Effects of prolonged and repeated immersions on heart rate variability and complexity in military divers

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ABSTRACT

Background: The influence of prolonged and repeated water immersions on heart rate variability (HRV) and complexity was examined in 10 U.S. Navy divers who completed six-hour resting dives on five consecutive days. Pre-dive and during-dive measures were recorded daily.

Methods: Dependent variables of interest were average heart rate (HR), time-domain measures of HRV [root mean square of successive differences of the normal RR (NN) interval (RMSSD), standard deviation of the NN interval (SDNN)], frequency-domain measures of HRV [low-frequency power spectral density (psd) (LFpsd), low-frequency normalized (LFnu), high-frequency psd (HFpsd), high-frequency normalized (HFnu), low-frequency/high-frequency ratio (LF/HF)], and non-linear dynamics of HRV [approximate entropy (ApEn)]. A repeated-measures ANOVA was performed to examine pre-dive measure differences among baseline measures. Hierarchical linear modeling (HLM) was performed to test the effects of prolonged and repeated water immersion on the dependent variables.

Results: Pre-dive HR ($P=0.005$) and RMSSD ($P<0.001$) varied significantly with dive day while changes in SDNN approached significance ($P=0.055$). HLM indicated that HR decreased during daily dives ($P=0.001$), but increased across dive days ($P=0.011$); RMSSD increased during daily dives ($P=0.018$) but decreased across dive days ($P<0.001$); SDNN increased during daily dives ($P<0.001$); LF measures increased across dive days (LFpsd $P<0.001$; LFnu $P<0.001$), while HF measures decreased across dive days (HFpsd $P<0.001$; HFnu $P<0.001$); LF/HF increased across dive days ($P<0.001$); ApEn decreased during daily dives ($P<0.02$) and across dive days ($p<0.001$).

Conclusion: These data suggest that the cumulative effect of repeated dives across five days results in decreased vagal tone and a less responsive cardiovascular system.

INTRODUCTION

Divers in the United States military undergo physical, mental and emotional stresses as part of their training and day-to-day duties. A military diver's tasks can be repetitive over hours or days, and for some divers, require extended time underwater. While standards of practice are in place to protect the health of the diver, the influence of prolonged and repeated immersion on the function of the physiological systems both during and after immersion is poorly delineated. Thus, expanding the knowledge base of how underwater immersion influences physiological functioning would enhance health-monitoring practices.

Heart rate (HR) variability (HRV) is a non-invasive measure of cardiovascular functioning, as well as a measure of autonomic nervous activity [1]. These metrics can monitor aspects of training load, adaptation and fatigue [2-5]. The metrics of HRV can be sorted into three separate categories:

1) time-domain measures;
2) linear frequency-domain measures; and
3) nonlinear dynamics (e.g., complexity).

Common time-domain measures include the mean of the R-R intervals (also termed NN interval and related to mean HR), the standard deviation of the NN intervals (SDNN), and the square root of the mean squared

KEYWORDS: heart rate variability; complexity; cardiac dynamics; water immersion

difference between NN intervals (RMSSD). Typical frequency-domain measures are power at high frequencies (HF), low frequencies (LF) and very low frequencies (VLF, which will not be discussed in this paper). Nonlinear dynamics include measures of approximate entropy (ApEn), detrended fluctuation analysis, and Poincaré plots, but only ApEn will be discussed in this paper. Short-term RMSSD [6] and HF power directly reflect parasympathetic activity [7,8]. Meanwhile, LF power is mediated by a combination of sympathetic and parasympathetic activity [7]. Nonlinear dynamics, though less well delineated physiologically, are useful adjuncts to time- and frequency-domain measures [9]. ApEn describes the conditional probability of specific patterns between a selected finite time series and the next incremental comparison. A higher probability indicates a lower complexity (i.e., more regularity in the pattern) and a smaller ApEn value. From a functional perspective, a decrease in ApEn is interpreted to indicate a less adaptive system [10,11].

Immersion in thermoneutral water results in fluid shifts, and blood volume is redistributed from the legs and abdomen to the thorax. The increase in thoracic blood volume alters cardiac regulation and regional autonomic nervous system activity. While a reduction in HR has been observed in response to water immersion [12,13], other studies have reported no changes [14,15]. However, these differences could be the consequence of any number of factors, including body position, water temperature, or the degree of water immersion (head out vs. complete water immersion).

Concurrently, a reduction in muscle sympathetic nerve activity and systemic vascular resistance may occur, with no appreciable change in blood pressure [16,17]. The change in cardiac regulation is mainly attributed to a shift toward parasympathetic dominance, as indicated by increased HF power and time-domain measures such as RMSSD during short- [18, 19] and long-duration water immersion [20].

After egress from the water following six hours of immersion, plasma volume and stroke volume are reduced by 10%-12% [21] and are not fully recovered by 16 hours post-immersion. Ankle-brachial index is also suppressed up to 16 hours after immersion egress [22], suggesting a prolonged change in vascular responsiveness. Although there is a significant decrease in ventricular filling, resting supine HR and BP are not different between pre- and post-immersion, but a shift toward greater sympathetic control (i.e., increased LF HRV and decreased HF HRV and ApEn) is evident when faced with an orthostatic challenge [21]. If immersion effects are cumulative, then resting cardiac regulation may also be affected after repeated immersions, and these compounding effects may be detrimental to performance and/or diver safety.

While physiological responses to short-term water immersions have been well documented [13,19,23-26], studies investigating the HRV and complexity response during long-duration water immersions have been limited. To our knowledge, responses to repeated long-duration immersions have not been studied, and better understanding of these effects may help provide useful and vitally important information regarding how stresses associated with repeated long-duration immersions affect cardiac control. To address current gaps in knowledge, divers were studied before and during six-hour immersions repeated once a day for five days in a controlled, swimming pool environment. "Immersion" throughout this paper refers to shallow-water (about 15 feet) dives with divers breathing air from demand regulators. We hypothesized that:

1) pre-dive (i.e., prior to entering the water) time-domain and frequency-domain measures of HRV and HR complexity would decrease across dive days;
2) time-domain and frequency-domain measures of HRV would increase during individual dive days while measures of complexity would decrease; and
3) time- and frequency-domain measures of HRV and complexity would decrease across dive days.

METHODS

Subjects and experimental protocol

Ten experienced military divers participated in the study; their physical characteristics are presented in Table 1. All were healthy, active, normotensive and non-smoking U.S. Navy Divers who were not taking any medications or supplements. Testing was completed at the Navy Experimental Diving Unit in Panama City, Florida, from 2009-2010. Approval was obtained from the Institutional Review Board of the Navy Experimental Diving Unit. Each subject gave written informed consent, and all procedures conformed with the Declaration of Helsinki.

As part of a larger study, participants underwent medical screening and physical examination, skinfold body fat measurement, and determination of maximal

Table 1: Subject demographics

subject	age	weight (kg)	height (cm)	BMI	body fat %	VO_{2max}	Hb	Hct
Mean	34	84.6	178.8	26.4	19	52.6	15.4	45
(SD)	(10)	(6.6)	(6.1)	(1.4)	(4)	(9.2)	(0.8)	(3)

BMI – body mass index; VO_{2max} – maximal oxygen uptake; Hb – hemoglobin; Hct – hematocrit

oxygen uptake rate (VO_{2max}). They then completed a dive week consisting of six-hour immersions repeated daily for five consecutive days, with 18 hours on the surface between dives. Participants were asked to refrain from the use of alcohol for 48 hours, caffeine and strenuous exercise for 24 hours, and all food or drink except for water for two hours preceding their daily arrival. Except for these restrictions, subjects ate and drank ad libitum while outside the laboratory.

UHM style is to not use spaces in representing degrees

Before each dive electrocardiogram (ECG) measure-ments (Dash 3000, General Electric, Milwaukee, Wisconsin) were obtained after subjects lay supine for at least 20 minutes in a laboratory (air temperature 22°C-24°C) adjacent to the pool. Data were sampled at 400 Hz using custom-built Labview software (National Instruments, Austin, Texas). Participants then began their six-hour dives.

During immersion, each participant sat at the bottom of a 15-foot-deep pool (32-33°C) wearing a T-shirt, shorts and a weight belt to maintain negative buoyancy with no physical or psychological stress. They were instructed to sit completely still in a chair and remain quiet during the dive. Movies or music were played during the dive. Divers breathed surface-supplied air using a MK 20 breathing apparatus (Ocenco, Pleasant Prairie, Wisconsin), and wore a custom 3-lead ECG (MSDCS, UFI, Morro Bay, California).

Lead II ECG was collected at 400 Hz throughout the duration of the dive. To mitigate water infiltration effects on the ECG signal, 6-cm diameter gel electrodes (Red Dot, 3M, St. Paul, Minnesota) were waterproofed with benzoin, waterproof tape, and moleskin, as described by Hoar, et al. [27]. Divers had a 10-minute break after three hours, when they surfaced to eat a small, standardized lunch (2.2 MJ: 24% fat, 64% carbohydrate, 12% protein; 500 mL liquid) while standing in chest-deep water.

Analysis of HR and HRV

The cleanest five-minute epoch was selected from minutes 16-30 and 46-60 of each hour within the six-hour dive (a total of 12 epochs/dive) to calculate measures of HR and HRV. Custom Matlab software using a robust QRS complex detection algorithm [28,29] was used for R-wave peak detection from all selected ECG segments. Manual correction of R-wave peak locations was performed when necessary. An RR interval time series was calculated at 4 Hz by cubic spline interpolation. Other measures were calculated from the RR time series.

Time-domain measures were HR, RMSSD and SDNN. Frequency-domain measures were power spectral density (psd) in the LF (0.04–0.15 Hz) and HF (0.15–0.4 Hz) bands (LFpsd and HFpsd, respectively), normalized LF (LFnu) and HF (HFnu) where the normalizing factor was the total power from the two bands, and the LF/HF ratio. The psd of HRV data were obtained using the Welch periodogram method (Matlab version 7.9, Natick, Massachusetts). A 512-point fast Fourier transform (FFT), giving a frequency resolution of 0.004 Hz, was applied to data filtered with a 360-point Hamming window and no overlapping segments. The non-linear measure of complexity was approximate entropy (ApEn).

Statistical analysis

To examine changes in HR and HRV measures predive (i.e., prior to entering the water for each dive day; hypothesis 1), a separate repeated measures analysis of variance (ANOVA) was performed for each variable. Huynh-Feldt corrections were applied when sphericity was violated and a Bonferroni adjustment was used to determine statistical differences between dive days. Statistical significance was set a priori at $\alpha=0.05$. Linear regression against Dive Day was applied to variables that indicated significant main effects to increase the confidence with which the results can be interpreted.

For in-water data, hierarchical linear modeling (HLM) with fixed slope and random intercept was performed to determine the effect of prolonged and repeated immersions on HR and HRV, both across epochs within dives (hypothesis 2) and across dive days (hypothesis 3). The repeated measures design of the study protocol violated the non-independence assumption of ordinary least squares regression, making this more common analytic technique inappropriate. Further,a repeated measures analysis of variance approach would have been severely limited by the presence of incomplete/unbalanced data. However, HLM is a regression technique that can accommodate nested/non-independent data by accounting for the shared variance among lower-level observations that are nested within upper-level observations. In the case of the present study, variables such as epoch and dive day (lower-level) are nested within diver (upper-level). HLM can accommodate incomplete or unbalanced data [30], ameliorating the loss of data and power that would otherwise result from listwise deletion. No form of imputation or other method of replacement for missing data was conducted, as simulation studies suggest that HLM without imputation may retain as much or more statistical power [31]. An average of 12.3% of data was missing across the five dive days (18.4% on Dive Day 1; 3.3% on Dive Day 2; 10% on Dive Day 3; 10% on Dive Day 4; 20% on Dive Day 5). These values indicate the percentage of total missing samples due to noise on the ECG and do not represent individuals having been absent from an entire testing session.

Unconditional (empty) models were first estimated – no predictors were entered – from which we calculated intraclass correlations (ICC; intercept variance divided by total variance) to determine the proportion of variance in HR and HRV accounted for by between-person differences: that is, the degree to which outcome measures were more similar within a diver as compared to outcome measures across divers. Fixed effects models with random intercepts (and fixed slopes) were then estimated to test for patterns of change in HR and HRV within and across dive days. Predictor variables entered into the models included epoch (within-dive) and dive day. Both variables were centered such that the values for the first epoch and for the first dive day were set to zero. Both age and fitness (VO_{2max}) are known to affect HRV [32] and thus, were used as co-variates in the models. Person-averages of the pre-dive values of the dependent variable were also included as co-variates in the models. Age and fitness were grand-mean centered, as they were assessed only once. Fitness would not be expected to change meaningfully across one week. Distribution checks of the dependent observations and of their associated residuals revealed non-Gaussian distributions for several of the models. Log-linear transformations resolved normality issues for all variables except for LFnu. Therefore, the analyses were repeated on the log-transformed variables. The person-average per-dive values of the dependent variables were also log-transformed to maintain the consistency of units and clarity of the relationships between these variables and predicted outcomes.

Finally, to test whether the nature of within-dive changes in HRV remained consistent across days, a third model that included a dive day by epoch interaction was also estimated. However, in no instance was model fit improved with the addition of the interaction, so only the main effects models were retained. Of primary interest were the fixed effects of day and epoch and their interaction on the measures of HR and HRV. Physiological responses are included in the Results section, with specific HLM modeling information included in Appendix A.

RESULTS

Subject demographics are presented in Table 1.

Pre-dive surface HR and HRV measures between dive days: repeated-measures ANOVA

Results of the repeated-measures ANOVAs used to determine differences in pre-dive HR and HRV values among days are presented in Table 2. Overall, HR increased ($P=0.005$) and HFpsd decreased ($P=0.025$) significantly across dive days, and the decrease in SDNN approached significance ($P=0.055$). Linear contrasts indicated a significant increase in pre-dive HR ($P=0.003$, $\eta2=0.696$) and significant decreases in pre-dive SDNN ($P=0.041$, $\eta2=0.425$) and RMSSD ($P=0.005$, $\eta2=0.646$) across dive days.

HR and HRV measures during immersion: hierarchical linear modeling

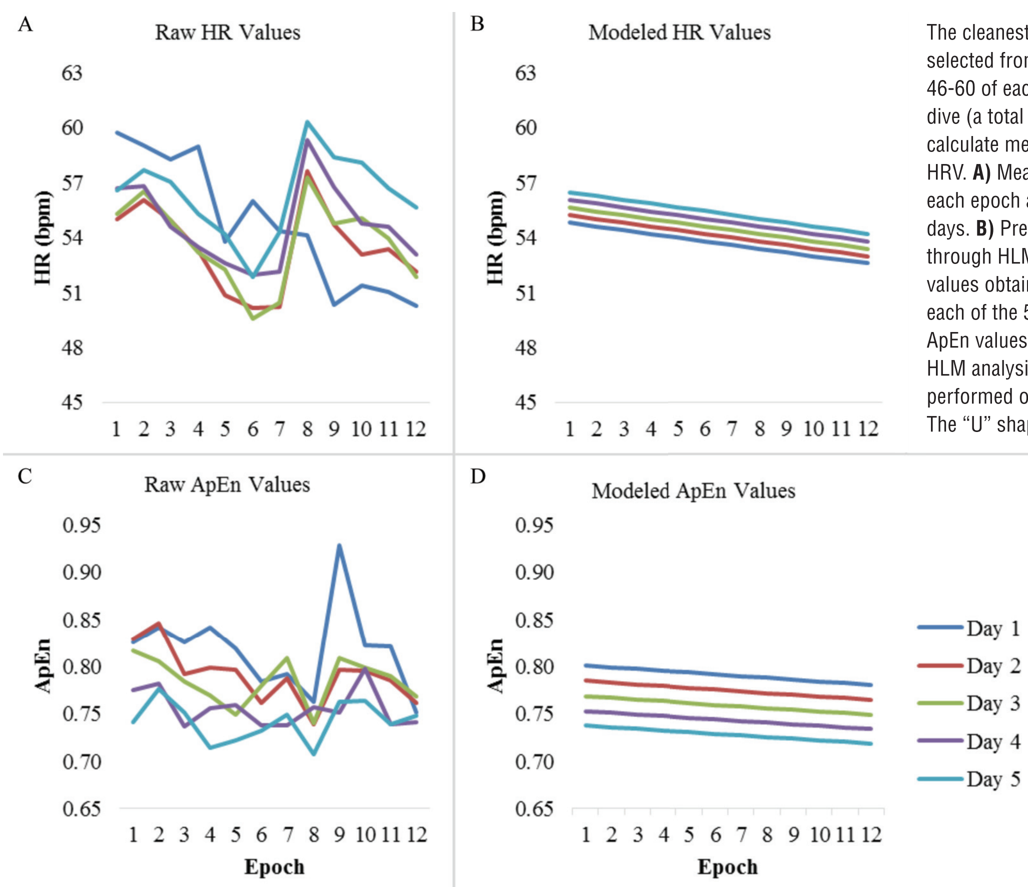
Refer to Appendix A for specific HLM modeling results. Both raw and predicted (via HLM) HR and ApEn values across epochs and between dive days are presented in Figure 1. HLM predicts the best-fit linear behavior across

Table 2: Pre-dive analysis of HR and HRV measures across dive days indicate a significant effect of dive day on HR, RMSSD and HFpsd

	DIVE DAY 1	DIVE DAY 2	DIVE DAY 3	DIVE DAY 4	DIVE DAY 5
HR	51 ± 8 [a b]	53 ± 9	53 ± 10	54 ± 8	54 ± 8
RMSSD	106.7 ± 57.3 [c]	82.1 ± 40.8	83.8 ± 46.3	76.1 ± 36.3	73.8 ± 36.3
HFpsd	4.390 ± 4.348	2.813 ± 2.732	2.723 ± 2.690	3.199 ± 2.507	3.368 ± 3.248
Hfnu	0.320 ± 0.075	0.298 ± 0.167	0.373 ± 0.204	0.272 ± 0.156	0.257 ± 0.108
LFpsd	8.331 ± 6.109	7.140 ± 6.209	5.236 ± 4.603	9.025 ± 6.387	10.727 ± 10.622
LFnu	0.680 ± 0.075	0.701 ± 0.167	0.627 ± 0.204	0.728 ± 0.156	0.743 ± 0.108
LF/HF	2.292 ± 0.823	3.356 ± 2.380	2.868 ± 2.885	4.036 ± 3.199	4.203 ± 3.976
ApEn	1.115 ± 0.119	1.153 ± 0.046	1.146 ± 0.085	1.176 ± 0.060	1.136 ± 0.092

HR – heart rate; SDNN – standard deviation of the normal R-R intervals; RMSSD – square root of the mean squared differences between adjacent normal R-R intervals; HFpsd – high-frequency power spectral density; HFnu – high-frequency normalized units; LFpsd – low-frequency power spectral density; LFnu – low-frequency normalized units; LF/HF – low-frequency high-frequency ratio; ApEn – approximate entropy.
Values are means ± SD.
[a] Denotes statistically significant difference between DD1 and DD2 at α=0.05. [b] Denotes statistically significant difference between DD1 and DD5 at α=0.05. [c] Denotes main effect between days.

Figure 1: Mean and predicted HR and ApEn values taken during pre-dive and throughout immersion and predicted from HLM.



The cleanest 5-minute epoch was selected from minutes 16-30 and 46-60 of each hour within the 6-hour dive (a total of 12 epochs/dive) to calculate measures of mean HR and HRV. **A)** Mean HR values obtained at each epoch across each of the 5 dive days. **B)** Predicted HR values obtained through HLM analysis. **C)** Mean ApEn values obtained at each epoch across each of the 5 dive days. **D)** Predicted ApEn values obtained through the HLM analysis. All analyses were performed on log transformed data. The "U" shape in the raw-values correspond to the 10-minute lunch that each diver was provided at 3 hours.

Table 3: Directional changes observed across dives, dive days and at pre-dive

	pre-dive	HLM across dives (epochs)	HLM across dive days
HR	↑	↓	↑
SDNN	↓	↑	-
RMSSD	↓	↑	↓
HFpsd	-	-	↓
HFnu	-	-	↓
LFpsd	-	-	↑
LFnu	-	-	↑
LF/HF	-	-	↑
ApEn	-	↓	↓

HR – Heart rate; SDNN – Standard deviation of normal R-R intervals; RMSSD – Square root of the mean squared differences between adjacent normal R-R intervals; HFpsd – High-frequency power spectral density; HFnu – high-frequency normalized units; LFpsd – low-frequency power spectral density; LFnu – low-frequency normalized units; LF/HF – low-frequency high-frequency ratio; ApEn – approximate entropy.
↓ denotes a statistically significant decrease ($p<0.05$)
↑ denotes a statistically significant increase ($p<0.05$)
- denotes a non-significant change

time points using hierarchical variables. Although the raw values fluctuate across epochs and vary among days, HLM extracts the linear behavior of the variables of interest. It is important to note that the "U" shape observed in this figure corresponds to the 10-minute lunch (in chest-deep water) that each diver was provided at the three-hour mark.

HR increased across dive days (P=0.011), but decreased from the beginning to the end of a dive (P=0.001). Thus, compared to the first dive day, HR at the outset of a dive was higher on later dive days, but tended to decrease across epochs within each dive. A summary of the significant changes and their directionality is presented in Table 3.

Time-domain measures of HRV: SDNN did not change across dive days but increased across epochs within a dive (P<0.001). RMSSD decreased across dive days (P<0.001), but increased within each dive (P=0.018).

Frequency-domain measures of HRV: Both HFpsd (P<0.001) and HFnu (P<0.001) decreased across dive days, but did not change across epochs within dives. In contrast to the high-frequency results, LFpsd (P<0.001) and LFnu (P<0.001) increased overall across dive days, but similarly to the high frequency results, did not change across epochs within dives. The main effects model suggested an overall increase in LF/HF across dive days (P=0.001), but no significant changes across epochs within each dive.

Non-linear dynamics. A graphical representation of ApEn across epochs and across dive days is presented in Figure 1. ApEn decreased overall across dive days (P=0.001) and across epochs within dives (P=0.019).

DISCUSSION

While the physiological response to immersion has been previously researched, the cumulative effect of multiple dive days is a novel observation. The primary finding of this study was the effect of repeated, six-hour shallow-water immersions on cardiac control as indicated by changes in HR and HRV. The post-hoc analysis of pre-dive HR and HRV data indicated that the effects of six-hour immersions with 18-hour dry recovery periods compound across days, and have a significant impact on cardiac control (hypothesis 1). The HLM analysis indicated that HR decreased throughout individual dives while time-domain measures of HRV increased (hypothesis 2). The HLM analysis across dive days (hypothesis 3) indicated an increase in HR and decrease in time-domain HRV measures. We observed a decrease in ApEn values across epochs and across dive days, suggesting diminished complexity and less adaptability.

HR increased and SDNN and RMSSD decreased between pre-dive measures, indicating that there is a cumulative effect of immersion on cardiac control, even at 18 hours following the end of immersion for each dive day. Together with those changes, the decreasing HF and increasing LF HRV measures across dive days suggest that parasympathetic influence diminished throughout the week, likely due to residual reductions in plasma volume [21,22].

During water immersion, fluid from the intracellular and interstitial compartments shifts to the extracellular compartment, and blood from the extremities is redistributed to the central circulation. Consequently, plasma volume, central venous pressure and stroke volume increase [12,17] resulting in a decrease in regional sympathetic outflow and bradycardia [13,16,24]. Findings from the HLM analysis agree with previous literature and suggest an increase in vagal tone during immersion [13,18-20,33] that may stem from baroreflex activation [34] or direct excitation of cardioinhibitory neurons from atrial natriuretic peptide [20,35]. Increases in RMSSD and SDNN but no changes in HF- or LF power across epochs may indicate that cardiac sympathetic regulation was not changed and that alteration in SDNN was mainly induced by the parasympathetic nervous system. The observed changes may have been even more significant without the 10-minute break in the middle of the immersion, which seemed to provide a partial "reset" (see Figure 1 left panel) for HRV and complexity.

In contrast to the increased parasympathetic influence over time within immersions, our data support a decrease in parasympathetic influence across dive days (i.e., the increased parasympathetic influence during immersion was attenuated with repeated immersions). This attenuation is represented through a decrease in HF measures, an increase in LF measures, and an increase in LF/HF. Florian, et al. [21] previously investigated the effects of a single six-hour dive on post-dive autonomic and hemodynamic function. They reported decreases in plasma volume, stroke volume, calf blood flow, and orthostatic tolerance. The decline in orthostatic tolerance was measured through augmented tachycardia and a larger drop in both systolic and diastolic blood pressure during post-dive head-up-tilt compared with pre-dive measures. Hypovolemia as well as cardiovascular and neuroendocrine adjustments accounted for the decrease. Boussuges, et al. [22] also showed reductions in plasma volume and stroke volume and an increase in peripheral vasoconstriction, hence sympathetic nerve activity, as evidenced by a decrease in the ankle brachial index that persisted to the next day. Thus, residual dehydration and increased sympathetic tone across dive days may explain the reduced parasympathetic influence during subsequent immersions. We acknowledge that central volume and baroreceptor reflex could play a role in the HR response, and such factors should be further explored [36].

The decrease in ApEn values across epochs and across dive days was one of the more interesting findings. We observed:

1) a decrease in ApEn across epochs while both SDNN and RMSSD increased; and

2) a decrease in ApEn and RMSSD across dive days.

While changes in ApEn values often follow the same directional changes seen in SDNN and RMSSD, other research suggests there is no significant correlation between ApEn and HRV measures [37]. This is likely due to the multifactorial relationship between complexity and linear measures of variability [10]. Independent directionality between non-linear and linear HRV metrics are not surprising if they measure different aspects of cardiac behavior. A decrease in ApEn highlights the stress on the cardiovascular system when the body is immersed for an extended period of time and insufficient time for complete recovery before the cycle is repeated. A decrease in complexity has been shown to reflect a decrease in working capacity and a decline in the ability to adapt to stress [10]. Ultimately, our observation of an increase in time-domain HRV measures across epochs combined with a decrease in ApEn values respectively indicates a more variable but less complex pattern of cardiac behavior. These findings suggest a less responsive cardiovascular system that could prove detrimental to post-immersion performance for an unknown period of time. Several studies have suggested that a decrease in ApEn reflects a lower adaptive capacity of the cardiac system [10,11,38] and a reduction in cardiac adaptability raises concern over how prolonged and repeated immersions may eventually affect physical and cognitive performance.

Boussuges, et al. [22] previously suggested that fluid replacement strategies are needed post-dive since preload and cardiac output remained significantly lower 16 hours post-immersion. Additional changes in hemodynamic measures suggest that divers are not fully recovered 16 hours after a six-hour dive [22]. Current results are consistent with those findings, as some HR and HRV metrics were not fully recovered after the 18-hour window between dives in this study. The accumulated stress of multiple dives might have reduced the adaptability of the cardiovascular system, a reduction measured as a decrease in ApEn. Although we do not believe that any hydration therapy would negate the effects of a single or repeated bout of prolonged immersion, it is plausible that a hydration intervention might attenuate these effects (note that the divers in this study were encouraged to rehydrate orally between dives. Thus, simple oral rehydration may not suffice). Furthermore, our study is limited by not being able to assess changes in plasma volume during the course of the dive and prevents any evidence based comparison to the findings reported by Boussuges, et al. [22]. The decrease in ApEn across epochs and repeated dive days should be a focus of future research.

Although these findings provide a valuable indication of the impact of repeated, long-duration dives on HR and HRV, replication with a larger sample could provide further benefit. Specifically regarding the present results, a larger sample of divers would provide greater variation (and so power to detect differences) in person-level predictors such as fitness and age. Greater precision in the magnitude of the fixed effects (regression coefficients) would also likely be observed. Nevertheless, small sample sizes within relatively simple fixed-effects hierarchical linear models such as those employed here do not appear to negatively impact most of the factors associated with model interpretation [39].

In addition, allowing participants to surface after three hours to consume a meal adds several complexities to our findings and warrants additional investigation. Alternatively, given the length of dive and experimental trials each day [14], not providing a small meal may have also altered responses across the week. Future studies should investigate the effects of repeated long-duration dives on cognitive changes and baroreflex sensitivity and should specifically assess how these measures correlated with the observed changes in HR and HRV. Although it may appear that data from space flight and bed rest may seem comparable [40,41], it is plausible to say that researchers need to better understand the differential impacts of these stressors on the physiological system. Each of these conditions have their own unique physical demands and thus, their own unique physiological responses.

CONCLUSION

The current data provide valuable insight into the autonomic response associated with repeated long-duration dives. Prolonged and repeated immersions significantly impacted pre-dive measures of SDNN and RMSSD between days, while RMSSD, ApEn and HF HRV measures decreased across days while the divers were in the water. Those changes across dive days were coupled with increases in HR, LF HRV measures, and LF/HF. Throughout each immersion, HR and ApEn decreased while SDNN and RMSSD increased.

Further research should aim to better understand the underlying mechanisms of HR and HRV phenomena and how these changes are represented in time-domain, frequency-domain, and non-linear measures of HRV and complexity during repeated long-duration immersions. Further exploring the changes observed in and out of the water, during rest, exercise, and into recovery in response to repeated long-duration immersions will provide valuable insight into the physiological responses to the acute and chronic stresses of immersion. Insights obtained through simple non-invasive measurement of ECG may prove valuable when assessing and monitoring the health of professional divers.

■

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Appendix A: Summary of results from HLM analysis of dive data

	fixed effects	intercept	day	epoch	pre-dive	age	fitness	— random effects — level 1	level 2
HR	Coeff	3.625	0.008	-0.004	0.184	-0.004	-0.004	0.008	0.002
	(SE)	(0.457)	(0.003)	(0.001)	(0.103)	(0.002)	(0.002)	(0.001)	(0.001)
	p	0.000	0.011	0.001	0.121	0.069	0.059	0.000	0.111
SDNN	Coeff	3.194	0.005	0.012	0.194	-0.006	0.014	0.062	0.087
	(SE)	(1.443)	(0.008)	(0.003)	(0.331)	(0.015)	(0.015)	(0.004)	(0.051)
	p	0.069	0.531	0.000	0.580	0.717	0.365	0.000	0.090
RMSSD	Coeff	2.416	-0.041	0.008	0.261	-0.006	0.006	0.067	0.179
	(SE)	(1.373)	(0.008)	(0.003)	(0.301)	(0.020)	(0.020)	(0.004)	(0.104)
	p	0.129	0.000	0.018	0.419	0.782	0.784	0.000	0.087
HFpsd	Coeff	5.405	-0.200	0.027	0.594	-0.327	-0.835	0.281	0.756
	(SE)	(7.123)	(0.044)	(0.032)	(0.489)	(1.470)	(2.072)	(0.018)	(0.442)
	p	0.477	0.000	0.402	0.270	0.831	0.701	0.000	0.087
Hfnu	Coeff	3.488	-0.273	-0.016	0.491	0.156	-1.190	0.197	0.089
	(SE)	(2.551)	(0.036)	(0.027)	(0.310)	(0.367)	(0.672)	(0.012)	(0.054)
	p	0.220	0.000	0.539	0.164	0.686	0.127	0.000	0.098
LFpsd	Coeff	-1.539	0.180	0.049	0.913	-0.003	0.581	0.363	0.313
	(SE)	(4.907)	(0.049)	(0.036)	(0.441)	(1.033)	(1.304)	(0.023)	(0.186)
	p	0.764	0.000	0.179	0.084	0.998	0.672	0.000	0.092
LFnu	Coeff	-1.559	0.102	0.005	0.332	-0.096	0.407	0.031	0.015
	(SE)	(1.020)	(0.015)	(0.011)	(0.239)	(0.146)	(0.268)	(0.002)	(0.009)
	p	0.178	0.000	0.611	0.213	0.536	0.180	0.000	0.099
LF/HF	Coeff	-5.268	0.375	0.022	0.383	-0.172	1.620	0.359	0.176
	(SE	(3.547)	(0.049)	(0.036)	(0.257)	(0.500)	(0.940)	(0.022)	(0.106)
	p	0.188	0.000	0.545	0.185	0.743	0.136	0.000	0.097
ApEn	Coeff	-0.078	-0.050	-0.014	0.507	-0.075	0.024	0.010	0.002
	(SE)	(0.393)	(0.008)	(0.006)	(0.296)	(0.056)	(0.104)	(0.001)	(0.001)
	p	0.849	0.000	0.019	0.143	0.231	0.827	0.000	0.131

HR – heart rate; SDNN – standard deviation of the normal R-R intervals; RMSSD – square root of the mean squared differences between adjacent normal R-R intervals; HFpsd – high-frequency power spectral density; HFnu – high-frequency normalized units; LFpsd – low-frequency power spectral density; LFnu – low-frequency normalized units; LF/HF – low-frequency/high-frequency ratio; ApEn – approximate entropy. Values are means ± SD. Day – represents the analysis across dive days. Epoch – represents the analysis of dive data performed across epochs.

HIERARCHICAL LINEAR MODELING

HR and HRV measures during Immersion

The intraclass correlation coefficient (ICC) for the unconditional HR model was 0.46 (intercept variance = 0.0072, residual variance = 0.0086), meaning that approximately 54% of the observed variability in HR was within subjects. Although none of the co-variates achieved the significance threshold of $P<0.05$, age and VO_{2max} achieved marginal significance ($P=0.07$ and 0.06 respectively) as predictors, and were both negatively associated with HR. A summary of the significant changes and their directionality is presented in the table above.

Time-domain measures of HRV

The ICC for the uncon-ditional SDNN model was 0.59 (intercept variance = 0.0932, residual variance = 0.0634), meaning that approximately 41% of the observed variability in SDNN was within subjects. RMSSD decreased across dive days ($P<0.001$), but increased within each dive ($P=0.018$).

The ICC for the unconditional RMSSD model was 0.60 (intercept variance = 0.1536, residual variance = 0.0700), meaning that approximately 40% of the observed variability in RMSSD was within subjects.

Frequency-domain measures of HRV

The ICC for the unconditional HFpsd model was 0.74 (intercept variance = 0.8341, residual variance = 0.2921), meaning that approximately 26% of the observed variability in HFpsd was within subjects. The ICC for the unconditional HFnu model was 0.35 (intercept variance = 0.1154, residual variance = 0.2179), meaning that approximately 65% of the observed variability in HFnu was within subjects.

The ICC for the unconditional LFpsd model was 0.63 (intercept variance = 0.6411, residual variance = 0.3718), meaning that approximately 37% of the observed variability in LFpsd was within subjects.

The ICC for the unconditional LFnu model was 0.34 (intercept variance = 0.0173, residual variance = 0.0343), meaning that approximately 66% of the observed variability in LFnu was within subjects. The ICC for the unconditional LF/HF model was 0.35 (intercept variance = 0.2186, residual variance = 0.3996).

Non-linear dynamics (ApEn)

The ICC for the unconditional ApEn model was 0.22 (intercept variance = 0.0031, residual variance = 0.0111), meaning that approximately 78% of the observed variability in ApEn was within subjects.

■