0001$	Incidence of DCS increases in dives with high workload **NOT** diving related $P = 0.62$
Suit (Dry)	30.9%	19.0%	$P < 0.0001$	Incidence of DCS increases in dives with Dry suit **BUT** In these cases by an increase in diving exposure $P = 0.0002$
Thermal comfort (confortable)	96.9%	94.02%	$P = 0.08$	**NO** influence on bubbles
Feeling before the dive (rested or tired)	93.4%	90.9%	$P = 0.14$	**NO** influence on bubbles
Divers related problem (no problem)	96.6%	94.4%	$P = 0.96$	**NO** influence on bubbles
Equipment malfunction	95.6%	97.2%	$P = 0.08$	**NO** influence on bubbles
No use of alcohol before diving	61.6%	57.6%	$P = 0.17$	**NO** influence on bubbles

Some risk factors increase the prevalence of DCS without any influence of bubble formation; these aspects could influence the effects of similar amount of bubbles.

Use of oral contraceptive pill (OCP) appeared to reduce the risk.

Another intriguing case is the effect of visibility on bubbles and DCS; our data in fact show that high visibility increases bubble formation (by an increase of depth, time, and GF facilitated by the good diving condition) but DCS prevalence is higher with low visibility. This also seems to indicate that even in the presence of lower bubble grades, the stress effect induced by low visibility, may increase deco-stress and bubble susceptibility.

Conversely (and somewhat more classically) it must be noted that some risk factors do indirectly cause an increase in bubble formation and DCS cases, by an increase in depth, diving time and GF facilitated by fair water temperature, dry suit use, and/or excellent visibility.

However, the most important data of our study come from the analysis of the 320 DCS cases. The most notable observation is that, although the analyzed dives implied inert gas saturation levels well within the currently adopted "safety limits," the current decompression algorithms clearly show a very significant "gray area" in their ability to predict DCS, demanding further research and a more "patho-physiological" approach to decompression.

The majority of DCS cases recorded in our DB (73.7%) actually occurred in a GF-value range between 0.70 and 0.90, that is in an area where the diver has correctly followed the indications of the adopted decompression model, without any omission of safety stop, ascent rate etc.

Data showed that only eight out of 320 DCS cases showed a Gradient Factor >1, which means that only 2.5% of these cases would have been "predicted" by the utilized algorithm.

All the other cases would have been considered unpredictable, unexpected or, as they are now frequently defined, "undeserved."

TABLE 4 | GF in DCS cases.

Numbers of cases	Percentage	GF
8	2.5	>1
14	4.4	>0.9
120*	**37.5**	**>0.80; <0.90**
116*	**36.2**	**>0.70; <0.80**
46	14.4	<0.70
10	3.4	<0.60
3	0.94	<0.50

Only eight cases could be "predicted" by the model algorithm, all the other cases recorded in our DB would have been considered "undeserved."
**The majority of cases 236 (73.7%) presented GF values between 0.70 and 0.90.*

Furthermore, all the eight "deserved" DCS cases involved fast and slow tissues indicating a better capacity to predict an excess of saturation in these compartment as compared to medium tissues. This is conversely confirmed by the observation that the fast compartments were involved in the DCS cases in a lower percentage than their incidence as the lead compartment in the total DAN Data Base.

The majority of DCS cases that we analyzed actually involved medium HT tissues with computed inert gas super-saturation levels well below the "accepted" and "safe" M-values.

Considering the involvement of many biological and physiological parameters such as endothelial function (Theunissen et al., 2013, 2015), hydration (Gempp et al., 2009), vascular and lymphatic response (Hugon et al., 2009; Balestra, 2014), to mention only a few of the more recently studied variables, we believe that more research efforts are now necessary to further clarify these aspects of the complex pathophysiology of decompression.

We maintain that the reliability limit of the so far adopted dive computer validation protocols has been reached and that the new frontier is to further improve the ability to customize safe decompression limits according to physiological variables, be it pre-determined and based on available scientific evidence such as the data mentioned above or, in a foreseeable future, by a proper "diver-dive computer" interaction facilitated by real-time physiological sensor-assisted technologies. Furthermore the recent discovery of unexpectedly significant circulating bubbles in breath

TABLE 5 | Description of leading tissues grouping and their involvement in DCS.

Fast leading tissues group		Medium leading tissues group	Slow leading tissues group
From 4 to 18.5 HT		From 27 to 38.3 HT	From 54.3 to 635 HT
Leading tissue	**Mean maximum GF in DCS**	**Percentage of cases in DCS**	**Percentage of cases in DAN DB**
8	0.99	1.2	2.59
12.5	0.91	1.9	5.73
18.5	0.79	12.8	16.21
27	0.77	26.6	27.55
38.3	0.76	49.4	42.44
54.3	0.81	7.5	4.47
77	0.68	0.6	1.02
SUMMARY LTG IN DCS VS. LTG DB (*P* = 0.0005)			
Fast tissue	0.83	15.9	24.5
Medium tissue	0.72	75.9	70.0
Slow tissue	0.69	8.1	5.5
STATISTICAL ANALYSIS OF LTG IN DCS VS. LTG DB			
Fast tissues grouping	vs.	Medium tissues grouping	$P = 0.0008$
Fast tissues grouping	vs.	Slow tissues grouping	$P = 0.0005$
Medium tissues grouping	vs.	Slow tissues grouping	Ns

Is appears that the algorithm can correctly predict inert gas accumulation only in the fast and slow compartments.
This is confirmed by the lower prevalence of fast tissue involvement in the DCS group than in the DAN DB, while the medium HT compartments were more significantly involved in the recorded DCS cases.

hold diving causing DCS (Cialoni et al., 2016) requires us to extend the DAN DSL DB also the Breath Hold Divers Community.

CONCLUSION

In conclusion the first analysis of the DAN DB shows clearly that most dives were made in a Time and Depth “safe zone.” Interestingly certain risk factors appear to be related to DCS but not to significantly influence bubble formation, confirming that such risk factors may affect the individual response to similar bubble levels.

Our data also indicate that the current algorithms are well focused to predict the maximum allowed GF-value (and therefore the decompression risk) in fast compartments but are deficient in identifying the correct maximum GF in the medium compartments, which appear to be prevalent in the DCS cases analyzed in this study.

The DAN Europe DSL DB analysis can provide important data to improve recreational diving safety and this will further improve with the continuing entry of data in our DB allowing for an increasingly valid and complete data analysis.

ETHICS STATEMENT

All experimental procedures were conducted in accordance with the Declaration of Helsinki (World Medical Association, 2013) and were approved by the Academic Ethical Committee of Brussels (B200-2009-039). All methods and potential risks were explained in detail to the participants. All personal data were handled according to the Italian Law on privacy. Written informed consent was obtained from all the participants.

AUTHOR CONTRIBUTIONS

DC: Contributions to the conception and design of the work, performed laboratory studies, contributions to the acquisition, analysis, or interpretation of data for the work; wrote the submitted manuscript. MP: Contributions to the conception and design of the work, performed laboratory studies, contributions to the acquisition, analysis, or interpretation of data for the work. CB: Contributions to the conception and design of the work, Oversaw the research program, reviewed the manuscript. AM: contributions to the conception and design of the work, Oversaw the research program, reviewed the manuscript.

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Recreational Diving Practice for Stress Management: An Exploratory Trial

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Background: Within the components of Scuba diving there are similarities with meditation and mindfulness techniques by training divers to be in a state of open monitoring associated with slow and ample breathing. Perceived stress is known to be diminished during meditation practice. This study evaluates the benefits of scuba diving on perceived stress and mindful functioning.

Method: A recreational diving group (RDG; $n = 37$) was compared with a multisport control group (MCG; $n = 30$) on perceived stress, mood, well-being and mindfulness by answering auto-questionnaires before and after a 1-week long UCPA course. For the diving group, stability of the effects was evaluated 1 month later using similar auto-questionnaires.

Results: Perceived stress did not decrease after the course for the MCG [The divers showed a significant reduction on the perceived stress score ($p < 0.05$) with a sustainable effect ($p = 0.01$)]. An improvement in mood scale was observed in both groups. This was associated to an increase in mindfulness abilities.

Conclusions: The practice of a recreational sport improves the mood of subjects reporting the thymic benefits of a physical activity performed during a vacation period. The health benefits of recreational diving appear to be greater than the practice of other sports in reducing stress and improving well-being.

Keywords: perceived stress, recreational diving, sport, stress, mindfulness

INTRODUCTION

Chronic stress is a common complaint for middle-aged populations. Stress can be defined as a pattern of cognitive appraisal, physiological responses and behavioral tendencies that occur in response to a perceived imbalance between situational demands and the resources needed to cope with them. Chronic stress can occur in response to everyday stressors that are ignored or poorly managed. The reaction of individuals to chronic stress is theorized in the general alarm syndrome (Selye, 1956) and allostasis theories (McEwen, 2004), contributing to high biological cost featuring the allostatic load (Chrousos, 2009). Excessive chronic stress, which is constant and persists over an extended period of time, can be psychologically and physically debilitating. The consequences of chronic stress are serious. It constitutes a public health problem by increasing morbidity and mortality (Sayers, 2001). It notably contributes to anxiety and depression, which increase the risk of heart disease (Anderson and Anderson, 2003). In the professional sphere, it impacts job performances (Scullen et al., 2000). For the individual, it induces emotional and mood disturbance (Głebocka, 2016) and decreases well-being and quality of life (Palgi, 2013). Whether any biological marker for chronic stress is validated, robust questionnaires are available for assessing the level of perceived stress of subjects. Namely, the Cohen perceived stress scale (PSS; Cohen et al., 1983) allows a linear measurement of perceived stress, with the threshold found to be related to mental disorders (Collange et al., 2013).

It is well-known that each individual reacts in a specific way when they are exposed to a stressful situation. The individual's alteration depends on many variables, particularly when studied in the psychology of health (Bruchon-Schweitzer, 2002) and positive psychology (Martin-Krumm and Tarquino, 2011). The described concepts take into account how the subject's history and personality contribute to the assessment of the situation and what the subject will do (social support, stress and control of the perceived situation), the regulation by cognitive adjustment (*coping* centered on the task) or emotions (defuse the situation) that the subject will feel and the type of response that they subsequently implement (Bruchon-Schweitzer, 2002).

Within this frame of reference mindfulness must be taken into account. Mindfulness is a state of consciousness resulting from intentionally focusing one's attention on the present moment, without judging the experience that unfolds moment after moment (Kabat-Zinn, 2003). It is considered as one of the mind's natural resources, present in all individuals to varying degrees. The *Mindful* subject is a subject who chooses to consciously receive what is happening to his/her conscience with an attitude of openness, receptivity and non-judgment, allowing him- or herself not to be imprisoned by negative effects.

To deal with chronic stress and to improve stress management is challenging. Psychological fitness aimed at regulating emotions and mood disorders to enable improved effectiveness under stress and faster recovery from psychological stress (Bates et al., 2010; Mullen, 2010). Many programs exist that are centered on stress and emotional regulation by using breathing and relaxation exercises. Particularly meditation exercises allow mindfulness functioning to develop and improve the abilities for emotion regulation and for coping with stress (Chiesa and Serretti, 2009). One of the main concerns for such training is the need for the subject's regular compliance for the practice. Daily compliance is key for training efficacy.

Practicing physical activity, aerobic or anaerobic, has also been proposed for a long time as an ecological method to reduce stress and to improve emotion regulation (Paluska and Schwenk, 2000). Moreover, physical activity has an indisputable interest in the prevention and treatment of mental disorders associated with anxiety (Fox, 1999; Penedo and Dahn, 2005) and beyond that, there are somatic benefits, notably for cardiovascular diseases (Paffenbarger et al., 1986; Penedo and Dahn, 2005). These benefits are all the more important when the practice is regular (Petruzzello et al., 1991): The practice of moderate activity (at least 3 h per week) or intense activity (at least 20 min three times a week).

Scuba diving as a recreational sport has seen a tremendous rise ever since the diving structures have become more organized and offer access to reliable equipment. Every year, more than 260,000 people dive as part of the French Federation of Studies and Aquatics Sports. Most of the studies in this particular field tend to focus on the dangers and incidents of the sport, but not many actually cover the benefits of scuba diving on health in general and on stress management in particular. Yet immediately after diving, many divers experience a salutogenesis effect from the sport described as a state of well-being. Theoretically, from a psychological point of view, the analysis of scuba diving suggests that it favors experiencing a state of full consciousness and of openness associated with slow and ample breathing. Moreover, the homogenous stimulation of somaesthetic and proprioceptive captors during diving could improve senses perception, movement and body sensations. These psychological characteristics are close to those developed during meditation, suggesting that a scuba diving exercise could be considered as a meditation exercise inducing a state of mindfulness and that the repetition of diving should develop mindfulness functioning for regular divers.

Altogether, these elements suggest that the mechanisms of salutogenesis created by meditation in a broader aspect, could indeed be transcribed into scuba diving. Given how beneficial mediation is toward stress management, one could argue that scuba diving, even at a recreational level, could improve any diver's mental health by its virtue in stress management.

The final aim of the study is to investigate the effect of a week's scuba diving training on the level of perceived stress by comparing it with the effect of doing another sports training for a week. The effect on perceived stress level is also examined in terms of developing mindfulness skills. It is also hypothesized that the benefits of a week's scuba diving course on the level of perceived stress lasts 1 month.

MATERIALS AND METHOD

Subjects

Two middle-class populations registered at the same leisure sports club were included at the same period of the year: one population sample registered for a 1-week recreational scuba diving course (37 subjects, diving group) and one population sample registered for a 1-week multisport course (30 subjects, control group).

Potential participants were excluded from the samples if (i) they were undergoing medical treatment for psychological issues at the time of the study or (ii) they had taken part in a stress management program prior to recruitment for the current study.

Recruitment and Data Collection

Subjects received a cover letter supported by the leisure sports club's respective board, inviting participation in the study and stating the exclusion criteria. Medical staff assisted the subjects' recruitment.

The study was conducted in accordance with all applicable regulatory requirements, including the 1996 version of the Declaration of Helsinki and approved by the French Health Service's ethics committee. All volunteers provided written informed consent before participation.

Protocol

We conducted an open non-controlled and non-randomized interventional study. The exploratory protocol included two similar sessions of standardized auto-questionnaires for both groups (baseline and post-training sessions). A third session 1 month after the training program was added for the diving group only (post 1-month training session) using the same questionnaires.

At each session, subjects fulfilled auto-questionnaires in a "paper and pencil" booklet. They were asked to respond to socio-demographic questions at the first session only. For divers only, questions were added at session one about usual diving practice and at the post-1 month training session about life events that occurred during the month that followed the end of the course.

For each subject, the intervention was a training course lasting 1 week. It included 6 days of sports practice. For divers, diving was carried out using air at a maximum depth of 40-meters, with a maximum of two dives a day. For the multisport group (the non-divers), the course included kayaking, mountain climbing or hiking with a maximum of two activities a day.

Measures

Primary outcome measure: The Perceived Stress Scale (PSS; Cohen et al., 1983) is a 14-item scale designed to assess subjects' appraisal of how stressful their life situation feels to them. Each self-descriptive statement was evaluated using a five-point Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree). The PSS is recommended for assessing non-specific appraisal because it is found to predict better stress-related psychological symptoms and physical symptoms compared with commonly used life event scales. The most appropriate stress-threshold would be a score strictly superior to 27 for anxiety and 28 for depression (Collange et al., 2013).

Four auto-questionnaires evaluated psychological states as secondary outcome measures.

The Freiburg Mindfulness Inventory-14 is a short form with 14 items developed for people without any background knowledge in mindfulness (Walach et al., 2006; Trousselard et al., 2010). It constitutes a consistent and reliable scale evaluating the state of mindfulness and two subfactors (Kohls et al., 2009): acceptance as an ability to embrace unwanted thoughts and feelings as an alternative to experiential avoidance and being present, which characterizes being in non-judgmental contact with environmental events as they occur. Each self-descriptive statement was evaluated using a four-point Likert scale ranging from 1 (strongly disagree) to 4 (strongly agree).

The Body Connection Scale (BCS, Price and Thompson, 2007) is a 20-item scale designed to assess body awareness with two-faceted sensory awareness and bodily dissociation. Sensory awareness evaluated the ability to identify and experience inner sensations of the body and the overall emotional/physiologic state of the body such as bodily changes/responses to emotions and/or environment (12 items). The concept of bodily dissociation is characterized by avoidance of internal experience. Bodily dissociation has experiential aspects including normal everyday experiences, such as distraction from bodily experience or emotional disconnection (8 items). Each self-descriptive statement was evaluated using a four-point Likert scale ranging from 0 (strongly disagree) to 3 (strongly agree).

Warwick-Edinburgh Mental Well-Being Scale (WEMWBS; Tennant et al., 2007; Trousselard et al., 2016) covers both affective constructs, including the experience of happiness and constructs representing psychological functioning and self-realization (Keyes, 2007). WEMWBS comprises 14 items relating to the previous week, with responses that range from (1) "none of the time" to (5) "all of the time."

Mood was evaluated using the abbreviated version of the Profile of Mood States (POMS; Shacham, 1983). It consisted of an adjective checklist of 37 items that range from (0) "not at all" to (4) "extremely." The subjects were asked to answer according to their present mood. Six factors were then calculated: anxiety-tension, depression-dejection, anger-hostility, fatigue-inertia, vigor-activity and confusion-bewilderment.

Statistical Analysis

All data, expressed as mean (SD), were treated as ordinal data except for gender and marital status.

For each psychological variable, the distribution normality was tested as the variance homogeneity was also tested (Levene Test). In general, the normality and homogeneity conditions were respected (exceptions will be reported case-by-case). For every questionnaire, the internal coherence was tested using the Cronbach Test (Cronbach and Meehl, 1955). It appeared that each questionnaire showed an acceptable psychometric quality (Cronbach alpha $\geq$0.7).

The effect of the sport's training was carried out separately using student tests on primary and secondary outcomes. For the 1-month persistence on diving effects, analyses of variance (ANOVA) with a time session (three sessions) were performed as within subjects' effect. This was done separately for the primary outcome and each secondary variable. For significant effects, *post-hoc* analyses using Newman-Keuls were applied. When data are expressed as categorical or percentages, Chi-square tests were used.

In some very specific cases (non-obvious distribution normality, non-homogeneity of variances), non-parametric tests were used. This is particularly true for the POMS questionnaire and its subfactors. We used the Wilcoxon test for paired samples, the Mann-Withney tests for independent samples and Friedman's ANOVA test for multiple paired samples.

All analyses were performed with SPSS 17.0 for Windows (SPSS GmbH Software, Munich). We judged $p < 0.05$ as significant. When $p < 0.1$, results were expressed as a tendency to a difference.

RESULTS

Participants

Table 1 describes socio-demographic characteristics according to the sample population.

TABLE 1 | Comparisons of the two samples population.

		Diver group M (*SD*)	Control group M (*SD*)	*P*-value (X^2 or *t*)
Sample size		37	30	
Age	Years	41.83;10.81	29.53;5.28	*<0.001*
	[Range]	[30–52]	[24–35]	
Gender	Male (%)	29 (78.37%)	8 (21.63%)	*<0.001*
	Female (%)	8 (21.63%)	23 (76%)	
Marital status	Couple (%)	23 (62.16%)	5 (16.66%)	*<0.001*
	Single (%)	14 (37.83%)	25 (83.33%)	
Educational level	Undergraduate studies (%)	3 (8.1%)	1(3.33%)	0.11
	Graduate studies (%)	34 (91.9%)	29 (96.67%)	
Perceived stress		35.1 (8)	36.8 (5.2)	0.31
POMS	Tension anxiety	5.6 (4.4)	4.4 (4.5)	0.27
	Depression	2 (3.3)	3.4 (5.5)	0.21
	Anger	3.5 (4.1)	2.6 (5.3)	0.39
	Confusion	2.9 (3.2)	3.6 (3.5)	0.36
	Fatigue	4.2 (4.1)	4.9 (4)	0.5
	Activity Vigor	13.2 (4.6)	11.1 (3.5)	*0.04*
	Negative Mood	18.4 (15.5)	19 (20.6)	0.88
Mindfulness	Total	38.4 (7)	35.9 (3.8)	0.08
	Presence	17.7 (3.3)	16.8 (2)	0.19
	Acceptation	20.5 (4.1)	19 (3.1)	0.11
Body connection	Conscience	20.9 (5.1)	23.6 (5.6)	*0.04*
	Dissociation	14.6 (6)	14.7 (3.4)	0.9
WEMWBS		50.7 (8.4)	47.8 (7.2)	0.14

M, mean; SD, Standard Deviation; X^2, Chi-square test; t, student test.
Italic p values indicated significant differences.

Concerning this diving sample population, 59.9% of participants have a level 2 diving level at best, 55% mentioned diving as a hobby; 64.9% have dived at least 20 times in the last 12 months and 16.2% dived more than 60 times in that timeframe. During the diving course, divers all dived with air at various depths, some of which might induce narcosis (when deeper than 30 meters). Fifty percent of the participants reported being to a depth where narcosis occurs.

As shown in **Table 1**, there is no notable difference for psychological profile at baseline session between the two groups: diver group exhibited a higher level of Activity Vigor at the POMS and a lower level of body conscience at the BCS-subscale. There is no significant difference between men and women, among young or older people (above the 33 years-old median) on the psychological scores.

83.78% of the subjects in the diver group exhibited scores above the clinical threshold of 27 and were considered stressed at baseline. 96.67% of the subjects in the control group were considered stressed at baseline; there is no difference for the number of subjects above the clinical threshold between the two groups ($X^2 = 2.94$, $p > 0.05$).

Sports Practice Effect on the Primary Outcome

The perceived stress significantly dropped after the recreational diving course (t-test, $p < 0.01$) whereas no significant change was observed after the multisport course (t-test, $p = 0.24$).

We observed a course-induced effect of the level of perceived stress ($F = 7.46$; $p = 0.02$). The drop in the perceived stress score is not different between groups (divers vs. control group: $F = 0.004$; $p = 0.95$). There is no interaction between the course-induced effect and the group ($F = 1.36$; $p = 0.25$).

Five subjects in the group of divers reduced their perceived stress level lower than the clinical threshold upon course completion, whereas only two subjects in the control group did ($X^2 = 3.91$; $p = 0.048$). The number of subjects decreasing their score under the clinical threshold was higher after the diving course (13.51%) than after the multisport courses (6.67%) ($X^2 = 3.91$, $p = 0.04$). The Number Needed to Treat (NNT) with diving to see a one-person benefit (under the threshold) is 8 against 17.

Sport Practice Effects on Secondary Outcomes

Table 2 compares the effects of the two sports courses for the mood subfactors and for the well-being. For the scores concerning depression, tension-anxiety, anger and confusion, a significant decrease was observed after the course for the two groups. Fatigue mood level only decreased for the control group ($t = 2.19$, $p = 0.03$). Activity-vigor mood level only increased for the control group after the course ($t = -2.37$, $p = 0.02$). Well-being scores significantly increase for both groups.

Table 3 showed differences on mind-body variables after the course for both groups. The diving course induced an increase

TABLE 2 | Comparisons for each sample between before and after the course.

	Diving group		Control group	
	M (*SD*)	***p***	**M (*SD*)**	***p***
Tension anxiety	2,14 (2.97)	*<0.01*	1.43 (2.38)	*<0.01*
Depression	1,06 (2.11)	*0.03*	1.5 (2.69)	*0.03*
Anger	1,17 (1.44)	*<0.01*	0.63 (1.27)	*0.04*
Confusion	1,53 (1.99)	*<0.01*	1.9 (2.68)	*<0.01*
Fatigue	4,22 (3.43)	>0.05	3.47 (2.77)	*0.03*
Activity vigor	13,94 (4.86)	>0.05	12.74 (4.51)	*0.02*
Well-being	52.92(8.34)	0.02	51.51(6.48)	*<0.01*

M, mean; SD, Standard Deviation.
Italic p values indicated significant differences.

TABLE 3 | Comparisons for each sample between before and after the course.

	Diving group		Control group	
	M (*SD*)	***p***	**M (*SD*)**	***p***
Mindfulness	39.65 (6.57)	*0.05*	37.27 (3.99)	*0.04*
Mindfulness-acceptation	21.62 (3.76)	*0.03*	19.86 (2.9)	0.1
Mindfulness-presence	18.06 (3.49)	0.46	17.41 (2.08)	*0.16*
Bodily conscience	19.51 (6.58)	0.1	24.36 (6.31)	0.33
Bodily dissociation	13.1 (5.03)	*0.05*	16.57 (4.55)	*<0.01*

M, mean; SD, Standard Deviation.
Italic p values indicated significant differences.

in global mindfulness functioning ($t = -1.97$, $p = 0.05$) with an improvement in the acceptation sub factor ($t = 2.29$, $p = 0.03$) associated to a decrease in bodily dissociation ($t = 1.99$, $p = 0.05$). The multisport course induced an increase in global mindfulness functioning ($t = -2.21$, $p = 0.04$) associated to an increase in bodily dissociation ($t = -1.99$, $p = 0.05$).

Persistence of the Diving Effects

Half of the divers filled out questionnaires 1 month after the course (48.65%) for this specific subsample and answered all three sessions.

For the primary outcome, a significant session effect was observed with a decrease in the perceived stress score ($F = 7.48$, $p < 0.01$). *Post-hoc* analyses show that the perceived stress score measured at baseline was higher than the scores at the end of the course ($p = 0.02$) and for 1 month afterwards (persistence: $p < 0.01$). No difference is observed between the scores at the end and 1 month after the course ($p > 0.05$).

On secondary outcomes diving effect persistence was also observed. For tension-anxiety score, results showed a significant effect of the sessions ($F = 7.49$, $p < 0.01$). *Post-hoc* analysis showed that the tension-anxiety score measured at baseline was higher than the scores at the end of the course ($p = 0.02$), and tended to remain lowered 1 month afterwards (persistence: $p = 0.06$). For bodily dissociation score, results showed a significant effect of the sessions ($F = 12.65$, $p < 0.01$). *Post-hoc* analysis showed that the bodily dissociation score measured at baseline was higher than the scores at the end of the course ($p = 0.01$) and tended to remain lowered 1 month afterwards (persistence: $p = 0.09$).

DISCUSSION

This study investigated the psychological effect of a 1-week scuba diving course compared to another 1-week sports course. It showed that this particular sport practice induced a decrease in perceived stress level, which was not observed after a multisport course. This improvement in divers' appraisal of how stressful their life situation feels to them reduced the risk for developing psychological symptoms of 13.51% for divers. In comparison, only 6.67% of the multisport practitioners decreased their score under the clinical threshold of the perceived stress scale. Moreover, such stress management improvement was only associated to an increase in well-being for diving practice. Concerning sport practice effects on mood, both sport courses decreased negative mood in terms of tension-anxiety, depression, anger, and confusion. However, they exhibited opposite effects on fatigue and activity-vigor moods: fatigue and activity-vigor were higher after the diving course whereas they decreased after the multisport course. The reported fatigue after a 1-week diving course appears to be considered as physical as it was not associated with any psychological symptoms that mimic depression or psychological fatigue. In line, the improvement in stress perception after the 1-week diving course appeared as a beneficial effect that lasted for at least a month.

Altogether, such improvements in mental health have already been described in literature (Paluska and Schwenk, 2000; Penedo and Dahn, 2005). However, comparisons between the types of physical activity are scarce. Our results suggest that different patterns of sport practice benefits exist in terms of stress regulation, improving well-being and fatigue. One of the explanations might involve a difference in a stronger mind-body connection depending on the sport activity. Indeed, we observed that the diving course improved mindfulness functioning, acceptance attitude and decreased bodily dissociation whereas the multisport course induced an increase in global mindfulness functioning associated with an increase in bodily dissociation.

Concerning mechanisms that could be involved in the diving benefits, a possible depth effects, implying narcosis, must be proposed although the psychological data recorded in this exploratory study do not allow to go further to confirm this statement. Furthermore, two main psychological mechanisms could be involved in the diving body-connection improvement: specific proprioceptive and somesthetic stimulations and deep breathing induced by the use of the pressure regulator. Body perception involves the processing of sensory information, or sensory integration and refers to this process by which the brain receives a message through the senses and transforms it into an appropriate behavioral response (Miller et al., 2007). It participates in mind-body connection. The motor activity during diving leads to singular sensory pattern by associating proprioceptive and somesthetic stimulations exerted to the entire body. If mind-body connection cannot be reduced to these peripheral proprioceptions, this latter contributes strongly to it.

In the strict sense, proprioception defines a coupling system that intervenes in the perception of movement (kinaesthesia) and body positioning (statesthesia). It constitutes a moto-proprioceptive loop leading to proprioceptive sensitivity, which allows sensitivity to the deep organs (bones, joints, muscles, ligaments). Data reinforces the importance of the loop in the genesis of self-perception: (i) there is reaffirming sensory flux specific to each movement that can be defined as a true signature; (ii) there is a continuous solicitation of this system by the form and deformations of the body which would allow the constitution of reliable invariants relative to the body, by convoking the body itself. These elements give proprioception a primacy in the sense of a primary meaning capable of calibrating others, of playing the role of a matrix. Proprioceptive stimulation when diving associating the sensory flows with the movement of the subject and the somesthetic pattern of stimulation may intervene in both the regulation of the postural tone and the bodily experience and thus may increase the perception of the Self (Roll, 2003). On the other hand, the breathing that the subject establishes during a dive is a deep, steady and slow breathing. Such respiration is known to increase heart rate fluctuations and sinus respiratory arrhythmia through a better balance in the autonomous nervous system (Shaffer et al., 2014). This autonomous functioning is associated with the maintenance of a physiologically efficient and highly regenerative inner state. This psychophysiological mode is conducive to healing and rehabilitation, emotional stability and optimum performance (McCraty et al., 1995, 1998). Although, we cannot conclude as to which specific component of diving creates these mind-body connection improvements. The difference observed between the two sport groups could be explained by an optimization of the respiratory and proprioceptive profits induced by diving.

This exploratory study suffers from multiple biases, starting with the population studied. Matching of sociological and demographical data, such as age, gender, education and psychological data could not be set up. Entries for diving practice show that middle-aged men are the most frequent. The opposite is observed for multisport courses which mainly included young females. Second, studying perceived stress and psychological factors can prove to be tricky, as they are variables that evolve on one's personal situation and evolve potentially in a big way during a period when one does not work. The "break effect" thus plays an important role in our results. Though we can still observe that divers are not less tired at the end of their courses, compared with the group control whose tiredness level decreases. This puts this "break effect" into perspective that we would have expected from both groups. Thirdly, the remanence effect is studied in a small group (18 of the 37 divers answered the final test). We can consider that this group may have appreciated the most benefits of this study, therefore answered 1 month after the diving sessions. Finally, the hourly volume of daily physical activities during the leisure club sport courses could not be compared exactly between the diving courses and multisport courses. These would be equivalent given the scheduled week program. Weather conditions during all considered diving courses were good enough for all dives to be completed.

CONCLUSION

Diving as a recreational activity offers multiple health benefits, such as a decrease in perceived stress and an improvement of multiple psychological factors associated with mindfulness abilities. There does not seem to be any modification of the perceived stress level in our group control, but psychological factors are also improved. The "holiday's effect" paired with a physical activity, both play a big role in both groups and we can thus confirm the studies that were previously made on the subject.

Well-controlled studies are needed to clarify the mental health benefits of diving; by keeping in mind that scuba diving is a risky activity if safety regulations are not adhered to, even though it has a polarity with meditation. A new diver would not be expected to be familiar with the aquatic environment; the immersion reflex and the use of specific equipment (with an unknown reliability) can be a source of stress. This can lead to decompression accidents, drowning or pulmonary edema. Nevertheless, it is relevant to discuss the potential applications of these preliminary results for preventing anxiety and depressive syndromes or for promoting recovery after a stressful period.

AUTHOR CONTRIBUTIONS

FB, MC, MB, CR, PB and MT was involved in the conception and trial design. FB, NL, RG, JR, and MT contributed reagents, materials, analysis tools. FB, GM and MT wrote the draft of the article and contributed to the refinement of the study protocol and provided expert insight.

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Safety Priorities and Underestimations in Recreational Scuba Diving Operations: A European Study Supporting the Implementation of New Risk Management Programmes

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Introduction: Scuba diving is an important marine tourism sector, but requires proper safety standards to reduce the risks and increase accessibility to its market. To achieve safety goals, safety awareness and positive safety attitudes in recreational scuba diving operations are essential. However, there is no published research exclusively focusing on scuba divers' and dive centres' perceptions toward safety. This study assessed safety perceptions in recreational scuba diving operations, with the aim to inform and enhance safety and risk management programmes within the scuba diving tourism industry.

Materials and Methods: Two structured questionnaire surveys were prepared by the organisation Divers Alert Network and administered online to scuba diving operators in Italy and scuba divers in Europe, using a mixture of convenience and snowball sampling. Questions in the survey included experience and safety offered at the dive centre; the buddy system; equipment and accessories for safe diving activities; safety issues in the certification of new scuba divers; incidents/accidents; and attitudes toward safety.

Results: 91 scuba diving centres and 3,766 scuba divers participated in the study. Scuba divers gave importance to safety and the responsiveness of service providers, here represented by the dive centres. However, they underestimated the importance of a personal emergency action/assistance plan and, partly, of the buddy system alongside other safety procedures. Scuba divers agreed that some risks, such as those associated with running out of gas, deserve attention. Dive centres gave importance to aspects such as training and emergency action/assistance plans. However, they were limitedly involved in safety campaigning. Dive centres' perceptions of safety in part aligned with those of scuba divers, with some exceptions.

Conclusion: Greater responsibility is required in raising awareness and educating scuba divers, through participation in prevention campaigns and training. The study supports the introduction of programmes aiming to create a culture of safety among dive centres and scuba divers. Two examples, which are described in this paper, include the Hazard Identification and Risk Assessment protocol for dive centres and scuba divers, and the Diving Safety Officer programme to create awareness, improve risk management, and mitigate health and safety risks.

Keywords: buddy system, accessories, incident, accident, awareness, prevention campaign, training, dive centre

INTRODUCTION

Problem

Scuba diving is a sport and recreational activity that has become one of the most important marine tourism sectors globally, with up to 1,000,000 certifications issued annually (Garrod and Gössling, 2008; Musa and Dimmock, 2013; Dimmock and Musa, 2015; PADI, 2017). Over time, training agencies have developed relatively safe standards for a subgroup of leisure divers who are not risk seekers, but rather passive observers of the natural environment and marine life (Whatmough et al., 2011). The development of safe standards has gone hand in hand with the mass commercialisation of the scuba diving sport (Dimmock and Cummins, 2013). In turn, the scuba diving industry has evolved to include a support system around people practising the sport (MacCarthy et al., 2006). Certifying agencies have created new education and training packages, and travel agencies and destinations have been accommodating the necessities of a more diversified market (Dimmock et al., 2013). Safety has become paramount to ensure that scuba diving could be increasingly accessible to new markets, new destinations could become within reach of scuba diving with minimal risks, and scuba diving could translate into a safe and relaxed adventure, in line with increasing market demands (Dimmock and Musa, 2015). Safety organisations, including the Divers Alert Network (DAN), are dedicated to research on scuba diving safety and medicine, campaigning, emergency medical assistance, education, prevention, mitigation, accessories, and insurance for diving operations and scuba divers.

Today, scuba diving is a relatively safe sport (Wilks and Davis, 2000; Divers Alert Network [DAN], 2017), meaning that there is still a residual risk, which is considered a so-called 'accepted residual risk.' So, accidents do still happen in scuba diving. In addition, safety issues related to scuba diving still receive negative attention by the media globally (Marroni et al., 1994; Wilks and Davis, 2000). In 2017 alone, there were at least 30 news headlines on scuba diving fatalities, featuring in websites such as bbc.com, cbc.ca, foxcarolina.com, and independentmail.com. These issues, coupled with the increased accessibility of the sport in new markets such as holiday makers, disabled people and children, calls for constant attention to and improvements in safety standards (Dimmock and Musa, 2015).

There is also an important difference to consider between professional and recreational diving. On the one hand, professional diving has established risk assessment, mitigation programmes, codes of practise and regulations since long ago and as a consequence, it has become a safe profession. On the other hand, recreational diving is based on self-responsibility, and therefore risk awareness and attitudes are determining factors for triggering dive accidents. Recreational scuba diving operations are exposed to a series of risks (**Figure 1**). These can develop into liability, incidents/accidents, injury or death of people affected. Risks must be considered for various locations and can involve a number of people, infrastructure, vessels, vehicles, the environment and services. Although certifying and safety agencies are in possession of data regarding risks that are reported directly by the recreational scuba diving industry, there is no published research focusing exclusively on scuba divers' and dive centres' perceptions toward safety. As there is still evidence of avoidable accidents in recreational diving in spite of a well-established education by the training agencies, we believe that this training is mainly focussed on skills and appropriate materials, and that more awareness of and proper attitude toward safety would help to reduce the number of such incidents and accidents.

Aims and Objectives of the Study

This is the first study that assesses safety perceptions in recreational scuba diving operations. The aim was to collect data on the safety perceptions of dive centres and scuba divers, to guide decision making concerning safety in scuba diving operations. Bottom–up information, generated not only by dive centres and professionals, but also by scuba divers, is important to ensure that perceptions of various role players in the recreational scuba diving industry are aligned, and to support and enhance the implementation of safety management programmes in recreational scuba diving operations.

More specifically, information was collected from dive centres in Italy and scuba divers in Europe for four purposes: to identify general safety perceptions at dive centres and among scuba divers; to assess whether information from dive operators aligns with information from scuba divers and address gaps; to propose ways to educate scuba divers on the real risks of diving; and to verify if the results from the study would justify introduction of programmes of risk mitigation and risk prevention in recreational scuba diving.

Safety Attitudes and Behaviour in Recreational Scuba Diving Operations

The literature offers elements supporting this investigation. On the one hand, these elements shed a positive light on the relationships between scuba diver training and safety behaviour, the link existing between environmental protection and safety, and the general safety attitudes and behaviour of scuba divers. For example, recent work by Ong and Musa (2012) shows that scuba divers see themselves as very responsible under water, checking their underwater position/orientation, carrying a surface marker buoy as a safety accessory, and monitoring their diving depth at all times. In addition, scuba divers

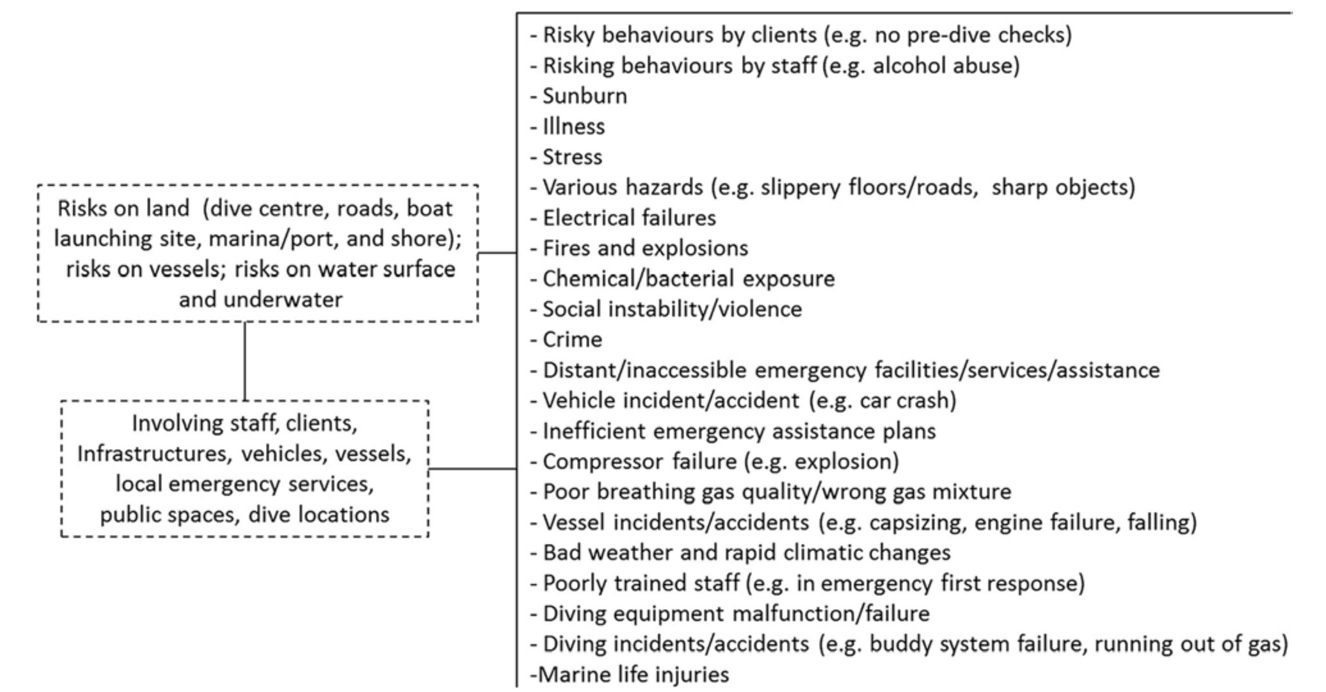

FIGURE 1 | Risks associated with recreational scuba diving operations. Source: Authors.

with better knowledge of diving practises and diving skills (e.g., buoyancy) show safer behaviour under water. Finally, scuba divers who are more environmentally responsible (avoiding contact with the substrate and with marine life) are safer as well. A study by Merchant (2011) relays the attitudes of scuba divers toward the use of equipment for safety; scuba divers appreciate the protective role of wetsuits, boots, gloves and hoods against hazardous interactions with marine life and cold temperatures. A recent investigation by DAN Europe, on the diving profiles of 39,000 dives from over 2,600 scuba divers in Europe, shows that scuba divers dive within the recommended 'safe zone' (not exceeding 30 m in depth) and that their average ascent rate is lower that the recommended safe speed; thus, scuba divers dive conservatively (Cialoni et al., 2017). These findings suggest that scuba divers tend to follow recommendations made by known diving safety agencies, as well as a tendency toward best practise and safety prioritisation.

Generally, divers' positive attitudes toward safety are linked with the performance and views shared by service providers, including dive centres. Research by O'Neill et al. (2000) and Ince and Bowen (2011) demonstrates how the attention to safety paid by dive centres has a strong impact on clients' perceptions, increasing satisfaction and conveying a feeling of trust. The studies show that, when selecting a dive centre, scuba divers give priority to its safety record and approach; in addition, they regard the appearance of the dive centre's premises, vehicles, staff and equipment as important in terms of assurance and safety standards. Andy et al. (2014) highlighted that, according to scuba divers, diving skills, including those in managing problems and those in leading dives, are crucial competencies that dive guides need to possess.

On the other hand, scuba divers may engage in risky behaviour, show limited interest in safety procedures and accessories, and are negligent in reporting accidents and incidents. The British Sub-Aqua Club has been stating for decades that generally, most scuba diving accidents would be easily avoidable if simple diving practise principles were followed (Cumming and Watson, 2017). Dive centres may be at fault by not enforcing safety regulations, not pursuing the best approach in managing risks, and sharing skewed views on safety with clients. A study by Bonnet et al. (2008) reports that 50% of divers interviewed in France regularly took risks while scuba diving, diving below depths allowed by their respective scuba diving certifications, and performing several dives in succession, resulting in nitrogen saturation. Recent work by Lafère et al. (2017) reports on how scuba divers can continue to adopt risky diving behaviours even after experiencing decompression illness, and after being diagnosed with risky physiological conditions and advised with contra-indications. The study highlights the importance of including the scuba diver psychological profile in diving risk research. Research by Wilks and Davis (2000) also argues that there are issues with the reporting of fatal and non-fatal accidents and incidents in the recreational scuba diving industry, due to limited access to information held by hospitals and medical surgeries. Of the reported accidents, some are not properly identified from hospital records, with the exception of decompression illness. Other literature highlights the controversial views by scuba divers with regard to the buddy system, which is normally accepted as a standard safety procedure of monitoring one another during diving, yet is either dismissed or disregarded by a good proportion of scuba divers (Coleman, 2007). The failure of the buddy system is reported as one of the main causes of accidents in scuba diving, as well as featuring consistently in scuba diving fatalities (Ranapurwala et al., 2017).

MATERIALS AND METHODS

Research Introduction and Handling of the Participants' Data

The research followed a quantitative, descriptive and non-experimental method of data collection, deploying a structured questionnaire survey for scuba diving operators and scuba divers. However, the first phase of the research was characterised by a qualitative pilot study collecting preliminary data to guide the structuring of the survey. With regard to the handling of the participants' data at any phase during this study, there was no instance in which the identity of the participants had to be made known; therefore, all data were treated anonymously. Further, no sensitive data were requested throughout the research, respecting the privacy of the participants. Last, by agreeing to take part in the research, the objectives of which were always clearly outlined upon invitation, the participants provided an informed consent and were free to leave the research, either by interrupting an ongoing interview or by deciding not to complete a questionnaire survey, at any moment.

Pilot Study for Survey Design

The pilot study took place in the Portofino Marine Protected Area (MPA), which is located in north-western Italy in the Liguria Sea. This location was selected based on its long scuba diving history and its popularity as a recreational scuba diving destination in Europe (Lucrezi et al., 2017). The MPA counts approximately 20 scuba diving centres distributed among various towns, and up to 80,000 dives are logged in the MPA annually (Pedemonte, 2017). During November 2015, dive centres in the Portofino MPA were visited by staff of DAN, and invitations to operators and scuba divers to participate in a face-to-face interview were extended. Three dive centres and 17 scuba divers participated in the interviews, which covered aspects including demographic profile; scuba diving experience; safety attitudes; scuba diving incidents/accidents; and risk prevention. Interview responses were transcribed verbatim and subjected to thematic analysis to guide the structuring of the questionnaire survey for the second phase of the research.

Survey Design and Structure

Based on the data collected in the first phase of the research, two structured questionnaire surveys were designed to target both scuba diving centres in Italy and scuba divers in Europe. Italian dive centres were selected for this study for two main reasons: Italy counts the greatest number of dive centres with active DAN membership in Europe; and Italy represents the main case study of the Green Bubbles project[1], a research project on sustainable scuba diving funded by the European Commission. The survey was prepared by medical and engineering staff at DAN (Europe and Southern Africa) in collaboration with engineers, marine biologists and environmental scientists participating in Green Bubbles.

The surveys included mostly close-ended questions, deploying a five-point Likert scale of importance (1 = No importance to 5 = Very important) or multiple-choice questions. The questionnaire directed at dive centres included questions on: dive centre characteristics; the importance of experience and safety aspects offered at the dive centre; influencing factors in the formation of dive groups and buddy pairs at the dive centre; the importance of equipment and accessories for diving; the importance of various aspects in the certification of new scuba divers; incidents/accidents at the dive centre; and safety attitudes. The questionnaire directed at scuba divers included questions on: demographic details and scuba diving experience; the importance of safety aspects when choosing a dive centre; influencing factors when choosing buddies; the importance of various forms of safety behaviour as a scuba diver; the importance of equipment and accessories for diving; scuba diving incidents/accidents; and safety attitudes.

Second Pilot Study and Final Administration to Dive Centres and Scuba Divers

Questions in both surveys were checked by a hired statistician for statistical validity. Following this cheque, the survey directed at scuba divers was tested through a pilot study, in which data were collected using a mixture of convenience and snowball sampling techniques. In the pilot study, the survey was made available online through KwikSurvey, and promoted for 10 days in Italy and Turkey using direct emails sent to scuba divers registered with DAN Europe, the DAN Europe web site, the social networks (Facebook), and newsgroups. Following the pilot study, both surveys were finally launched. The population of dive centres in Italy is approximately 1,000 (Italia Sub, 2018; C. Pellegrini personal communication), and that of certified scuba divers in Europe is 3,000,000 (European Underwater Federation, 2018), although it is not known how many of these individuals are active divers. Therefore, a mixture of convenience and snowball sampling (inviting centres and divers through the DAN Europe membership network and social networks like Facebook and Twitter) was adopted to maximise the outreach of the surveys to these populations. The surveys were made available online through KwikSurvey and Survey Monkey, in Italian for the dive centres, and in Italian, English, French, German, Spanish and Turkish for the scuba divers in Europe. Both surveys were launched at the end of 2015 and closed at the end of 2016.

Data Analysis

Data were captured in Microsoft Excel (2010) and all analyses were performed with Statsoft Statistica software (Version 13.2, 2016). Graphs were created with GraphPad Prism (Version 5, 2007). The profile of the participants (dive centres and scuba divers) and their responses were outlined through

[1] www.greenbubbles.eu

descriptive statistics, breakdown statistics and frequency tables. Responses from both groups (dive centres and scuba divers) were represented separately to identify commonalities and divergences. The results were worked up in order to be used later for actions concerning safety at dive centres and among scuba divers.

RESULTS

Pilot Study for Survey Design and Second Pilot Study

The majority of the participants in the pilot study (70%) were male and recreational scuba divers (95%), with some also possessing technical qualifications. About 80% had logged 51–200 total dives, and an additional 15% had logged over 200 dives. From the thematic analysis of the interviews with the participants (pooling views from dive centre operators and scuba divers), it is evident that the participants tended to be 'safe' divers and/or to promote 'safe' diving: they dived at depths ranging between 21 and 40 m; they dived mostly with a buddy; they towed a dive flag and used a surface marker buoy when needed; and they also carried an underwater torch with them. However, the participants also made a number of statements which highlighted the need for better investigation of perceived risks and safety procedures, risk identification and mitigation, and emergency action/assistance plans. For instance, although the majority of the participants claimed never to have experienced an incident/accident while scuba diving, a good proportion (30%) had mentioned at least one type of incident/accident. When asked what their greatest fears were in relation to scuba diving activities, the participants provided answers including drowning, decompression illness, and boating accidents. Last, when asked whether they were aware of any formal way of assessing risks and identifying hazards, the participants answered negatively. These answers provided the foundation for the development of the survey which was tested through the second pilot study.

A total of 204 scuba divers from Turkey and 187 scuba divers from Turkey participated in the second pilot study. Since the results from the second pilot study are similar to those of the final survey directed at scuba divers, the results from the second pilot study are not reported.

Final Survey to Dive Centres and Scuba Divers

A total of 91 scuba diving centres from Italy and 3,766 scuba divers from Italy, Germany, France and Turkey, among other countries, participated in the questionnaire surveys. The proportion of dive centres participating in the survey represented a suitable sample with a confidence level of 95% and a confidence interval of 9.8. The proportion of scuba divers participating was a suitable sample with a confidence level of 99% and a confidence interval of 2.1.

All dive centres catered for recreational divers, although 60% also accommodated technical and free divers. A similar proportion accommodated scuba divers with disabilities. Most dive centres offered boat dives (over 60%) and shore dives (33%). The scuba divers were mostly male (75%) and middle aged (57% were 41–60 years old) with a good level of experience. Nearly half of them had logged 100–500 dives, and their average certification level was advanced (43%). Good proportions were also instructors (31%). Whereas the majority (over 70%) of the divers were recreational, 34% possessed a technical qualification, and 23% defined themselves as 'technical divers.' Nearly all had dived outside their country of origin. The majority (58%) preferred to dive at depths between 19 and 30 m, with an additional 20% preferring to dive down to 40 m. Only 10% preferred depths beyond 40 m. Most dives were from a boat (57%).

A summary of important experience and safety aspects that should be provided at the dive centre, according to the two groups (dive centres and scuba divers), is represented in **Figure 2**. The most important aspect to all dive centres was breathing-gas quality, followed by staff experience, training provided, and available oxygen for first aid. Although the proximity of a treatment chamber was relevant for over half of the dive centres, it received the lowest importance score. In line with dive centres, scuba divers believed that breathing-gas quality and available oxygen for first aid were the most important elements to consider when choosing a dive centre. They also gave the least importance to the proximity of a treatment chamber, and less importance to training provided and whether the dive centre had insurance.

A summary of influencing factors in the formation of groups and buddy pairs according to dive centres and scuba divers is provided in **Figure 3**. Both groups agreed that gender, age, family relations and friendship were not influential. Dive centres believed recommendations by the instructors, experience and gas consumption of a potential buddy to be the best elements to consider. Scuba divers gave buoyancy skills, emergency skills and experience of a potential buddy the greatest importance, and did not think of instructors' recommendations as influential.

A summary of important accessories for diving according to dive centres and scuba divers is provided in **Figure 4**. Both groups considered the dive computer and the personal surface marker buoy as the most important accessories. Whereas over 50% of the dive centres gave importance to other accessories also, including a backup mask, whistle, knife, and a reel and guide line, scuba divers gave less importance to other accessories, with the exception of a whistle.

About 30% of the dive centres had dealt with scuba diving incidents/accidents, mostly with regard to decompression illness (60%), but also involving drowning (11%), equipment failure (11%), and boat accidents (9%; **Figure 5**). Dive centres perceived these events as the greatest risks in scuba diving (**Figure 5**). About 30% of the scuba divers had experienced a diving incident/accident, and twice this proportion had witnessed a diving incident/accident. Experienced and witnessed incidents/accidents involved equipment failure and splitting of the buddy pair (**Figure 5**). Other relevant incidents/accidents involved changes in weather conditions

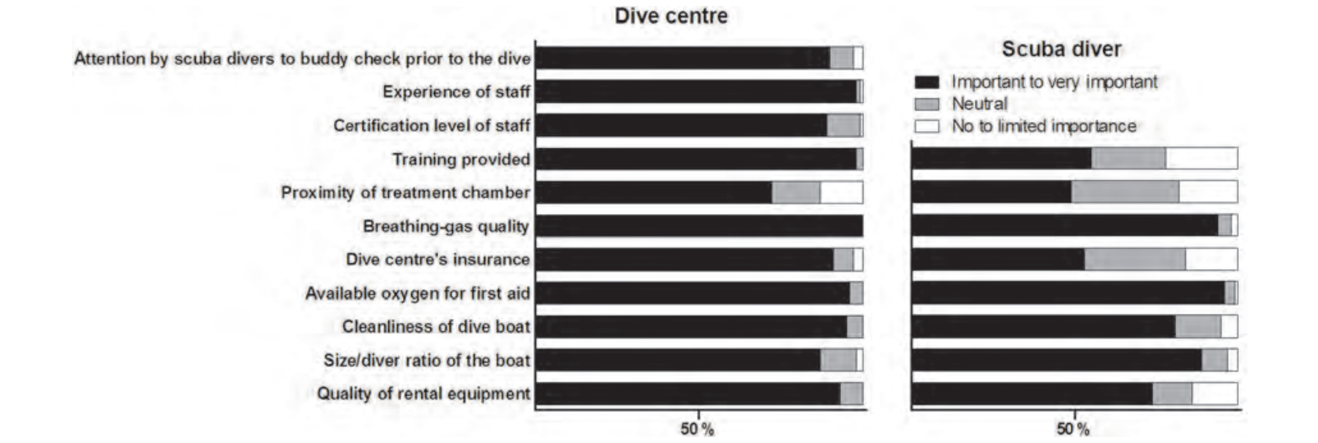

FIGURE 2 | The importance of experience and safety aspects that are offered at the dive centre according to the dive centres **(left)** and according to scuba divers **(right)**.

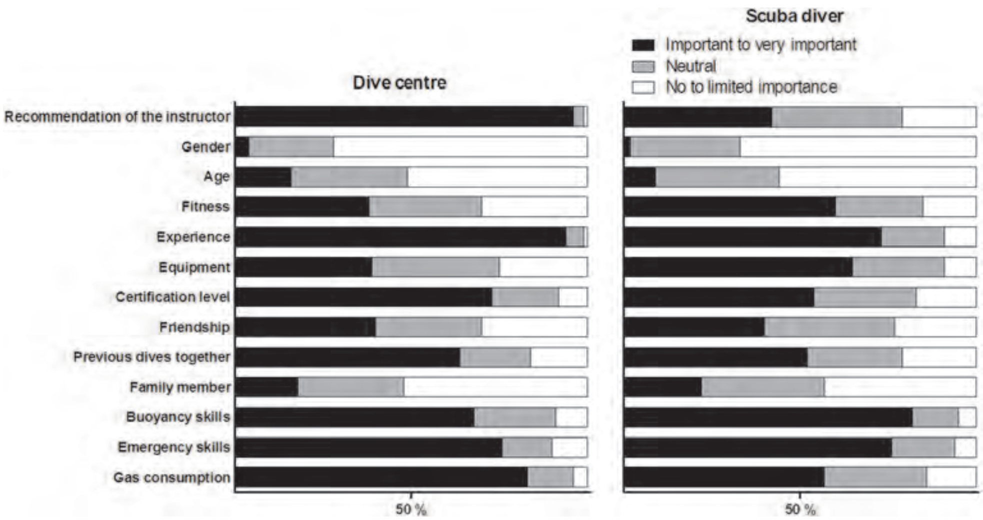

FIGURE 3 | Influencing factors in the formation of dive groups and buddy pairs at the dive centre according to the dive centres **(left)** and according to scuba divers **(right)**.

and interactions with hazardous marine life (**Figure 5**). Scuba divers were in greatest fear of equipment failure and decompression illness, but they were least concerned about hazardous marine life and the risk of drowning (**Figure 5**).

A summary of important aspects in the certification of new scuba divers, according to the dive centres, is provided in **Figure 6**. Most dive centres gave great importance to the basic aspects of diver training, particularly exercises such as clearing the mask, equalising, equipment assembly and buoyancy skills. They also valued emergency skills, learning how to use the dive computer, the buddy cheque and the importance of environmental protection. Although all dive centres had emergency action/assistance plans in place, about 10% did not have an active insurance plan for diving accidents and professional liability. They were actively involved in some safety campaigns, mostly related to the prevention of propeller incidents (33%), breathing-gas quality (32%) and keeping hydrated throughout the diving day (24%).

Figure 6 also provides information on the attitude toward safety by scuba divers. Most scuba divers felt that being in possession of dive insurance and drinking enough water during the diving day are very important safety aspects of being a diver. They also gave importance to possessing one's own diving equipment and to the buddy cheque. Nearly all dived with their personal equipment. All but 15% dived with a buddy; when doing a buddy cheque, 44% performed six or more controls, although a high proportion (55%) made fewer cheques and 1% made none. Divers gave importance to relying on the dive centre's emergency action/assistance plans for a dive location. Although over 80% possessed active diving insurance, a smaller percentage (60%) had a personal emergency action/assistance plan in place.

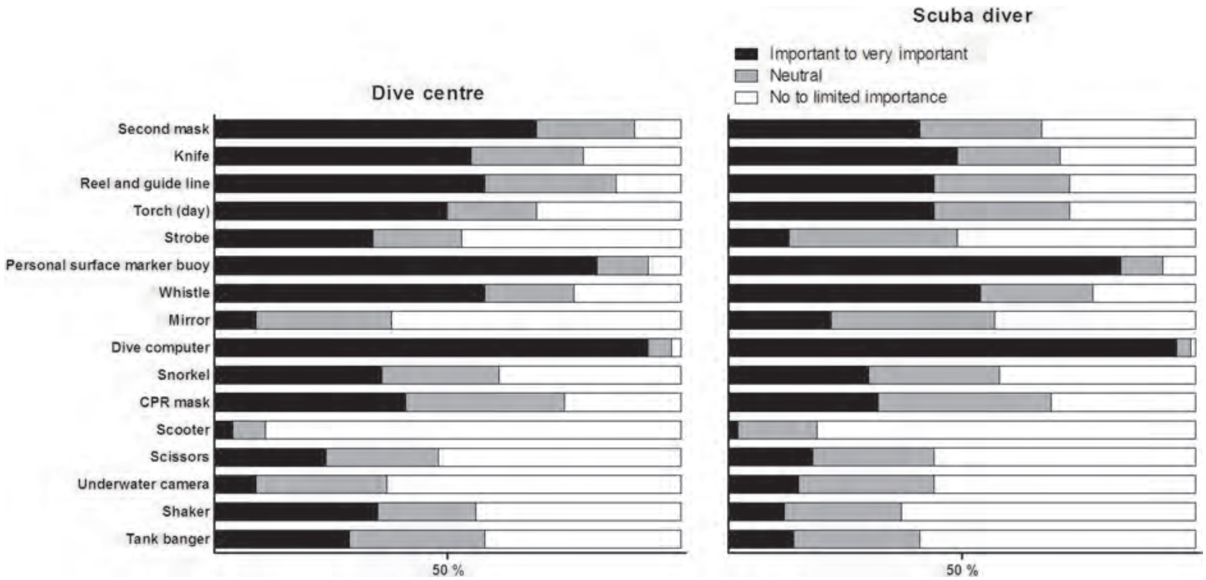

FIGURE 4 | The importance of various types of equipment and accessories for diving activities according to the dive centres **(left)** and according to scuba divers **(right)**.

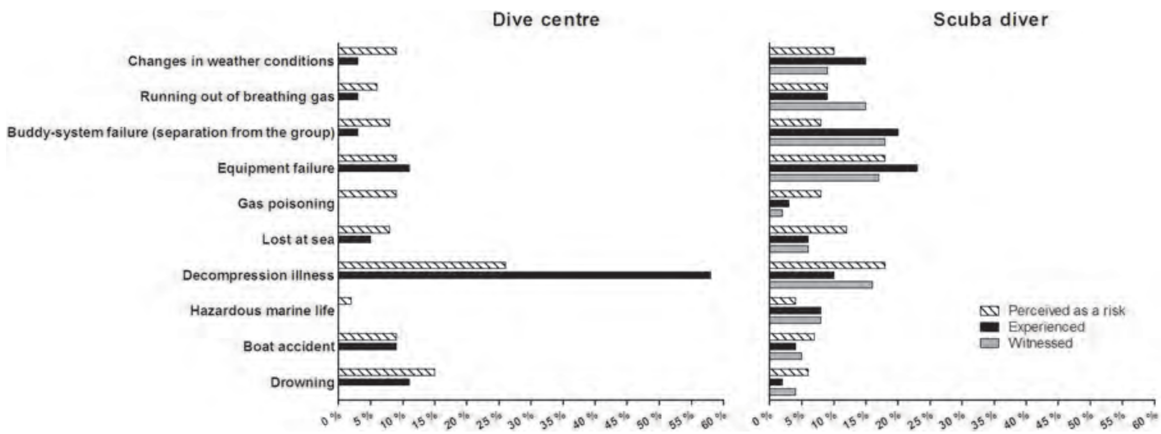

FIGURE 5 | Incidents or accidents related to scuba diving activities perceived as a risk and reported by the dive centres **(left)** and perceived as a risk, experienced and witnessed by scuba divers **(right)**.

DISCUSSION

Safety Perceptions: Recommended Actions

Below is a discussion of the results of this study, accompanied by a description of relevant actions to be taken. These actions, as well as the results they are based on, are listed in **Table 1**. The scuba divers were mostly middle-aged men with considerable diving experience and thus likely to engage in intensive, although not necessarily technical, diving activities. These divers valued the ability to rely on the dive centre's emergency action/assistance plans for a dive location. Given that ageing increases medical risks associated with scuba diving activities (Smerz, 2006; Ardestani et al., 2015), scuba diving operations face important implications for safety and risk management. The dive centres participating in this study offer a variety of services and cater for divers of different backgrounds and experience. However, the demographic profile of the participating scuba divers, and age range in particular, calls for a comprehensive understanding of risks associated with ageing in scuba diving, prevention actions and campaigns, and *ad hoc* emergency action/assistance plans, both on vessels and on land. Organisations such as DAN have been promoting publicly accessible campaigns of prevention and information online for ageing divers (Divers Alert Network [DAN] Europe, 2017c). However, more focus must be given to the matter, particularly considering that scuba divers of older generations may not be searching for safety information on the web but rather look for alternative, more

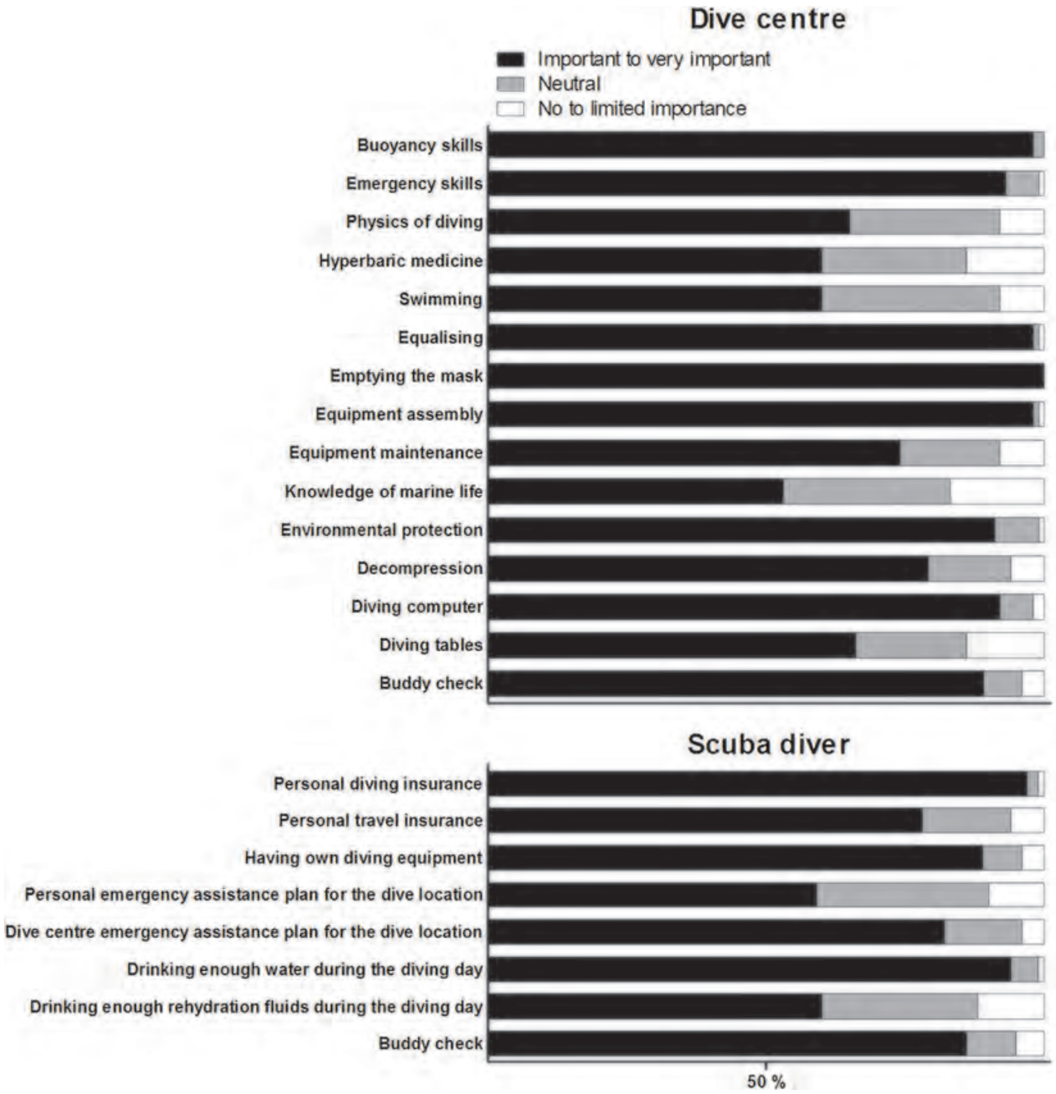

FIGURE 6 | The importance of various aspects in the formation of new scuba divers according to the dive centres **(top)** and the importance of various forms of safety behaviour according to scuba divers **(bottom)**.

accessible sources of information, such as the dive centre or magazines.

Data on the profile of the scuba divers confirmed the limited participation of female persons and youth in the sport, which is a common finding in scuba diving research (Garrod, 2008). The lack of participation by younger people in scuba diving activities, which is reflected in recent statistics from international certifying agencies like PADI (2017), can be addressed through scuba diving education campaigns in schools, an initiative that is gaining momentum in Italy (Progetto Scuola D'Amare, 2017). The re-inclusion of female persons in the scuba diving market can be foreseen as difficult, due to the withdrawal of women from the market following maternity. However, marketing strategies are being tested to ease such re-inclusion through the participation of children in educational activities at the dive centre while parents enjoy scuba diving (C. Cerrano, personal communication). The studied population reflected the actual population of members in DAN Europe (Cialoni et al., 2017; M. Pieri, personal communication), which was the main vector of promotion of the questionnaire survey. However, the limited participation by beginners in the study was evident. This limitation may be a result of improper communication between the organisation and beginners. Providing an understanding of the importance of participating in scientific research to improve safety standards in scuba diving should be part of the 'end of course' package for beginners. This could motivate young divers to participate in Citizen Science to support safety policy.

Dive centres and scuba divers acknowledged breathing-gas quality and available oxygen for first aid as the top safety services to be provided by dive centres, in line with other research (O'Neill et al., 2000; Ince and Bowen, 2011). Consequently, receiving the support of dive centres and scuba divers for air

TABLE 1 | Summary of actions based on the results from questionnaire surveys with dive centres and scuba divers.

Main results	Recommended action
Main market being characterised by middle aged people	Ageing and scuba diving information and prevention campaigns; *ad hoc* plans in place by dive centres
Main market being characterised by males	Understand lack of female participation in scuba diving and provide solutions
Limited participation by youth in scuba diving	Scuba diving education in schools
Limited participation of beginners in Citizen Science and research; no communication channel established with beginners	Introduce Citizen Science as part of 'end of course' package for beginners
Importance given by scuba divers and dive centres to breathing gas quality and to oxygen availability for first aid	Obtain support of divers for air quality control and on-board oxygen safety campaigns; ensure dive centres understand benefits of air quality analysis; ensure dive centres understand the importance of having oxygen on board
Personal emergency action/assistance plan underestimated by scuba divers	Develop a web based tool or mobile app providing a step by step guide for divers to have their own emergency action/assistance plan
Importance given by scuba divers to buoyancy skills and gas consumption when choosing as buddy; importance given by diving centre to buoyancy skills training	Promote buoyancy skills training and events
Importance given by scuba divers and dive centres to dive computer and surface marker buoy	Add a section on generic dive computer use together with classical decompression management chapters of training packages; more attention toward surface marker buoy deployment during training dives
Value of some safety-related accessories being underestimated by scuba divers	Add a dedicated section on safety-related accessories in equipment chapters of training packages
Value of daily fluid intake for rehydration being underestimated by scuba divers and dive centres	Hydration campaigns
Limited participation by dive centres in propeller injury and boat accident prevention campaigns	Propeller injury campaigns
Limited attention given by scuba divers and dive centres to hazardous marine life risks	Promotion of training courses for hazardous marine life injuries
Decompression illness perceived as top risk by scuba divers and dive centres and experienced as top accident/incident by dive centres	Safety campaign about decompression illness
Reported experienced and witnessed accidents by scuba divers greater than accident reports by scientific organisations	Encourage and facilitate dive incident and accident reporting
Equipment failure both experienced and perceived as a risk by scuba divers; importance given by scuba divers to equipment when choosing a buddy	Development of preventive maintenance programmes
Running out of gas and buddy separation either experienced or witnessed as top accidents/incidents; drowning, however, not perceived as a risk	Development of innovative devices to prevent/alert running out of gas, drowning, and buddy separation
Being lost at sea perceived as top risk by scuba divers	Development of innovative tools to prevent cases of lost divers at sea
Importance given by scuba divers and dive centres to pre-dive buddy cheque, but buddy cheque controls underestimated by scuba divers; buddy system failure experienced and witnessed as top accident/incident by scuba divers	Establish standard pre-dive buddy cheque procedures with the consensus of major dive organisations

quality control and on-board oxygen safety campaigns becomes a priority for safety agencies (Divers Alert Network [DAN] Europe, 2017c). A low proportion of dive centres seems to be actively involved in safety campaigns focusing on breathing-gas quality, whereas safety campaigns on oxygen on board vessels were not mentioned by the dive centres in this study. Dive centres need to be educated on the benefits of safety campaigning (e.g., on the risks of dehydration and propeller injuries). Safety campaigns covering different topics can either be a free initiative of the dive centre as well as an event promoted by safety organisations with the support of dive centres. Examples of the benefits of safety campaigning include better awareness and safety behaviour among staff and clients, and increased satisfaction and loyalty of clients.

Dive centres and scuba divers gave little importance to the proximity of a treatment chamber. In other contexts, such as in the Caribbean and the United States, scuba divers often wish to know where the nearest chamber is (F. Burman, personal communication). The perceptions of participants in this study could be a result of hyperbaric treatment being a worst case scenario that is out of dive centres' control, in comparison with other scenarios involving divers and dive centres more directly. They could also be due to the requirement for recompression being included in emergency action/assistance plans. Scuba divers also gave limited importance to whether a dive centre had valid insurance for diving accidents; they felt that the ability of the dive centre to offer emergency action/assistance would be more useful. Although the two elements appear to be disconnected, emergency action/assistance plans offered to divers by dive centres may be enhanced by insurance policies. It is important to raise awareness on the connexion between a dive centre's insurance and efficient emergency action/assistance plans. Paradoxically, scuba divers possessed active dive insurance, although they did not have a personal emergency action/assistance plan in place. This result may suggest that scuba divers do not take personal responsibility for safety and emergency action/assistance, and rather lay it on the dive centre and on safety agencies. Based on these outcomes, it is important to educate divers on assuming more responsibility in planning their own emergency response. The development of a web-based tool or mobile application, providing a step-by-step guide for divers to follow their own emergency action/assistance plan, seems like a reasonable approach for the diving community, which is becoming increasingly technology-driven.

Although dive centres and scuba divers did not agree on what is influential in the formation of buddy pairs and groups, all elements considered are worthy of attention. Experience of a potential buddy was given great importance by both groups. Scuba divers gave also importance to the buoyancy skills and emergency skills of a potential buddy (see also Andy et al., 2014). Even though dive centres felt that gas consumption would be more influential compared with these elements, they gave great importance to the latter as part of diver training. These results suggest how diver training should be restructured to give more attention to these aspects (Hammerton, 2017). When this is not possible, training should be integrated with events and workshops organised by safety and training agencies to raise awareness regarding buoyancy control and basic emergency first response. Examples include the Master Trim competition and the Oxygen Provider course launched by DAN Europe (Divers Alert Network [DAN] Europe, 2017a,b).

Modifications to diver training are also recommended in light of results related to diving accessories. Scuba divers and dive centres agreed that the dive computer and surface marker buoy are the most important accessories for scuba diving activities (Ong and Musa, 2012). This emphasis should be supported by re-categorising these accessories as 'must have' equipment, and by providing more information and training on their use. This can happen through the addition of a section on dive computer use together with classical decompression management chapters in training packages, and by dedicating more time to surface marker buoy deployment during training dives. Although dive centres appreciated the utility and value of other accessories, scuba divers did not. A dedicated section on safety accessories should be considered as a valuable addition to equipment-related chapters in scuba diver training packages.

The results of this study highlight limited participation in safety campaigns, including hydration campaigns, prevention of propeller incidents, and breathing-gas quality control. This was reflected in the little value given to aspects like daily fluid intake for rehydration during the diving day, perceptions of decompression illness, and limited attention given to marine life injuries. The overarching actions that can be taken are the promotion of safety campaigns and obtaining the support of dive centres to raise awareness (Divers Alert Network [DAN] Europe, 2017c). Campaigns on decompression illness should place an emphasis on statistics to reassure divers, yet retain key messages on the potential risks of the disease among scuba divers (Cialoni et al., 2017). The limited attention given to hazardous marine life can be addressed by information and the promotion of training courses for handling hazardous marine life injuries.

The data collected on perceived risks, incidents and accidents highlight the importance of safety campaigns to answer to the concerns of the diving community as opposed to top–down approaches that impose safety rules. Top–down approaches and regulations can make some sectors of the scuba diving industry (e.g., professional diving) very efficient. However, this system can only work if there is a legal obligation to follow such regulations. Recreational diving has no such regulations at all in many countries, and therefore the only alternative to achieve the same goal (acceptable risk and safe practise) is education and campaigns for awareness of risks, mitigation and proper attitudes toward safety. The data also point to communication and reporting gaps between the diving community and safety agencies. Figures of reported incidents/accidents that are either experienced or witnessed by scuba divers, as well as those experienced by dive centres, are noteworthy and greater than those featuring in accident reports by scientific organisations (Wilks and Davis, 2000; Cialoni et al., 2017). This gap needs to be addressed by encouraging and facilitating the reporting of dive incidents and accidents to safety agencies.

Scuba divers experienced equipment failure more frequently and perceived it as a greater risk compared with other incident/accident types; this result, reflected in the prime importance of equipment when selecting a buddy, is in line with other findings (O'Neill et al., 2000; Ince and Bowen, 2011). It is critical to give scuba divers better control and understanding of their equipment, together with the assurance that possible incidents/accidents would be faced with minimal safety repercussions. Preventive maintenance programmes run by safety organisations and certifying agencies and supported by scuba diving centres could serve these purposes. The programmes could be divided into levels, based on various degrees of experience. They could feature either as additions to certifying packages or as dedicated workshops (Eventbrite, 2017).

Alongside equipment failure, running out of gas was either experienced or witnessed as top incident/accident. However, drowning, which can be a direct consequence of running out of gas, was not seen as representing a major threat. Campaigns promoting the rule of thirds and other standard safety procedures aimed at preventing incidents/accidents concerning running out of gas (and consequent drowning) are strongly recommended. These can be introduced by safety organisations, although the importance of breathing-gas management must be stressed during training. The scuba diving industry can benefit from the development of innovative devices that alert divers and prevent them from running out of gas. Safety agencies, in collaboration with engineering companies, are working on prototypes to address this need (Altepe et al., 2017). Devices can be designed to serve a variety of alerting purposes, for example those concerning buddy separation or being lost at sea, both of which were either experienced or perceived as top incidents/accidents in this study.

In line with recent research (Ranapurwala et al., 2017), buddy-system failure was either experienced or witnessed as a main incident/accident. This result contrasts with the importance given by scuba divers to the pre-dive buddy cheque. In addition, although scuba divers gave importance to the proper selection of a buddy, the buddy system and pre-dive buddy cheque, the buddy cheque controls were underestimated. These results call for action regarding the control of buddy-system functioning

(Coleman, 2007; Ranapurwala et al., 2017). Pre-dive buddy cheque procedures should be created with the consensus of dive organisations to standardise buddy-system protocols and minimise risks during dives. The code of conduct of buddy pairs or diving groups needs to be enforced by certifying agencies and dive masters. Some notable agencies, for example Global Underwater Explorers and Unified Team Diving, give prime importance to pre-dive group cheques, but others may still require more emphasis to be placed on these procedures.

Overarching Actions to Enhance Safety Culture in Recreational Scuba Diving

Both dive centres' and scuba divers' attitudes require action on various levels, involving dive centres all the way up to safety organisations and certifying agencies. Based on data and discussions retrieved from the available literature, it is evident that overarching programmes and actions are required to manage safety risks during scuba diving operations, raise awareness of safety among scuba divers, educate and equip scuba divers with important knowledge for managing risks, and enhance the sustainability of scuba diving activities (Wilks and Davis, 2000; Coxon et al., 2008).

This set of actions is embodied in programmes such as the Hazard Identification and Risk Assessment (HIRA) and Diving Safety Officer (DSO) (Burman, 2015, 2016a,b; Divers Alert Network [DAN] Southern Africa, 2016). These programmes, which were launched in 2015 by DAN Southern Africa, are applicable to all infrastructure, vehicles, vessels, equipment, and services pertaining to scuba diving activities, with particular (but not exclusive) focus on recreational scuba diving operations. HIRA is a campaign designed by DAN to equip scuba diving operators with knowledge to identify and mitigate risks associated with their business, and to implement emergency action/assistance plans (Burman, 2015, 2016a, 2017; Divers Alert Network [DAN] Southern Africa, 2016). The DSO campaign is a programme educating and empowering competent people to enhance and oversee the implementation of the HIRA by raising awareness, enhancing risk management and mitigating health and safety risks at the dive centre and among scuba divers (Burman, 2016b). The overarching aim of both campaigns is to create a culture of safety among dive centres and scuba divers, which is underpinned by commitment toward safety, concerns for risks and their potential impacts, consciousness of risks, and a sustained effort to reduce or manage these risks. A culture of safety leads to the natural improvement of safety on behalf of scuba diving operations and, ultimately, scuba divers who see that the dive centre is prioritising safety (Burman, 2016b).

Study Limitations

There are a number of limitations associated with this study, which attention in order to properly interpret the results, and should be addressed in answering future research questions.

The study remained focused mainly on DAN members through convenience sampling, although the snowball sampling technique allowed the researchers to reach individuals beyond DAN members. The techniques of data collection online via convenience and snowball sampling hold the disadvantage of attracting a specific group of people, in this case, a potentially safety-aware group of scuba divers. In addition, many divers may have been guided to provide answers based on social norms, rather than based on their actual opinion and behaviour. This study used Likert scales in order to collect perception data from scuba divers. While Likert scales usefully provide information on the strength of a feeling or attitude, they also present some disadvantages (Maree and Pietersen, 2016). These include the provision of too simple answers, the provision of answers to questions even in cases where these have been misunderstood, and the limited number of options available in response to a given question, among others.

The psychological profile of scuba divers is an important component to measure in studies of this type; however, the extent to which psychological profile was assessed in this study only included safety attitudes and some self-reported behaviours (e.g., diving without a buddy). This study also focused on perceived risks, and its recommendations were based on these perceived risks. Perceived risk does not necessarily correlate with actual risk; therefore, the results in this study and the relevant interpretations must be considered with caution. An analysis of the influence of diver experience (e.g., technical divers vs. recreational divers) on perceptions was not within the scope of the paper. Indeed, the paper deals with universal recommendations based on scuba divers' perceptions, regardless of the specialisation level of scuba divers. However, future research should consider the importance of analytical techniques (e.g., regression analysis) aimed at assessing the influence of scuba divers' demographic and experience profile on their perception of safety and risk.

CONCLUSION

This study investigated perceptions about safety in recreational scuba diving operations. Safety attitudes at dive centres and among scuba divers were assessed to recommend actions aligning perceptions by dive centres and scuba divers on safety, and to enhance risk prevention and management during diving operations. The results demonstrate that the middle-aged, experienced market dominating scuba diving activities gives prime importance to safety and the responsiveness of service providers, represented here by the dive centres. However, scuba divers underestimate the importance of a personal emergency action/assistance plan and of safety procedures, including the buddy system. Scuba divers agree that some risks, for example those associated with running out of gas, deserve attention. Dive centres' perceptions of safety partly align with those of scuba divers. However, greater responsibility in raising awareness and educating scuba divers is required through participation in prevention campaigns and training. This study supports the introduction of overarching programmes such as the HIRA protocol for dive centres and scuba divers, and the DSO programme, intended to create awareness, improve risk management, and mitigate health and safety risks. These types of programmes have the goal of creating among dive centres and scuba divers a culture of safety, which is one aspect possessing the potential to support the sustainable future of recreational scuba diving.

ETHICS STATEMENT

This study was conducted in accordance with the Declaration of Helsinki (World Medical Association, 2013) and was approved by the Academic Ethical Committee of Brussels (B200-2009-039). No private personal information was asked from participants in the study. The data were handled according to the Italian Law on privacy.

AUTHOR CONTRIBUTIONS

SL: contributed to the acquisition, analysis, or interpretation of data for the work (surveys), and wrote the submitted manuscript. SE and MP: contributed to the conception and design of the work (surveys), to the acquisition, analysis, or interpretation of data for the work (surveys), and reviewed the manuscript. FB: contributed to the conception and design of the work (HIRA and DSO), to the acquisition, analysis, or interpretation of data for the work (surveys), and reviewed the manuscript. TO: contributed to the analysis of data for the work (surveys). DC and GT: contributed to the conception and design of the work (surveys), and reviewed the manuscript. AM: oversaw the research programme, and reviewed and approved the manuscript. MS: reviewed the manuscript.

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Patent Foramen Ovale (PFO), Personality Traits, and Iterative Decompression Sickness. Retrospective Analysis of 209 Cases

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Introduction: There is a need to evaluate the influence of risk factors such as patency of foramen ovale (PFO) or "daredevil" psychological profile on contra-indication policy after a decompression sickness (DCS).

Methods: By crossing information obtained from Belgian Hyperbaric Centers, DAN Emergency Hotline, the press, and Internet diving forums, it was possible to be accountable for the majority if not all DCS, which have occurred in Belgium from January 1993 to June 2013. From the available 594 records we excluded all cases with tentative diagnosis, medullary DCS or unreliability of reported dive profile, leaving 209 divers records with cerebral DCS for analysis. Demographics, dive parameters, and PFO grading were recorded. Twenty-three injured divers were tested using the Zuckerman's Sensation Seeking Scale V and compared to a matched group not involved in risky activities.

Results: 41.2% of all injured came for iterative DCS. The average depth significantly increases with previous occurrences of DCS (1st DCS: 31.8 ± 7.9 mfw; 2nd DCS: 35.5 ± 9.8 mfw; 3rd DCS: 43.4 ± 6.1 mfw). There is also an increase of PFO prevalence among multiple injured divers (1st DCS: 66.4% 2nd & 3rd DCS: 100%) with a significant increase in PFO grade. Multiple-times injured significantly scored higher than control group on thrill and adventure seeking (TAS), experience seeking, boredom susceptibility and total score.

Conclusion: There is an inability of injured diver to adopt conservative dive profile after a DCS. Further work is needed to ascertain whether selected personality characteristics or PFO should be taken into account in the clearance decision to resume diving.

Keywords: peer review, health care, diving, risk-taking, prevention, accident

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INTRODUCTION

Upon their ascent and in the hours after the dive, SCUBA divers expose themselves to possible nitrogen decompression problems. These problems (DCS: Decompression Sickness) are caused by gas bubbles formation in the blood vessels and/or supersaturated body tissues (Germonpre et al., 2015). Although, the precise mechanisms are not known, many provocating factors have been advocated in bubble formation or consequences (Carturan et al., 1999; Blatteau et al., 2008; Germonpre et al., 2009).

One of them, the Patency of Foramen Ovale (PFO), a condition that is present in about one third of the human population is a heritage of the fetal cardiac circulation (Hagen et al., 1984). It has been well associated to certain forms of DCS. Indeed, PFO is a pathway through which vascular gas emboli (VGE) can arterialize, given sufficiently favorable circumstances (such as large amount of VGE, PFO grading, straining maneuvers, delayed desaturation, etc.). Therefore, it seems to be a direct relationship between "cerebral" forms of DCS and PFO (Balestra et al., 1998, 2004; Germonpre et al., 1998; Ries et al., 1999; Cantais et al., 2003; Mitchell and Doolette, 2009; Wilmshurst et al., 2015).

Nonetheless, routine screening for PFO at the time of dive medical fitness assessment (either initial or periodic) is not indicated. However, consideration should be given to investigating for PFO if the diver has suffered from DCS, especially if the dive profile was not very ≪ provocative ≫ and if the DCS was characterized by cerebral, spinal, vestibulocochlear, or cutaneous manifestations (UHMS, 2011; Smart et al., 2015).

After the diagnosis of a PFO, considered likely to be associated with increased DCS risk (Odds Ratio Between 2.5 and 5.6; Bove, 1998; Germonpre et al., 1998; Torti et al., 2004), the diver may consider several options in consultation with a diving physician such as quitting diving, diving more conservatively (Examples include: reducing dive times to well inside accepted no-decompression limits; restricting dive depths to <30 m; performing only one dive per day; use of nitrox with air dive planning tools; intentional lengthening of a safety stop or decompression time at shallow stops; avoidance of heavy exercise and unnecessary lifting or straining for at least 3 h after diving, etc.) or PFO closure (Billinger et al., 2011).

This study aimed to examine the willingness of experienced recreational scuba divers in Belgium to comply with more conservative diving procedure after an initial DCS and a positive PFO diagnosis. These data will assist in evaluating the effectiveness of the medical counseling after a DCS, and the need for possibly stricter contra-indication related to "daredevil" psychological profile.

METHODS

Belgium counts about 10 hyperbaric centers, however only three of them have the expertise to treat injured divers: Antwerp (UZA), Brussels (Military hospital), and Charleroi (Civil hospital). This regional dispersion does not facilitate the data gathering on diving accidents. However, by crossing information obtained from the Hyperbaric Centers of Brussels and Charleroi, the DAN Emergency Hotline, the press and Internet diving forum's, it was possible to be accountable for the majority if not all DCS, which have occurred in Belgium from January 1993 to June 2013.

Although, full ethical review and approval was not required for this study in accordance with the national and institutional requirements, all 594 Belgian divers who suffered from DCS were reviewed in accordance with the Declaration of Helsinki. Each diver gave verbal consent for use of their case in studies where only group data are reported. In this study, when a case was identified for inclusion, the clinical information was loaded into a database that was stripped of individual identifiers.

For all the divers, we recorded several data such as age, gender, diving certification, number of dives performed, years of experience, previous history of dive accident, type of accident, circumstances of the accident, and presence of a PFO with grading (grade 0, no contrast passage at rest or after Valsalva strain; grade 1, no or slight (<20 bubbles) contrast passage at rest or after Valsalva strain; and grade 2, important (≥20 bubbles) contrast passage at rest or after Valsalva strain; Germonpre et al., 1998).

The majority of these cases are consulting with neurological symptoms. Cases with tentative diagnosis (minor, vague, and subjective symptoms not responding to proper treatment), medullary DCS (no significant correlation between the prevalence of PFO and the occurrence of spinal DCS; Germonpre et al., 2015; Balestra and Germonpre, 2016) or unreliability of reported dive profile were therefore excluded.

This left 286 divers records with cerebral DCS for analysis. From these, 239 medical files were available but only 209 contained all the necessary information for analysis.

From 2010 to 2013, 23 injured divers were tested with the Zuckerman's Sensation Seeking Scale V (Zuckerman, 1983). It has indeed been used in several studies to identify the sensation seeking and risk taking traits in risk sports (Dahlback, 1990; Cronin, 1991; Freixanet, 1991; Harding and Gee, 2008). Form V of the scale which is most used operates with a total scale and four subscales: thrill and adventure seeking (TAS), experience seeking (ES), disinhibition (Dis), and boredom susceptibility (BS). We used a French translation of Zuckerman's Sensation Seeking Scale. The translation was done by Carton et al in 1990 and tested for reliability and factor structure (Carton et al., 1990). They were then compared to a matched group of individual not involved in risky activities.

Statistical Analysis

Clinical recovery after 6 month (complete, mild or severe residual symptoms) and PFO grading were considered as a dependent variable and were analyzed using nonparametric testing of the difference in ranks. Characteristics related to the dive and clinical parameters were analyzed as independent variables and were analyzed with unpaired t-Test or repeated-measures ANOVA with Bonferroni *post-hoc*. All data passed the Kolmogorov-Smirnov test, allowing us to assume a Gaussian distribution.

GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, California, USA) was used as standard computer statistical package., A threshold of $P < 0.05$ was considered statistically significant. Data are presented as mean ± standard deviation (*SD*) unless precised otherwise.

RESULTS

In our sample, injured divers are mostly men (80.4%) being 40.5 ± 11.2 years old (15–68 years, median 42.3 years) with a dive experience of 9.6 ± 9.3 years (0–37 years, median 6 years) and 646 ± 101 dives (5–2,959 dives, median 300 dives).

Incident breakdown by diver qualification shows that no certification level, from novice to instructors, is immune to problems. When compared to an historical cohort (1995–2005; Lafere et al., 2009), instructors are overrepresented (26.9% of the injured divers vs. 8.6% of the diving population, One Way ANOVA, $p < 0.01$).

Ninety-six percent of the recorded dives were performed in flooded quarries and gravel pits as well as in dam's reservoirs [i.e., Fresh water depth (mfw)]. Although, there is no cave diving, visibility varies greatly depending on the quarry (between 1 and 10 m), the number of divers, and the nature of bottom, marble quarries being the clearest. Under the thermocline, which depth varies between 1 and 10 m according to the season, the temperature oscillates between 1 and 8°C all year. Above, the temperature oscillates between 1°C in winter and 18°C in the summer.

Dive profile and decompression were managed either according to a personnal dive computer (168/209—80.38%), US Navy dive table with timer and depth gauges (30/209—14.35%) or a customized dive table generated by a decompression software (11/209—5.26%). The two main used decompression models were the Uwatec^{TM} Bühlmann ZH-L8 ADT and the Suunto^{TM} RGBM. However, statistical analysis fail to demonstrated any difference in DCS severity or outcome between these two decompression models. Dives were characterized by a maximal depth of 33.8 ± 9 mfw (14–60 mfw, median 34 mfw) and a total dive time of 39.5 ± 13 min (13–89 min, median 39 min). From the 87 available dive-profile printouts, we observed a dive profile error in 40% of cases (normal saturation/inadequate offgassing, mainly due to low/out-of-air situation due to poor planning), a "logical" cause of decompression incident in 20% of cases (increased saturation/"normal" offgassing or increased or "normal" saturation/insufficient offgassing as in cases of strenuous effort or cold water diving) and finally in 40% of the cases the accident is declared undeserved. These dive parameters or profile errors seem to depend much more on the level of acquired skills, than on the teaching system as no statistical difference in DCS severity or outcome was observed between PADI vs. CMAS (One Way ANOVA, $p = 0.488$).

Without knowing the number of dives carried out, it is difficult to calculate the incidence of the accidents. However, from a previous study (Lafere et al., 2009), this number was estimated at 1,042,618 dives per year. This gives us an estimated incidence for DCS in Belgium of 0.73/10,000 dives.

From the 209 injured diver records analyzed, 125 (59.8%) were treated for a first episode of cerebral DCS (1st DCS), 70 (33.5%) for a second episode (2nd DCS) and 14 (6.7%) for a third one (3rd DCS). With regard to biometric data (age, body mass index), smoking habits, no significant differences were found between DCS subgroups. However, there was a significant difference in the number of dive done each year between the "first-time" and the "multiple-times" injured divers, which seem to be the more active. Applied treatment did not differ between groups but for the number of additional hyperbaric oxygen session (HBOT). Outcomes are resumed in **Table 1**.

It is important to note that 41.2% of all injured came for iterative DCS. Further analysis **Figure 1** shows that the average depth of the causal dives significantly increases with the previous occurrences of DCS (1st DCS: 31.8 ± 7.9 mfw; 2nd DCS: 35.5 ± 9.8 mfw; 3rd DCS: 43.4 ± 6.1 mfw; $p < 0.0001$). Total dive time is not different (1st DCS: 39 ± 13 min; 2nd DCS: 38 ± 11 min; 3rd DCS: 46 ± 16 min; $p = 0.458$).

All divers underwent echocardiography, either transthoracic (TTE) or transesophageal (TEE), with the use of agitated saline for contrast in order to assess the presence or absence of

TABLE 1 | Long-term outcomes of 209 divers with cerebral decompression sickness.

		1st DCS (*n* = 125)	2nd DCS (*n* = 70)	3rd DCS (*n* = 14)	*p*
Dives/year		40 ± 2.7 [5–168, median 34]	70 ± 8.1 [25–200, median 60]	74 ± 8.9 [20–125, median 77]	<0.0001
Treatment received		1 ± 0.07 USN TT6 [0–2, median 1]	1 ± 0.14 USN TT6 [0–2, median 1]	1 ± 0.17 USN TT6 [0–2, median 1]	0.75
		7 ± 0.8 HBOT [0–20, median 6]	11 ± 1.3 HBOT [4–18, median 10]	8 ± 1 HBOT [2–12, median 8]	0.04
Outcome	**Complete resolution**	73.6%	27.1%	0%	
	Mild residual symptoms	19.2%	57.1%	85.7%	0.08
	Severe residual symptoms	7.2%	15.7%	14.3%	
Resume diving		81.6%	84.3%	0%	NA
Delay to resume diving		4.2 ± 0.7 months [0.5–15, median 5]	3.5 ± 0.6 months [1–8, median 5.5]	NA	0.97

Mild residual symptoms are mild paresthesia, weakness, residual pain or some impairment of daily activities. Severe residual symptoms are difficulty walking, paralysis, uncompensated vertigo, or speech disorders. USN TT6, US Navy Treatment Table 6, i.e., 2.8 ATA, 100% oxygen for 285 min with air break. HBOT, Hyperbaric oxygen session 2.5 ATA, 100% of oxygen for 70 min without air break. Data are presented as mean ± standard error on mean (SEM).

patency of the foramen ovale. The prevalence of PFO in these groups (**Figure 2**) increased with the occurrence of iterative DCS (1st DCS: 66.4% 2nd & 3rd DCS: 100%). Also, at the second and third DCS, all injured divers had increased their permeability, from zero to grade 1 or 2 PFO or from 1 to grade 2 PFOs. The difference of TEE/TTE score on an ordinal scale was statistically significant (Wilcoxon signed rank test, $p = 0.023$).

On the Sensation Seeking Scale V, multiple injured scored higher than all other groups on TAS, ES, BS, and Total score (**Table 2**). The differences were significant in relation to control group on all scales except for Dis. Between the two diver groups only TAS difference was significant.

DISCUSSION

One of the most remarkable observation was undoubtedly that despite all divers receiving medical counseling about diving safety and DCS prevention when resuming diving (consisting of a 1 h consultation with schematic drawing of PFO and bubble possible pathways and risks), more than one third of the diver were admitted for iterative DCS. This might be explained by two hypotheses.

First, there is the legitimate question of either the effectiveness of preventive measures, or the implementation of these measures when resuming diving after an accident. On one hand, studies that have evaluated procedures to reduce nitrogen load after

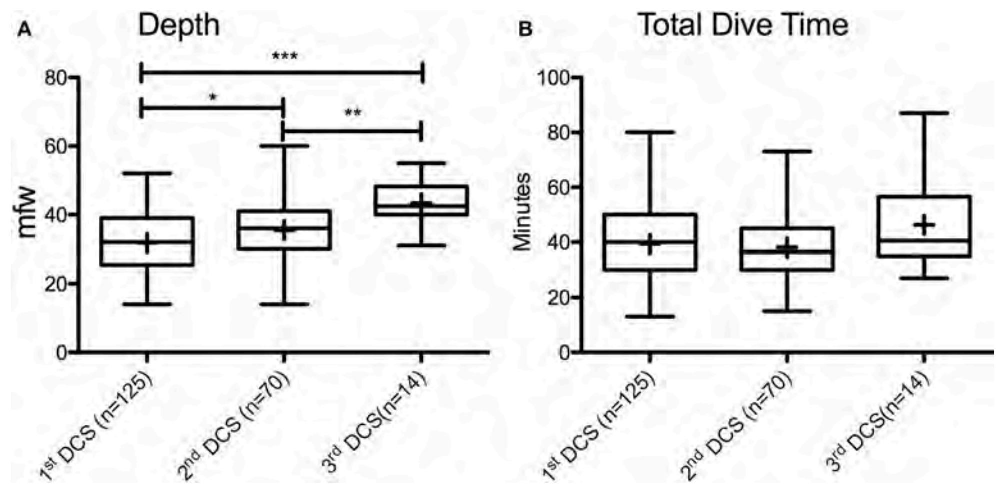

FIGURE 1 | Box and whisker plots of variation in dive parameters (**A**. Depth; **B**. Total dive time) according to decompression sickness occurrence (DCS), indicating median, 25–75 percentiles and minimum and maximum observations; "+" marked in the boxes indicate the means; $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

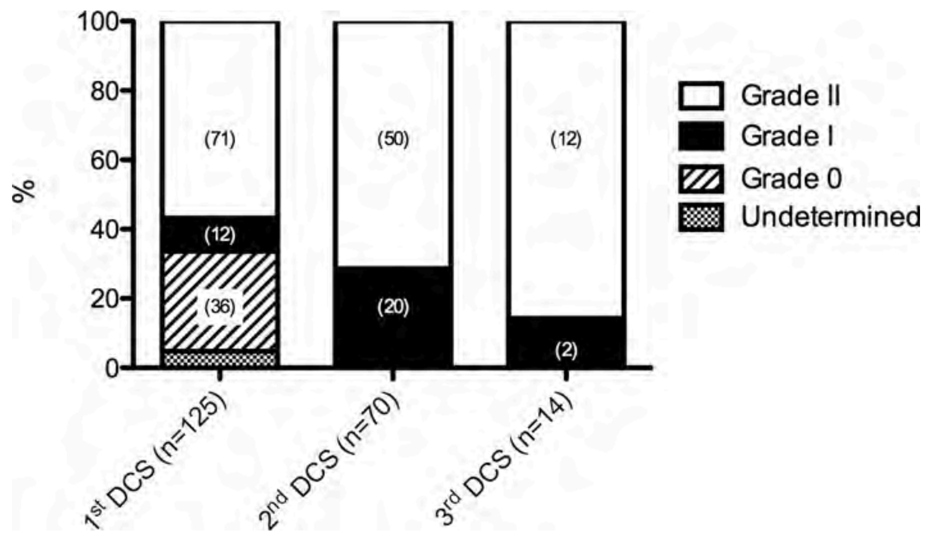

FIGURE 2 | Prevalence of PFO (patent foramen ovale). Grade 0, no PFO—no contrast passage at rest or after Valsalva strain; Grade 1, no or slight (<20 bubbles) contrast passage at rest or after Valsalva strain; and Grade 2, important (≥20 bubbles) contrast passage at rest or after Valsalva strain. DCS, decompression sickness. Numbers in parentheses are total number of cases.

TABLE 2 | Sensation seeking scale V mean scores of divers with multiple DCS ($n = 7$), compared with first timer DCS ($n = 16$) and control group ($n = 23$).

	1st DCS ($n = 16$)	Multiple DCS ($n = 7$)	Control group ($n = 23$)	p	1st DCS vs. control	Multiple DCS vs. control	1st DCS vs. Multiple DCS
TAS	7.44 ± 2.03	8.71 ± 2.03	4.35 ± 1.59	<0.001	<0.001	<0.001	<0.01
ES	6.06 ± 1.77	7.71 ± 1.78	5.31 ± 2.10	<0.01	<0.05	<0.01	ns
Dis	5.22 ± 2.41	4.85 ± 1.95	4.7 ± 1.87	0.816	ns	ns	ns
BS	4.44 ± 1.79	5.71 ± 1.98	4.26 ± 1.96	<0.05	<0.05	<0.05	ns
Total	21.7 ± 6.8	27 ± 4.2	18.4 ± 3.7	<0.01	<0.05	<0.01	<0.01

Total score correspond to the sum of subscale: thrill and adventure seeking (TAS), experience seeking (ES), disinhibition (Dis), and boredom susceptibility (BS).

a first episode of DCS appeared to reduce the probability for subsequent DCS (Klingmann et al., 2012; Honek et al., 2014a,b). On the other hand, it has to be noted that subgroup analysis shows that multiple time injured divers dived significantly more than the average diver with 70 ± 38 dives/year and 74 ± 33 dives/year for 2nd DCS and 3rd DCS group, respectively. Moreover, the average depth of the causal dives significantly increases with subsequent occurrences of DCS (1st DCS: from 31.8 ± 7.9 mfw for the first DCS to 43.4 ± 6.1 mfw for the third DCS). This suggests that having had a decompression accident does not seem to constitute a sufficient argument to modify a diver's underwater behavior.

Indeed, whereas all sportsmen seek physical sensations, they not necessarily do so by voluntary adopting behaviors known to be dangerous (Lafollie and Le Scanff, 2007). However, injured divers score very high not only on TAS but also on ES, which means that they are eager to seek new unusual experiences in all areas of life. Divers also score unusually high on boredom susceptibility. On the disinhibition scale there are no differences with control. High disinhibition scorers enjoy partying but are not willing to take the risk of making a fool of oneself or becoming a social misfit. The high TAS and ES scorers may be more used to take risks. For high TAS scorers there is a constant risk of severe injury or death. For high ES scorers there may be the risk of becoming a drug addict and the social consequences that go with it (Breivik, 1997). Although, the number of multiple injured divers is small, they scored significantly higher on TAS than any other groups, giving support for the notion of physical risk taking. Indeed, many diving accidents are at least in part attributable to failure to follow correct procedures. As has been stated for over 50 years in the British Subaqua Club incident report: "most of the incidents reported within this document could have been avoided had those involved followed a few basic principles of safe diving practice" (BSAC, 2016). However as Harding & Gee mention in their study, the preferred way to examine the role of personality as a predisposing factor in DCS would be to measure variables before as well as after the incident. Without such data the possibility of the experience having an effect on supposedly stable personality characteristics cannot be ruled out (Harding and Gee, 2008). Nonetheless, one observation may confirm the importance of behavioral issues. Indeed, the monthly breakdown of accidents shows that Belgian diver dives all year round independently of the season. As in other databases, BSAC for example, 50% of the reported incidents have occurred in the summer period (June–September), however with an unusual January peak in the winter period. This anomaly can possibly be explained either by a more significant number of divers continuing their activity during the winter months, or more probably by the more rigorous winter we had in 1997, 1998, 2009, 2010, 2012 compared to other years. Indeed, when these years are excluded, distribution follows a Gaussian pattern. When planning a dive in cold water or in conditions that might be strenuous, dive tables requires the divers to assume a depth that is 3 m deeper than the actual depth. Nonetheless none of the injured divers during this particular period have adapted their decompression schedules. Another argument seems to confirm the importance of behavior. Normally one star divers are limited in depth. Indeed, in Belgium, they cannot dive deeper than 15 m (20 m when accompanied by an instructor). Yet the average depth of their accidents is 24.1 ± 9.1 mfw (17–43, median 24). In the same way, the maximum depth allowed in quarry, gravel pit and lake's reservoirs is 40 m pushed for seasoned divers (four stars divers and instructors) to 60 m in case of air diving. Yet the average depth of instructor accidents is 41.4 ± 9.7 mfw (27–69, median 40.5). These faulty dive profiles may reveal some hidden psychological motive or a potential self-destructive attitude questioning diver's capacity to understand and to cope with specific risk.

The second hypothesis relies with the patency of foramen ovale (PFO). Our results show an increase of PFO prevalence among multiple injured divers with furthermore, also an increase in PFO grade. Since this is a retrospective study, a selection bias cannot be fully excluded, which would mean our results are just an incidental finding. However, there are arguments why this would not be the case. Indeed, a statistical correlation has been shown between ischemic cerebral incidents in diving (cerebral DCS) and large PFOs (grade 2). The same is true for PFO and "unexplained" stroke (Van Camp et al., 1993; McGaw and Harper, 2001). No such correlation has been demonstrated for small PFOs (grade 1). Moreover, a prospective follow-up study has documented the increase in PFO size in humans (Germonpre et al., 2005). This is an important finding, as the authors stated it, because it may imply that increased susceptibility to neurologic DCS could develop over time. According to our results this seems to be the case.

Finally, although not statistically significant, it has to be noted that the risk of residual symptoms, mild or severe, seems to increase with the number of DCS. This might be explained by several mechanisms. The more frequently, and the more deep you dive, the more serious DCS one risks; alternatively, there may be a depletion of the physiological “reserve neurological capacity” with each new accident, the potential for healing being reduced every time. This can explained why injured divers focus on the simplistic idea that they need “to get fixed” and why technical diving organizations even have recommended preventive PFO closure in order to undertake high-risk dive training. Anecdotally, it should be noted that two divers of our series had benefited from a PFO closure within the 10 years preceding their second accident. During the ultrasound control, they both had a grade 2 PFO despite the device being in place. Studies with long-term follow-up of PFO closure among divers therefore appear mandatory. In the meantime, safe diving is something to be learned, not something that can be implanted (Germonpre, 2015).

There are some inherent limitations to this study, mainly concerning the representativeness of the divers in our database. First, we cannot be sure that we do not have a full record of all types of incident. If a hyperbaric center is not involved and if those involved do not declare their accident, then it will go unrecorded. It is impossible to assess just how many incidents are unrecorded.

Although, not the only sports divers’ federation in Belgium, the Belgian chapter of CMAS (FEBRAS) is by far the largest group, and 83.3% of the divers from our database are affiliated with them. There are many similarities between the two populations. First, the average age is similar (40.5 $\pm$ 11.2 vs. 40.6 $\pm$ 11 years). Secondly, from the data obtained from our patients, the accident victims performed a total of 65,134 dives over a cumulative period of 1,063 years, yielding to an average number of 51 $\pm$ 45 dives/year (5–200, median 45). Based on retrospective data obtained from CMAS affiliated dive clubs, this number is consistent with the Belgian Underwater Federation estimation of 45–50 dives/year.

The certifications breakdown between the two populations is also very similar. There are some differences, as our database does not contain any divers with no certification and has a significant over representation of instructors. This might be explained by the fact that instructors carried out the greatest number of dives by far [64 $\pm$ 42 dives/year, (5–200, median 95)] as well as the deepest dives [39.8 $\pm$ 11.3 mfw, (24–61, median 37)]. This seems also logical, as seasoned divers, who naturally achieve higher ranking in their respective organizations through the years, constitute a large part of the examined cohort.

Finally, women constitute 19.6% of the cohort, from novice to instructor. Unfortunately, we do not know the proportion of woman affiliated to the FEBRAS. However, this figure is coherent with the literature, several commercial studies having shown, women fluctuate 1 year on the other around 25% of all active divers (Shapiro, 2003; Altman et al., 2005).

This is why this report should be treated as a sample and not as a definitive and complete record. However it gives a fair picture of the injured diver, dive parameters, risk factors and outcome in Belgium, which seems representative of the whole diving population.

Diver education toward save diving through adoption of conservative dive profiles to significantly reduce the risk of recurrent DCS is of paramount importance (Klingmann et al., 2012). However, from the results of the present study, it seems obvious that further work is needed to ascertain whether selected personality characteristics should be taken into account in the clearance decision to resume diving after a DCS because PFO remains a reason for caution where definitive recommendations still cannot be made (Germonpre, 2015).

Although, PFO is considered a risk factor for cerebral DCS in SCUBA divers, the primary cause of DCS, however, is the nitrogen bubble, not the PFO (Germonpre, 2005; Germonpre et al., 2005). Therefore the degrees of DCS risk reduction dependent on how the diver manages his/her dive and decompression to reduce the incidence of VGE (Germonpre, 2015) which depends on the behavioral capacity to comply with more conservative dive profile. From the present study, it is clear that diving safety is something to be learned.

AUTHOR CONTRIBUTIONS

DC and PL provided substantial contributions to the conception and design of the work; and the acquisition, analysis, and interpretation of data for the work. PL has drafted the work and revised it critically for important intellectual content. PG and CB revised it critically for important intellectual content. Final approval of the version to be published was the responsibility of PL, PG, and CB.

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Commercial Divers' Subjective Evaluation of Saturation

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Commercial saturation diving involves divers living and working in an enclosed atmosphere with elevated partial pressure of oxygen (ppO_2) for weeks. The divers must acclimatize to these conditions during compression, and for up to 28 days until decompression is completed. During decompression, the ppO_2 and ambient pressure are gradually decreased; then the divers must acclimatize again to breathing normal air in atmospheric pressure when they arrive at surface. We investigated 51 saturation divers' subjective evaluation of the saturation and post-decompression phase via questionnaires and individual interviews. The questions were about decompression headaches and fatigue; and time before recovering to a pre-saturation state. Twenty-two (44%) of the divers who responded declared having headaches; near surface (44%) or after surfacing (56%). 71% reported post-saturation fatigue after their last saturation, 82% of them described it as typical and systematic after each saturation. Recovery was reported to normally take from 1 to 10 days. The fatigue and headaches observed are compatible with divers' acclimatization to the changes in ppO_2 levels during saturation and decompression. They appear to be reversible post- decompression.

Keywords: saturation diving, diving fatigue, headache, hemoglobin, long term health effects, relative hypoxia

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INTRODUCTION

Commercial saturation diving in the North Sea started with the emergence of the offshore oil and gas industry in 1969. The North Sea has depths from 100 to 180 meters of sea water (msw) that became the standard range of manned underwater operations. In 1980, the interest shifted to deeper diving in Norway and in Brazil. A series of contracts was awarded in Norway for validating diver interventions to 300–350 msw. During this period, outstanding developments were conducted at the Norwegian Underwater Technology Centre (NUTEC) in Bergen and several deep saturation dives were performed in the Norwegian fjords (Hope et al., 2005a).

However, at the turn of the 90s, the Norwegian media raised the issue of potential long-term health effects of deep diving. This became a national debate and in 1993, the Godøysund conference concluded that standard saturation diving in the Norwegian continental shelf should be limited. Today, NORSOK-U100 standards (Standards Norway, 2014) that regulates manned underwater operations in Norway limits standard operations to 180 msw.

The limitation of diving depths in Norway was not the end of deep diving. The expertise was transferred from Norway to Brazil by companies like Comex that were operating internationally.

In Brazil, deep diving to 200–240 msw became a routine (Hope et al., 2005b). The key factors for preserving divers' health identified during the Brazilian operations were: (a) selection on experience, (b) selection on fitness, and (c) progressive adaptation to increasing depths (Vivaqua, 2017).

A second conference held in Norway in 2005 revisited the 1993 Godøysund conclusions. This conference first accounted for the evolution of diving procedures and the success of Brazilian operations. It also permitted separating the issues of the potential neurological effects due to long exposures to elevated partial pressure of oxygen (ppO_2), the effect of circulating venous gas on the lung function (Thorsen et al., 1995), and oxygen effects. Oxygen effects were further split into oxygen pulmonary toxicity (Thorsen et al., 1990, 1993) and changes in blood hemoglobin concentration (Hofso et al., 2005). During the conference, the results of the Examination of Long Term Health Impact of diving (ELTHI) study were presented (Murray et al., 2004). The ELTHI study failed to detect any long-term health effects except for welder divers.

Saturation procedures use higher than normal levels of oxygen in the breathing gas, as illustrated in **Figure 1** for a typical saturation profile. We therefore postulated that some of the symptoms reported during and after decompression from saturation were related to relative hypoxia when the diver returns to surface; breathing normobaric air with a ppO_2 of 21 kPa. The rationale for this relative hypoxia was based on the succession of two oxygen exposures. In the first step, during saturation, the divers become acclimatized to elevated levels of oxygen. This is supported by observations of a reduction in red blood cell counts. Drops in hematocrit and hemoglobin concentrations have also been measured in divers after a period of saturation, although the values remained within normal range (Thorsen et al., 2001; Deb et al., 2017; Kiboub et al., 2018a,b).

In a second step, by the end of the decompression, the ppO_2 decreases and the oxygen content in the chamber breathing gas must be kept below 23% to prevent the risk of fire. Depending on the diving procedures, this happens at 12–13 msw and lasts for 16–21 h until the chamber reaches surface pressure. At the end of the decompression, this drop of breathing gas ppO_2 is perceived by the body as hypoxia (De Bels et al., 2012; Kiboub et al., 2018a).

Our objective in this study was to assess signs and symptoms of hypoxia in saturation divers surfacing from decompression in the North Sea, by implementing an evaluation questionnaire.

MATERIALS AND METHODS

Ethics Statement

This study involved professional divers working in the North Sea, with TechnipFMC as a diving contractor. The study protocol was approved by the Academic Ethical Committee of Brussels (B20-2009-039) and TechnipFMC Diving and Health services in Norway and United Kingdom divisions. Data collection was conducted according to the Declaration of Helsinki principles for ethical human experimentation (World Medical Association, 2013). All participants agreed in writing on individual consents before inclusion in the study. Data collected from the questionnaires were included into an Excel database. The database complied with the 2018 European general data protection regulations; it was fully anonymized, and the anonymization was irreversible.

Worksite

The monitoring sessions were conducted onboard the Diving Support Vessel (DSV) Deep Arctic between 2015 and 2016. The study covered two operations; one in the Norwegian sector at 110 msw storage depth (121 msw working depth) and the other in the United Kingdom sector at 136 msw storage depth (155 msw working depth). In the two sectors, the divers used the same breathing gasses and equipment and performed the same type of work (well-intervention). However, the saturation procedures differed slightly because of local regulations.

Study Group

The study group consisted of male certified commercial saturation divers. All divers who were cleared for diving by the hyperbaric nurse during the pre-dive medical examination, were eligible for participation. The divers were organized in teams of three men. Each team was involved in one excursion per day during their 12 h-shift. The divers' shift time was fixed over the saturation period, and the shifts were distributed over the day as the vessel operated a two-bells system for 24 h coverage. Bell run time was limited to 8 h and diver's in-water time to 6 h; with a 30-min restitution break inside the bell in the middle of the in-water time. **Table 1** describes the study group demographics, total and saturation diving experience and body-mass index (BMI).

Saturation Procedures

Saturation was conducted using heliox (helium and oxygen mixture) as breathing gas. Two saturation procedures were used, NORSOK U-100 saturation procedures (Standards Norway, 2014) in Norway, and the TechnipFMC standard saturation procedures in the United Kingdom; which only differ in the final decompression.

During compression, the chamber ppO_2 was maintained between 21 and 45 kPa. The divers were pressurized to storage depth at a rate of 1 m/min. At storage depth, the chamber ppO_2 was kept between 38 and 42 kPa. During the excursions, the breathing gas ppO_2 varied from 60 to 80 kPa. During decompression, the chamber ppO_2 was kept between 46 and 50 kPa in Norway and between 48 and 52 kPa in the United Kingdom (difference of 2 kPa, or 4%). Close to surface, the chamber oxygen percentage was kept between 21 and 23% in both procedures.

The decompression durations were 5 days and 4 h in the Norwegian project and 5 days 13 h in the United Kingdom project. The decompression durations differed by 9 h (5%). The maximum saturation durations are limited to 14-days bottom time in Norway and 28-days total time in the United Kingdom. The actual saturation durations varied according to the operational needs.

Questionnaire

The study was part of a larger project of divers' monitoring, aiming at collecting information on divers'

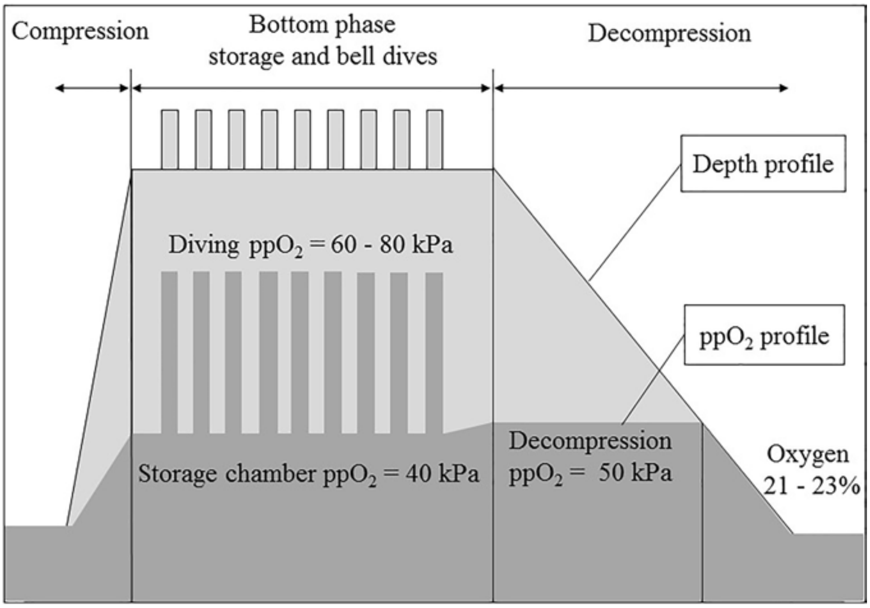

FIGURE 1 | A typical saturation profile (compression, storage and bell dives, decompression at constant partial pressure of oxygen -ppO_2- and at constant oxygen percentage) and the corresponding ppO_2 profile.

TABLE 1 | Subjects (*n* = 51) biometric information and experience expressed in mean (range).

	Age [years]	Total diving [years]	Saturation diving [years]	BMI [kg/m^2]
Mean (range)	46.1 (31 to 61)	21.8 (6 to 42)	15.9 (2 to 39)	26.5 (21.0 to 32.3)

high pressure nervous syndrome (HPNS) symptoms, fatigue, heat and cold exposure, stress, sleep, and hydration. The study was based on a questionnaire focusing on the diver's subjective evaluation of oxygen acclimatization:

- Diver's experience of headaches during or after decompression,
- Diver's subjective evaluation of post-saturation fatigue,
- Time required to return to normal pre-saturation state regarding the factors above,
- Diver's strategy to cope with return to normal life.

The questionnaire included boxes to tick-off and, when relevant, a visual scale using a line of 10 cm (0 to 10) allowing a continuous evaluation. The divers were asked to complete the questionnaire within 12 h after surfacing from decompression. Some then went into structured interviews with the investigator in a separate room in the vessel's hospital, one at a time. The objective was to provide explanations if needed and ensure that all the questions were answered. The interviewer did not influence or change the divers' answers at any stage.

Transthoracic Venous Gas Bubble Detection

Transthoracic ultrasound echography and Doppler examinations for circulating venous gas bubble detection were conducted within 2 h of the end of decompression. A Mindray M7 echocardiograph (Mindray, Shenzhen, China), equipped with a 2.5 MHz linear array transducer was used. Each diver was at rest on a bed in supine position for 3 min, before his heart was examined in an apical four chamber view as described in Bulwer and Rivero (2010). After the first examination, the subject performed a series of three squats prior to a second examination to detect potential bubbles released after the effort. Venous bubbles were also monitored using the Doppler. Several video sequences of 250 frames were registered for each diver, and used for the bubbles count. The Eftedal-Brubakk bubbles grading system was to be referred to in the eventual presence of bubbles (Eftedal and Brubakk, 1997).

Statistical Analysis

Different statistical tests were adopted depending on the nature of the data. Two-sided Fisher exact tests were applied with categorical data. Pearson's test was conducted to examine correlations between age and recovery time. *P*-values < 0.05 were considered significant.

RESULTS

The diving operations were concluded without any incidents. A total of 51 divers were invited to participate in the study, and all of them accepted and answered the questionnaire ($n = 29$ in the Norwegian sector, and $n = 22$ in the United Kingdom sector). The average saturation duration for the divers involved in the study was 19.7 ± 6.5 days. No bubbles were detected after saturation in any of the divers, either in the ultrasound images or by Doppler. The questions' response rates and results are described in **Tables 2**, **3**. There was no correlation between age and recovery time (correlation coefficient = 0.023).

DISCUSSION

Although the storage and diving depths were different in the Norwegian and United Kingdom sectors, the same breathing gasses and equipment were used; and the work scope was similar. The decompression durations and chamber ppO_2 were comparable; and no bubbles were detected, suggesting that the decompression stresses in the two sectors were also similar. Therefore, the data for the two saturation procedures in the Norwegian and United Kingdom sectors were merged in further discussion.

Oxygen Levels in Saturation

The use of oxygen in diving is a trade-off between positive and negative effects. Saturation procedures use elevated oxygen content. The principle is that a high ppO_2 in the breathing gas mixture increases the inert gas gradient and accelerates its elimination during decompression. The disadvantage is the negative effect of elevated oxygen levels on the pulmonary function, and its potential toxicity on the central nervous system (Davis et al., 1983; Manning, 2016).

The ppO_2 values used in commercial saturation diving have been empirically set. For instance, the chamber ppO_2 at storage

TABLE 2 | Questions related to headaches.

Questions	Participant's number	Scores	Response percentage (%)
Q1: During this last decompression, have you experienced headaches?	34	Yes = 11 No = 23	32 68
Q2: During this last decompression, if you had headache, when did the symptoms declare?	11	Near surface = 6 After surfacing = 5	55 45
Q3: During this last decompression, if you had headache, grade its severity on a scale from 1 (light) to 10 (severe)	10	4 (grades 1 to 2) 6 (grades 6 to 9)	40 60
Q4: Usually, do you experience headache during or after decompression?	34	Never = 14 Sometimes = 10 Often = 5 Always = 5 Sometimes + often = 15	41 29 15 15 44
Q5: Usually, if you had headache, when do the symptoms declare?	16	Near surface = 9 After surfacing = 7	44 56
Q6: Usually, if you had headache, how long does the headache last?	13	Few hours = 3 Half a day = 3 One day = 4 More than 1 day = 3	23 23 31 23
Q7: When back home after a saturation, do people around you say you look pale?	20	Yes = 19 No = 1	95 5

TABLE 3 | Questions related to post-saturation fatigue.

Questions	Participant's number	Scores	Response percentage (%)
Q8: After this last saturation, have you experienced fatigue?	24	Yes = 17 No = 7	71 29
Q9: After this last saturation, if you experienced fatigue, grade its severity on a scale from 1 (light) to 10 (severe)	17	(Mean ± SD) 3.4 ± 1.92	100
Q10: Usually, after a saturation, do you experience fatigue?	22	Yes = 18 No = 4	82 18
Q11: Usually, if you experienced fatigue, is it physical or mental?	19	Physical = 17 Mental = 2	89 11
Q12: Usually, after a saturation, grade your well-being or mood on a scale from 1 (bad) to 5 (normal) and 10 (excellent)	22	(Mean ± SD) 6.18 ± 1.56	100
Q13: Usually, after a saturation, grade your alertness on a scale from 1 (bad) to 5 (normal) and 10 (excellent)	22	(Mean ± SD) 6.23 ± 1.91	100
Q14: Usually, after a saturation, how many days does it take to return to normal?	26	(Mean ± SD) 4.31 ± 2.92	51

depth, which is currently around 40 kPa, came after a chamber with an external regeneration system had a pipe rupture. The chamber dropped from 150 to 70 msw before the chamber operators could close the skin valves. At the time, it was though that if a chamber could drop half its depth, then the chamber ppO_2 should be twice the normal value for the divers to avoid hypoxia. And it has remained thus since.

Post-saturation Headaches

Headaches were frequently reported by the end of decompression. The headache score was 32% (question Q1). When combining the groups "often" and "sometimes" in question Q4, the score became 44%, no difference was found between Q1 and Q4 (P = 0.25). The reported severity of the headaches differentiated two groups of divers; with one group describing headaches as light (grades 1 to 4) and the other group describing them as severe (scores 6 to 10). This last group also represented divers who reported being prone to migraines, thus there could be a link between their sensibility and the severity of the post-saturation headache effects.

The onset of headaches occurred near surface (44%) and after decompression (56%), which coincided with the reduction of the chamber ppO_2 or the switch to atmospheric 21 kPa ppO_2. The headache occurrences thus appeared to be synchronized with the changes of inhaled ppO_2. This is consistent with a reactive cerebral vasodilation due to hypoxia.

Post-saturation Fatigue

Most divers reported a feeling of fatigue, lasting up to 10 days after the end of decompression. It is reasonable to expect divers performing intense efforts 8 h a day, for several consecutive weeks, to experience physical fatigue. Several divers involved in night shift dives also mentioned the time required to readjust their circadian rhythms. However, if 71% of the divers reported post-saturation fatigue after their last saturation (question Q8), they also described it as typical and systematic (82% in question Q10). There is no difference between Q8 and Q10 (P = 0.5). The feeling of fatigue was presented in the following way:

- The feeling of fatigue was declared to be more physical (82%) than mental (question Q11). This feeling was generally described as a limitation to effort, like becoming breathless when climbing stairs.
- The sense of fatigue remained within moderate levels on a scale from 1 (light) to 10 (severe) [mean $\pm$ standard deviation (SD) = 3.4 $\pm$ 1.92 in question Q9].
- The overall well-being or mood, graded on a scale from 1 (bad) to 10 (excellent), was not affected (6.18 $\pm$ 1.56 in question Q12).
- The capacity to focus or mental alertness, graded on a scale from 1 (bad) to 10 (excellent), was generally unaffected (6.23 $\pm$ 1.91 in question Q13). However, one diver mentioned difficulty to concentrate post-saturation.

Recovery From Post-saturation Fatigue

All the divers indicated a recovery process and reversible symptoms. However, their post- saturation sense of fatigue could last from 1 to 10 days (4.31 $\pm$ 2.92 days in question Q14). One diver reported a recovery in 3 days by comparing his bicycling performance on a regularly used circuit. After returning home, the divers adopted different strategies to manage this feeling of fatigue. Some said they immediately caught up with life and got intensely involved in sport, social life and auxiliary business. Others reported they preferred to take a relaxing week.

An often-depicted relative paleness after decompression was observed on most of the divers. Some divers confirmed that this paleness was noticed by their families after their return home (question Q7, 95%). The divers transferring from the chamber to surface instantly switched from breathing heliox to air. It is conceivable that their bodies may react to the relative drop in oxygen at this point. The transient isobaric counter diffusion and the counter fluxes of helium and nitrogen might have momentarily disturbed alveolar ventilation and reduced oxygen exchanges. The deprivation of sunlight may also contribute to the paleness of the divers.

A study was conducted simultaneously onboard the same vessel in 2016, where hemoglobin and erythropoietin (EPO) levels were measured pre-saturation, immediately post-saturation and 24-h post-saturation (Kiboub et al., 2018a). An increase in EPO was registered over the initial 24-h post-saturation. As EPO regulates erythrocyte production, a post-saturation increase in EPO may counteract the hypoxia perceived after decompression. EPO may thus contribute to the reversible nature of the symptoms observed in this study.

Possible Evolution of Saturation Procedures

Considering that most of the saturation procedures used in the offshore industry were designed empirically in the 90s, knowledge obtained via research may contribute to their improvement. The benefit would not primarily be divers' safety, since saturation diving is already relatively safe (1 decompression sickness per 1,000 exposures) (Petroleum Safety Authority Norway [PTIL], 2018) but rather the divers' well-being.

During decompression from saturation, the chamber ppO_2 is linked to the ascent rate (Kot et al., 2015). An experimental saturation dive was performed in Norway in 2004 where a lower chamber ppO_2 protocol was used. The authors reported a case of decompression sickness and neurological deficit after this dive (Thorsen et al., 2006). The margin for changing ppO_2 is thus narrow, but there may still be room for improvement. Currently, the ppO_2 used for the diving mixtures are specified within 60 to 80 kPa. This refers to the US Navy diving manual used in the early 70s, that authorized large excursion distances. These excursion distances have since been restricted to safer values, but the ppO_2 has remained constant. It is possible that diving mixtures might be redefined according to new experience in the diving industry. The results from the present study may help mitigate some concerns of diving long-terms effects, by showing that some symptoms appear to be related to oxygen acclimatization with reversible short-term effects.

This study has some limitations. When the interviewer was present, questions were answered with 100% compliance. However, in the interviewer's absence some answers were missed. This is a known limitation; as already shown by other authors in evaluation of other aspects of saturation (Dolan et al., 2016).

The data concerning time for recovery after saturation diving were based on the divers' recollection, and not assessed for the dive after which the questionnaire was completed. Future studies should address the progress of post-saturation recovery concerning symptoms of readjustment to normal life.

CONCLUSION

We conclude that post-saturation diving effects on headaches and fatigues include a dimension that is compatible with acclimatization to the higher than normal levels of oxygen experienced in saturation. This assumption is consistent with the hemoglobin concentration changes measured in similar conditions and supported by the subjective evaluation of saturation by the divers as assessed by the questionnaire. These effects appeared reversible post-decompression.

It may be that parts of the alleged long-term effects of saturation diving, developed in 1993 at the Godøysund Conference, are in reality short-termed, reversible effects, associated with oxygen acclimatization.

AUTHOR CONTRIBUTIONS

JI, CB, and ØL conception and design of research. JI, FK, and ØL collection of data. JI, FK, IE, and CB analysis and interpretation of data. All participated in the manuscript writing, review, and final approval.

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Executive Functions of Divers Are Selectively Impaired at 20-Meter Water Depth

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Moving and acting underwater within recreational or occupational activities require intact executive functions, since they subserve higher cognitive functions such as successful self-regulation, coping with novel situations, and decision making; all of which could be influenced by nitrogen narcosis due to elevated partial pressure under water. However, specific executive functions that could provide a differentiated view on humans' cognitive performance ability have not yet been systematically analyzed in full-water immersion, which is a research gap addressed within this approach to contribute to a better understanding of nitrogen narcosis. In this study, 20 young, healthy, and certified recreational divers participated and performed three different executive-function tests: the Stroop test (Inhibition), the Number/Letter test (Task switching), the 2-back test (Updating/Working memory), and a simple reaction time test (Psychomotor performance). These tests were performed once on land, at 5-meter (m) water depth, and at 20-meter (m) water depth of an indoor diving facility in standardized test conditions (26°C in all water depths). A water-proofed and fully operational tablet computer was used to present visual stimuli and to register reaction times. Performance of the simple reaction time test was not different between underwater and land testing, suggesting that reaction times were not biased by the utilization of the tablet in water immersion. Executive functions were not affected by the shallow water immersion of 5-m water depth. However, performance scores in 20-m water depth revealed a decreased performance in the incongruent test condition (i.e., an index of inhibitory control ability) of the Stroop test, while all other tests were unaffected. Even though only one out of the three tested cognitive domains was affected, the impairment of inhibitory control ability even in relatively shallow water of 20-m is a critical component that should be considered for diver's safety, since inhibition is required in self-control requiring situations where impulsive and automatic behavior must be inhibited. Our interpretation of these selective impairments is based on a discussion suggesting that different neural networks within the central nervous system, which process specific executive functions, are affected differently by nitrogen narcosis.

Keywords: nitrogen narcosis, inert gas narcosis, water immersion, hyperbaric environment, SCUBA, cognition, human performance

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INTRODUCTION

Breathing air at increased ambient pressure can provoke inert gas narcosis (IGN) that affects the human nervous system, including alterations of cognitive functions, motor control, and mood states (Bennett and Rostain, 2003; Clark, 2015). Nitrogen narcosis is the most prominent form of IGN in recreational divers and it is thought that associated cognitive and motor impairments can increase the risk for incidents and reduce working performance (Kneller et al., 2012). In unexpected and dangerous situations, being it in underwater or other exceptional environments, an intact executive control system is necessary to guarantee not only a fast but also a correct decision. Executive abilities allow quickly adaptation to new requirements by shifting the mind set while simultaneously inhibiting inappropriate behaviors (Jurado and Rosselli, 2007). Such abilities might be necessary, e.g., in out-of-air situations in which it is required to quickly adapt to the unexpected situation by shifting attention to the new situation and the urge to ascent uncontrolled to the surface must be inhibited while evaluating the action possibilities. Moreover, information from internal sensory systems (e.g., urge to breath) along changing information from the environment (e.g., current depth, buoyancy status, position of the buddy) must be held in working memory, while constantly updating these information due to permanently changing states. Although several executive functions have been reported and different classifications and concepts have been postulated (Banich et al., 2000; Jurado and Rosselli, 2007; Banich, 2009; Diamond, 2013), it is thought that executive functions serve as the basis for higher cognitive control processes such as decision making and self-regulation and are thus extremely important for human performance and safety in extreme environments. However, specific executive functions (further definition on what is meant by specific is provided below) have not been investigated so far at water depths that are relevant for recreational divers, even though it would be of particular interest for diving safety and for further understanding of narcosis to reveal whether and to which degree specific executive control processes are impaired due to nitrogen narcosis.

Besides the problem that the existing knowledge regarding human performance and cognitive impairments under water is controversial and performance outcomes possibly biased by emotional, situational and other factors (c.f. Baddeley, 2000; Freiberger et al., 2016; Lafere et al., 2016), little attention has been paid to shallower water depths (Petri, 2003), i.e., pressure less or equal than 4–5 absolute atmosphere pressure (ATA; 1 ATA corresponds to 760 mmHg or to 1.01325 bar), which is commonly thought as the critical threshold to clearly identify nitrogen narcosis (Braubakk and Neuman, 2003; Clark, 2015). More specifically, detrimental effects on cognition and psychomotor performance, measured by a computer, as well as by means of objective measures of brain cortical arousal, have been detected already at 1.5–5 ATA in the hyperbaric chamber and in real-water immersion (Poulton et al., 1964; Petri, 2003; Balestra et al., 2012; Dalecki et al., 2012, 2013; Freiberger et al., 2016; Lafere et al., 2016; Germonpré et al., 2017), and changes in brain cortical arousal were even reported to be present 30-min after surfacing (Balestra et al., 2012; Lafere et al., 2016; Germonpré et al., 2017) Other reports suggest that a considerable amount of human performance decrements during diving might be attributed to open-water situations (Nevo and Breitstein, 1999; Baddeley, 2000). However, shallow-water immersion in controlled test conditions (i.e., not in open water) affects human cognitive processing (e.g., psychomotor speed and mental rotation ability) even when psychological factors (e.g., anxiety and mood) are eliminated as possible biasing factors (Dalecki et al., 2012, 2013). However, whether specific executive functions are impaired shallower than the critical threshold has not yet been studied.

Another important methodological issue is the kind of performance testing since cognitive performance has been tested across different studies with various test methods, definitions, task demands, and task complexities in different depths and test conditions, which often lead to ambiguity of the underlying cognitive concept under investigation. More specifically, most tests deployed in the past, e.g., classical neuropsychological tests such as card sorting, visual search, trail making tests, and multitasking and mathematical tests, tap into multiple-cognitive resources and involve non-executive processes (Miyake et al., 2000; Friedman et al., 2008; Miyake and Friedman, 2012). Recent advancements in cognitive psychology and neuroscience, however, suggest that executive processes, which underlie higher cognition, could be measured relatively isolated with more specificity by distinguishable executive functions (EF) tests. Those are thought of being more sensitive to alterations in brain functions and psycho-pathological states compared to classical neuropsychological tests (Miyake et al., 2000; Friedman et al., 2008; Miyake and Friedman, 2012). It is assumed that EFs comprise a set of lower-level cognitive processes necessary for successful self-regulation, coping with novel situations, complex planning, and decision making that are primarily localized but not exclusively in the prefrontal cortex of the brain (Banich, 2009; Miyake and Friedman, 2012; Snyder et al., 2015). Three executive functions measured by specific tests that were frequently used in the past served as the basis for an influential and relatively new cognitive model, the unity-diversity model, which assumes that executive functions share some commonalities but are nevertheless separable and represent their unique functions (Miyake et al., 2000). This idea is supported by neurophysiological evidence that defines the existence of a superior cognitive control network, which is reflected by common neural activation patterns and by domain-specific activation patterns (Niendam et al., 2012). Based on this model (Miyake et al., 2000; Friedman et al., 2008; Miyake and Friedman, 2012), the commonalities and behavioral differences are characterized by three core aspects of cognitive control: the ability to update relevant information in the working memory, to switch between different tasks and rule sets, and to inhibit responses to dominant, prepotent stimuli; all of which can be measured relatively isolated by specific test procedures (Snyder et al., 2015) and which are thought to form the basis for higher functions such as problem solving, reasoning, and planning (Diamond, 2013). There are different tests available to capture those specific executive functions by computer-based procedures including reaction time and error scores measures. Three of the most frequently used tests, which were used within the present approach, are the Stroop test as an index of the inhibitory control ability, the n-back test as an index of working memory capacity and the Number/Letter test as an index of the so called set-shifting ability (e.g., Miyake et al., 2000; Diamond, 2013). However, for all the tests, numerous variants for different research or clinical demands exist.

As outlined above, specific EFs have not been investigated in water immersion at depths that are relevant for recreational divers (i.e., <5 ATA), which is a research gap that is addressed with the current experiment. We argue that measuring EFs provide advantages in measuring cognitive performance in real underwater conditions compared to classical neuropsychological tests or other tasks that tap into multiple and non-cognitive processes. They are well described in terms of their neural substrate; they are frequently used, clearly defined, provide good sensitivity, and specificity, use scant testing time, and provide objective measurers such as reaction time and error rates (if measured by a computer); which are all aspects that make the tests ideally suited for investigating cognition in environmental circumstances with restricted test possibilities such as underwater. The first aim of the present approach was, therefore, to investigate the three core EFs in real water immersed conditions from the unity-diversity perspective with the purpose of providing a differentiated view of underwater cognitive performance. The second and complementary aim was to increase the ecological validity (compared to hyperbaric chamber testing) by analyzing cognitive performance computer based and in real-water immersion while holding test conditions standardized and controlled.

Therefore, we questioned (i) whether we could identify performance decrements of executive functions elicited by relatively shallow water immersion of 5- and 20-meter (m) fresh water depth and (ii) whether a detailed and separated analysis of specific executive function reveals cognitive deteriorations more differentiated than other tasks that activate multiple executive control processes.

MATERIALS AND METHODS

Participants

The requirement for participating in this study was a valid SCUBA (self-contained underwater breathing apparatus) diving license per European norm EN 14153-2, a valid medical fitness certificate, and a minimum of 10 dives, which at least two dives had to be deeper than 20-m water depth. Twenty qualified divers (4 females) aged 30 ± 8.7 years having a mean self-stated diving experience of 349 ± 516 (range was between 10 and 2000; and the median was 61) logged dives participated in the present experiment. Prior to the experiment, all participants were fully informed of the purpose of the study and filled an informed consent form. The test protocol followed the rules of the Helsinki declaration and was pre-approved by the ethics committee of the Deutsche Gesellschaft für Psychologie.

Measurements of Cognitive Functions, Psychomotor Speed, Heart Rate, and Ventilation

For all tests, we used a waterproof Windows based tablet computer (Alleco®, Finland) with a touchscreen being seven inches and framed in an aluminum case. Through a special liquid within a leaf that is put over the screen, the touch screen was fully operational underwater. Thus, this tablet computer served as the visual stimuli presentation within cognitive tests and as the stimulus response device registering finger presses at the touchscreen at specified regions (more details are provided below). The advantage of this tablet is that the fingers were placed on the screen, and finger presses were the same in water immersion and on land, i.e., due to the incompressible liquid between the screen and the leaf, the forces required to press the button were the same in all conditions. Thus, water viscosity should not affect reaction-time measurements, which was confirmed with an additional reaction time test (see results, **Figure 1**). For stimuli delivery and response measures, we used the software Presentation (Neurobehavioral Systems®, United States). Every four seconds, heart rate, and tank air pressure were recorded and digitally stored with a commercially available diving computer (Galileo Sol; Uwatec®, Swiss). Heart rate was averaged only for the time frame in which the tests were performed underwater (about 10 min). We additionally estimated the pressure adjusted amount of air (i.e., corrected to normobaric condition) breathed within one minute (i.e., l/min) by using the gas-pressure change in the tank within a 1-min period. This ventilation was than averaged for the complete time frame of executing the cognitive tests. For the baseline measures, no heart rate and ventilation were monitored due to the limited usability of the diving computer to measure without any elevated atmospheric pressure.

Based on Miyake et al. (2000), three executive function tests that are thought to form the core basis of higher cognitive functions, were used to measure inhibitory control (Inhibition), task shifting (Shifting), and working memory (Updating). All tasks were modified in terms of their application on a tablet computer and restricted time for testing in 20-m water depth (i.e., to avoid a decompression dive and consider limited air supply).

Inhibition

This test is a modified and computerized short version of the classical Color-Word Stroop test (Stroop, 1935), which is thought to reflect inhibitory or interference control ability (e.g., MacLeod, 1991; Friedman and Miyake, 2004). As depicted in **Figure 1A**, the test consists of a congruent and an incongruent color-word condition. In the congruent condition, presented words [German: blau and rot (blue and red)] were printed in the same color as the semantic meaning of the word (i.e., the word blue was printed in blue color). In the incongruent condition, the presented word was not in the same color as the semantic meaning of the word (e.g., the word blue was printed in red colors). In this incongruent color-word condition, the prepotent response to react to the written word must be inhibited since it is required to react to the color of the ink. This results in

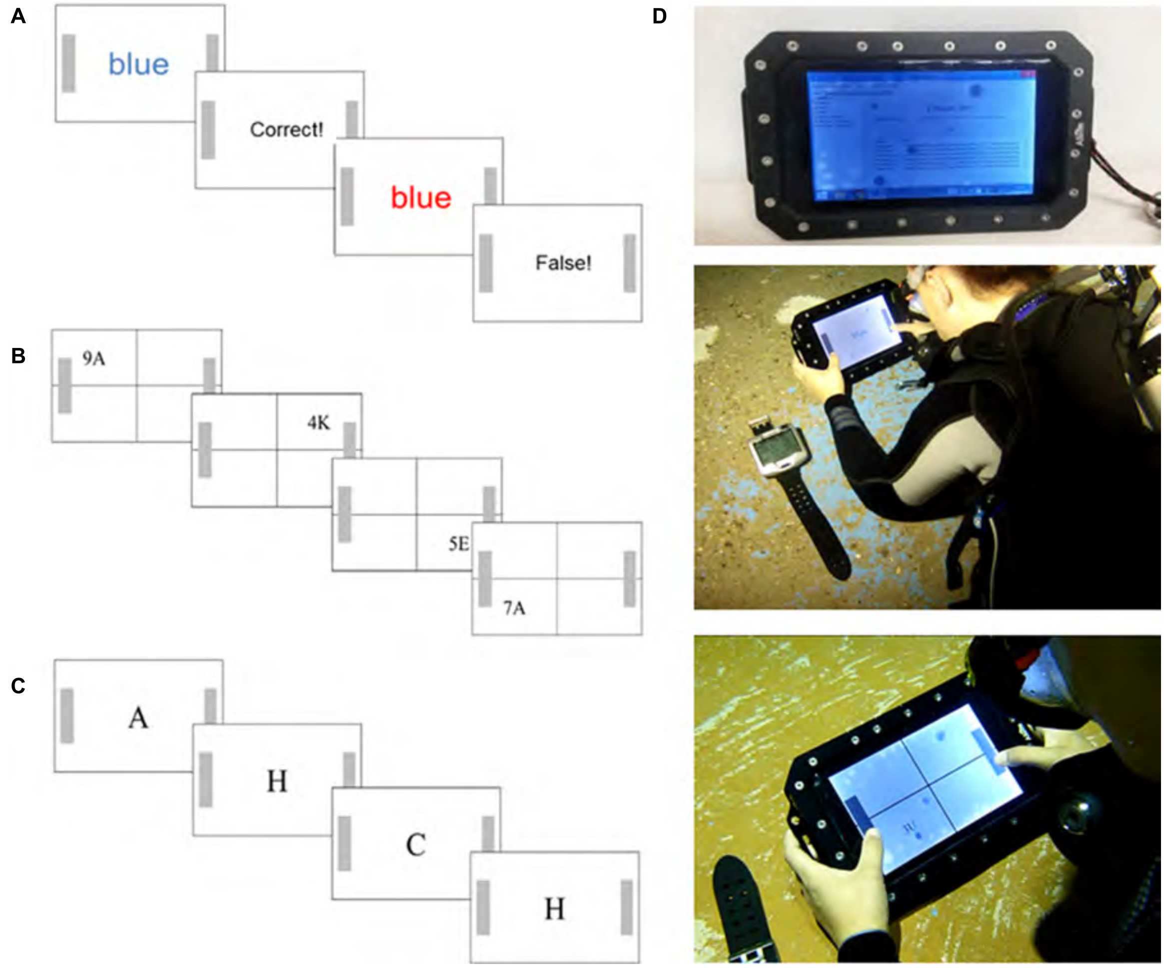

FIGURE 1 | Test sequence and test setup: Example stimuli sequence of **(A)** the Stroop test, **(B)** the Number/Letter Test, and **(C)** the 2-back test. **(D)** Photography of the underwater tablet computer, the diving computer, and a diver performing the tests underwater. More details are presented within the text.

an interference effect reflected by increased reaction time scores (compared to the congruent condition) and it is thought to index the ability to inhibit a dominant response (e.g., Aron, 2007). The test consisted of a presentation of 64 words, 32 were congruent, and the other 32 were incongruent, which were presented randomly. Each word was presented for a maximum of 2500 ms (if not responded) and followed by feedback (correct or false response) and the next target word was presented with an inter stimuli interval (ISI) between 800 and 1600 ms. Participants were instructed to press the right button when the word meaning corresponded to the color red and the left button if not corresponding and vice versa for the blue color. Reaction times were computed by calculating the mean value and excluding reaction times lower than 100 and higher than 2000 ms. Additionally, reaction times were separately calculated for the congruent and incongruent trials, and the "Stroop effect" was calculated by subtracting the mean reaction time of the congruent trials from the incongruent trials. Wrong responses (errors) for both stimuli categories (congruent and incongruent) were also counted.

Shifting

To measure the executive function shifting ability, a modified and shorter version of the Number/Letter task (Rogers and Monsell, 1995) was used, which consisted of presenting Number/Letter pairs in one of four quadrants at the tablet's screen in a clockwise direction beginning at the upper-left quadrant (see **Figure 1B**). When the Number/Letter pair was presented in the upper two quadrants, participants had to decide whether the number was uneven or even and had to press either the left (even) or right (uneven) button, respectively. When the Number/Letter pair was presented in the bottom quadrants, they had to decide whether the letter was a consonant or a vowel and had to press the left button for the consonant or the right bottom for the vowel. Thus, the tests contained two trials (i.e., Number/Letter pairs) in which the task rule was constant (i.e., upper quadrants) and two trials in which the task rule switched (i.e., from the upper right to the bottom right quadrant and from the bottom left to the upper-left quadrant). It is thought that the switch between the task rules provokes increased reaction times due to the mental shift from one rule to the other, i.e., the shifting ability

(Rogers and Monsell, 1995). In total, 64 pairs were presented, and each pair was presented for a maximum for 3000 ms, the ISI was 300–500 ms, and the letter-number pairs were randomly presented. Reaction times were computed by calculating the mean value and excluding reaction times lower than 100 and higher than 2000 ms. Additionally, reaction times were calculated separately for the conditions having no switch in the task rule (No-Switch) and for the condition with a switch in the task rule (Switch). The "Switch-Cost" was computed by subtracting the mean reaction times of the No-Switch trials from the Switch trials. Wrong responses (errors) for both stimuli categories (Switch and No-Switch) were also counted.

Updating

A modified and short version of the 2-back task was used to measure the executive function of memory updating (Kirchner, 1958). As depicted in **Figure 1C**, at the screen, a row of letters was presented consecutively whereas each letter was presented solely for 500 ms and replaced by another letter with an ISI of 1000 ms. Participants were required to indicate by button press (right press for right handers and left press for left handers) when a letter was presented that has already been presented two letters previously. If the displayed letter was not presented two letters previously, no reaction was required. Thus, this test requires to actively maintain two letters in the working memory while continuously updating the working memory with a new letter, i.e., the first letter in the sequence has to be removed from the memory and must be replaced by a new letter (e.g., Kane et al., 2007). There were 60 letters presented in the test, which 20 letters were target letters and letter presentation sequences were different in each condition. The 2-back task mean reaction time was computed for all successfully identified letters, while the amount of false alarms (i.e., button press although no target letter) and the missed targets (i.e., no bottom press although a target letter) were counted.

Psychomotor Function

A simple one-choice reaction time task was used to measure psychomotor function. A red-small square was presented in the middle of the screen for a maximum of 500 ms. After the button response was performed as fast as possible, the next square was randomly presented with an ISI between 800 and 1600 ms. There were 32 squares presented, and participants were required to press the target button at the screen with the right thumb (left thumb for left handers). Reaction times were computed by calculating the mean value and excluding reaction times lower than 100 and higher than 1000 ms.

Experimental Procedure

The experiment occurred in an indoor-diving facility. The water temperature at any depth was 26°C, and the maximum water depth was 20 m with excellent visibility. Participants, after arriving at the test location, were asked to fill in questionnaires regarding their diving experience and other anthropometric data. Then, the cognitive tests were explained by the investigator and a test trial including all tests, and the full amount of test stimuli was performed by each participant to minimize learning effects from baseline testing to the first underwater test condition.

The subsequent experimental sequence was as follows: Before the baseline land measures were performed, the diving equipment consisting of standard SCUBA diving equipment (buoyancy control device, breathing regulator, 10-liter tank, and 3-mm neoprene suit, mask, fins, and diving computer) were mounted. Baseline measures were performed in a seated and comfortable position and participants wore the diving mask, breathed through the regulator, and wore earmuffs. All cognitive tests were performed with the tablet computer and in the same sequence: The order was the reaction time test (RT-Test) followed by the Stroop test, the Number/Letter test, and the 2-back test. Before each test, a short familiarization trial (8 stimuli) was provided to be certain of task understanding and 30 s breaks were included between each test. After baseline measurements, participants were equipped with the diving gear and the belt for heart-rate measurements. All 20 participants performed the tests in the same sequence and the same rest breaks once on a platform at 5-m water depth and once at 20-m depth in an almost lying position (**Figure 1D**). There were 11 participants pseudo-randomly assigned to start at 5 m and 9 others started at 20 m. This randomization was chosen to average out possible learning effects, that could occur between the baseline measure and the first test either at 5-m or at 20-m water depth. After the descent either to 5 or to 20 m and before task execution, participants could freely swim in a slow pace underwater for 5 min to ensure familiarization with the environment. This procedure resulted in a dive timeline for the group that started at 5 m (referred to as 5–20 m group hereafter) in starting the first test about seven min. (1 min. descent, 5 min. fin-swimming, one min. task preparation) after leaving the surface, 10 min of testing at 5 m, about two min. descent to the 20 m depth, 5 min. of fin-swimming, 1 min. task preparation, 10 min. of testing, and 2 min. of ascent and 3 min. safety stop at 3 m (i.e., the full dive lasted about 40 min. for each participant). The same procedure was performed by the group that started at 20 m (referred to as 20–5 m group hereafter) with the difference that the descent to 20 m took about 2 min. more, and the safety stop was included in the 5 min. fin-swimming at 5 m.

Statistical Analysis

All variables were checked for a violation of normal distribution using the Shapiro–Wilks test. In the case of normally distributed variables, mixed ANOVAs with a between factor group (5–20 m group/20–5 m group) and with repeated measures on the factor condition (Baseline/5 m/20 m) were performed to reveal any influence of water immersion, increased pressure in 20-m depth and to observe whether the time of exposure (i.e., different effects between the group that performed the tasks beginning at 5 m and the group beginning at 20 m) had any effect on task performance. Significant results were further explored by Bonferroni-corrected pairwise comparisons (*t*-tests). Effect sizes were estimated according to Cohen (1988) by partial eta-squares (η_p^2), where $\eta_p^2 > 0.01$ indicates a small effect, $\eta_p^2 > 0.06$ indicates a medium effect and $\eta_p^2 > 0.14$ indicates a large effect. In the case of non-normally distributed variables, Friedman tests with the same factor for the condition effects were performed, and Mann–Whitney-U tests for all variables were performed between groups. Since heart rate and ventilation were only recorded in water immersion and data were normally distributed, dependent t-tests between 5- and 20-m conditions for both variables were used.

RESULTS

The t-tests for heart rate at 5 m (83.29 ± 16.82 bpm) and ventilation (10.51 ± 3.12 l/min) revealed that they were not significantly (both $p > 0.05$) different to the same measurements at 20 m (79.02 ± 12.49 bpm; 10.18 ± 2.60 l/min), which indicated that participants were not differently physically active in both test conditions. Psychomotor speed, as tested by the RT-test at the beginning of the experiment was not significantly different between test conditions [$F(2,36) = 2.18$; $p = 0.12$], although reaction times slowed underwater by about 10 ms at 5 m and 12 ms at 20 m. There were no group [$F(1,18) = 0.41$; $p = 0.52$] and no condition*group interaction effects [$F(2,36) = 0.059$; $p = 0.94$].

Figures 2, **3**, **4** illustrate the separated analysis of the three executive function tests with **Figures 2A,B**, depicting

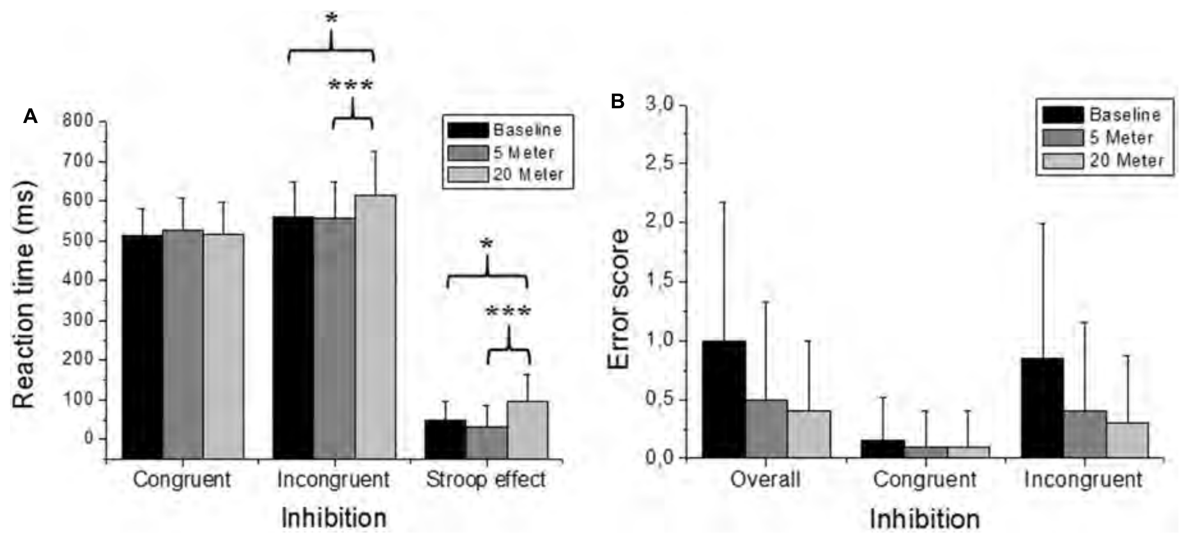

FIGURE 2 | Stroop task performance (i.e., inhibition). Depicted are mean reaction times and the mean errors. **(A)** Stroop task performance of the congruent and the incongruent stimuli and the resulting Stroop effect. **(B)** "Overall" represents all errors independent of the stimuli, while the separated errors for the congruent and incongruent stimuli are depicted as well. Values representing the arithmetic mean and error bars indicate the corresponding standard deviations. *Denotes $p < 0.05$ and ***$p < 0.001$.

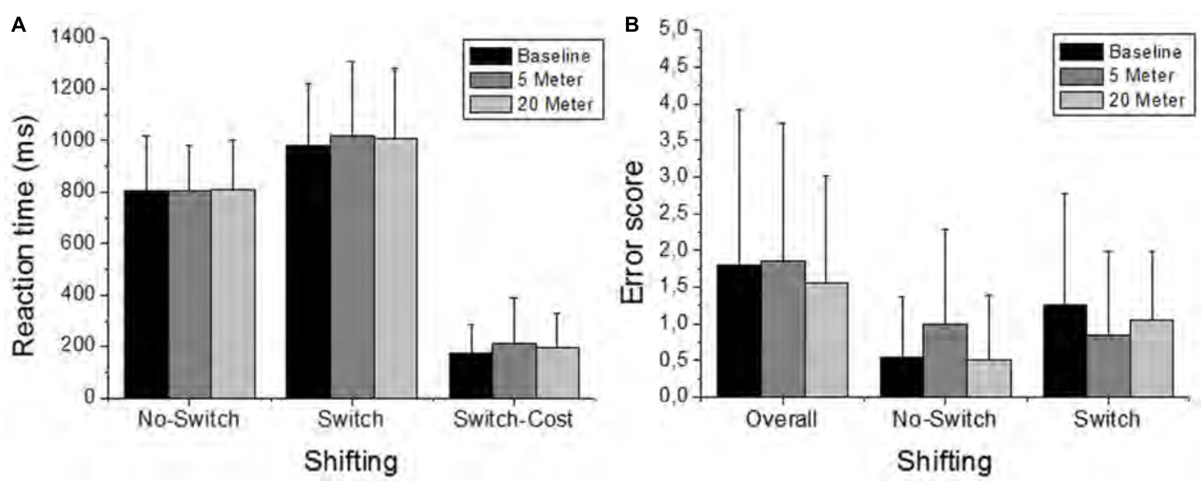

FIGURE 3 | Number/Letter task performance (i.e., shifting). Depicted are reaction times and the error scores. **(A)** Reaction times of the No-Switch trials and the Switch trials and the corresponding Switch-Costs. **(B)** "Overall" includes the errors that occurred in the complete test and separated errors for the different trials (No-Switch and Switch) are depicted separately as well. Values representing the arithmetic mean and error bars indicate the corresponding standard deviations.

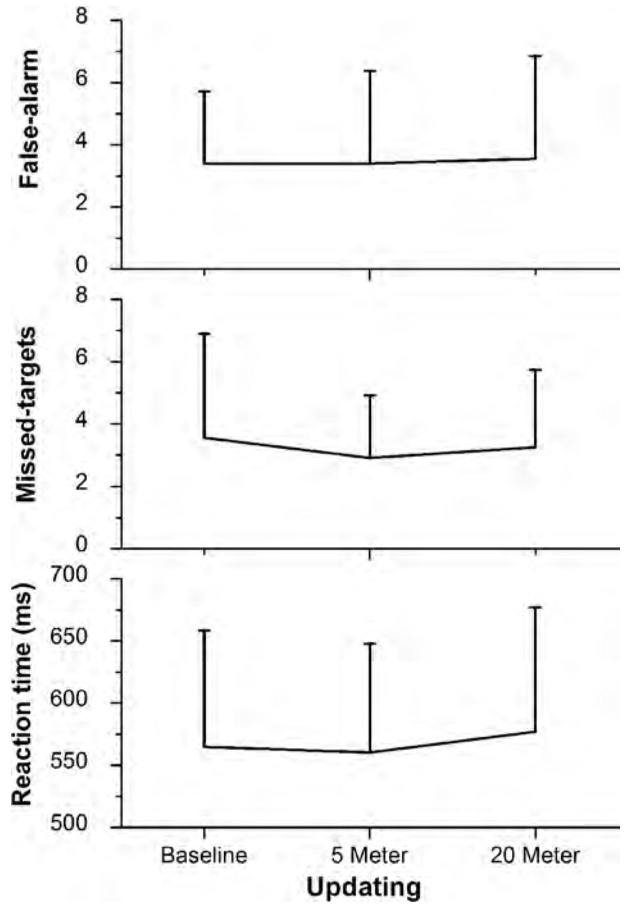

FIGURE 4 | 2-back task performance (i.e., updating) with the lower panel representing the reaction time, the middle panel representing the amount of missed target letters, and the upper panel representing the false-alarm rate. Values representing the arithmetic mean and error bars indicate the corresponding standard deviations.

the Stroop test performance (i.e., inhibitory-control ability). In addition, **Table 1** depicts all variables of the Stroop task performance separated for each group. Reaction times of the simple condition (i.e., congruent stimuli) were not affected by water immersion or by increased water depth (ANOVA condition effect was $F(2,36) = 0.46$; $p = 0.63$). There was also no group [$F(1,18) = 0.20$; $p = 0.89$] and no condition*group interaction effect [$F(2,36) = 0.49$; $p = 0.61$]. However, ANOVA for the reaction times of the incongruent trials yielded a highly significant main effect with high effects sizes [$F(2,36) = 7.92$; $p = 0.001$; $\eta_p^2 = 0.30$]. Moreover, groups did not differ in task performance [$F(1,18) = 0.003$; $p = 0.95$] and there was no interaction effect between condition and group [$F(2,36) = 0.45$; $p = 0.64$] *Post hoc* comparisons revealed that reaction times were higher (i.e., slower response) in 20-m water depth compared to the dry baseline test ($p = 0.037$; +51 ms, and 9.2% slower) and to the 5-m water immersion condition ($p = 0.001$; +55 ms, and 9.1% slower). No differences were detected between the 5-m condition and baseline condition ($p > 0.05$). The pure measure of inhibitory control (i.e., the Stroop effect) confirmed these findings, since the ANOVA main effect [$F(2,38) = 9.74$; $p < 0.001$; $\eta_p^2 = 0.39$] was highly significant. Again, no group effect [$F(1,18) = 0.12$; $p = 0.72$] and no condition*group interaction [$F(2,36) = 1.90$; $p = 0.16$] emerged. Accordingly, *post hoc* measures revealed that inhibition at 20 m was significantly inferior to the baseline measure ($p = 0.014$) and to the 5-m depth ($p < 0.001$), while the measurements at 5 m compared to baseline were not different ($p > 0.05$). Friedman's ANOVA for the error scores of the congruent trials ($\chi^2 = 0.40$; $p = 0.81$), the incongruent trials ($\chi^2 = 2.91$; $p = 0.23$) and for the overall error ($\chi^2 = 4.49$; $p = 0.10$) detected no statistical differences in the amount of error between test conditions. Moreover, we found no differences between the groups in all Stroop task related error scores (all $p > 0.05$).

The ability to switch between task rules (i.e., shifting ability) was not influenced by shallow-water immersion (5 m) or by deeper-water immersion (20 m), since in all three, ANOVAs condition effects on the reaction time of the variables Switch, No-Switch, and Switch-Cost were not significant (all three had $p > 0.05$; **Figure 3A**). There were also no group or condition*group interaction effects for all variables (all $p > 0.05$; **Table 2**). Additionally, Friedman's ANOVA could not detect significant differences in the amount of error between test

TABLE 1 | Reaction times and error scores of the Stroop task separated by condition and experimental group.

Reaction times of the Stroop test (Inhibition)									
Group	**Congruent Baseline**	**Congruent 5-m**	**Congruent 20-m**	**Incongruent Baseline**	**Incongruent 5-m**	**Incongruent 20-m**	**Stroop effect Baseline**	**Stroop effect 5-m**	**Stroop effect 20-m**
5–20 m	513.7 ± 82.6	519.8 ± 85.3	524.1 ± 94.7	564.1 ± 109.8	559.9 ± 111.1	603.6 ± 138.2	50.3 ± 59.1	40.4 ± 41.8	79.6 ± 72.6
20–5 m	509.3 ± 46.6	530.5 ± 81.2	504.6 ± 68.6	558.1 ± 46.0	553.2 ± 69.6	623.4 ± 81.4	48.7 ± 29.8	22.6 ± 67.9	118.7 ± 54.6
Error scores of the Stroop test (Inhibition)									
Group	**Congruent Baseline**	**Congruent 5-m**	**Congruent 20-m**	**Incongruent Baseline**	**Incongruent 5-m**	**Incongruent 20-m**	**Overall Baseline**	**Overall 5-m**	**Overall 20-m**
5–20 m	0.18 ± 0.40	0.09 ± 0.30	0.18 ± 0.40	0.36 ± 0.67	0.18 ± 0.40	0.27 ± 0.46	0.54 ± 0.68	0.27 ± 0.46	0.45 ± 0.52
20–5 m	0.11 ± 0.33	0.11 ± 0.33	0.00 ± 0.00	1.44 ± 1.33	0.66 ± 1.00	0.33 ± 0.70	1.55 ± 1.42	0.77 ± 1.09	0.33 ± 0.70

Depicted are means and standard deviations.

TABLE 2 | Reaction times and error scores of the Number/Letter task separated by test condition and experimental group.

Reaction times of the Number/Letter task (Shifting)									
Group	No-Switch Baseline	No-Switch 5-m	No-Switch 20-m	Switch Baseline	Switch 5-m	Switch 20-m	Switch-Cost Baseline	Switch-Cost 5-m	Switch-Cost 20-m
5–20 m	822.6 ± 259.3	837.8 ± 203.3	804.3 ± 230.2	992.4 ± 275.3	1048.6 ± 308.1	986.9 ± 281.7	169.7 ± 129.2	210.7 ± 149.4	182.5 ± 119.8
20–5 m	782.1 ± 157.0	766.5 ± 140.5	817.2 ± 143.9	967.5 ± 205.6	983.0 ± 271.5	1032.1 ± 276.2	185.4 ± 88.8	216.5 ± 208.2	214.8 ± 151.4
Error scores of the Number/Letter task (Shifting)									
Group	No-Switch Baseline	No-Switch 5-m	No-Switch 20-m	Switch Baseline	Switch 5-m	Switch 20-m	Overall Baseline	Overall 5-m	Overall 20-m
5–20 m	0.45 ± 0.93	1.09 ± 1.37	0.36 ± 0.92	1.36 ± 1.74	0.36 ± 0.67	0.90 ± 0.83	1.81 ± 2.52	1.45 ± 1.69	1.27 ± 1.42
20–5 m	0.66 ± 0.70	0.88 ± 1.26	0.66 ± 0.86	1.11 ± 1.26	1.44 ± 1.33	1.22 ± 1.09	1.77 ± 1.64	2.33 ± 2.06	1.88 ± 1.55

Depicted are means and standard deviations.

TABLE 3 | Reaction times (RT) and error scores of the 2-back task separated by test condition and experimental group.

Reaction times and error scores of the 2-back task (Updating)									
Group	RT Baseline	RT 5-m	RT 20-m	Missed-targets Baseline	Missed-targets 5-m	Missed-targets 20-m	False-Alarm Baseline	False-Alarm 5-m	False-Alarm 20-m
5–20 m	567.5 ± 112.9	559.7 ± 99.9	578.9 ± 121.5	4.45 ± 4.18	3.81 ± 2.18	3.72 ± 2.57	3.09 ± 2.21	3.90 ± 3.04	3.72 ± 3.49
20–5 m	561.6 ± 69.1	561.2 ± 74.7	575.0 ± 72.7	2.44 ± 1.50	1.77 ± 1.09	2.66 ± 2.39	3.77 ± 2.53	2.77 ± 2.94	3.33 ± 3.24

Depicted are means and standard deviations.

conditions (all three had $p > 0.05$; **Figure 3B**) and *U* tests detected no significant differences between groups ($p > 0.05$). The same accounts for the ability to update information in working memory as measured by the 2-back test (Updating ability). Neither the condition effect of the ANOVA for the 2-back's reaction time was significant nor were the group or condition*group interaction significant ($p > 0.05$; **Figure 4** and **Table 3**). Moreover, Friedman tests for the false-alarm and the missed-targets parameters revealed no significant influences of water immersion to 5 or 20 m water depth compared to land (both $p > 0.05$, **Figure 4**) and there were no differences between groups (all $p > 0.05$).

DISCUSSION

The central purpose of the present approach was to investigate cognitive performance in shallow water immersion by using executive-function tests. To the best of our knowledge, this study is the first that measured specific executive functions at 20-m water depth. Separated analysis of the three specific executive functions revealed that only the inhibitory control ability, measured by the Stroop test, was influenced by the 20-m water depth, while the switching and updating abilities were not affected. More specifically, incongruent reaction times were increased without statistically significant changes in error-rates, which suggests that the participant's cognitive system slowed and held accuracy constant, i.e., there is no substantial evidence for a change in strategic behavior in the speed-accuracy setting as has been observed in other studies (Fowler et al., 1985; Sparrow et al., 2000). However, descriptively at 20-m water depth error rates in the Stroop test decreased compared to baseline conditions, which suggests a slight change in the speed-accuracy setting, although not significant ($p = 0.10$ for overall error of the Stroop test). As depicted in **Table 2**, this decrease was more pronounced for those participants that started at 20-m water depth and less for the group that started at 5-m water depth, which points toward slight learning effects rather than changes in strategic behavior. However, given our experimental design, small sample size, and the respective analysis, we cannot completely exclude that a change in strategic behavior might have occurred.

The reaction-time task revealed that reaction times that are normally not affected at these pressure levels are not different in water immersion and on land, thus providing evidence that the use of the touch screen of the tablet computer did not systematically bias reaction time registrations in full water immersion and different depths. The experiment was conducted in a relatively safe environment with constant temperature and visibility, and heart rate and ventilation were at low rates and not different between experimental conditions. Thus, it is unlikely that anxiety or physical activity might have influenced task performance. Consequently, the biasing factors of open water that have often been reported have not influenced our findings. Nevertheless, caution is still needed, since the cognitive component of anxiety has been neglected in this approach, and it is still unclear whether and which kind of anxiety affects all or only some executive processes (Eysenck et al., 2007; Derakshan and Eysenck, 2009), a factor that warrants further investigation.

Generally, our findings agree with observations that cognitive performance deteriorates in water depth shallower than the usually accepted nitrogen narcosis threshold of 4 ATA (Poulton et al., 1964; Petri, 2003; Dalecki et al., 2012, 2013). Because those observations with respect to our study were either detected with specific tests measured with a computer in the pressure chamber (Petri, 2003) or with a computer in shallow water immersion (i.e., 5 m) (Dalecki et al., 2012, 2013), the necessity for investigating cognitive functions in real water immersion in combination with sensitive and specific computer-based tests is confirmed. However, it should also be noted that only two out of the three tested cognitive domains were influenced by 20-m, which suggests that central parts of the cognitive control system retained their full functionality. The most interesting finding was that the inhibitory control ability was significantly impaired in 20-m water depths by about 9%. The inhibitory control ability is strongly involved in behavior, which is important to the safety and accident prevention in extreme environments. According to Diamond (2013), inhibition involves the ability to control behavior, thoughts, and emotions in situations where strong internal predispositions must be overrun. A lack of inhibitory control capacity would allow us to be driven by environmental stimuli and internal emotions that pull us in uncontrolled and possibly dangerous directions. Inhibitory control enables individuals to choose how to react and how to behave (Diamond, 2013); thus, it is necessary and active, e.g., in underwater out-of-air situations where the internal drive to ascent uncontrolled to the surface must be controlled (i.e., inhibited). Therefore, it is an important observation and of practical value for diving safety that the inhibitory control ability is already impaired at water depth of 20 m.

However, although the data analysis did not yield any group or condition*group interaction effects, it should be considered that our experimental paradigm might include that the effects are partially blurred due to learning/practice effects and different time of exposure to nitrogen partial pressure. If learning effects have occurred from baseline to the first measure then the overall decrease in performance of the first underwater condition (i.e., either 5 or 20-m) might underestimate real effects. Moreover, one group started the cognitive tests at 20-m water depth, while the other group started at 5-m water depth, i.e., the 5–20 m group, had one more chance to improve task performance due to learning. Thus, the reported effects at 20-m water depth of the 20–5 m group might underestimate the real effects and overestimate the effects for the 5–20 m group. Indeed, reaction times and error rates of the incongruent part of the Stroop test and the Stroop effect (cf. **Table 1**) indicate that this was the case for the Stroop test, and a similar pattern occurred in the Number/Letter task (cf. **Table 2**), i.e., slower performance and more errors for the 20–5 m group in 20-m compared to the 5–20 m group. Therefore, this discussion accounts in the same way not only for the Stroop test but also principally for the 2-back and the Number/Letter test. Nevertheless, there were no significant group and no condition*group effects, which indicates that if learning effects occurred they were not substantial and did not bias our central conclusion. In addition, it is well known that time of exposure, independently from environmental condition, at 33 m water depth influence the critical flicker fusion frequency (CFFF), an objective measure of nitrogen narcosis, cortical arousal, and performance, with changes observable even up to 30 min. post-dive (Balestra et al., 2012; Hemelryck et al., 2013; Lafere et al., 2016). From this perspective one might argue that task performance of the 20–5 m group should have been deteriorated in 5-m, which was statistically not the case. The reason for this could either be related again to practice effects or mean that the executive function tests used in this approach were not sensitive enough to detect such longer lasting changes as has been done with the CFFF paradigm on 33-m dives. Alternatively, the pressure induced effects of nitrogen in 20-m were not enough to provoke those changes. However, due to the relatively small sample size within each group, despite any significance, caution on these factors is still needed and warrants more research, e.g., by a higher sample size, elevated water depths, combined behavioral (executive function testing), and more objective measures (CFFF) along a careful consideration of the testing order and exposure time.

Due to the absence of cognitive declines at only a water depth of 5 m, nitrogen narcosis is most likely the factor that has caused performance decrements due to elevated nitrogen partial pressure at 20 m. Our finding of selective impairments of executive functions is difficult to reconcile with theories of nitrogen narcotic effects on humans information processing capacity such as the general cognitive slowing model (Fowler et al., 1985) (i.e., different arousal), the evolutionary hypothesis (Kiessling and Maag, 1962), or the multiple processing model that has been recently proposed (Dalecki et al., 2013). Our results contrast with the latter study. Dalecki et al. (2013) found that the simple (congruent stimuli), but not the complex (incongruent stimuli), Stroop-task performance slowed already at 5-m water immersion. In contrast, only the incongruent condition was affected at 20-m water depth in the present study. One possible explanation, however, is that the error rates of the simple-test conditions were significantly affected in the Dalecki et al. (2013) study, while we found no substantial error-rate changes. Thus, a change in the speed-accuracy setting could have emerged in the simple condition of their study, while it has probably not occurred in the present approach.

A Hypothetic Explanation of Selective Executive Function Impairments

Due to a lack of directly comparable studies and the difficulty to integrate our results within other models, we propose an alternative explanation of our results and several other recent findings of IGN effects by considering current insights in the mechanisms of IGN, neuroimaging approaches, and cognitive models of the human executive control system. We suggest that elevated nitrogen partial pressure due to water immersion of 20 m might have selectively impaired neuronal networks that are differently engaged in executive processes (Niendam et al., 2012). As has been shortly addressed in the introduction, meta-analysis indicates that different executive functions, subserving higher cognitive functions, involve the activation of a common frontal-cingulate-parietal-subcortical neural network. For each executive function, a unique activation pattern can be identified in distinction to other executive functions (Niendam et al., 2012), a finding which is supported by behavioral reaction time analysis using confirmatory factor analysis (Miyake et al., 2000). Considering this unity-diversity idea, our results suggest that 20-m water immersion does not generally affect the neural network, subserving all three EFs (i.e., the common areas) and rather exhibits its influence only onto specific neural structures that are unique for the Stroop task performance. The anterior cingulate cortex (ACC) is one brain structure that is strongly involved in conflict monitoring, as it is required in the incongruent (and not in the congruent) condition of the Stroop test but not necessarily in the 2-back and task-switching tests (Botvinick et al., 2001; van Veen et al., 2001; Owen et al., 2005; Mansouri et al., 2009). More specifically, the ACC is involved in detecting and resolving competing and simultaneously active representations (e.g., competing color and word meaning processing) and signals the need for attentional control to the dorsolateral prefrontal cortex (Kühn et al., 2016). In contrast, the switching between two tasks (i.e., successively as in the task-switching task) or within working memory tasks (i.e., as in the 2-back task), the involvement of this ACC function is minimized and other brain structures are involved (Dreher and Grafman, 2003; Owen et al., 2005).

Such different involvement of specific brain areas, however, cannot explain why they should be differently affected by nitrogen narcosis without considering new research that combines functional neuroimaging and the molecular basis of neurotransmitter within specific brain areas and insights from IGN mechanisms: There is accumulating evidence that inert gases act competitively at the level of cellular proteins, supporting a protein-binding theory, and thus modulate the regulation of the nigro-striatal pathway, which is involved in cognitive processes (Franks and Lieb, 1984; Abraini et al., 1998; review in Rostain et al., 2011). This pathway is primarily regulated by excitatory glutamatergic neurotransmitters and gamma amino butyric acid (GABA) inhibitory neurotransmission and responsible for dopamine levels in the striatium (Rostain et al., 2011). Furthermore, it has been shown that increased nitrogen pressure can decrease glutamate and dopamine levels and increase serotonin in rats (Vallée et al., 2009; Vallee et al., 2009). Therefore, these and other results show that nitrogen narcosis can disturb the glutamatergic pathways by GABA neurotransmission induced reduction of glutamate (Rostain et al., 2011), which in turn affects the functions of cortical structures. The ACC has been identified to accumulate glutamate faster than other brain regions when stimulated, e.g., within pain perception or cognitive tasks involving conflict monitoring such as in the Stroop task (Hutchison et al., 1999; Mullins et al., 2005; Taylor et al., 2015). Considering that a recent study found that pain perception is reduced during a simulated 50-m dive (Kowalski et al., 2012) and that ACC is strongly involved in pain perception (Rainville et al., 1997; Hutchison et al., 1999; Büchel et al., 2002), the ACC may be one brain area that is highly sensitive to neurotransmitter alterations induced by elevated nitrogen pressure. Complementary to this discussion, the step-by-step mechanism of anesthetic action could be a potential explanation for the differential effects of nitrogen on different executive functions (Colloc'h et al., 2007). They revealed that the anesthetic agents, xenon and nitrous oxide first bind to brain intracellular proteins with large hydrophobic cavities and disrupt functions of the targeted proteins, which in turn is sufficient to provoke symptoms of the early stage of anesthesia. Only the following step(s) with higher gas concentration involves gas-binding of smaller cavities (such as the NMDA receptor) leading to surgical anesthesia. It is thought that such a step-by-step mechanisms also accounts for other inhaled anesthetics and other receptors such as the $GABA_A$ receptor (Colloc'h et al., 2007). Since it is also well known that different brain regions are differently deactivated during anesthesia, possibly due to different kinds and density of target receptors (Kaisti et al., 2003; Franks, 2008), this might also be responsible for differential onset and symptoms related to nitrogen narcosis. Thus, cognitive functions and other functions (e.g., pain perception) associated with ACC activity or possibly other sensitive brain structures might be influenced by narcosis earlier than other functions that are controlled by other brain structures. This in turn might also explain the selective impairment of executive functions within this approach and other studies that found selective impairments within a specific domain at a given hyperbaric environment.

However, although this hypothesis might be an interesting avenue for further research by applying behavioral tests that are clearly identified in terms of their neural substrate, it requires further methodological developments by establishing neuroscientific techniques within hyperbaric settings and/or ideally in real water immersed conditions. Clear inferences from behavioral measures to its neural substrate without neurophysiological measures should still be handled cautiously. Nevertheless, it appears that new avenues for IGN research in the field from a neuroscientific perspective is necessary by combining behavioral measures, recent developments in wireless electroencephalography (EEG) devices, and further objectives measures such as the CFFF. This might provide new insights into this field of research when technical advancements allow water-proof measurements, which has already been demonstrated with EEG (Schneider et al., 2014).

Limitations

Since our approach is the first that addressed executive processes under "real-life" conditions, it has also some limitations. First, as has been already discussed, our experimental method cannot completely exclude practice effects, which should be excluded in future studies, e.g., by extensive learning prior to varying the experimental condition (e.g., Germonpré et al., 2017) or by an additional control group performing the same tests in the same and time-matched sequence without water immersion. In line with this, time of exposure should be additionally emphasized, e.g., by exposing subjects to only one water depth or by the application of between-subject instead of within-subject experimental designs. Second, the cognitive tests developed for this purpose were shortened to a minimal level of visual stimuli within each test to be certain that three different executive functions can be tested in a short time frame. Consequently, this might have provoked increased random noise in the reaction time outcome. Complementary to this limitation, the unity-diversity idea proposed by Miyake et al. (2000) was developed, besides other reasons, because of the so-called task-impurity problem, which means that even well-defined executive function tasks and scores derived from those tests still include systematic variance and measurement errors (i.e., random noise in the data) from non-executive processes (Miyake and Friedman, 2012). However, to account for this problem within real-life and extreme environmental conditions would require a fundamental other-experimental approach that is out of the scope of this study. Another limitation is that our environment was relatively safe, which makes it still difficult to transfer conclusions to varying conditions of open-water situations. Our experimental design was established to explore cognitive performance in different water depths compared to land by a computer, but it would be desirable for future studies to observe possible longer-lasting effects as has been shown by the use of the CFFF paradigm (Balestra et al., 2012), which would, however, also need a different experimental procedure. Additionally, neuropsychological measures are generally susceptible to changes in the strategic behavior, motivational drive, and more specifically, practice effects in executive function tests have been shown to vary between test subjects and depend on many factors (Calamia et al., 2012). Thus, given this limitation, it would be worthwhile for future research to combine computer-based approaches to study behavioral and longer-lasting effects with parallel recordings of objective measures such as CFFF and neuroimaging methods in extreme environments. This might be useful to capture a sophisticated view of human cognitive performance and brain cortical function beyond that what can be revealed by each method alone. Lastly, other executive functions that were not captured by our tests could be measured along other aspects such as the interactive effects of increased or decreased partial pressure of oxygen, nitrogen, and carbon dioxide (e.g., exercise) on executive functions (Freiberger et al., 2016; Brebeck et al., 2017) and the role of diving experience given our heterogeneous sample regarding diving experience. However, based on our experimental findings of selective executive function impairments by an approach that was the first within this field of research, more specific theory-driven hypothesis could be derived in future studies, e.g., to test specific other important functions that are associated with ACC activity such as emotional regulations (Bush et al., 2000), which are known to be influenced by nitrogen narcosis (Jurado and Rosselli, 2007; Löfdahl et al., 2013).

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of 'Ethical Principles of Psychologists and Code of Conduct, APA, Deutsche Gesellschaft für Psychologie' with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the ethics committee of the 'Deutsche Gesellschaft für Psychologie'.

AUTHOR CONTRIBUTIONS

FS contributed to the design of the study, performed the data acquisition, analysis, interpreted the data and wrote the manuscript. MD substantially contributed to the design of the study, data analysis and interpretation. Moreover, MD critically revised the manuscript and approved the final versions and its content.

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Oxygen Toxicity and Special Operations Forces Diving: Hidden and Dangerous

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In Special Operations Forces (SOF) closed-circuit rebreathers with 100% oxygen are commonly utilized for covert diving operations. Exposure to high partial pressures of oxygen (PO_2) could cause damage to the central nervous system (CNS) and pulmonary system. Longer exposure time and higher PO_2 leads to faster development of more serious pathology. Exposure to a PO_2 above 1.4 ATA can cause CNS toxicity, leading to a wide range of neurologic complaints including convulsions. Pulmonary oxygen toxicity develops over time when exposed to a PO_2 above 0.5 ATA and can lead to inflammation and fibrosis of lung tissue. Oxygen can also be toxic for the ocular system and may have systemic effects on the inflammatory system. Moreover, some of the effects of oxygen toxicity are irreversible. This paper describes the pathophysiology, epidemiology, signs and symptoms, risk factors and prediction models of oxygen toxicity, and their limitations on SOF diving.

Keywords: oxygen toxicity, CNS-toxicity, pulmonary toxicity, diving, closed-circuit rebreather

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INTRODUCTION

Military diving, especially within the domain of the Special Operations Forces (SOF), is one of the most extreme forms of diving. Depending on the task, this type of diving demands different equipment, procedures and training and, therefore, it is totally unlike commercial or civilian diving. For SOF divers, range and endurance, high mobility and stealth, are of utmost importance. To facilitate these requirements, the most commonly used equipment is the closed-circuit oxygen rebreather (O2-CCR): a comprehensive overview of the historic aspects of CCR diving is already published (Donald, 1992; Acott, 1999; Butler, 2004).

A CCR is fundamentally different from any open-circuit or semi-closed diving system. Instead of releasing exhaled air to the surrounding environment, it is recirculated within the apparatus. Any exhaled carbon dioxide (CO_2) is "scrubbed" by a chemical mixture, often various hydroxides, for instance NaOH and $Ca(OH)_2$. The efficacy of scrubbers is beyond the scope of this review, but factors like granule size, ambient temperature, and humidity greatly affect and sometimes limit scrubber efficiency. CCRs can be used with air, mixed gas, or pure oxygen. There is no exhaled gas under the form of bubbles and gas consumption is very much limited, increasing the possible autonomous dive time. In case of a breathing gas composed of pure oxygen, a second substantial difference compared to a regular SCUBA or mixed gas rebreather can be noted: the oxygen diver has no accumulation of nitrogen or other inert breathing gas and, therefore, no decompression limits. To facilitate recirculation, the breathing gas is temporarily stored in a "counter lung" before inspiration. The volume of this counter lung limits the tidal volume (TV) and maximum minute volume (MMV) of the SOF diver. The limitation of TV and MMV and possible saturation of the soda lime can cause retention of CO_2 (Arieli et al., 2006a,b). This greatly affects the development of oxygen toxicity (see below).

Although many studies have investigated the toxic effects of oxygen on both the central nervous system (CNS) and the pulmonary system, the question remains how applicable those studies are for SOF divers. Much of the research was conducted with animals; although this has greatly contributed to our understanding of physiological processes, the results cannot always be extrapolated to humans (Robinson et al., 1974; Bryan and Jenkinson, 1988; O'Collins et al., 2006). Secondly, much of the available data on humans is rather old, and many of these experiments will never be replicated today due to our current viewpoint on research ethics. Nevertheless, these historical studies give some insight into the (life-threatening) dangers that oxygen poses for man (Donald, 1992; Acott, 1999). Since technological advances (such as, the capacity of "scrubbing" CO_2) influence the occurrence of oxygen toxicity, many of these older studies cannot be used to determine the threshold or safe limits or oxygen exposure. Lastly, most human experiments were performed in rest in a recompression chamber, the so-called "dry dives." Although dry dives enable researchers to administer oxygen in partial pressures above 1 ATA (equal to 101.3 kPa), the effects of oxygen are not the same as in an actual dive. Several studies have shown that submersion can alter the physiologic reaction to breathing gases (Donald, 1992; Kerem et al., 1995; van Ooij et al., 2011).

However, even when taking these limitations into consideration, some relevant data on oxygen toxicity in diving are still available. The aim of this paper is to summarize the pathologic effects of oxygen (mainly on the CNS and pulmonary system) and their operational consequences for SOF divers.

CENTRAL NERVOUS SYSTEM TOXICITY

The phenomenon of CNS toxicity is commonly referred to as the Paul Bert effect, named after the French physiologist who first described it (Bert, 1878). In many dry dive experiments, Bert showed that oxygen is toxic and potentially lethal for many organic species including seeds, fungi, insects, and several small mammals. Others published similar results, showing that CNS toxicity was dependent on the inspired partial pressure of oxygen (PO_2) and the time exposed. In 1910 Bornstein was probably the first to expose two human volunteers in a dry dive setting to hyperbaric oxygen at a PO_2 of 2.8 ATA (equal to 283.7 kPa) for 30 min without any complaints (Acott, 1999).

The tolerance for oxygen in dry dives is much higher than in wet dives (Donald, 1992). In an immersed setting, a PO_2 above 1.4 ATA can lead to nausea, numbness, dizziness, twitching, hearing and visual disturbances, unconsciousness and convulsions (Harabin et al., 1995). In humans, no oxygen induced convulsions have been described with a PO_2 lower than 1.3, although susceptibility to oxygen toxicity has a high interpersonal and intra-individual variability (Donald, 1992; Arieli et al., 2008). While convulsions can occur without any prior symptoms, visual disturbances generally precede convulsions (Curley and Butler, 1987; Arieli et al., 2006a). Reports on the incidence of CNS toxicity vary greatly, ranging from 1 in 157,930 CCR dives to approximately 3.5% of the CCR dives (Harabin et al., 1995; Walters et al., 2000; Arieli et al., 2002). This may be attributed to different exposures in time and depth, or to different definitions of the symptoms or, perhaps, because the covert nature of SOF diving precludes the reporting of precise incidences.

Pathogenesis and Risk Factors

Although the exact mechanism is not fully understood, currently, the most plausible explanation is related to an overflow of reactive oxygen species (ROS) in the brain after an increase of cerebral blood flow (CBF) (Visser et al., 1996a; Koch et al., 2008). Due to the increased PO_2 in plasma there is an auto oxidation of nitric oxide ($^{\cdot}NO$) to several ROS, of which peroxynitrite ($ONOO^-$) is the most important (Goldstein and Czapski, 1995; de Groot et al., 2004). ROS cause angiotensin-II induced vasoconstriction via activation of non-phagocytic NAD(P)H oxidase (Weissmann et al., 2000; de Groot et al., 2004; Nguyen Dinh Cat et al., 2013). However, at the same time, endothelial and neuronal nitric oxide synthase (eNOS and nNOS), which are responsible for vasodilatation, are increased (Hoehn et al., 2003). The net result of both processes is vasoconstriction and a reduction of CBF up to a certain "breaking point." Possibly due to depletion of the radical oxygen scavenger system, vessels dilate and increases CBF (Chavko et al., 1998; Demchenko et al., 2002; Eynan et al., 2014). Simultaneous to this increase in CBF, the cortical electroencephalography (EEG) activity increases (Bean and Coulson, 1971; Visser et al., 1996b). This increase in CBF and cortical EEG activity precedes convulsions (Bean and Coulson, 1971; Visser et al., 1996a,b; Demchenko et al., 2001; Koch et al., 2008). The exact mechanism though which ROS cause convulsions is not entirely clear. ROS are believed to directly affect various ionic conductance that regulate cell excitability, as well as disrupting chemical synaptic transmission (Manning, 2016). The role of superoxide dismutase (SOD), catalase and other scavengers in the brain, predominantly on the function of the hippocampus, remains to be elucidated. However, animal experiments have shown that modulating the N-methyl-D-aspartate (NMDA) and N-nitro-L-arginine (NNA) system alters the susceptibility (Eynan et al., 2014; Manning, 2016). When any prodromal symptoms are encountered and the PO_2 is lowered, convulsions may be avoided (Arieli et al., 2008).

Despite extensive research to identify risk factors in CNS toxicity, most studies were on animals and the relevance for oxygen diving in humans remains unclear. Oxygen toxicity is dependent on both PO_2 and time, i.e., the time of onset of symptoms is shorter when the PO_2 is higher (Donald, 1992; Arieli et al., 2002). An increase in end-tidal partial pressure of carbon dioxide ($PetCO_2$), either by "CO_2 retainers" (divers with a delayed or altered response to hypercapnia) or due to exercise, also increases susceptibility to oxygen toxicity (Arieli et al., 2001; Koch et al., 2013). Dehydration and starvation prolongs the latent period to onset of convulsion in rats, but the pathophysiological mechanism is unclear (Bitterman et al., 1997). In a small study, a ketogenic diet in divers increased oxygen tolerance, but the mechanism for neuroprotection remains unknown (Valadao et al., 2014). Adding nitrogen or helium to the inspired gas, as well as adding periods of breathing air (commonly called "air breaks") to the oxygen exposure, protect against convulsions in dry dives, but the feasibility in oxygen diving is limited (Hendricks et al., 1977; Bitterman et al., 1987; Harabin et al., 1988; Arieli et al., 2005, 2008). An overview of pharmacological agents and vitamins that protect or sensitize has recently been published (Jain, 2017). A few pharmacological agents are worth mentioning here: scopolamine and cinnarizine (agents frequently used to prevent and treat motion sickness) do not seem to either attenuate or sensitize oxygen toxicity (Bitterman et al., 1991; Arieli et al., 1999). Caffeine is effective in delaying convulsions in rats, but its efficacy in humans has yet to be confirmed (Bitterman and Schaal, 1995). Due to the unpredictability and operational limitations, relying on pharmacological agents for extending SOF diving is not currently relevant.

Prediction Model and Variability

The first prediction model (published by Harabin et al.) was based on 661 CCR dives (Harabin et al., 1995). This was later refined by Arieli et al. who based their model on 2,039 CCR dives and which remains the most accurate model to date (Arieli et al., 2002). The chance of oxygen toxicity (as Z-score in a normal distribution) in any dive can be estimated by: $Z = [\ln(t) - 9.63 + 3.38 \times \ln(PO_2)]/2.02$. Note that this equation includes PO_2 (in kPa) and time (in min) as variables. The recovery time (similar to "surface interval" in air dives: the time for a diver to neutralize the oxygen stress) is based on experiments in rats and estimated by: $K_t = K_e \times e^{-0,079t}$, where K_t is regarded as a cumulative oxygen toxicity index at time t (in min) and K_e the "toxicity dose" at the end of exposure. However, to our knowledge, no studies have tested the efficacy of these models in humans.

Although the methodology of the model is sound, a considerable intra and interpersonal variability in oxygen toxicity still remains. In an effort to identify the military divers at risk, the "oxygen tolerance test" has long been advocated, where subjects were exposed to breathing 100% oxygen at 2.8 ATA for 30 min (Butler and Knafelc, 1986). However, after evaluation, this was proven obsolete because it lacked predictive value and many navies now refrain from using this test (Visser et al., 1996b; Walters et al., 2000). A similar test for CO_2, the Read test, has also proven ineffective (Arieli et al., 2014). Although the ability to detect CO_2 by divers can be trained, it is unknown whether this reduces the incidence of oxygen toxicity (Eynan et al., 2003, 2005). To our knowledge no valid test is available to screen for oxygen tolerance.

Operational Consequences

Even though the pathophysiological mechanisms and risk factors are not yet clarified, there is a clear depth (PO_2) and time relationship. Oxygen toxicity of the CNS is a rare but potentially life-threatening complication of exposure to high PO_2, which can occur without prodromal symptoms. If mild symptoms do occur and can be timely recognized, convulsions may be avoided by reducing depth. However, delayed progression to convulsions after reducing oxygen exposure have been described.

In sports diving, the PO_2 limit recommendations ranges from 1.4 to 1.6 ATA (Lang, 2001). The limits for military SOF diving are different, due to differences in the equipment and the amount of "acceptable risk" (**Table 1**) (Vann, 1988; United States Department of the Navy NSSC, 2008). The model from Arieli et al. allows 24 min at a PO_2 of 2.5 ATA when accepting a risk of maximum 5% of CNS toxicity (Arieli et al., 2002). The widely used US Navy Diving Manual allows a single-depth exposure to a PO_2 of 2.5 ATA for 10 min (United States Department of the Navy NSSC, 2008). However, these high risks are only taken with the proper training, equipment and safety precautions, and only when the operational demands leave no alternative options. Incidence reports of oxygen toxicity using the Arieli model are lacking, most likely due to the covert nature of SOF diving.

PULMONARY OXYGEN TOXICITY

In 1899, the Scottish pathologist, James Lorrain Smith, published the pathological effects of increased inspiratory oxygen tension on several small animals (Lorrain Smith, 1899). In these classic experiments, mice and larks were exposed to increasing pressures of oxygen for long periods of time. Besides several episodes of CNS toxicity, most of the animals perished because of hypoxia as a result of insufficient ventilation due to acute or chronic lung inflammation. Compared to CNS toxicity, a lower partial pressure of oxygen is required to cause symptoms, but the exposure time has to be much longer (hours to days). Exposure above 0.5 ATA is regarded as potentially damaging for the pulmonary system. In humans, early symptoms include tracheobronchial irritation with retrosternal pain and coughing (Klein, 1990). Longer exposures damage the tracheal mucosa with impaired mucus clearance (Sackner et al., 1975). These complaints precede changes in lung function tests, such as, a decrease in vital capacity (VC), but have a low predictive value due to high variability (Klein, 1990). The incidence in divers is unknown, since no studies have investigated the epidemiology.

TABLE 1 | Single-depth oxygen exposures.

Depth (fsw/msw)	Limit US Navy (United States Department of the Navy NSSC, 2008) (min)	Risk of CNS toxicity (Arieli et al., 2002)(%)	Limit Arieli et al. <5% (min)
25/7.7	240	13.1	83
30/9.2	80	6.3	63
35/10.7	25	2.4	48
40/12.3	15	1.7	38
50/15.3	10	1.8	24

Depth in feet of sea water (fsw) and meters of sea water (msw). The limits are printed as in the US Navy Diving Manual with (in the third column) their associated risk for CNS toxicity based on the model of Arieli et al. (2002). The last column shows the bottom time when accepting a maximum risk of CNS toxicity of 5%.

Pathophysiology and Risk Factors

Pulmonary oxygen toxicity (POT) can be divided into two phases. The first exudative phase (**Figure 2**, left side) is marked by local inflammation with capillary and endothelial edema, a decrease of type I alveolar cells, and an influx of inflammatory cells (Miller and Winter, 1981; Bryan and Jenkinson, 1988; Demchenko et al., 2007). These changes are reversible and the lung returns to its normal state (**Figure 1**) if the inspired oxygen pressure is reduced below 0.5 ATA. In the following proliferative phase (**Figure 2**, right side) fibroblasts and type II alveolar cells infiltrate the inflamed endothelia. With continuing inflammation, this ultimately leads to alveolar fibrosis and a four- to fivefold increase of thickness of the air-blood membrane and, as a consequence, loss of diffusion capacity (Kapanci et al., 1972; Robinson et al., 1974). These changes are irreversible. The rate at which these changes occur is directly related to the inspired PO_2 and can occur as early as 3 h at a PO_2 of 3 ATA during a dry dive (Winter and Smith, 1972; Klein, 1990).

When divers are immersed, many physiologic processes are altered. Circulating volume is redistributed due to the hydrostatic pressure on the body and peripheral vasoconstriction when immersed in cold water, both resulting in volume shift and intrathoracic pooling (Norsk et al., 1985; Choukroun et al., 1989; Pendergast and Lundgren, 2009). Even though the mammalian diving reflex lowers the heart rate, the net result of both processes is pulmonary hypertension, because the cardiac output is increased as a result of the Frank-Starling mechanism (Dahlback et al., 1978). The increased blood flow in the lung recruits apical fields (compared to exercise), but also stiffens the lung (Choukroun et al., 1983; Pendergast and Lundgren, 2009). Beside these effects on lung circulation, the intrathoracic pooling and pulmonary hypertension triggers the baroreceptors in the right atrium, which increases diuresis through an increased vasopressin release (Norsk et al., 1986; Boussuges et al., 2007, 2009). Lastly, the position of the diver in the water (horizontal or vertical) and the position of the breathing apparatus compared to the body (deeper or lower than the diver) also influences perfusion of the lung and breathing dynamics (Badeer, 1982; Taylor and Morrison, 1991). As a result of all of the above processes, gas exchange in the lung during immersion is substantially different from that during dry dives (Prefaut et al., 1978; Taylor and Morrison, 1990; van Ooij et al., 2011).

Very few studies have reported risk factors for developing POT in divers, let alone in SOF divers. Most of the results are derived from dry dive experiments. Many of these studies use a decrease in VC as a marker to determine the amount of POT; however, the validity of this measurement is questioned (see below). Shykoff reported that exercise in an immersed setting and repeated exposure increases POT (Shykoff, 2008a,b). To our knowledge, no other risk factors have been identified. In animal studies, scavengers (such as, SOD and catalase) were shown to protect lung tissue against the overload of oxygen radicals; however, this effect has not been confirmed in humans (Kimball et al., 1976; Frank et al., 1978; Potter et al., 1999).

Prediction Model and Variability

In military and commercial diving, the current standard for determining the maximum pulmonary oxygen exposure in

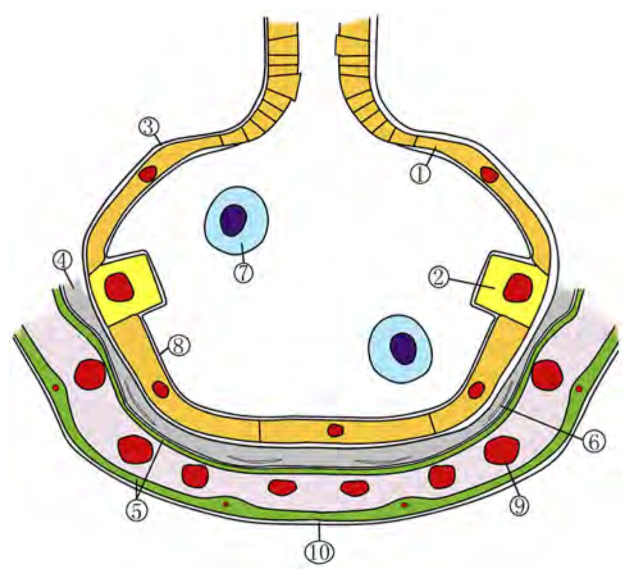

FIGURE 1 | Schematic representation of the normal alveolocapillary region. 1, alveolar type 1 cell; 2, alveolar type 2 cell; 3, basement membrane; 4, interstitium; 5, capillary endothelial cell; 6, fibroblast; 7, alveolar macrophage; 8, surfactant layer; 9, red blood cell; 10, capillary base membrane. Adapted with permission from van Ooij et al. (2013).

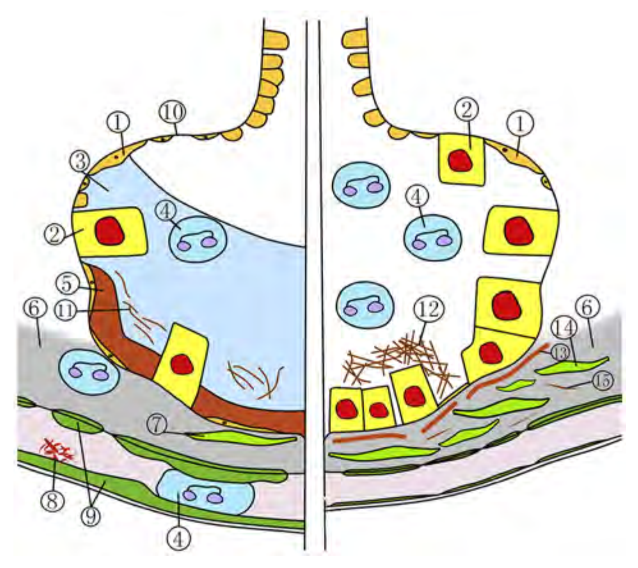

FIGURE 2 | Exsudative stage (left) and proliferative stage (right) in pulmonary oxygen toxicity. 1, type 1 alveolar cell; 2, type 2 alveolar cell; 3, alveolar edema; 4, neutrophil; 5, hyaline membrane; 6, edematous interstitium; 7, fibroblast; 8, fibrin thrombus; 9, swollen capillary endothelial cell; 10, denuded basement membrane; 11, alveolar fibrin formation; 12, collagen fibers deposition; 13, incorporation of hyaline membrane; 14, fibroblastic proliferation; 15, interstitial fibrin.

diving is “units of pulmonary toxicity dose” (UPTD). One UTPD equals the amount of damage caused by breathing 1 min of 100% oxygen at 1 ATA (Bardin and Lambertsen, 1970). The basic concept of UPTD is that a certain threshold (amount of oxygen molecules) is required to cause local damage, which can be measured by a decrease in VC. For instance, an exposure of 615 UPTD causes the VC to decrease 2% in 50% of the divers, while 1425 UPTD lowers the VC by 10% in 50% of the divers (Clark and Lambertsen, 1970; Wright, 1972). Since the 1970s, many studies have further refined the basic model (Clark et al., 1999). To calculate the amount of UPTD, the following equation must be solved: $UPTD = t \times [0.5/(PO_2-0.5)]^{-5/6}$, with PO_2 in ATA and time in minutes. Arieli et al. published an improved equation to more accurately determine the decrease in VC in a dry setting, based on data from several studies that included exposures with humans: $\Delta VC = 0.0082 \times t^2(PO_2/101.3)^{4.57}$; please note that, here, PO_2 is in kPa and time is in hours (Eckenhoff et al., 1987; Clark et al., 1991; Arieli et al., 2002). The “cumulative units of pulmonary toxicity dose” (CPTD), the “oxygen toxicity unit” (OTU) and derived equation for repetitive exposure (REPEX) were introduced to include recovery and facilitate multi-day exposures, but was never validated in divers (Hamilton, 1989). Arieli et al. also published an updated equation to estimate the recovery of lung volume, which was extrapolated from data derived from animal experiments performed in a dry setting (Arieli et al., 2002). There is no consensus which model is the most valid to plan SOF operations.

The main flaw in the UPTD concept and the derived equations is the change in VC as the sole indicator to determine oxygen stress. VC has a circadian rhythm and there is a strong intra and interpersonal variability when measuring lung volumes (Hruby and Butler, 1975; Harabin et al., 1987). Ventilation during anaesthesiology with a high PO_2 is known to influence VC, possibly due to absorption atelectasis (O’Brien, 2013). Whether this also occurs in SOF divers, or how long this endures after diving, is unknown. Recent findings have proven that immersion itself alters VC regardless of oxygen stress (Shykoff, 2005; van Ooij et al., 2011, 2012). Since the UPTD model was derived from dry dives, the above-mentioned factors are not taken into account. Although the original authors recognized the limitations of the UPTD model, more advanced diagnostic measurements were either too difficult to perform or were unavailable in the 1960s/1970s (Bardin and Lambertsen, 1970).

Operational Consequences

POT is more insidious than CNS toxicity; it affects the oxygen divers in long shallow-water dives or when recurrently exposed. The current prediction model (UPTD) was developed in dry setting during a time when capabilities to measure lung parameters were limited. Newer parameters, such as, the ratio between diffusion capacity of carbon monoxide and nitric oxide ($DL_{NO/CO}$), fraction of exhaled nitric oxide (FE_{NO}) or volatile organic compounds (VOCs), might be more accurate in determining POT, but these tests have yet to be validated (Shykoff, 2008a,b; Caspersen et al., 2011; van Ooij et al., 2014b,a, 2016; Vermeulen et al., 2016). Especially the VOCs are of interest because, in the field of pulmonology, this noninvasive diagnostic modality is increasingly utilized for diagnosing asthma, acute respiratory distress syndrome and lung cancer (Bos et al., 2014, 2016; Boots et al., 2015). However, until a new valid parameter to determine POT has been established, the UPTD model remains the gold standard, despite its limitations.

As equipment improves and dive times are extended, SOF divers might be increasingly exposed to a level at which irreversible damage may occur. The Royal Netherlands Navy currently dives with the LAR 5010 by Dräger and within the limits given by NATO (Allied Diving Publication), which are highly similar to the US Navy Diving Manual (United States Department of the Navy NSSC, 2008). Oxygen exposure is limited to 450 UPTD per day and 2250 UPTD per week. A single exposure up to 1425 UPTD is regarded the absolute maximum and only to be used in exceptional circumstances with sufficient medical support available (i.e., recompression facilities and medical capacity within the operational theater). The Royal Netherlands Navy Diving Medical Center performs yearly dive medicals on all Dutch SOF divers according to and surpassing the standard of the European Diving Technology Committee (Wendling and Nome, 2004). In a recent 20-year longitudinal cohort study, we found no significant changes in pulmonary function and diffusion capacity of SOF divers compared to other Navy divers or non-divers (Voortman et al., 2016). Tetzlaff et al. published similar results (Tetzlaff et al., 2005). Yearly exercise tolerance testing shows $VO_{2\,max}$ values regularly surpassing 50 ml/kg/min and all divers remain fit for diving duties during their career. This may be due to either sufficient “recovery” time between extreme dives, or because exposures are not severe enough to cause irreversible damage. Although current monitoring does not show any deleterious effects, it remains necessary to continue this monitoring of long term health effects as the level of exposure in recent years has increased.

OTHER PATHOPHYSIOLOGIC CHANGES

While CNS toxicity and pulmonary toxicity have been described as separate entities in this review, their occurrence may be more closely related. In addition to cold, stress and physical activity, CNS toxicity activates the sympathetic nervous system, which in animal experiments leads to pulmonary edema though the pulmonary venule adrenergic hypersensitivity response (Winklewski et al., 2013). Hyperoxia, even at normobaric conditions, induces many physiological changes which are often not fully understood. In addition, the clinical relevance of these changes and impact on SOF diving remains to be elucidated. Although this paper does not aim to give a full review of all known pathophysiological effects of oxygen in divers, the effects on sight and exercise tolerance are important in the context of SOF diving. For further reading of the effects of hyperoxia on other parts of the body we suggest the work of Bennett and Elliott (Brubakk and Neuman, 2003).

Ocular Toxicity

Visual acuity is of crucial importance to SOF divers. Visual complaints are a frequent side-effect of daily clinical treatments in recompression chambers (hyperbaric oxygen therapy: HBOT). Transient myopia with up to 0.25 dioptres loss for each week exposed to high oxygen pressures can occur, but generally resolves after a few weeks (Butler et al., 2008). Apart from one case report, hyperopic myopia has not been reported in oxygen divers (Butler et al., 1999). Although extreme HBOT exposures can cause irreversible cataract or keratoconus, this has not been described in divers (Palmquist et al., 1984; Butler, 1995; McMonnies, 2015). These effects of oxygen on the ocular system are probably irrelevant for SOF divers, as oxygen pressures are generally much lower and exposure is less frequent compared with daily HBOT in patients for several weeks.

Exercise Tolerance

There are several reports on fatigue and reduced exercise tolerance after high oxygen exposures (Comroe et al., 1945; Lambertsen, 1978; Shykoff, 2005). Divers complained about retrosternal pain or the inability to "give their full" for several days. To what extent this is a subjective complaint, or limits (diving) performance, is unknown. Although the mechanism behind these complaints is not fully understood, generalized oxidative stress depletes the scavenger system and leads to lipid peroxidation of the cell membranes causing cell damage (Ferrer et al., 2007; Perovic et al., 2014). After diving, because there is an upregulation of glutathione peroxidase (GPx) and catalase activity in lymphocytes, the inflammatory system may also be involved (Ferrer et al., 2007). Damage and dysfunction of erythrocytes has been described after hyperbaric hyperoxic exposure and in saturation divers, its effect on exercise tolerance is unknown (Dise et al., 1987; Hofsø et al., 2005). To what extend performance is impaired in SOF divers after oxygen diving remains to be confirmed.

SUMMARY

In diving and hyperbaric environments, oxygen toxicity has been a topic of interest for over a century. Although many human experiments are not reflecting current equipment or procedures anymore, the results do illustrate the damaging potential of oxygen. Diving with high partial pressures of oxygen can result in acute life-threatening neurologic complications or irreversible pulmonary structural changes. However, the extent to which these problems occur in oxygen diving remains unknown, due to the lack of studies on humans during immersion, and/or epidemiologic studies.

In SOF diving, where 100% oxygen rebreathing diving systems are frequently used, operational demands and health risks are taken into account when planning dives. All current limits or diving tables with high PO_2 possess a certain quantity of "acceptable" risk. The question arises as to whether civilian or commercial divers should use the same limits as SOF divers.

To develop more accurate prediction models, we need to identify the pathophysiological mechanism of oxygen toxicity and the factors that, subsequently, increase or decrease the risk to various parts of the body. This is complicated by the covert nature of SOF diving, limiting publication of data. Also, in view of the considerable inter- and intra-personal variability, perhaps the future of oxygen diving requires real-time individual monitoring of early symptoms of oxygen toxicity, such as, CBF or exhaled VOCs, to protect humans from the harmful effects of oxygen when diving.

Current Limits on Oxygen Exposure in the Royal Netherlands Navy

Central Nervous System Oxygen Toxicity

Divers exposed to a PO_2 above 1.3 ATA should be considered to be at risk for developing CNS toxicity. An estimation of the chance of CNS toxicity in diving, as Z-value in a normal distribution with t in minutes and PO_2 in kPa, can be made (Arieli et al., 2002). There is no consensus regarding a "maximum acceptable risk."

$$Z = \frac{\ln(t) - 9.63 + 3.38 \times \ln(PO_2)}{2.02} \tag{1}$$

Pulmonary Oxygen Toxicity

Any PO_2 above 0.5 ATA is regarded as toxic for the pulmonary system. The amount of "units of pulmonary toxicity dose" (UPTD), with t in minutes and PO_2 in ATA, can be calculated with the function below (Bardin and Lambertsen, 1970). Many authorities regard an exposure of 615 UPTD as the "maximum safe exposure for a single dive".

$$UPTD = t^{-1.2}\sqrt{\frac{0.5}{PO_2 - 0.5}} \tag{2}$$

AUTHOR CONTRIBUTIONS

TW student of RvH: Acquisition and review of literature, drafting, and revising manuscript. PJvO co-promotor of TW: Review of literature, help with theoretical framework, writing, and reviewing concept manuscripts. RvH promotor of TW: Review of literature, help with theoretical framework, writing, and reviewing concept manuscripts.

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ORIGINAL RESEARCH
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Thirty-five Day Fluoxetine Treatment Limits Sensory-Motor Deficit and Biochemical Disorders in a Rat Model of Decompression Sickness

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According to the OECD statistical base for 2014, anti-depressants will, on average, be distributed at a rate of 62 daily doses per 1,000 inhabitants for the 25 countries surveyed (Health at a glance: Europe 2014; OECD Health Statistics; World Health Organization and OECD Health Statistics, 2014). Divers must be concerned. On another hand, divers are potentially exposed to decompression sickness including coagulation inflammation and ischemia, which can result in neurological lesions or even death. The purpose of this study is to assess whether chronic treatment with anti-depressants may represent a contraindication to the practice of an at-risk activity, such as, scuba diving, or even presents a benefit by attenuating the severity of the symptoms. We study for the first time the effect of a 35-day fluoxetine treatment (20 mg/kg) on the occurrence of decompression sickness in laboratory rats ($n = 79$). Following exposure to the hazardous protocol, there is a significant correlation between the type of treatment and the clinical status of the rats in favor of a better clinical prognosis for the rats treated with fluoxetine with a significantly higher number of No DCS status and a lower number of Severe DCS status in the Flux, compared to Controls. The treatment modifies the rat performances both significantly and favorably during the physical and behavioral tests, just like their biological and biochemical constants. After decompression, rats under treatment display lower sensory-motor deficit and lowers biochemical disorders. From a biological point of view, we conclude fluoxetine should not be seen as a contraindication for diving on the basis of anticipated increased physiological risk.

Keywords: mitochondrial DNA, venous gas emboli, oxidative stress, oxygen, depression, capillary leak

INTRODUCTION

Gas embolism following an at-risk decompression induces disseminated coagulation, systemic inflammation, and ischemia, which can cause neurological disorders or even death.

In animals, we have been able to demonstrate (Blatteau et al., 2012, 2015; Vallee et al., 2016) that fluoxetine administered at a high dose before hyperbaric exposure presents a curative interest in the event of the occurrence of decompression sickness. Several hypotheses could explain the beneficial effect of fluoxetine on DCS. Firstly, fluoxetine administered in a single high dose (50 mg/kg) has anti-inflammatory properties, as is shown by a reduction in the circulating level of inflammatory markers (Branco-De-Almeida et al., 2011; Blatteau et al., 2012, 2015). A reduction in infiltration of neutrophils and preservation of the number of platelets and red blood cells are also observed (Blatteau et al., 2012). CVA and DCS are accidents which both include ischemia. The anti-inflammatory action of fluoxetine in both cases could counter the ischemic process. Secondly, fluoxetine has been proved to be effective in post-CVA recovery (Chollet et al., 2011) due to activation of the mTOR signaling pathway, which plays an important role in cell growth and proliferation and control of synthesis of the proteins required for synaptogenesis and dendritogenesis. Thirdly, the inhibition of NMDA (NR2A) channels by fluoxetine limits the glutamatergic excitotoxicity induced by non-apoptotic cell death. This non-voltage dependent inhibition could therefore be neuroprotective (Szasz et al., 2007). Finally, the inhibition of SERT transporters, which are present on the neuronal membranes, increases the serotonin level in the synaptic cleft, which is the source of its action as an anti-depressant (Branco-De-Almeida et al., 2011), but they are also present on platelet membranes and have an anti-coagulant effect (Halperin and Reber, 2007). Blood thinning by SSRIs would therefore be beneficial in limiting prothrombotic processes. Conversely, a decompression accident affecting the brain or the spinal cord could be aggravated by a hemorrhagic transformation. Administration of fluoxetine also has the effect of dilating the small cerebral (Ungvari et al., 1999; Sanchez-Ortiz et al., 2007), and cutaneous (Lin, 1978) arteries as has been demonstrated in the rat.

Other arguments seem to indicate that fluoxetine would be an aggravating factor in DCS. Initially the inhibition of the TREK 1 channel causes dissociation of the C-terminal domain from the cell membrane and prevents the passage of potassium ions (Chen et al., 2007). This counters the reduction in the neuroprotective cell excitability exercised by TREK 1. Our previous studies, which blocked the TREK 1 channel, either by using native KO mice (Vallee et al., 2012), or by internalizing the channel pharmacologically with spadin (Vallee et al., 2016), to have "anti-depressed" mice models, have highlighted increased susceptibility to decompression sickness with an unfavorable prognosis for survival (Vallee et al., 2012, 2016). Then, Baek et al. (2015) observed that in mice treated for more than 14 days, fluoxetine increased inflammatory intestinal problems, contrary to the anti-inflammatory action observed during a single acute dose. Finally, fluoxetine also has hepatotoxic side effects because it is metabolized in the liver by demethylation into norfluoxetine (Johnson et al., 2007).

Also, it was important to respond to the toxicity of a chronic treatment by fluoxetine on the risk of DCS. In fact, fluoxetine, an SSRI (selective serotonin reuptake inhibitor) initially distributed under the brand, Prozac (Lilly France S.A.S.), is a chronically prescribed anti-depressant. Depression, affecting 3–5% of the global population (Lepine and Briley, 2011) raises a very serious question: is taking an anti-depressant a contraindication to practicing an at-risk activity such as, scuba diving? Depression is not as such a contraindication to the practice of scuba diving, even if this activity requires good psychic stability given the risks it carries. However, we will not focus on the attentiveness aspect this sport requires for monitoring parameters such as, depth control or adherence to decompression time. Conversely this work concerns the effect of anti-depressant medication in the context of decompression sickness. This study is the first to assess the somatic effect of a chronic treatment with fluoxetine at the normal doses on the occurrence of decompression sickness in laboratory rats.

The purpose of this study is to assess whether chronic treatment with anti-depressants may represent a contraindication to the practice of an at-risk activity, such as, scuba diving, or even presents a benefit by attenuating the severity of the symptoms. On a rat model treated with fluoxetine for 35 days, we measured the clinical and biochemical effects of hyperbaric exposure causing DCS.

MATERIALS AND METHODS

The experimental design can be follow in **Figure 1**.

Animals and Ethical Statement

All procedures involving experimental animals were in line with European Union rules (Directive 2010/63/EU) and French law (Decree 2013/118). The Ethics Committee of the Institut de Recherche Biomédicale des Armées approved this study. According to our Animal Care Committee, a scoring system inspired by Swiss veterinary guidelines was implemented to ensure the welfare of animals. For each animal, a dedicated observer scored the stress or pain (from 0 to 3) relating to specific

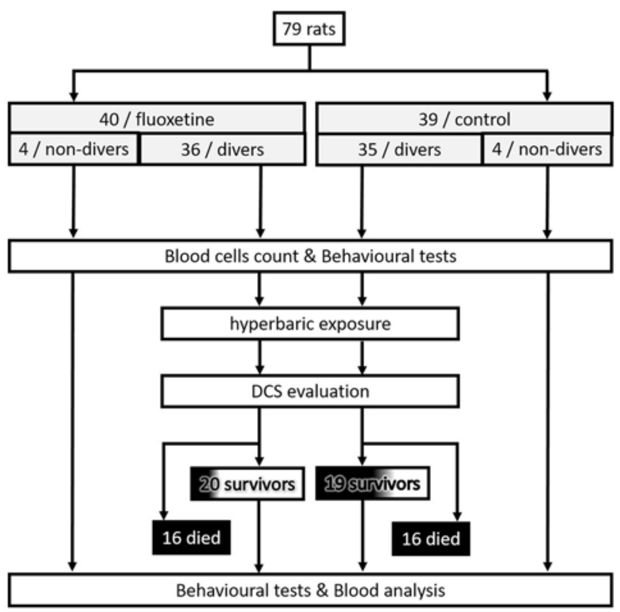

FIGURE 1 | Flowchart describing the experimental design.

criteria listed on a form (see Supplementary Material for more information). Degree 3 pain (very painful) in one case or a total score of 12 in the table were the ethical endpoints. On this sheet, the most commonly found were: vocalizing, aggression or withdrawn behavior, reduction in exploratory behavior, licking, closed eyes, tears, bubbles in the eyes, high respiratory rate, runny nose, fur bristling, labored breathing, convulsions, paralysis, difficulty moving, and problems with the fore or hind limbs (classified as motor disorders). In this study, no score reached 12 and there was no need to cull the animal based on these criteria. Actually, animals displaying Degree 3 convulsions died very rapidly. At the end of the experiment, rats were anesthetized first with halothane (5% in oxygen, Halothane, Belamont, France) in order to gain time and to minimize stress, and then with an intraperitoneal injection of a mixture of 16 mg/kg xylazine (Rompum® 2%, Bayer Pharma) and 100 mg/kg ketamine (Imalgène® 1000, Laboratoire Rhône). Our investigator (NV) is associated with Agreement Number 83.6 delivered by the Health and Safety Directorate of our department, as stated in the French rules R.214-93, R-214-99, and R.214-102.

Rats were housed in an accredited animal care facility. Rats were kept in group cages (two per cage) both during rest and during the experiments and maintained on a regular day (6:00 am–6:00 pm)/night (12 h) cycle. Food (AO3, UAR) and water were provided ad libitum and the temperature was kept at $22 \pm 1^{\circ}$C.

The rats were separated into two groups: 39 control rats (Ctrl) and 40 rats (Flux) treated with an anti-depressant daily. The Flux rats drank water supplemented with fluoxetine (Fluoxetine Zentiva, Laboratoires Sanofi, 20 mg dispersible tablets) for 36 days. The tablets were diluted so as to correspond to a dose of 20 mg/kg of fluoxetine. The dose was regularly adjusted depending on the volume of water drunk and the weight of the rats. Drinking bottles were weighed every day to control hydration, and changed every 2 days. The rats were all weighed once a week to check their weight gain. They were 12 weeks old at the time of the hyperbaric protocol.

Hyperbaric Exposure

Samples of eight rats (four per cage) from the flux pool and the ctrl pool were subjected to the hyperbaric protocol in a 200-l tank fitted with three observation ports. The rats were free to move around the cage.

The compression protocol involved two ramps of pressure increase, first at 0.1 atm/min up to 1 atm, followed by 1 atm/min up to 9 atm; 9 atm corresponds to the pressure where animals were kept for 45 min before decompression. The decompression rate was 60 atm/min up to the surface. Compression and decompression were automatically controlled by a computer linked to an analog/digital converter (NIUSB-6211, National Instrument, USA) with two solenoid valves (Belino LR24A-SR, Switzerland) and a pressure transmitter (Pressure Transmitter 8314, Burket Fluid Control System, Germany). The software was programmed on a DasyLab (DasyLab National Instrument, USA) by our engineer. The software also controlled the temperature and oxygen rate. Compressed air was generated using a diving compressor (Mini Verticus III, Bauer Comp, Germany) coupled to a 100-l tank at 300 bars. The oxygen analyzer was based on a MicroFuel electrochemical cell (G18007 Teledyne Electronic Technologies/Analytical Instruments, USA). The temperature inside the tank was monitored using a platinum resistance temperature probe (Pt 100, Eurotherm, France).

Water vapor and CO2 produced by the animals were captured with soda lime (<300 ppm captured by the soda lime) and seccagel (relative humidity: 40–60%). Gases were mixed by an electric fan. The day–night cycle was respected throughout.

Behavior and Clinical Observations

At the end of decompression, the rats were first observed over 30 min in an open field. The possible occurrence and the time to onset of the following manifestations were recorded: respiratory distress, moving difficulties, convulsions, and death. Specific motor sensory tests were performed 30 min after the hyperbaric exposure. Beam walk, righting reflex, dynamic weight-bearing (DWB) assessment, and thermal stimulation tests were performed within 2 h of the dive. Beam Walk and DWB tests were also performed 2 weeks before the dive.

The beam walk performance was tested using seven wooden planks of different widths (7.7–1.7 cm), which were 1.5 m long and elevated 110 cm above the floor. Rats were placed on the widest plank and the ability to cross the plank without the feet slipping in two trials was observed. The procedure was repeated on successively narrower planks. The narrowest plank that a rat could cross without slipping was recorded i.e., No.7 for 1.7 cm, No.6 for 2.7 cm, No.5 for 3.7 cm, No.4 for 4.7 cm, No.3 for 5.7 cm, No.2 for 6.7 cm, and No.1 for 7.7 cm.

The righting reflex was elicited by holding the rat in one hand and turning it over on its back 7 or 8 cm above a covered table surface. The way the animal tried to regain its original position with its feet down was studied. To avoid injuring the rats when testing for righting, the animals with a motor score below 3 were tested for their ability to turn by dropping them from a height of 2 or 3 cm. Righting: 0 No attempt to right itself, 1 Weak or delayed attempt to right or rights itself in the direction of the roll, 2 Normal righting counter to the direction of roll.

The DWB distribution was assessed by a biometric floor instrumented cage (DWB, Bioseb Development, Vitrolles, France). The device consisted of a Plexiglas box (width 22 $\times$ length 22 $\times$ height 30 cm) with a calibrated weight transducer pad composed of 44 $\times$ 44 captors (TEKSCAN, Boston, MA). The rat was allowed to move freely within the box for 4 min each. Using a synchronized video recording and a scaled map of the stimulated captors, each of the rat's paws was validated by an observer and identified as a unique paw. The pressure exerted by each paw (in grams) was only measured when the four paws were in contact with the biometric floor and then normalized by the total weight of the rat. Ratios distinguishing the forepaws vs. hind paws and the right vs. left side were calculated to assess the weight-bearing distribution: (1) the sum of the right and the left forepaws (F) was normalized by the sum of the right and the left hind paws (H) (F/H ratio); (2) the left forepaw (LF) was normalized by the right forepaw (RF) (LF/RF ratio); (3) the left hind paw (LH) was normalized by the right hind paw (RH) (LH/RH ratio). The time spent on three paws and on four paws (in s) was also measured to determine the solicitation of the paws in postural stability. The time period spent on four paws was normalized by the time period spent on three paws (4P/3P ratio).

The thermal stimulus was delivered using the Hargreaves technique (7371 Plantar Test from Ugo Basile S.R.L. Biological Research Apparatus, Comerio, Italia). Rats were placed in a clear Plexiglas box resting on an elevated glass plate. Following acclimatization, a radiant beam of light at 60°C was positioned under the hind paw, and the average time for the rat to remove the paw from the thermal stimulus over three trials was electronically recorded in seconds as the paw withdrawal latency (PWL). The intensity of the beam was set to produce basal PWL's of ∼8–10 s. A maximal PWL of 25 s was used to prevent excessive tissue damage due to repeated application of the thermal stimulus.

Anesthesia and Sacrifice

All rats were anesthetized 120 min after surfacing (after behavioral and clinical tests) first with isoflurane 4% (Isoflo, Axience SAS, France) to minimize stress, and then by intraperitoneal injection of a mixture of 16 mg/kg xylazine (Rompum® 2%, Bayer Pharma, Germany), 100 mg/kg ketamine (Imalgene® 1000, Rhône laboratory, France) and 1.65 mg/kg of acepromazine (Calmivet®, Vétoquinol, France).

Rats were kept for aortic blood sampling for biochemistry and then sacrificed by injecting pentobarbital (200 mg/kg ip, Sanofi Santé, France).

Full Blood Analysis

Blood cells counts were analyzed in 15 μL samples taken from the tip of the tail and diluted in the same volume of 2 mM EDTA (Sigma, France). Blood tests were carried out using an automatic analyzer (ABCvet, SCIL Animal Care Company, France) on samples taken 30 min before the dive and again 120 min afterwards. The second test values were corrected according to the hematocrit variation.

Under general anesthesia and 2 h after the end of the hyperbaric protocol, blood samples were collected from the abdominal aorta with a blood collection set (Vacutainer Brand, Becton Dickinson, Meylan, France) for biochemistry, cytokines, and circulating oligonucleotides analysis.

Blood biochemistry [Na^+, K^+, Ca^{2+}, creatinine kinase, glucose, blood urea nitrogen (BUN), creatinine, transaminase, bilirubin, albumin, globulin, total proteins, lactate, cholesterol, triglycerides] was conducted with automatic analyzers (Vetscan VS2, Abaxis Veterinary Company, France; Reflovet Plus SCIL Animal Care Company, France; and Accutrend Plus, Roche Diagnostic, USA) on lithium heparin (4 mL, Lithium Heparin 68 I.U., BD Vacutainer, Becton Dickinson, Plymouth, UK) blood samples. Hemolytic samples were rejected.

Circulating Oligonucleotides

Circulating oligonucleotides were quantified from blood samples (EDTA tubes: 2 mL, K2E 3,6 mg, BD Vacutainer, Becton Dickinson, Plymouth, UK). 3 detection processes were performed.

The levels of circulating oligonucleotides in the blood plasma were measured (1) in accordance with the method previously used in the laboratory (Vallee et al., 2013) including double centrifuging before the extraction/purification phase, which was followed by a qPCR targeting a mitochondrial locus (marked mDNA), and which includes (2) the measurement by spectrophotometry post-extraction usually used to adjust the parameters of the qPCR, and (3) by direct measurement at the different stages (centrifuging/extraction/amplification) using a fluorochrome targeting the double strands of DNA without distinction.

Quantification of oligonucleotides as previously performed (Vallee et al., 2013) in blood, required immediate centrifuging for 10 min at 1,500 g at 4°C, and a second one of supernatants for 10 min at 20,000 g at 4°C. The samples collected were stored at −80°C until analysis.

Five microliters of synthetic DNA (Yakima Yellow-BHQ-1™ probe, Eurogentec, Seraing, Belgium), for internal normalization, was added to 140 μL plasma samples before extraction. DNA was extracted using a QIAcube automat (Qiagen, Venlo, Nederland) and the NucleoSpin RNA Virus kit (Macherey-Nagel, Düren, Germany), according to the manufacturer's instructions.

Quantitative PCR was carried out with a LightCycler 480 II (LightCycler 480 Software Release; Roche Diagnostics, Mannheim, Germany) on 2 μL DNA extract added to 8 μL reaction mixture. For the negative controls, water was substituted for the extract. The reaction mixture for mitochondrial DNA amplification contained 1 μL water, 1 μL primers F and R (1 μM; Eurogentec, Seraing, Belgium), and 5 μL of an amplification mixture SyberGreen (Go Taq qPCR Master Mix 2X, Promega, Madison, USA), mitochondrial primers [mitochondrial primer forward (F) (5′ ACC TCG ATG TTG GAT CAG 3′); mitochondrial primer reverse (R) (5′ TAG ATA GAA ACC GAC CTG G 3′)]. The reaction mixture for internal control amplification contained 2 μL water, 1 μL primers delivered by the manufacturer (Eurogentec, Seraing, Belgium), and 5 μl of an amplification mixture SyberGreen (Go Taq qPCR Master Mix 2X, Promega, Madison, USA). Thermocycler settings were: initialization step 94°C/10 min (denaturation step 94°C/15 s; annealing 55°C/20 s; and elongation step 72°C/35 s) × 45 cycles; pre-melting 95°C/10 s, melting 55°C/10 s then up to 95°C, cooling 37°C/30 s. All assays were carried out in duplicate. Specificity was checked by melting curve analysis, as described previously.

DNA double strand fluorometric measurements were performed on samples after both centrifugation and extraction. Four microliters of superrnatant or extract was mixed with 196 μL of a reaction mixture (QuantiFluor ONE dsDNA Dye, Promega, Madison, USA), incubated according to the manufacturer's instruction, and measured with Quantus Fluorometer (Promega, Madison, USA).

Spectrophotometric quantification from 1.5 μL DNA extract was performed after extraction using Nanodrop-1000 and its software (Thermo Scientific, Wilmington, USA).

Clinical Status

The Lethal DCS status includes rats which died in the 2 h following the end of the hyperbaric protocol. The Severe DCS status was attributed when the rat presented severe locomotor signs in the form of paresia or paralysis of at least 1 limb and/or a beam walk test score reduced by at least two points. The DCS status includes rats without serious motor symptoms, with high transaminase values (±10% of extreme values for the controls) suggesting the presence of bubbles in the liver (L'abbate et al., 2010; Vallee et al., 2016). The other rats were considered as No DCS.

Statistical Analyses

Individual blood cell count data were calculated as the percentage change from baseline (the measurement before hyperbaric exposure). Numerical data points are expressed as mean and standard deviation. A contingency table was used for independence and association tests coupled with the χ^2 significance test. Different groups were compared using the Mann-Whitney (MW) test and matched comparisons within groups were analyzed using the Wilcoxon (W) test. Multiple comparisons were performed using the Kruskal-Wallis test followed by the Bonferroni-Dunn *post-hoc* test. The Kolmogarov-Smirnov test was used to compare the distribution of water consumption over the time of treatment. The significance threshold was 5% and *p*-values below were assumed to indicate significant differences.

RESULTS

Monitoring of the Animal Population

Over the 36 days of treatment, the Flux rats drank more than the Ctrl rats in terms of distribution with a lower consumption in the first 5 days and then a higher consumption from the 10th day of treatment (Kolmogorov–Smirnov$_{Ctrl/Flux}$, $n = 39/40$, $p < 0.0001$) and also in terms of average quantity per day (Beverage: Ctrl = 33.1 ± 1.5 mL, Flux = 35.2 ± 3.7 mL; MW$_{Ctrl/Flux}$, $n = 39/40$, $p < 0.0001$).

The weights of the rats subjected to the hyperbaric protocol were similar (Weight: Ctrl = 348.3 ± 19.2 g, Flux = 340.1 ± 22.4 g; MW$_{Ctrl/Flux}$, $n = 35/36$, $p = 0.099$). Weight had no incidence on the clinical data (KW$_{SevereDCS/DCS/noDCS/Lethal}$, $n = 13/6/20/32$; $p = 0.151$).

Clinical Status of the Rats Following the Dive

In the control rats exposed to the hyperbaric protocol (**Figure 2**), there was 45.7% mortality at 2 h (Lethal DCS), 28.6% of rats presented symptoms of severe but not lethal DCS (Severe DCS), 11.4% moderate DCS, and 14.2% of rats No DCS. In the rats treated with fluoxetine, 44.4% of animals died, 8.3% were classified Severe DCS, 5.5% DCS, and 41.6% No DCS.

There is a significant correlation between the type of treatment and the clinical status of the rats (contingency table $\chi^2_{Ctrl/Flux} = 9.424$, $n = 35/36$, $p = 0.021$), in favor of a better clinical prognosis for the rats treated with fluoxetine with a significantly lower number of No DCS status in the Ctrl and a significantly lower number of Severe DCS status in the Flux.

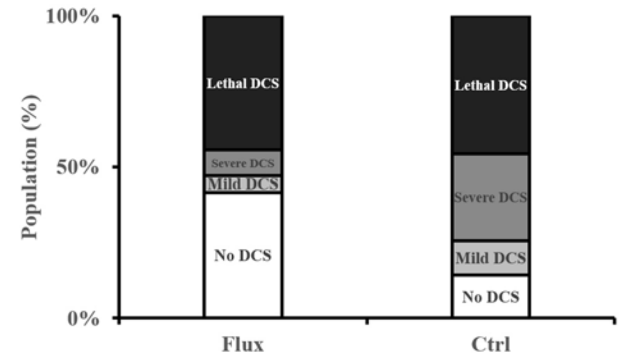

FIGURE 2 | Clinical status after the hyperbaric protocol exposure.

Clinical and Behavioral Analyses

Before the dive the Flux rats experienced slightly more difficulties of significance on moving on the beam (**Figure 3A**) above the void (Beam: Ctrl = 6.88 ± 0.32 vs. Flux = 6.55 ± 0.50; MW$_{Ctrl/Flux}$, $n = 35/36$, $p = 0.0002$). Following the dive, the performance of Ctrl and Flux survivors had deteriorated with a global locomotor impairment (Beam: Ctrl = 4.94 ± 2.00 vs. Flux = 5.85 ± 1.18; Ctrl, W$_{before/after}$, $n = 19/19$, $p < 0.0001$; Flux, W$_{before/after}$, $n = 20/20$, $p = 0.039$) more severe in the Ctrls (delta Beam: Ctrl = 2.00 ± 2.05 vs. Flux = 0.75 ± 0.37; MW$_{Ctrl/Flux}$, $n = 19/20$, $p = 0.039$). As expected, the levels of performance deterioration were higher in the Severe DCS rats than in the DCS and No DCS and No Divers rats, all treatments included (delta Beam: Severe DCS = 3.46 ± 1.61, DCS = 0.50 ± 0.54, No DCS = 0.25 ± 0.64, No Divers = 0.37 ± 0.74: KW$_{SevereDCS/DCS/NoDCS/NoDivers}$, $n = 13/6/20/8$, $p < 0.0001$).

The performance deterioration levels for the righting reflex test (**Figure 3B**) are higher in Severe DCS rats than in No DCS rats, all treatments included (delta reflex: No DCS = 0.00 ± 0.00 vs. Severe DCS = 0.30 ± 0.630: KW$_{SevereDCS/DCS/noDCS/NoDivers}$, $n = 13/6/20/8$; $p = 0.042$), without there being any difference related to the treatment.

For the infrared stimulation (**Figure 3C**) in the palm of the hind paw, reduced algesic sensitivity in the No Divers Ctrl rats was observed compared to the No Divers Flux rats only for the left paw (InfraRed stimulation on left paw; L Paw No Divers: Ctrl = 12.22 ± 3.78 s vs. Flux = 5.97 ± 1.61 sec; KW$_{Ctrl/NoDiversCtrl/Flux/NoDiversFlux}$, $n = 19/4/20/4$; $p = 0.040$;), but without significant lateralization in non-exposed rats (No divers MW$_{CtrlR/L}$, $n = 4/4$, $p = 0.312$; MW$_{FluxR/L}$, $n = 4/4$, $p = 0.191$). This difference did not appear in rats that had dived, whatever the treatment or symptoms (Left Paw: Ctrl KW$_{NoDivers/SevereDCS/DCS/NoDCS}$, $n = 4/10/4/5$, $p = 0.208$, Flux KW$_{NoDivers/SevereDCS/DCS/NoDCS}$, $n = 4/3/2/15$, $p = 0.258$; Right Paw: Ctrl KW$_{NoDivers/SevereDCS/DCS/NoDCS}$, $n = 4/10/4/5$, $p = 0.930$, Flux KW$_{NoDivers/SevereDCS/DCS/NoDCS}$, $n = 4/3/2/15$, $p = 0.137$). Globally, there is no greater difference between the no diver rats and the divers (Left Paw MW$_{CtrlDivers/NoDivers}$, $n = 19/4$, $p = 0.080$; Left Paw MW$_{FluxDivers/NoDivers}$, $n = 20/4$, $p = 0.121$; Right Paw MW$_{CtrlDivers/NoDivers}$, $n = 19/4$, $p = 0.598$; Right Paw MW$_{FluxDivers/NoDivers}$, $n = 20/4$, $p = 0.510$).

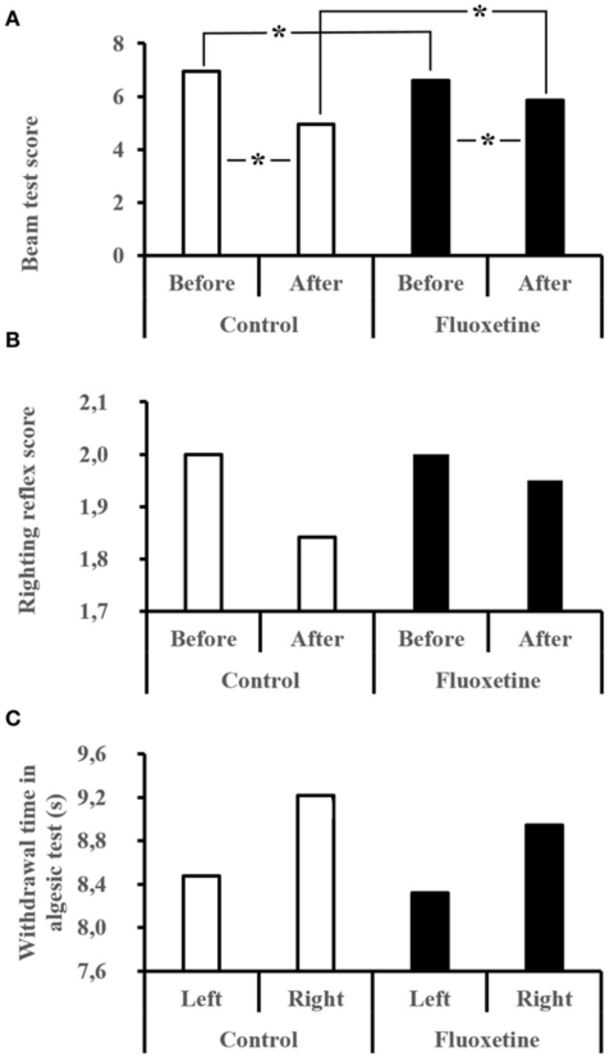

FIGURE 3 | (A–C) Behavioral performances after the hyperbaric exposure. **(A)** Performance for the beam test. The best score is 1 and the worst is 7. **(B)** Righting reflex. The best score is 2 and the worst is 0. **(C)** Algesia test performed with infrared stimulation on left and right hind paws. Average time for the rat to remove the paw from the thermal stimulus. *Significant difference ($p < 0.05$).

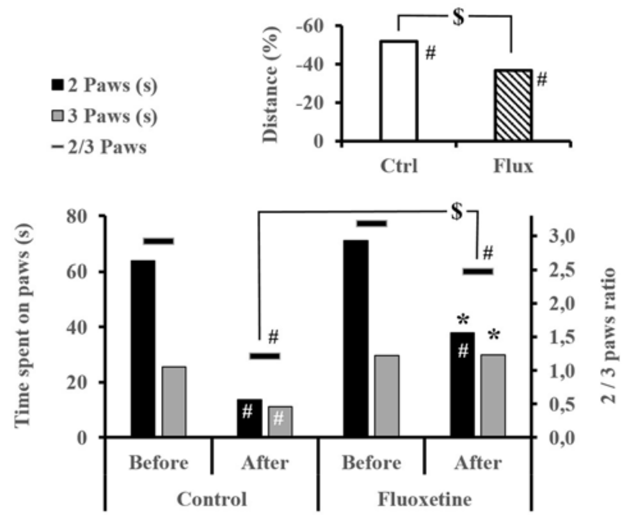

FIGURE 4 | Exploratory behavior by video tracking. Time spent on 2 or 3 paws and its ratio, and the mean distance walk in each group, within the video box for 4 min. #Intragroup significant difference ($p < 0.05$) between before and after the dive. *Significant difference ($p < 0.05$) between groups after the dive for the evolution of the same parameter. $Trend ($p < 0.01$) at the intergroup level.

Before the dive, the treatment did not influence exploratory behavior (**Figure 4**), in terms of time passed on 2 or 3 paws (2 paws $KW_{Ctrl/Flux}$, $n = 39/40$,$p = 0.247$; 3 paws $KW_{Ctrl/Flux}$, $n = 39/40$, $p = 0.499$; ratio 2/3 Paws $KW_{Ctrl/Flux}$, $n = 39/40$, $p = 0.590$) or, in terms of distance covered (distance $KW_{Ctrl/Flux}$, $n = 39/40$, $p = 0.499$). Performance were significantly deteriorated after the dive, except for the time spent on 3 paws in the flux pool (**Figure 4**). Globally the Severe DCS rats have inhibited exploratory behavior in comparison to the No DCS (2 paws $KW_{SevereDCS/DCS/NoDCS}$, $n = 13/6/20$, $p = 0.001$; ratio 2/3 paws $KW_{SevereDCS/DCS/NoDCS}$, $n = 13/6/20$, $p < 0.0001$). At intragroup level, this same difference is only found in non-treated rats (Ctrl 2 paws $KW_{NoDivers/SevereDCS/DCS/NoDCS}$, $n = 4/10/4/5$, $p = 0.011$; Ctrl ratio 2/3 paws $KW_{NoDivers/SevereDCS/DCS/NoDCS}$, $n = 4/10/4/5$, $p = 0.006$). During video tracking after the dive, the surviving Flux rats explored their space more significantly than the surviving Ctrl rats because they spent more time on two or three paws with a tendency to travel further (2 paws $KW_{Ctrl/Flux}$, $n = 19/20$,$p = 0.014$; 3 paws $KW_{Ctrl/Flux}$, $n = 19/20$, $p = 0.005$; ratio 2/3 Paws $KW_{Ctrl/Flux}$, $n = 19/20$, $p = 0.096$; distance $KW_{Ctrl/Flux}$, $n = 19/20$, $p = 0.068$).

Full Blood Count

Before the dive a mild effect from the chronic treatment with fluoxetine may be seen. Also it appears that the Flux animals have increased Mean Corpuscular Volume (KW_{MCV}, $n = 39/40$, $p = 0.029$) and Mean Corpuscular Hemoglobin (KW_{MCH}, $n = 39/40$, $p = 0.030$), which could be linked to a liver disorder (Blann and Ahmed, 2014). Furthermore, no significant rheological predisposition has been observed (in terms of prior Full Blood Count) related to the occurrence of an accident with hindsight, particularly concerning the hematocrit/hydration.

The second sampling carried out after the hyperbaric exposure showed rheological changes (**Figure 5** and **Table 1**).

After the dive an increased hematocrit was observed, i.e., a hemoconcentration in all the animals, which has involved correcting the count values (marked *) for each individual. A significant difference was revealed between the raw values related to clinical status with a higher hemoconcentration in the Severe DCS compared to the No DCS (HCT: No DCS = 55.5 ± 13.7%; Severe DCS = 67.0 ± 13.4%; $KW_{NoDivers/SevereDCS/DCS/NoDCS}$, $n = 8/13/6/20$, $p = 0.047$), which seems to support a threshold value of severe pathology in these precise experimental conditions. However, there is no significant difference in the proportion of this variation which can be directly attributed to the treatment ($KW_{Ctrl/Flux/CtrlNoDivers/FluxNoDivers}$, $n = 19/20/4/4$,

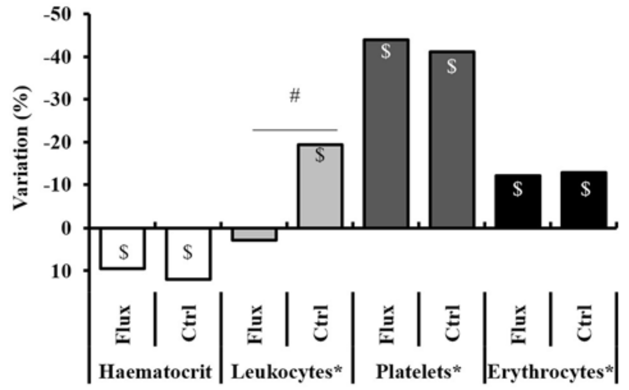

FIGURE 5 | Full blood count variation, between before and after the hyperbaric exposure. Ctrl, control; Flux, Fluoxetine; ND, non-diver. *Corrected according to the individual hematocrit variation. $^{\$}$Significant difference ($p <$ 0.05) between before and after the dive for the same parameter. $^{\#}$Significant difference ($p < 0.05$) between groups after the dive for the evolution of the same parameter.

TABLE 1 | Full blood count variation after hyperbaric exposure.

***p*-Values for before/after comparisons (Wilcoxon test)**	**Ctrl** *n* = 19		**Flux** *n* = 20		**Ctrl ND** *n* = 4		**Flux ND** *n* = 4	
Hematocrit	**<0.0001**	↑	**<0.0001**	↑	**0.029**	↑	**0.029**	↑
Leucocytes*	**0.003**	↓	0.0867	⟷	0.100	⟷	0.100	⟷
Platelets*	**<0.0001**	↓	**<0.0001**	↓	**0.029**	↓	**0.029**	↓
Erythrocytes*	**<0.0001**	↓	**<0.0001**	↓	0.100	⟷	0.100	⟷
MCH	0.066	⟷	**0.0004**	↓	0.146	⟷	0.559	⟷
MCV	**<0.0001**	↑	**<0.0001**	↑	**0.027**	↑	**0.026**	↑

*Corrected according to the individual hematocrit variation.
Ctrl, control; Flux, Fluoxetine; ND, non-diver.
Bold numbers note significant variations.

$p = 0.593$) or the clinical data ($KW_{NoDivers/SevereDCS/DCS/NoDCS}$, $n = 8/13/6/20$, $p = 0.145$), which suggests that the variation in the hematocrit is potentially due to the stress of the second sample collection or the hyperbaric protocol (simulated or real) on the one hand, and also to the effects of the hyperbaric exposure on the other hand.

Post-dive and after an individual correction of values due to the hematocrit variation, there is a reduction in the number of circulating platelets in all groups, without there being a significant difference in their raw value or the proportion of these variations between the different groups (Platelet count: $KW_{Ctrl/Flux/CtrlNoDivers/FluxNoDivers}$, $n = 19/20/4/4$, $p = 0.646$; $KW_{Nodivers/SevereDCS/DCS/NoDCS}$, $n = 8/13/6/20$, $p = 0.766$; Platelets%: $KW_{Ctrl/Flux/CtrlNoDivers/FluxNoDivers}$, $n = 19/20/4/4$, $p = 0.613$; $KW_{Nodivers/SevereDCS/DCS/NoDCS}$, $n = 8/13/6/20$, $p = 0.678$), which suggests that these variations are potentially due to the stress of the sample collection or the hyperbaric protocol (simulated or real).

There is a reduction in the number of circulating red cells only post-dive, without there being a significant difference in their raw value or the proportion of these variations between the different groups (RBC count: $KW_{Ctrl/Flux/CtrlNoDivers/FluxNoDivers}$, $n = 19/20/4/4$, $p = 0.657$; $KW_{Nodivers/SevereDCS/DCS/NoDCS}$, $n = 8/13/6/20$, $p = 0.645$; RBC%: $KW_{Ctrl/Flux/CtrlNoDivers/FluxNoDivers}$, $n = 19/20/4/4$, $p = 0.832$; $KW_{Nodivers/SevereDCS/DCS/NoDCS}$, $n = 8/13/6/20$, $p = 0.550$), which suggests that these variations are potentially due to the stress of the hyperbaric protocol (real). It is similar for hemoglobin.

A significant reduction in the number of circulating leukocytes is observed in the Ctrl but not in the Flux, without differences appearing in the No Diver rats. In proportion, the reduction in the number of circulating leukocytes is much less significant ($KW_{Ctrl/Flux/CtrlNoDivers/FluxNoDivers}$, $n = 19/20/4/4$, $p = 0.024$) in the surviving Flux ($2.78 \pm 33.2\%$) than in the surviving Ctrl ($-19.3 \pm 23.7\%$) or the No Diver Flux. Factually, this variation cannot be attributed directly to the hemoconcentration, or to the stress caused by handling, but to the hyperbaric protocol (real) and the consequences of it on the one hand, and to the effect of fluoxetine on the other hand since there is no difference in proportion linked to the clinical status ($KW_{Nodivers/SevereDCS/DCS/NoDCS}$, $n = 8/13/6/20$, $p = 0.228$).

When the clinical data is examined, the main post-dive rheological difference lies in the raw value of the hematocrit, which is higher in the Severe DCS compared to the NoDCS. When focus is placed on the effect of the treatment, fluoxetine seems to reduce the recruitment of circulating leukocytes after hyperbaric exposure.

Blood Biochemistry

Following the hyperbaric protocol the biochemistry was carried out using blood taken from the vena cava. No significant difference was observed between the two groups of non-exposed and NoDCS rats. It was similar when the NoDivers rats were compared between themselves (Ctrl and Flux), apart from the globulin which was higher in the Flux than the Ctrl (globulin NoDivers $KW_{Ctrl/Flux}$; $n = 4/4$; $p = 0.019$) which is seen as a mild (pro)inflammatory syndrome or liver failure.

Globally (**Figure 6** and Supplementary Table 2 for statistical analysis) the DCS rats present a low level of total proteins suggesting either hemodilution (but there is an increase in the hematocrit) or a protein leak due to a capillary leak or a renal lesion. These animals also present high levels of uric acid, transaminases and lactate, which taken together could be linked to hemoconcentration. Taken independently, the latter three elements suggest a defect in glomerular filtration, hepatic impairment and anaerobic functioning, respectively. As was expected, the SevereDCS presented irregularities for the same elements with, in addition, lower concentrations of albumin, creatinine, and globulin (a toxic, hydrophobic breakdown product of red blood cells that is bound to and carried by albumin), which accompanied the global reduction in the level of total proteins already recorded in the DCS statuses, confirming the capillary leak or glomerular lesion. A glomerular lesion could also explain the high levels of circulating urea. In addition, the high levels of transaminases lead to a suspicion of a liver disorder, possibly with the presence of bubbles in the liver. More seriously,

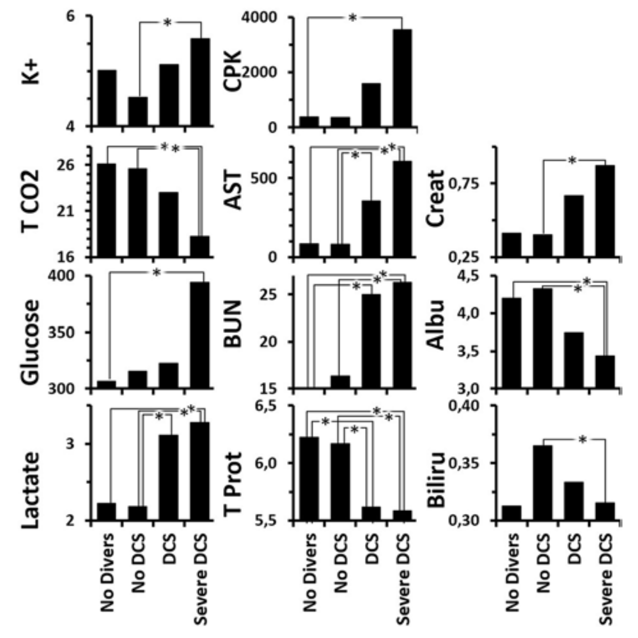

FIGURE 6 | Analysis of blood biochemistry differences after the hyperbaric protocol according to the clinical status of rats. K+ (mmol/L), T CO_2 (mmol/L), Glucose (mg/dL), Lactate (mmol/L), CPK/Creatinine Kinase (U/L), AST/Transaminase (U/L), BUN/UREA (mg/dL), Total Protein (g/dL), Creatinine (mg/dL), Albumin (g/dL), Bilirubin (mg/dL). *Significant difference in *post-hoc* analysis ($p < 0.05$) (see Supplementary Table 2 for statistical details).

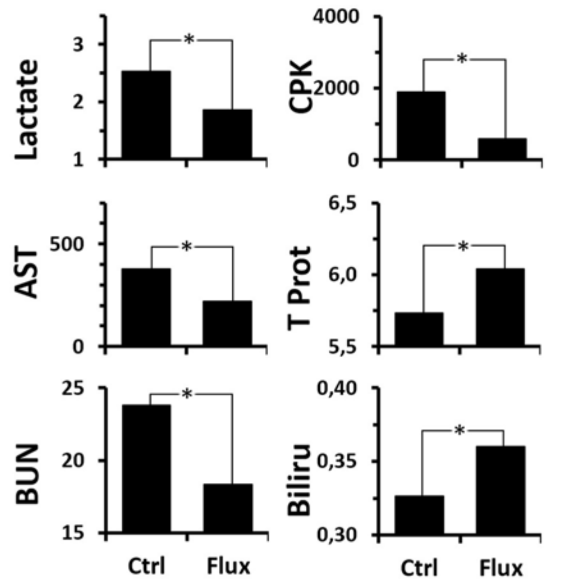

FIGURE 7 | Analysis of blood biochemistry differences after the hyperbaric protocol according to rat treatments. Lactate (mmol/L), CPK/Creatinine Kinase (U/L), AST/Transaminase (U/L), BUN/UREA (mg/dL), Total Protein (g/dL), Bilirubin (mg/dL). *Significant difference ($p < 0.05$) (see Supplementary Table 3 for statistical details).

the joint reduction in the TCO_2 and the increase in the levels of glucose and lactate lead us to consider a defect in the respiratory chain accompanied by cell breakdown, explained by high levels of potassium and CPK (muscle or heart straining) on the other hand. It all suggests a multi-organ failure.

When more particular interest is taken in the actual effect of the chronic treatment with fluoxetine on the survivors (**Figure 7** and Supplementary Table 3 for statistical analysis), a global improvement in the biochemical profile is observed compared to the Ctrl. Initially it appeared that recourse to anaerobic respiration (production of lactate) is less pronounced. It also appears that the hepatic and renal malfunctions (transaminases and urea) associated with capillary leaks (Total Protein, Bilirubin) and, more generally, the muscle and cell stress (CPK) are less significant.

Inflammatory Cytokines

The non-exposed rats treated with fluoxetine tend to present lower levels of circulating IL-1 beta than animals which did not receive treatment, whether non-exposed or not (IL-1 beta: $KW_{Ctrl/Flux/CtrlNoDivers/FluxNoDivers}$, $n = 19/20/4/4$, $p = 0.091$; / *post-hoc* $_{CtrlNoDivers/FluxNoDivers}$ $p = 0.016$;/*post-hoc* $_{Ctrl/FluxNoDivers}$ $p = 0.044$).

Circulating Oligonucleotides

In order to understand better the interest of the different assay methods, it was necessary to present the results according to two clinical classifications, the first technique (**Figure 8A** and Supplementary Table 4 for statistical analysis) being more restrictive than the second (**Figure 8B** and Supplementary Table 5) covering all the rats which had decompression sickness (All DCS = DCS + Severe DCS). So it appears generally that, amongst the animals exposed to the decompression protocol, the animals which experienced decompression sickness (SevereDCS, DCS, or All DCS) present higher circulating DNA values than animals that are unscathed (NoDCS) (**Figures 8A,B** and Supplementary Tables 4, 5), whatever the method envisaged.

However, it should be noted that there is a discordance in the DNA measurements between the measurements made with the Quantus fluorometer measuring the total double strand quantity (histograms with dots), which is expressed as the trend AllDCS>NoDCS>NoDivers, and those that target the mitochondrial DNA (black histograms) which tends to conclude that NoDivers>AllDCS>NoDCS **(Figures 8A,B)**.

By taking a more particular interest in the effect of the treatment in the survivors (**Figure 8C** and Supplementary Table 6 for statistical analysis), it is generally noted that the Ctrl rats have higher levels of circulating oligonucleotides compared with rates having received fluoxetine, or No Diver rats.

DISCUSSION

Like previous studies the dive protocol has indeed caused cases of DCS (Pontier et al., 2008; Blatteau et al., 2012, 2015; Vallee et al., 2013; De Maistre et al., 2016), globally seen as an alteration in physical and behavioral performances accompanied by a deterioration of biological constants, all groups included. However, chronic treatment with fluoxetine modifies

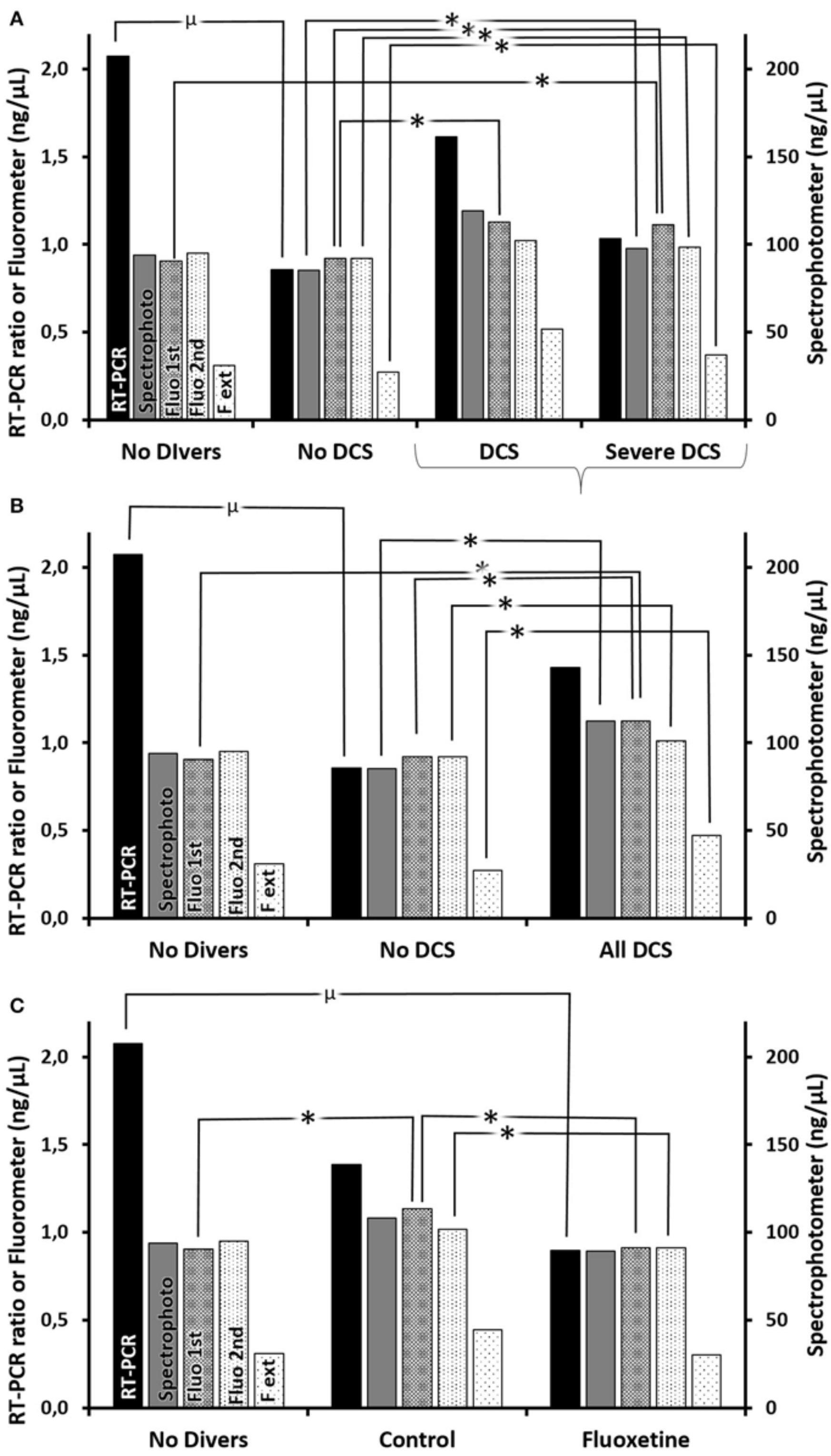

FIGURE 8 | (A–C) Circulating oligonucleotides according to clinical status **(A)** with all DCS together **(B)** or according to treatment. **(C)** Quantifications of oligonucleotides were performed (1) by RT-PCR (black histograms) including double centrifuging before the extraction/purification phase, which was followed by a qPCR targeting mDNA and an internal standard (i.e., ratio), and which includes (2) (gray histograms) the measurement by spectrophotometry (nanodrop) post-extraction usually used to adjust the parameters of the qPCR, and (3) (histograms with dots) by direct fluorimetric measurement at the different stages (1st centrifuging → Fluo 1st, 2nd centrifugating → Fluo 2nd, extraction/amplification → F ext) targeting the double strands of DNA without distinction. *Significant difference, or μ a trend, in *post-hoc* analysis ($p < 0.05$) (see Supplementary Tables 4–6 for statistical details).

the rat performances both significantly and favorably during the physical and behavioral tests, just like their biological and biochemical constants, following exposure to the hazardous protocol. It does not, however, offer such as, beneficial effect as a high dose of fluoxetine of 50 mg/kg administered on an *ad hoc* basis (Blatteau et al., 2015).

Before the dive a mild effect from the chronic treatment with fluoxetine can be seen. Also, it appears that the Flux animals have an increased Mean Corpuscular Volume and a Mean Corpuscular Hemoglobin, as well as increased globulin levels. This resulted in a mild (pro) inflammatory syndrome (also in line with the increase in pro-inflammatory IL-1 betas) or mild liver failure. This mild inflammation could explain why the algesic sensitivity of the left hind paw was more pronounced in No Dive treated rats. In addition, this would explain their difficulty in moving on the narrow plank above the void (reduction in agility). For all that, their exploratory behavior was not affected when it involved moving in a less restrictive area (flat surface). However, this could also be due to a sampling effect.

Following the hyperbaric protocol and from a clinical point of view, the rats presenting DCS (whether Ctrl or Flux) had inhibited exploratory behavior and reduced motor and locomotor scores for the beam test. Nevertheless, this exploratory behavior was significantly less degraded in rats treated with fluoxetine, which means that the rats were either less stressed as a result of (a removal of inhibition by) their anti-depressant treatment or suffered less damage than the Ctrl rats and therefore were more able to move about. This corresponds to the description by Kaur and Kulkarni (2002), which describes fluoxetine as limiting the immobility induced by hyperalgesia. This is comparable to the previous study by Blatteau et al. (2015) even though the fluoxetine was administered acutely. This result is interesting because a loss of sensitivity (nociception) is regularly observed in humans after decompression sickness, and is found again in the P DCS rats.

Generally and rheologically, the protocol producing DCS has increased the hematocrit and significantly reduced the number of circulating erythrocytes and leukocytes, as well as the number of circulating platelets. This is generally attributed to prothrombotic phenomena and an inflammation causing diapedesis. However, the reduction in the number of leukocytes was significantly lower in the Flux rats, which implies that fluoxetine would reduce diapedesis, as has been observed in previous studies (Blatteau et al., 2012, 2015; Vallee et al., 2016). These effects are generally attributed either to the direct interaction of bubbles circulating with the platelets, or to the abrasion of the endothelial cells by these circulating bubbles (Nossum et al., 1999, 2002). In parallel, the question is whether there is a pathological hematocrit value as described in this work with the Severe DCS group. This situation was previously described in the work of Musallam et al. (2013) where the effect on mortality was noted beyond the hematocrit thresholds of 0.48 in women and 0.52 in men, and the effect were considerably higher for values exceeding 0.54.

The biochemical analyses show a capillary leak, a symptom observed in humans in critical cases, which would explain the recorded hemoconcentration (Gempp et al., 2013, 2014).

The stress of decompression is felt most particularly in the cells via the measurement of circulating oligonucleotides, which is more significant in the rats with decompression sickness. Fluoxetine again seems to improve this constant whatever the DNA measurement considered. The increase in circulating oligonucleotides was initially attributed to the destruction of cells by bubble abrasion following necrotic phenomena (Vallee et al., 2012). When considering total double strand DNA it is seen that Divers without accidents have more circulating DNA than No Divers, which reinforces the previous theory and also suggests that the presence of bubbles is possible and that it can break down some cells without necessarily being pathological for the organism. In humans, we sometimes talk about "Bubble-resistant Subjects," referring to individuals in whom high levels of circulating bubbles are detected but who do not develop symptoms. In a contradictory way, this work also shows that the Divers without accidents had less mitochondrial DNA (this time) than No Divers, which suggests that this DNA is specifically broken down by the hyperbaric exposure. Given that the other technique for DNA detection (total double strand) is less specific than a sequence, the only hypothesis that we could put forward at this stage would be premature mitochondrial death linked to oxidative stress (see Shokolenko et al., 2009; Resseguie et al., 2015) causing the breakage of amplified sequences linked to the hyperbaric exposure and their high oxygen contents. In particular, this hypothesis relies on the over-production of ROS (Reactive Oxygen Species) and HSP (Heat Shock Protein) in a dive (Eftedal et al., 2013) and the impact of these components on the integrity of the DNA strands (Shokolenko et al., 2009; Hobani, 2016; Rima et al., 2016), as well as the work of Cacciuttolo et al. (1993) and Witte et al. (2014) recounting a direct effect of hyperoxia on the breakdown of DNA strands in the cells of mammals. In fact, the oxidative stress targets the genome guardians and particularly the repeated sequences rich in guanine, particularly telomeres (Thilagavathi et al., 2013a,b; Mishra et al., 2016). This result therefore raises the question of the toxicity of hyperoxic hyperbaric and normobaric exposures in humans (see O'reilly, 2001), whether therapeutic or not.

The acute dose of fluoxetine (50 mg/kg), as opposed to the lower chronic dose of 20 mg/kg, delivers better neuroprotection against decompression sickness when the TREK 1 channels have previously been blocked by spadin (Vallee et al., 2016): they inhibit NMDA-R receptors, regulate the inflammatory effects, and have analgesic properties. This same acute dose of 50 mg/kg administered alone seems to have less effect (Blatteau et al., 2012, 2015). However, it seems more advantageous than the chronic dose used in this study, with them all remaining effective. This would confirm a major, dose-dependent effect of fluoxetine potentializing its anti-inflammatory effect.

It could be concluded that the Ctrl DCS rats face a clinical assessment tending toward multi-organ failure in the most severe cases, which is countered by fluoxetine in Flux rats. The presence of disseminated coagulation and cell destruction in the widest sense justifies the initial appearance of bubbles (DCS), which causes a progressive inflammatory syndrome, which defines decompression sickness. The beneficial effect of fluoxetine is noted here. It predicts a favorable clinical prognosis for DCS and would compensate for the loss of neuroprotection due to the inhibition of TREK 1, as it has been able to be shown in previous works (Vallee et al., 2012; Blatteau et al., 2015).

CONCLUSION

Apart from the psychic effects, fluoxetine taken for 35 days does not have a negative incidence on the rate of DCS in the rat model. On the contrary, it improves the results of behavioral tests and the rheological and biochemical results, following the protocol producing decompression sickness. The effects of chronic treatment with fluoxetine are similar to those for an acute dose, but they seem to be dose-dependent. The fact that fluoxetine is unlikely to exacerbate the pathology associated with decompression would not allow the inclusion of treatment with fluoxetine, and possibly and more generally treatments with SSRIs, in the list of contraindications for scuba diving. This will add weight to further investigations on humans particularly epidemiological.

AUTHOR CONTRIBUTIONS

JB and NV conception and design of research; CC, SD, and NV performed experiments; CC and NV analyzed data; CC, JB, SD, JA, LC, JR, and NV interpreted results of experiments; NV prepared figures; CC and NV drafted, edited, and revised manuscript; CC, SD, JA, LC, JB, JR, and NV approved final version of manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fphys.2017.00604/full#supplementary-material

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Angiotensin Converting Enzyme Inhibitor Has a Protective Effect on Decompression Sickness in Rats

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Introduction: Commercial divers, high altitude pilots, and astronauts are exposed to some inherent risk of decompression sickness (DCS), though the mechanisms that trigger are still unclear. It has been previously showed that diving may induce increased levels of serum angiotensin converting enzyme. The renin angiotensin aldosterone system (RAAS) is one of the most important regulators of blood pressure and fluid volume. The purpose of the present study was to control the influence of angiotensin II on the appearance of DCS.

Methods: Sprague Dawley rats have been pre-treated with inhibitor of angiotensin II receptor type 1 (losartan; 10 mg/kg), angiotensin-converting enzyme (ACE) inhibitor (enalapril; 10 mg/kg), and calcium-entry blocker (nifedipine; 20 mg/kg). The experimental groups were treated for 4 weeks before exposure to hyperbaric pressure while controls were not treated. Seventy-five rats were subjected to a simulated dive at 1000 kPa absolute pressure for 45 min before starting decompression. Clinical assessment took place over a period of 60 min after surfacing. Blood samples were collected for measurements of TBARS, interleukin 6 (IL-6), angiotensin II (ANG II) and ACE.

Results: The diving protocol induced 60% DCS in non-treated animals. This ratio was significantly decreased after treatment with enalapril, but not other vasoactive drugs. Enalapril did not change ANG II or ACE concentration, while losartant decreased post dive level of ACE but not ANG II. None of the treatment modified the effect of diving on TBARS and IL-6 values.

Conclusion: Results suggests that the rennin angiotensin system is involved in a process of triggering DCS but this has to be further investigated. However, a vasorelaxation mediated process, which potentially could increase the load of inert gas during hyperbaric exposure, and antioxidant properties were excluded by our results.

Keywords: renin-angiotensin system, calcium channel blocker, decompression illness, vasomotion, animal model, angiotensin converting enzyme inhibitor, angiotensin receptor antagonist

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INTRODUCTION

Decompression sickness (DCS) is the most serious danger for Self-Contained Underwater Breathing Apparatus (SCUBA) divers. It is a systemic pathology displaying a wide range of symptoms including minor ones such as skin rashes up to more serious clinical outcomes like neurological damage, cardiac collapse and death (Vann et al., 2011). Spinal cord DCS, which is the more severe form of DCS, represents 40–45% of total DCS cases and manifests in a broad array of symptoms that can result in severe morbidity, life-long disabilities, and even death. Among these most serious cases, 20–30% of divers will suffer definitive sequelae. DCS is strongly connected with vascular bubble formation resulting from supersaturation during inadequate decompression (Eftedal et al., 2007). However, it has been stated that only 13% of its appearance can be explained by these venous gas emboli (VGE) alone (Ljubkovic et al., 2010), and even high amounts of circulating bubbles do not necessarily lead to DCS (Bakovic et al., 2008). This shows that although necessary the presence of bubbles is not sufficient to evoke DCS.

One possible, and repeatedly hypothesized, mechanism could rely on the well-documented diving-induced impairment of vascular function (Brubakk et al., 2005; Lambrechts et al., 2013a; Mazur et al., 2016). In line with this hypothesis, administration of nitric oxide (NO) donors decreases both the amount of circulating bubbles (Dujić et al., 2006; Møllerløkken et al., 2006) and the probability of DCS (Wisløff et al., 2004), while blockade of NO production increases the probability of DCS (Wisloff et al., 2003; Mazur et al., 2014). In addition, pretreatment with the PDE5 inhibitor sildenafil led to increased occurrence of DCS (Blatteau et al., 2013) which suggests that the level of circulating NO rather than its vasodilating action could be involved in the development of DCS. However, circulating levels of NO assessed after diving failed to show any modification. Indeed, blood nitrate concentration was not different after a single open water sea dive with air or nitrox (Marinovic et al., 2012; Lambrechts et al., 2013b; Theunissen et al., 2013) compared with before the dive.

The renin angiotensin aldosterone system (RAAS) is one of the most important regulators of blood pressure, fluid volume, and sodium and potassium balance. Angiotensin II (Ang II) is produced by the conversion of Angiotensin I (ANG I) by the angiotensin converting enzyme (ACE), an enzyme which is abundant in the lungs, bound to endothelial cells and is also found in many other organs including the kidney. Increased levels of serum ACE have been reported after decompression and at even higher levels following DCS (Thorsen et al., 2006). Although not the only peptide formed by RAAS, Ang II is the major active metabolite and exerts its effects via AT1 and AT2 receptors. Stimulation of AT1 receptors by Ang II is responsible for Ang II evoked contraction of smooth muscle cells, enhancement of sympathetic outflow and release of aldosterone by the adrenal gland. AT1 receptor may also increase oxidative stress and indirectly reduce bioavailabilty of the greatly vasoactive NO (Newsholme et al., 2010), promote formation of endothelial microparticules (Yang et al., 2014) and inflammation-related processes (Pacurari et al., 2014). AT2 receptors stimulate the generation of procoagulant microparticles (Cordazzo et al., 2013) and promote thrombosis in the microcirculation (Senchenkova et al., 2010). All of these have been proven to trigger diving-related physiological changes. Indeed, altered vascular permeability (Gempp et al., 2013), platelet aggregation (Pontier et al., 2012), release of microparticles and inflammation (Thom et al., 2015), as well as oxidative stress (Mazur et al., 2016) have been evidenced post-dive. Recently, we found that plasmatic concentration of AngII was decreased after the dive in asymptomatic rats but not animals which suffered DCS (Mazur et al., 2016). This led us to hypothesize that maintaining the concentration of circulating Ang II could be part of the mechanisms leading to DCS.

That is why in this study we decided to control its influence on appearance of DCS events after a simulative dive *in vivo* in male Sprague Dawley rats. ANG II was blocked on two different levels: production by long-term angiotensin converting enzyme inhibitor with enalapril and its direct action through angiotensin II receptor type 1 by long-term inhibition of AT1 with losartan. Treated rats were compared with the control not-treated group. To further assess whether vasomotion-related mechanisms are involved we included the effect of long-term calcium-entry blockade, assessed by nifedipine treatment, which would show non-ANG II, non-NO regulation of vasomotion and its importance in decompression sickness appearance after a simulative *in vivo* dive.

METHODS

All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (National Institutes of Health Publication No. 85-23, revised 1996), and national laws from the French Ministry of Agriculture. They were formally approved by the Université de Bretagne Occidentale animal research ethics committee.

Animals

Eighty-three male Sprague Dawley rats were obtained from Janvier SAS (France) 5 weeks before exposure to pressure. Before treatment the rats were allowed to become accustomed to the facility for a week. They were housed in an environmentally controlled room (temperature 21 ± 1°C, relative humidity 27 ± 16%, 12–12 h light-dark cycle) and fed standard rat chow.

Animals were randomly divided into six groups. Four groups, based on pharmaceutical intervention, were compared post-dive with the fifth, untreated diving control group for susceptibility to DCS. To control for the influence of either diving itself or treatment before diving on plasmatic markers, an additional untreated non-diving control group ($n = 8$) was included.

Drugs

The treatment lasted 4 weeks and drugs were administrated orally in drinking water. Enalapril (Enalapril EG 20 mg) as an ACE inhibitor ($n = 15$) and losartan (TEVA) as an AT1 receptor antagonist ($n = 15$) were dissolved directly in tap water and given at the dose of 10 mg/kg/day, a dose that has been demonstrated to cause maximal inhibition of ACE activities or blockade of

AT1 receptors (Matsuyama and Kitani, 1996). Nifedipine (Sigma Aldrich, Paris, France) as a Ca^{2+} channel blocker ($n = 15$) was administrated at the dose of 20 mg/kg/day (Cao et al., 2010). Due to its low in-water solubility this drug was first dissolved in absolute ethanol (final alcohol concentration 1.2%vol.vol^{-1}). To discount any confounding influence of alcohol, an additional alcohol control group ($n = 15$) was created, where 1.2% vol.vol^{-1} ethanol was added to drinking water given to rats. Fresh solutions of all drugs were prepared daily and consumption was monitored. Once per week body weight and systolic blood pressure were measured. After 4 weeks of treatment the rats were placed in a 130-liter hyperbaric chamber (Comex, Marseille, France) and performed a simulated dive.

Blood Pressure Measurement

Resting caudal artery Systolic Blood Pressure (SBP) was obtained indirectly by the tail photoplethysmographic technique (IITC INC/Life Science Instruments, Woodland Hills, USA). All rats underwent 1 week of habituation to the procedure prior to the experiment. Measurements were made in front of a radiator at 29–30°C to vasodilate the tail artery. In each group baseline SBP was measured once during the treatment period and then again on the day before each compression protocol. Presented values of SBP are the mean of three separate readings obtained during the last measurement. Mean SBP values after the treatment, but before compression, were compared with those obtained in control animals.

Simulated Dive Protocol

Because both age and weight are known to influence the probability of DCS (Buzzacott et al., 2016), animals used in these experiments were all the same age (12 weeks old) and similar weights (450 ± 50 g) on the day of the experiment. The simulated dive protocol has been previously described (Mazur et al., 2016). Briefly, rats from each group were placed in a dry hyperbaric chamber. Compression and decompression were at a rate of 100 kPa.min^{-1}. Diving rats were compressed with air up to 1000 kPa absolute pressure and remained under this pressure for 45 min. Three decompression stops were performed during the ascent: 5 min at 200 kPa, 5 min at 160 kPa, and 10 min at 130 kPa. Total duration of the hyperbaric exposure was 83 min. All dive depths were monitored using a modified personal dive computer (Puk, Mares, Rapallo, Italy). This protocol has been used in our previous experiments and reliably induces about 63% of DCS. A non-diving control group of rats was similarly confined, (but not exposed to elevated ambient pressure), and observed for 1 h before physiological investigation. Following the observation period the rats were anesthetized by intramuscular injection of ketamine (80 mg/kg) and xylazin (15 mg/kg). After blood and organs had been collected the rats were euthanized with pentobarbital.

An additional 48 control rats from a previous experiment were added to the 15 control rats in this study, thus raising the control to treatment ratio to 4:1. These rats underwent the same diving protocol, were handled the same way, had similar weight and were the same age on the day of the simulative dive.

Classification of DCS

Animals were scored as having DCS only when displaying one or more symptoms of: respiratory distress, difficulty walking, paralysis, or convulsions (Arieli et al., 2009). The classification of DCS and analytical method were decided *a priori* to the experiment. To minimize the potential for animal suffering a pain/distress scale was approved by the Animal Research Ethics Committee and no rats displayed signs of distress at or above the level where early euthanasia was required. In nearly all non-fatal cases of DCS the rats appeared to recover fully by the end of the observation period, to the point where they were indistinguishable from rats without DCS. Other studies using an ED-50 rat model have classified their outcome variables as DCS vs. No-DCS, whereby the marginal cases were combined with the dead, or the classification was Dead vs. Not-Dead, where the marginal cases were combined with the asymptomatic. In this study the ternary classification of "No DCS" (no symptoms), mild "DCS" (with at least one symptoms excluding death within the observation period) or severe DCS ("Death" within 1 h) was retained to maximize statistical power, as described by Buzzacott et al. (2014).

Blood Sampling and Plasmatic Markers

Blood samples were collected immediately following anesthesia or death in a 2 mL eppendorf tube with 30 µl 7.5% EDTA as an anticoagulant, centrifuged at 1000 g and 4°C for 15 min, aliquoted and stored at −80°C until the assay. To explore which regulation loop was involved in DCS appearance we measured different biomarkers in plasma from the non-diving, diving with "No DCS" and mild "DCS" groups. Thiobarbituric Acid Reactive Substances (TBARS) was chosen as an indicator of oxidative stress and Interleukin 6 (IL-6), a pleiotropic cytokine associated with the inflammation process. Additionally, levels of angiotensin II (ANG II) as a peptide hormone that causes vasoconstriction and ACE an enzyme responsible for conversion of angiotensin I (ANG I) to its active form ANG II was measured to track the influence of the renin-angiotensin system on the appearance of DCS. Plasmatic concentrations were determined using commercially available ELISA assay kits; TBARS Assay Kit (Cayman Chemical Company, Ann Arbor, MI), ACE Assay Kit (EIAab, Wuhan, China) and Il-6 Rat ELISA Kit provided by ABCAM, (Paris, France). All ELISA samples were run in duplicate in accordance with the manufacturer's instructions.

Statistical Analysis

Data were analyzed using SAS ver. 9.3 (SAS, Cary, North Carolina). An ordinal logistic regression model was constructed as shown in Equation 1, using a cumulative logit function appropriate for ordinal, polychotomous dependent variables.

$$DCS = \beta_0 + \beta_1 TREATMENT + \beta_2 WEIGHT \quad (1)$$

Where DCS was 0 = asymptomatic, 1 = alive for 1 h but with signs of DCS, 2 = dead within 1 h. WEIGHT was the weight of each rat on the day of compression in grams, TREATMENT indicates whether each rat was treated with enalapril, losartan, or not treated (control). A similar but separate model was created for nifedipine which was compared with the alcohol group and also to the non-treatment control group. Data from kit measurements were analyzed using Statistica software (ver. 10, StatSoft France, 2011). Differences between the concentrations of plasmatic ACE, ANG II, TBARS, and IL-6 after the diving simulation were first analyzed for normality. If data were parametric we proceeded with one way ANOVA. Upon identifying significant differences in the ANOVA, a Dunnett *post-hoc* test was used to interrogate relevant parameters. For non-parametrically distributed results we ran ANOVA with a Kruskal–Wallis test. Data were considered significant at $p < 0.05$ and reported as means ± SE for indicated samples.

RESULTS

Blood Pressure Measurements

Systolic Blood pressure decreased in all rats that received antihypertensive drugs. It was significantly lower after the administration of enalapril (98 ± 2.3 mmHg) when compared with the control group (120 ± 2.2 mmHg, $p < 0.01$). Losartan (112 ± 2.9 mmHg) and nifedipine (112 ± 2.2 mmHg) showed a tendency to decrease SBP and the alcohol treatment did not change the blood pressure (117 ± 2.7 mmHg).

DCS Outcome

The diving protocol induced 60% DCS in the diving control group (**Figure 1**). Death appeared in 77% of the affected rats before the end of the observation period (usually during the last minutes of ascent or within 30 min after hyperbaric exposure). The remaining 33% of the affected rats all survived the observation period. The additional control group from a previous experiment was not significantly different in DCS outcome to the 15 control rats in this experiment, therefore these groups were merged. Due to its narrow range between animals, body weight had no significant influence upon DCS outcome in this experiment. Pre-treatment with enalapril lowered the ratio of DCS when compared with combined diving controls ($p = 0.01$). Enalapril also appeared to be significantly protective compared to losartan, which did not differ from the combined diving controls. Treatment with nifedipine did not significantly alter the appearance of DCS (**Figure 2**). The outcome of DCS in the alcohol group was also no different when compared with the combined diving control rats, which indicates that ethanol itself had no influence on the appearance of DCS (Buzzacott et al., 2015).

ACE, ANG II, TBARS and IL-6 Assays

Plasma ACE values are presented in **Figure 3**. Levels of ACE were not significantly different in control diving rats from the non-diving control group. We observed significantly lower post-dive concentrations in animals treated with losartan ($p = 0.02$), nifedipine ($p < 0.10$), or alcohol ($p < 0.10$) when compared with non-diving controls. Conversely, treatment with enalapril did not change the post-dive level of ACE when compared with either non-diving or diving controls.

Active forms of ANG II values are shown in **Figure 4**. The diving group did not differ significantly when compared with the non-diving group. Similarly, neither enalapril nor losartan modified post-dive levels of ANG II. Plasmatic concentration of ANG was significantly higher after the dive in rats that received alcohol alone or alcohol and nifedipine than in the diving control group ($p = 0.01$). However, nifedipine did not show a significant effect when compared with the alcohol group, which indicates that alcohol itself significantly elevated levels of ANG.

TBARS values are shown in **Figure 5**. Their level was significantly elevated among all diving groups when compared with non-diving control rats ($p < 0.04$), But there was no difference between treated groups.

IL-6 levels (**Figure 6**) were measured only in the diving groups. IL-6 did not significantly change in enalapril or losartan treated rats when compared with the diving control group. Nifedipine treatment stimulated a significant decrease of IL-6 levels when compared with the alcohol group ($p = 0.001$).

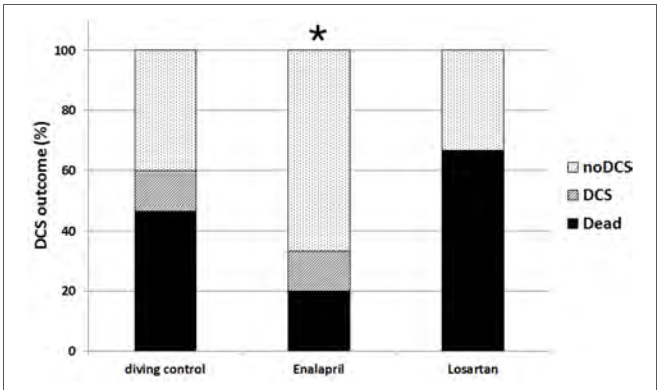

FIGURE 1 | Appearance of DCS among control rats and rats treated with enalapril and losartan. Appearance of DCS among the control rats and rats treated with enalapril and losartan expressed as a percentage of the total number of the rats in each group. Histogram in black represents the rats suffering severe DCS, stripes are rats with mild DCS and dots are the asymptomatic group *$p < 0.05$.

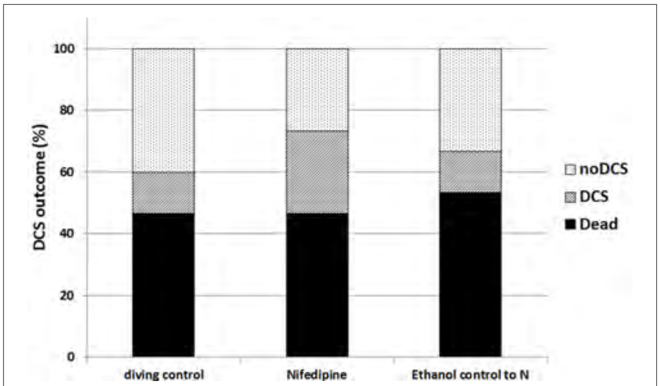

FIGURE 2 | Appearance of DCS among control rats, additional control alcohol group and rats treated with nifedipine. Appearance of DCS among the control rats, additional control alcohol group and rats treated with nifedipine expressed as a percentage of the total number of the rats in each group. Histogram in black represents the rats suffering severe DCS, stripes are rats with mild DCS and dots are the asymptomatic group. There was no statiscally significant difference between groups.

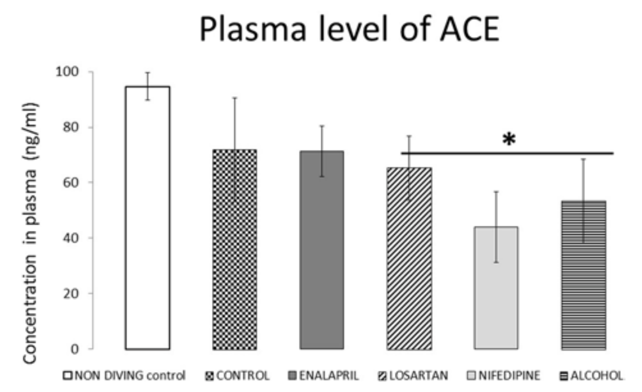

FIGURE 3 | ACE plasma levels for all diving groups with treatment compared with diving control and non-diving rats. ACE plasma levels expressed as ng/ml (mean ± SEM), for all the diving groups with treatment compared with diving control and non-diving rats. *$p < 0.05$.

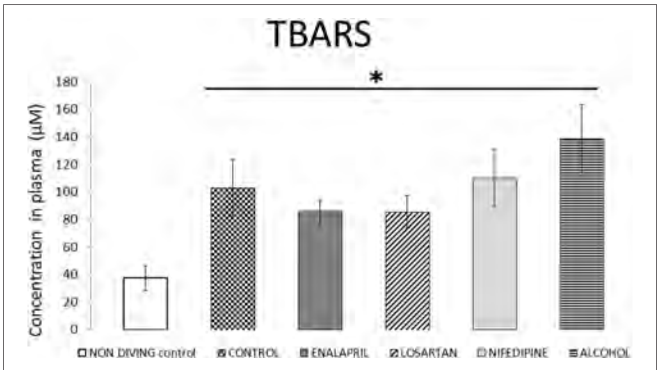

FIGURE 5 | TBARS plasma levels for all diving groups with treatment compared with diving control and non-diving rats. TBARS plasma levels expressed as μM (mean ± SEM), for all the diving groups with treatment compared with diving control and non-diving rats. *$p < 0.05$.

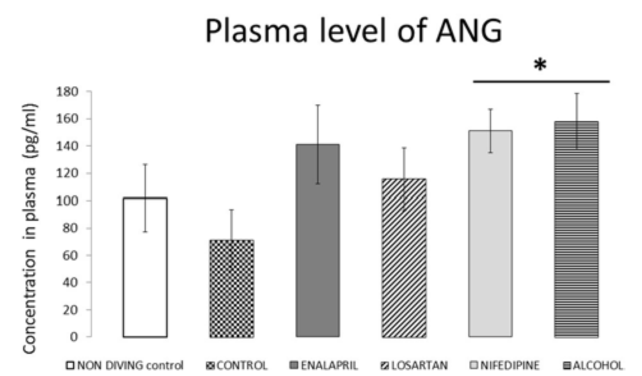

FIGURE 4 | ANG plasma levels for all diving groups with treatment compared with diving control and non-diving rats. ANG plasma levels expressed as pg/ml (mean ± SEM), for all the diving groups with treatment compared with diving control and non-diving rats. *$p < 0.05$.

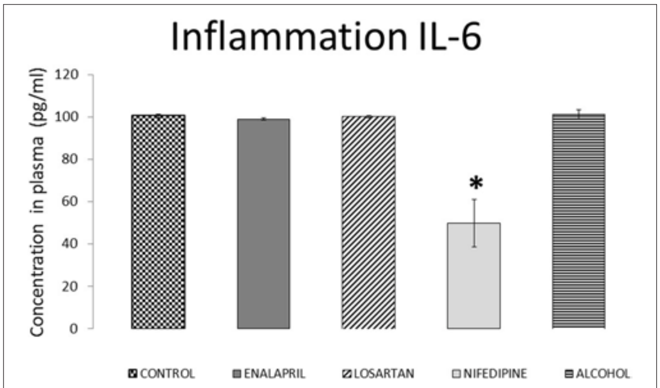

FIGURE 6 | IL-6 plasma levels for all diving groups with treatment compared with diving control. IL-6 plasma levels expressed as pg/ml (mean ± SEM), for all the diving groups with treatment compared with diving control. *$p < 0.05$.

Alcohol itself did not differ from basal values when compared with the diving control group ($p = 0.76$).

DISCUSSION

The main focus of this study was to assess whether the renin angiotensin system has an influence on the appearance of DCS. Additionally, by chronic administration of substances which inhibit either the formation of Ang II or its action on the vessels, or promotes vasomotion independently of the RAAS and NO-cGMP pathways, we aimed to confirm (i): whether the RAAS could be involved in the development of DCS and, if so, (ii): by which mechanisms.

A decrease in systolic blood pressure indicated that all of the drugs were efficiently administrated. However, because enelapril decreased SBP significantly more than either losartan or nifedipine we cannot exclude the hypothesis that lower blood pressure could have been protective against DCS. Also the effect obtained can be a dose related process thus we cannot exclude the scenario that other drugs would influence the outcome of DCS when administrated in a higher doses.

Among the drugs influencing the renin-angiotensin system, the angiotensin converting enzyme inhibitor enalapril appeared to significantly decrease the ratio of accidents while the angiotensin receptor blocker (ARB) losartan did not change the outcome. Such a divergent effect between these two drugs has been recently discussed (Strauss and Hall, 2016). Indeed, the authors claimed that although both drugs lower blood pressure, ACE inhibitors but not ARBs may also produce a pressure independent benefit and reduce the risk of myocardial infarction in people with cardiovascular risk factors. Similarly, we found that only the ACE inhibitor prevented DCS. This difference in susceptibility to DCS may be explained by the differently acting mechanisms of those drugs. Indeed, enalapril block the formation of angiotensin II and, thus, lowers or suppress its concentration while losartan blocks the effect through the AT1 receptor only. Our initial hypothesis leading this study was based on a previous work by our group which showed that the same hyperbaric protocol is associated with decreased plasmatic concentration of Ang II in animals with no signs of DCS while unchanged in the ones which suffer DCS (Mazur et al., 2016). Because ANG II is responsible for a number of diverse actions in the body, many of them connected with diving-physiology such as increased oxidative stress (Newsholme et al., 2010), control of inflammation-related processes (Davis, 2006) and participation in the thrombotic process (López-Farré et al., 2001), we thus hypothesized that keeping the post-dive concentration of ANGII low enough could prevent DCS. To do so, we compared the effect of enalapril with losartan, which do not act on ANGII concentration. That in the present study the plasmatic concentration of Ang II is unchanged in the control group, in which 60% of the animals suffer DCS, confirms these previous data. However, in the present study both enalapril and losartan failed to decrease plasmatic concentration of ANGII. Indeed, according to previous studies, plasmatic ACE level should have increased after enalapril treatment (Costerousse et al., 1998) while plasmatic level of ANG II decreased (Zhang et al., 2012). Though this confirms that the SRAA is probably involved in the triggering of DCS; it also indicates that enalapril acted through other mechanism(s) that the lowering of AngII.

Diving-induced oxidative stress is well-documented. Previous data indicate that the production of reactive oxygen species (ROS) already increases during the stay at depth (Wang et al., 2015a), and that it is further increased by decompression stress in a dose-dependent manner (Mazur et al., 2016). Based on these observations, it was hypothesized that increased oxidative stress could participate to the development of DCS. In our present study, levels of TBARS increased among all diving groups regardless of the treatments, which is consistent with that diving-induced oxidative stress. ANG II may locally produce superoxide-mediated vascular dysfunction (Newsholme et al., 2010) and is known as a pro-oxidative substance (Durand and Lombard, 2013). It has been proven that drugs used in the treatment of cardiovascular risk factors and especially ACE inhibitors have anti-inflammatory properties by acting as antioxidants. They prevent lipoprotein oxidation and nitric oxide quenching (Osiecki, 2004). However, in our study not only TBARS were not decreased by our treatments, but they were also not significantly different in the enalapril treated group than in the others despite significantly different DCS outcomes. This suggests that the protective effect of enalapril treatment is not due to its antioxidant effect. More generally, this result is in line with data from our team showing that the protective effect of anti-agregant is not associated with decreased post-dive oxidative stress (Lambrechts et al., 2015) and that pre-treatment with antioxidant did not prevent the occurrence of DCS in rats (Wang et al., 2015b), thus pointing a lack of relationship between oxidative stress and DCS.

Because inflammation has been repeatedly reported after diving, we also assessed whether enalapril treatment could act through its anti-inflammatory action. Previous studies showed that IL-6 is increased with DCS (Ersson et al., 1998; Blatteau et al., 2012; Bao et al., 2015) but not asymptomatic individuals (Chen et al., 2011; Blatteau et al., 2012). In our study rats treated with enalapril did not have modified levels of this biomarker. Treatment with nifedipine appeared to reduce levels of IL-6. This effect, however, did not change the outcome of DCS among nifedipine treated rats, suggesting that inflammation through IL-6 was not solely responsible for DCS. However there are many other markers which could be involved in establishing the inflammatory effect and they have been not studied here, thus we cannot definitively exclude this hypothesis.

Another property of ACE inhibitors not shared by ANG receptor blockers is their preventive effect on the breakdown of bradykinin. Bradykinin exerts an antithrombotic action through inhibition of both platelet aggregation and circulating PAI-I levels and is a potent stimulator of tissue plasminogen activator. Patients with chronic heart failure that have been treated with ACE inhibitors had reduced fibrinogen and endothelial von Willebrand factor levels when compared with baseline (Gibbs et al., 2001). Decreases in post-dive platelet count have been previously shown (Pontier et al., 2008; Ostrowski et al., 2011). Additionaly pre-treatment with an antiplatelet agent (abciximab) pretreatment has a strong protective effect on decompression risk by significantly improving DCS outcome (Lambrechts et al., 2015). Finally, increased PAI-1 levels (Eftedal et al., 2012) have been previously reported in rats in a context of decompression sickness. Thus, an improvement in prothrombotic state through increased bioavailability of bradykinin following treatment with ACE inhibitors would be consistent with the protective effect of enalapril but not losartan. Furthermore, ACE inhibition increases the release of nitric oxide (NO), which administration has been shown to prevent DCS in animals (Wisløff et al., 2004), through the accumulation of bradykinin (Cheetham et al., 2000). The effects of bradykinin have not been previously studied in either diving or DCS but could be an interesting new path to investigate.

Finally, we also assessed whether the influence of the RAAS on DCS could be related to its influence on vascular tone. We aimed to inhibit contraction through different pathways. Treatment with nifedipine, a specific blocker of L-type voltage dependent Ca^{2+} channels, aimed to inhibit direct vasoconstriction by influx of extracellular Ca^{2+} whereas blockade of the effect of ANG II, a secondary messenger dependent pathway, was obtained by losartan and enalapril. Neither losartan nor nifedipine altered the likelihood of DCS when compared with the combined diving control group, suggesting that the protective effect of enalapril probably not relies on vasodilating properties.

The main finding of this study is that chronic treatment with enalapril significantly protected rats from suffering DCS while neither losartan or nifedipine treatments influenced the outcome of the dive. The mechanism by which enalapril exerted this positive influence is still unclear but we may exclude antioxidant and anti-inflammatory properties as well as increased gas load induced by increased vasorelaxation. We instead hypothesize that this effect could be due to the influence of enalapril on bradykinin. This has to be further investigated before the influence of ANG II upon developing of DCS will be understood.

AUTHOR CONTRIBUTIONS

AM, AG, JD, MT, and FG: conception and design of research; AM, JL, ED, MB, and FG: performed experiments; AM, AG, and

FG: analyzed data; AM, AG, MT, and FG: interpreted results of experiments; AM: prepared figures; AM, MT, and FG: drafted manuscript; AM, AG, JL, MB, MT, and FG: edited and revised manuscript; AM, AG, JL, JD, ED, MB, MT, and FG: approved final version of manuscript.

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