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Cardiovascular and autonomic responses to physiological stressors before and after six hours of water immersion

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Florian JP, Simmons EE, Chon KH, Faes L, Shykoff BE. Cardiovascular and autonomic responses to physiological stressors before and after six hours of water immersion. *J Appl Physiol* 115: 1275–1289, 2013. First published August 15, 2013; doi:10.1152/japplphysiol.00466.2013.—The physiological responses to water immersion (WI) are known; however, the responses to stress following WI are poorly characterized. Ten healthy men were exposed to three physiological stressors before and after a 6-h resting WI (32–33°C): *1*) a 2-min cold pressor test, *2*) a static handgrip test to fatigue at 40% of maximum strength followed by postexercise muscle ischemia in the exercising forearm, and *3*) a 15-min 70° head-up-tilt (HUT) test. Heart rate (HR), systolic and diastolic blood pressure (SBP and DBP), cardiac output ($\dot{Q}$), limb blood flow (BF), stroke volume (SV), systemic and calf or forearm vascular resistance (SVR and CVR or FVR), baroreflex sensitivity (BRS), and HR variability (HRV) frequency-domain variables [low-frequency (LF), high-frequency (HF), and normalized (n)] were measured. Cold pressor test showed lower HR, SBP, SV, $\dot{Q}$, calf BF, LFn_{HRV}, and LF/HF_{HRV} and higher CVR and HFn_{HRV} after than before WI ($P < 0.05$). Handgrip test showed no effect of WI on maximum strength and endurance and lower HR, SBP, SV, $\dot{Q}$, and calf BF and higher SVR and CVR after than before WI ($P < 0.05$). During postexercise muscle ischemia, HFn_{HRV} increased from baseline after WI only, and LFn_{HRV} was lower after than before WI ($P < 0.05$). HUT test showed lower SBP, DBP, SV, forearm BF, and BRS and higher HR, FVR, LF/HF_{HRV}, and LFn_{HRV} after than before WI ($P < 0.05$). The changes suggest differential activation/depression during cold pressor and handgrip (reduced sympathetic/elevated parasympathetic) and HUT (elevated sympathetic/reduced parasympathetic) following 6 h of WI.

water immersion; orthostatic tolerance; static exercise; cold pressor; heart rate variability; autonomic nervous system

ALTHOUGH PHYSIOLOGICAL RESPONSES during water immersion (WI) are well documented, less is known about the residual effects in air following WI (46). Thermoneutral WI induces an increase in central blood volume and plasma volume (PV) (24, 39, 48) resulting from *1*) fluid shift from the interstitial and intracellular fluid compartments to the extracellular compartment (10, 66) and *2*) redistribution of blood volume from the legs and abdomen to the chest (23, 26). Consequently, excretion of fluid and electrolytes is augmented, together with suppression of levels of the fluid-regulating hormones renin, angiotensin II, aldosterone, and AVP to normalize blood volume (10, 48). Autonomic and hemodynamic variables are similarly affected during WI. Muscle sympathetic nerve activity (MSNA) and norepinephrine (NE) concentrations are reduced (38), cardiac output ($\dot{Q}$) and stroke volume (SV) are increased, blood pressure (BP) is unchanged, and systemic vascular resistance (SVR) is reduced (66). Short-duration (5–30 min) resting head-out or complete WI (42–44, 55) increases heart rate (HR) variability (HRV), particularly the high-frequency (HF) component, indicating a shift toward enhanced parasympathetic control. The parasympathetic shift is further augmented during 6 h of WI (60).

Physiological responses to WI may reduce physical performance and orthostatic tolerance after egress from the water (22, 54, 58). Indeed, the release of hydrostatic pressure following WI elicits acute hypovolemia (46, 48), and post-WI physiological responses in several reports indicate modulation of autonomic function (38, 46, 54) and changes in cardiac or vascular function (3, 4, 11). After WI, resting HR and BP remain unchanged compared with pre-WI values (4, 46) and $\dot{Q}$ is unchanged (46, 57) or reduced (4). Boussuges et al. (4) showed that the increase in SVR and reductions in preload, SV, $\dot{Q}$, and total arterial compliance can persist for up to 16 h following WI; however, whether these changes are related strictly to hypovolemia or to direct or indirect effects on autonomic function is unknown. Moreover, the autonomic and hemodynamic responses to stress (other than orthostatic) have not been studied previously.

Afferent and efferent reflex pathways can be characterized and effects of environmental adaptations (i.e., WI, spaceflight, and bed rest) on neural and cardiovascular responses can be determined by employing stressors such as the cold pressor test, static handgrip to fatigue, and passive upright tilt. The cold pressor test assesses reflex pathways originating from cold nociceptors in the skin and involving central vasomotor centers through sympathetic and pressure responses (17, 68). Static handgrip to fatigue elicits increases in BP, HR, and MSNA (56), with two mechanisms primarily responsible for neural and cardiovascular responses: *1*) feedforward control (central command), by activation of the cardiovascular center via descending central neural pathways, and *2*) feedback control (exercise pressor reflex), emanating from mechano- and metaboreceptors and their associated group III and IV afferent fibers in skeletal muscles (53, 56). Upon initiation of passive tilting, ~300–500 ml of blood are translocated from the chest to the dependent regions, leading to a reduction in venous return and SV. To counteract the reduction in SV and to maintain BP and cerebral perfusion, the baroreflex reduces vagal activity to the heart and increases sympathetic activation, contributing to tachycardia and arterial vasoconstriction (2).

Given that a change in blood volume and autonomic function can alter the responses to stress and since adaptation to environments that produce changes similar to those seen during WI have shown altered responses to stress (17, 34), it is likely that WI also affects physiological responses to these stressors. To address the gap in knowledge about autonomic and cardio-

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Table 1. *Subject characteristics*

Characteristic	Value
Age, yr	34 ± 10 (19–44)
Height, cm	179 ± 6
Weight, kg	85 ± 7
BMI, kg/m^2	26 ± 1
Body fat, %	19 ± 4
Maximal O_2 uptake, ml·kg^{-1}·min^{-1}	53 ± 10
SBP, mmHg	124 ± 8
DBP, mmHg	76 ± 6
Total cholesterol, mmol/l	4.63 ± 0.82
HDL, mmol/l	1.24 ± 0.41
LDL, mmol/l	2.72 ± 0.66
Triglycerides, mmol/l	1.17 ± 0.66
Glucose, mmol/l	4.89 ± 0.35
Hb, mg/dl	15 ± 1
Hct, %	44 ± 3

Values are means ± SD, with range in parentheses; $n = 10$ subjects. See *Glossary* for abbreviations.

vascular effects immediately after WI, we examined responses to the following stressors before and after a 6-h WI: *1*) cold pressor, *2*) static handgrip at 40% of maximum voluntary contraction (MVC) followed by postexercise circulatory arrest in the exercising arm, and *3*) 15 min of 70° head-up tilt (HUT). At rest and during the three stressors, we measured multiple hemodynamic variables and spontaneous baroreflex sensitivity, as well as time-domain and linear and nonlinear frequency-domain measures of HRV. We hypothesized that, following WI, cardiovascular and cardiac autonomic responses to the three stressors would be altered and that orthostatic tolerance during HUT would be diminished.

Glossary

α_1	Short-term fractal scaling component
ANP	Atrial natriuretic peptide
ApEn	Approximate entropy
BP	Blood pressure
BPV	Blood pressure variability
BRS	Baroreflex sensitivity
CBF	Calf blood flow
CVR	Calf vascular resistance
DBP	Diastolic blood pressure
DFA	Detrended fluctuation analysis
FBF	Forearm blood flow
FFT	Fast Fourier transformation
FVR	Forearm vascular resistance
Hct	Hematocrit
HF	High frequency
HR	Heart rate
HRV	Heart rate variability
HUT	Head-up tilt
LF	Low frequency
MAP	Mean arterial pressure
MSNA	Muscle sympathetic nerve activity
MVC	Maximal voluntary contraction
NE	Norepinephrine
$\dot{Q}$	Cardiac output
PDM	Principal dynamic mode
PEMI	Postexercise muscle ischemia
PNS	Parasympathetic nervous system
PSD	Power spectral density
PV	Plasma volume
RMSSD	Root-mean square of successive differences of RRI
RRI	RR interval
SBP	Systolic blood pressure
SDNN	Standard deviation of normal-to-normal R waves
SNS	Sympathetic nervous system
SV	Stroke volume
SVR	Systemic vascular resistance
WI	Water immersion

METHODS

Subjects

Ten healthy men participated in the study; their physical characteristics at screening are presented in Table 1. All participants were experienced military divers with an average of 10 yr of diving experience. They were healthy, active, normotensive nonsmokers who were not taking any medications that would affect responses in the study. Each subject underwent medical screening that included complete blood count, complete metabolic panel, lipid profile evaluation, urinalysis, physical examination, skinfold body fat measurement, and determination of maximal O_2 uptake. Approval was obtained from the Institutional Review Board of the Navy Experimental Diving Unit. Each subject gave written informed consent, and all procedures conformed to the Declaration of Helsinki.

Study Design

The study design is shown in Fig. 1. Subjects abstained from alcohol for 2 days, caffeine and strenuous exercise for 1 day, and food and drink (except water) for 2 h before reporting to the laboratory in the morning. Subjects wore running shorts and T-shirts for all visits. Each subject underwent physiological testing before and after a 6-h WI. All physiological testing was completed in a laboratory (air temperature 22–24°C) adjacent to the immersion tank. After completing pre-WI testing (see *Protocol for Pre- and Post-WI Testing*), each subject received a standardized snack, submitted a urine sample for measurement of urine specific gravity, emptied his bladder, and was weighed. A condom catheter was applied to collect urine during the dive. Subsequently, each subject was immersed in the tank, surfaced after the 3rd h for a 10-min lunch break while still immersed to

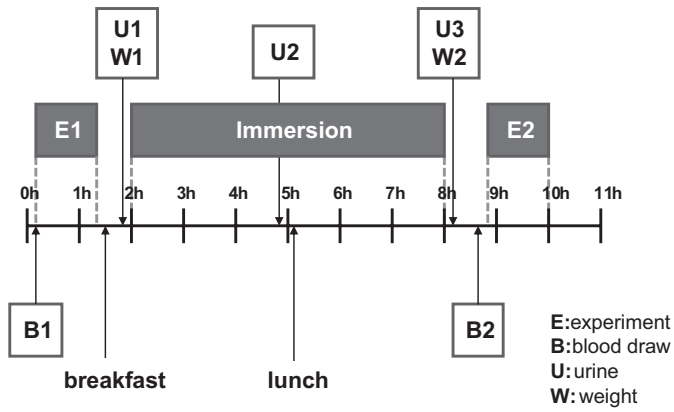

Fig. 1. Water immersion and experimental timeline. Experiments (E1 and E2) include baseline measurements and cold pressor, static handgrip, and head-up tilt tests. Urine was collected before (U1), during (U2), and after (U3) immersion. Subjects were weighed (W) and blood was drawn (B) before and after immersion. A standardized breakfast and lunch were provided before immersion and after 3 h of immersion, respectively.

midchest, and then returned to complete the WI. After surfacing at the end of the exposure, each subject emptied his bladder into a container; this post-WI volume plus that collected via his condom catheter is taken as his total urine output for the dive. A final weight was taken after post-WI urination and after the subject dried completely. The difference between pre- and post-WI weights represents the weight lost during the WI.

Protocol for Pre- and Post-WI Testing

Subjects lay supine on a tilt table with their arms outstretched; the tilt table (model 9505-345, Bailey) was modified to support a person's arms at the level of his heart when he is tilted up. Subjects were then instrumented for measurement of HR (electrocardiogram), BP (Finometer Pro, Finapres Medical Systems), and limb blood flow (venous occlusion plethysmography). $\dot{Q}$ was obtained from a Finometer or by echocardiography, as described in *Hemodynamic Measurements*. For measurement of handgrip MVC, the subject, using his left hand, briefly squeezed a custom-built handgrip device three times at maximal effort; the highest force generated was used as the MVC. After instrumentation and a 15-min adaptation period, a venous blood sample was obtained from the left antecubital vein for analysis of glucose, NE, aldosterone, atrial natriuretic peptide (ANP), AVP, Hb, and hematocrit (Hct). Hemodynamic measurements were then taken at rest and throughout the tests that followed. Total time of testing from the start of baseline measurements to the end of tilt testing recovery was 67 min.

Cold pressor test. Each subject placed his left hand in a 0–1°C mixture of ice and water for 2 min. Immediately following the test, the subject removed his hand from the ice water, and the hand was wrapped in a towel with a warming pack while recovery data were recorded. Subjects were instructed to relax, maintain normal breathing, and avoid the Valsalva maneuver and isometric muscular contraction throughout the test.

Static handgrip to fatigue. After a sufficient recovery period to allow all signals to return to basal values following the cold pressor test, baseline variables for the static handgrip test were recorded for 4 min. At the end of this period, the static handgrip test began. With use of a visual force feedback system, static handgrip with the left hand was maintained at 40% of pre-WI MVC until fatigue before and after WI. During exercise, the subjects were instructed to avoid the Valsalva maneuver as well as leg or abdominal muscle tension. When the achieved force declined to $<80\%$ of the target for ≥ 5 s, an upper arm cuff was inflated to 250 mmHg and the subject relaxed his hand. Two minutes of postexercise muscle ischemia (PEMI) in the exercising forearm followed, with 2 min of recovery after release of the cuff.

70° head-up tilt. Pretilt data were recorded for 5 min following a physiological stabilization period after the static handgrip. Each subject was then tilted 70° head-up from supine for 15 min or until symptoms associated with presyncope occurred or the subject requested termination of the test. Presyncope was defined as a rapid decrease in systolic BP (SBP) to <80 mmHg or a sustained SBP <90 mmHg associated with symptoms of light-headedness, nausea, or diaphoresis. Subjects were tilted back down to the horizontal position at the end of 15 min or, if presyncope occurred, to the Trendelenburg position ($-10°$) until hemodynamic stability was reached. Ten minutes of recovery were recorded in the supine position. Head-up tilt (HUT) time was limited to 15 min because of schedule constraints of testing and WI. Only data segments from periods of hemodynamic stability (i.e., excluding presyncope) were analyzed.

Water Immersion

All participants underwent a 6-h WI at the bottom of a 15-ft pool filled with comfortably warm water (32–33°C). They wore T-shirts and shorts, and weights were provided to maintain negative buoyancy. While sitting upright in a chair, each participant breathed surface-supplied air delivered with a MK20 breathing apparatus (Aga mask, Interspiro). The MK20 breathing apparatus uses a demand regulator that, once the pressure in the mouth is slightly below ambient water pressure at the regulator, delivers breathing gas at a pressure slightly greater than ambient pressure to minimize breathing resistance. The hydrostatic gradient in the chest of a seated submerged subject breathing via the MK20 apparatus is similar to that for seated, head-out WI.

After 3 h of WI, each subject returned to the surface to stand on a platform with head and shoulders out of the water for 10 min while consuming a small lunch with an energy content of 2.2 MJ (24% fat, 64% carbohydrate, and 12% protein) and 500 ml of liquid.

Hemodynamic Measurements

HR and arterial pressure. HR was derived from a five-lead surface electrocardiogram (Dash 3000, General Electric). Beat-to-beat arterial pressure was measured by photoplethysmography (Finometer) on a finger of the right hand. Finger pressure was calibrated to brachial artery pressure using the manufacturer's return-to-flow system. Beat-to-beat values of SBP, diastolic BP (DBP), and mean arterial pressure (MAP) were averaged for each 1-min time segment of cold pressor, handgrip, and HUT tests. Oscillometric brachial BP (model HEM-907XL, Omron) also was measured at the beginning of the monitoring period and after 15 min of supine rest.

Limb blood flow. Calf and forearm blood flow (CBF and FBF) were determined by venous occlusion plethysmography (model EC-6, Hokanson) on the calf during initial baseline, cold pressor, and static handgrip, and on the forearm for tilt baseline, HUT, and recovery from tilt. During each data-recording period, blood flow was acquired from three to four measurement cycles in succession. Limb vascular resistance [calf vascular resistance (CVR) and forearm vascular resistance (FVR)] was estimated as corresponding brachial MAP/CBF or brachial MAP/FBF.

$\dot{Q}$. During cold pressor and HUT testing, $\dot{Q}$ was assessed using transthoracic echocardiography (Acuson Cypress, Siemens). SV was determined from the flow velocity across the aortic valve (apical approach) and the diameter of the aortic orifice during systole (parasternal long axis). $\dot{Q}$ was calculated as SV·HR and expressed in liters per minute. SVR was calculated as MAP/$\dot{Q}$. During handgrip testing, $\dot{Q}$, SV, and SVR were taken from Finometer PRO Modelflow calculations (65).

Orthostatic tolerance was estimated by the maximum increase in HR ($+\Delta HR_{max}$) during HUT and by the orthostatic index (58) calculated from the change in HR and BP during HUT.

Time-Domain Analyses and Complexity Analysis of HRV

Time-domain HRV. Mean HR, root-mean square of successive differences (RMSSD) of RR intervals (RRI), and the standard deviation of normal-to-normal R waves (SDNN) were calculated. RMSSD mainly reflects the modulation of the parasympathetic system, and SDNN is an indicator of overall autonomic nervous system activity.

Baroreflex sensitivity. Baroreflex sensitivity (BRS) was estimated in the time domain according to the sequence method (49). Briefly, sequences during which the SBP and the RRI increased or decreased progressively over three or more consecutive beats were identified, and for each sequence, the slope of the linear regression line between SBP and RRI variations was used as an estimate of BRS. To be valid, a sequence was required to exhibit a change of ≥ 5 ms in RRI and ≥ 1 mmHg in SBP at each beat, and the correlation coefficient of linear regression was required to be ≥ 0.85. The reported value of the BRS index was the average of the slopes of the regression lines for valid sequences.

Approximate entropy. Approximate entropy (ApEn), a nonlinear statistical method used to assess the complexity of data, has been used to measure the loss of complexity of HRV in a variety of pathological and physiological conditions (51). ApEn determines the conditional probability of specific patterns between a selected finite time series and the next incremental comparison: the higher the probability, the

lower the complexity and the smaller the ApEn value. ApEn values were calculated from instantaneous RRIs using the embedding dimension $m = 2$ and the automatically selected threshold value r (6).

Frequency-Domain Analyses

A time-domain HRV signal was generated from the instantaneous RRI series at a uniform sampling rate of 4 Hz using cubic spline interpolation. The HRV signal was downsampled to 2 Hz, and mean and linear trends were removed. A BP variability (BPV) signal was generated similarly from SBP. These signals were transformed into the frequency domain. For each subject, HRV time-domain signal segments containing 360 points (3 min) were analyzed with power spectral density (PSD) and principal dynamic mode (PDM) methods. Since BPV also indicates sympathovagal interactions in humans (1), BPV was also investigated, but only using PSD.

PSD analysis of HRV and BPV. PSDs of HRV data were obtained using the Welch periodogram method (Matlab version 7.9, Natick, MA). 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. Mean spectral power in the low-frequency (LF, 0.04–0.15 Hz) and HF (0.15–0.4 Hz) bands and the LF-to-HF ratio (LF_{HRV}, HF_{HRV}, and LF/HF_{HRV}, respectively) were calculated. The same methodology with BPV yielded LF_{BPV} and HF_{BPV}. Power for LF_{HRV} and HF_{HRV} was also represented in normalized units (LFn_{HRV} and HFn_{HRV}). The HF band is thought to be dominated by cardiac parasympathetic nervous outflow, whereas the LF band is believed to be mediated by the cardiac sympathetic and parasympathetic nervous outflows. The ratio of LF to HF is generally taken as an indicator of balance between the two arms of the autonomic nervous system.

PDM analysis of HRV. PDM analysis was used in addition to PSD to assess sympathetic and parasympathetic dynamics during HUT. Unlike PSD, PDM accounts for the inherent nonlinear dynamics of HR control. Methodological details are described elsewhere (69). In this study, the optimal estimation error was found with nine Laguerre functions and a memory length of 60. PDMs are time-domain signals that are converted to the frequency domain via FFT. The two most dominant PDMs of HRV are considered to represent sympathetic and parasympathetic nervous system (SNS and PNS) activity.

BRS. The complex-valued transfer function between RRI and SBP was evaluated as the ratio of the cross-spectral density function of the two series to the PSD of the SBP series. The BRS gain (transfer function modulus) was determined by averaging the gain in the whole LF band (GainLF) regardless of the value of coherence (52) within the LF.

Blood Samples

Glucose, Hb, and Hct levels were determined immediately after blood collection (Rapidpoint 400, Siemens). Blood for all other analyses was centrifuged at 4°C and stored at −80°C until assay. Samples for NE, ANP, AVP, and 8-isoprostane were drawn into prechilled tubes containing EDTA. Blood for aldosterone was allowed to clot at room temperature for 30 min before centrifugation. Glucose was measured by the oxidase method; NE by HPLC; and aldosterone, ANP, AVP, and 8-isoprostane by immunoassay.

For calculation of PV, blood was drawn in a 2-ml sodium heparin tube for measurements of Hb and Hct using Rapidpoint 400 (Siemens). The relative change in PV (ΔPV) following WI was calculated from changes in Hb and Hct concentrations according to the Harrison modification of the Dill and Costill equation (25).

Statistical Analysis

Repeated-measures ANOVAs were conducted to determine the effect of WI on neural, hormonal, and hemodynamic variables. When appropriate, differences between factors were identified using the Bonferroni-Holm correction. All statistical analyses were performed using SAS version 9.2. The level of significance was set at $\alpha = 0.05$, and values are means ± SE.

RESULTS

Mean weight loss after WI, adjusted for food and fluid intake during WI, was 2.09 ± 0.09 kg. Urine production during WI was 1,000 ± 110 ml from 0 to 3 h and 590 ± 70 ml from 3 to 6 h, for a total of 1,590 ± 120 ml for the entire WI. Urine specific gravity did not change following WI (Table 2), but post-WI PV significantly decreased by 11.3 ± 1.2%.

Table 2 shows pre- and post-WI hormone and electrolyte concentrations, as well as resting hemodynamic data. Aldosterone, ANP, AVP, NE, glucose, and 8-isoprostane concentrations were similar before and after WI, and Hb and Hct concentrations significantly increased following WI. Resting SBP, SV, and CBF significantly decreased, CVR increased, and HR, DBP, $\dot{Q}$, and SVR remained unchanged following WI.

Cold Pressor

Hemodynamic and PSD HRV measurements before, during, and after cold pressor are presented in Figs. 2 and 3. BRS, BPV, and time-domain HRV measures are shown in Table 3.

Hemodynamic measurements. During cold pressor, HR, SBP, SV, $\dot{Q}$, and CBF were significantly reduced, and CVR was significantly increased following WI. Compared with pre-WI and baseline, the tachycardic response was blunted during the 1st and 2nd min of cold pressor.

HRV, BPV, and ApEn. Total HRV did not change after WI; however, post-WI LF/HF_{HRV} and LFn_{HRV} were lower and

Table 2. *Values of variables before and after 6 h of WI*

	Pre-WI	Post-WI
Weight, kg	85.7 ± 2.3	84.3 ± 2.3†
Urine specific gravity	1.014 ± 0.002	1.012 ± 0.001
Aldosterone, ng/dl	8.37 ± 1.74	4.68 ± 1.47
ANP, pg/ml	752 ± 131	706 ± 59
AVP, pg/ml	5.42 ± 1.03	5.48 ± 0.90
NE, nM	0.894 ± 0.114	1.151 ± 0.132
8-Isoprostane, pg/ml	24.57 ± 1.86	22.26 ± 2.09
Glucose, mg/dl	94.3 ± 4.1	90.6 ± 2.1
Hb, g/dl	14.57 ± 0.30	15.54 ± 0.31†
Hct, %	43.29 ± 0.88	46.39 ± 0.80†
HR, beats/min	54 ± 3	52 ± 3
SBP, mmHg	131 ± 2	124 ± 2†
DBP, mmHg	70 ± 2	70 ± 2
$\dot{Q}$, l/min	4.8 ± 0.3	4.4 ± 0.3
SV, ml/beat	91 ± 6	83 ± 5*
SVR, units	19.5 ± 1.2	20.7 ± 1.2
CBF, ml·100 ml^{-1}·min^{-1}	2.77 ± 0.57	1.66 ± 0.19*
CVR, units	43.6 ± 7.4	61.3 ± 7.7†
HUT time, min	15.0 ± 0.0	13.2 ± 1.0
Orthostatic index, units	52.1 ± 7.7	93.1 ± 12.9†
Δtilt HR_{max}, beats/min	31.1 ± 4.0	45.6 ± 5.1†
Δtilt SBP_{max}, mmHg	−28.9 ± 4.4	−38.2 ± 3.8†
Δtilt DBP_{max}, mmHg	−15.3 ± 2.2	−18.5 ± 2.2*
Maximum hand grip, kg	57.1 ± 2.5	55.5 ± 2.6
Hand grip duration, min	2.2 ± 0.3	2.2 ± 0.2

Values are means ± SE. Reductions in resting supine SBP, SV, and CBF and increases in CVR following WI suggest augmented peripheral sympathetic activation in response to PV contraction. Resting supine SBP and CBF decreased and CVR increased after WI. Decline in orthostatic index and altered hemodynamic responses during 70° HUT indicate reduced orthostatic tolerance. See *Glossary* for abbreviations. *$P < 0.05$, †$P < 0.01$ vs. pre-WI.

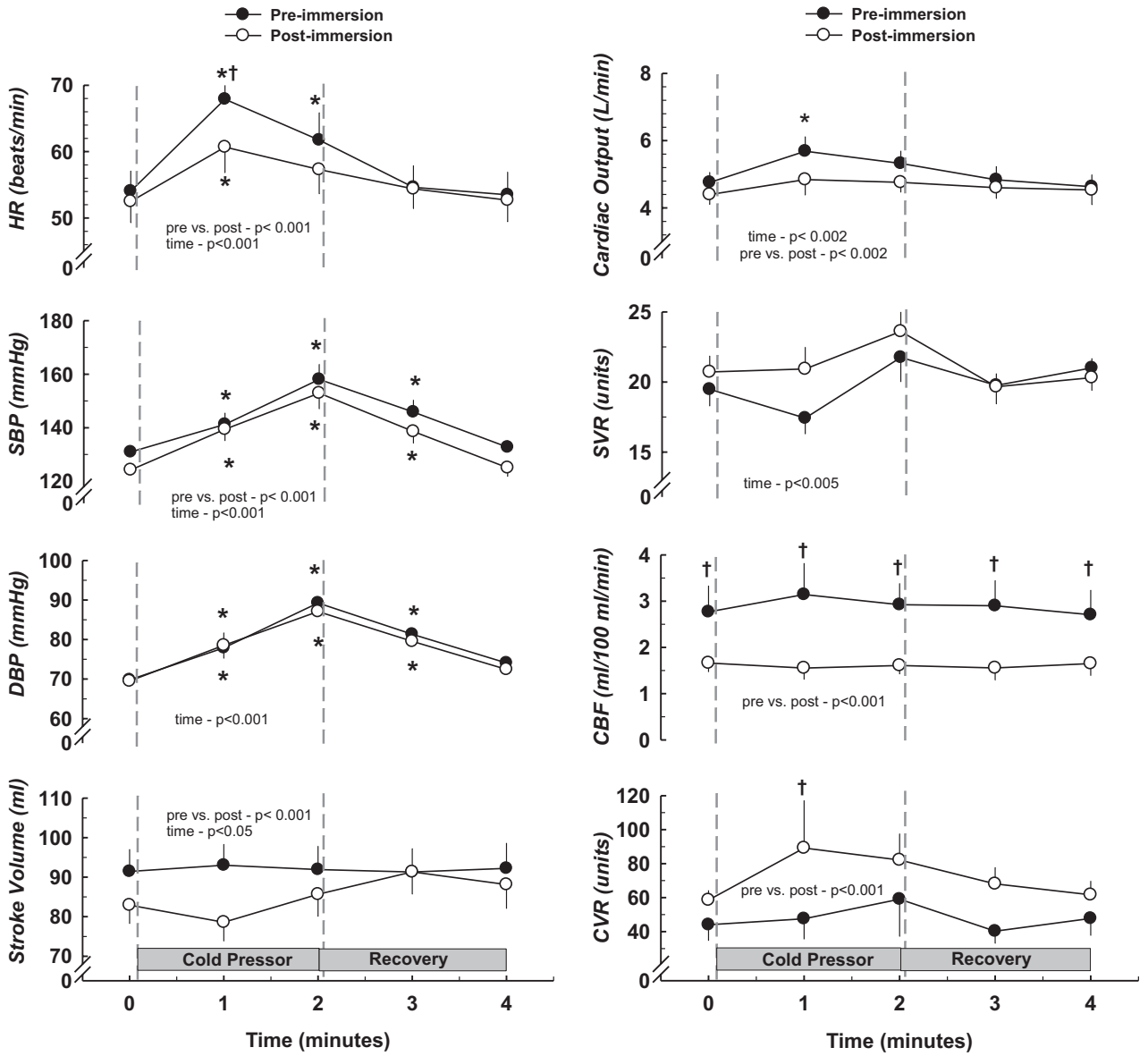

Fig. 2. Hemodynamic responses during cold pressor test before and after a 6-h water immersion. See *Glossary* for abbreviations. Values are group means ± SE. Statistical comparisons are noted on each plot. *$P < 0.05$ vs. baseline. †$P < 0.05$ vs. preimmersion. Postimmersion responses during cold pressor favored a lower cardiac output, resulting from reduced HR and stroke volume. Compared with preimmersion values, postimmersion CBF was reduced and CVR was increased throughout cold pressor and recovery.

HFn_{HRV} was higher than pre-WI values throughout cold pressor and recovery. The time-domain measure of BPV, SBP-SD, significantly increased from baseline during the 1st min of cold pressor in the post-WI trial only. After WI, ApEn decreased significantly during cold pressor and increased significantly after cold pressor.

BRS. BRS measured using the sequence technique did not change, but GainLF decreased similarly in both groups during cold pressor and returned to baseline values by the 1st min of recovery. Pre- and post-WI BRS responses were similar.

Static Handgrip

Maximum handgrip strength following WI was reduced by 3.1%, but the decrease was not statistically significant ($P = 0.054$). The time to fatigue during static handgrip testing (isoforce) was similar before and after WI (Table 2). Hemodynamic and PSD HRV measurements before, during, and after handgrip and PEMI are presented in Figs. 4 and 5. BRS, BPV, and time-domain HRV measures are shown in Table 4.

Hemodynamic measurements. At the same absolute and relative force, HR, SBP, SV, $\dot{Q}$, and CBF were significantly reduced and SVR and CVR were increased across the phases of handgrip testing. HR gradually increased during static handgrip, reached its peak at fatigue, and immediately returned to baseline values during PEMI before and after WI. SBP and DBP increased progressively during static handgrip, peaked at fatigue, and decreased but remained elevated compared with baseline during PEMI before and after WI. Although post-WI SV was significantly lower than pre-WI SV throughout testing, SV at maximum fatigue for each trial was significantly reduced

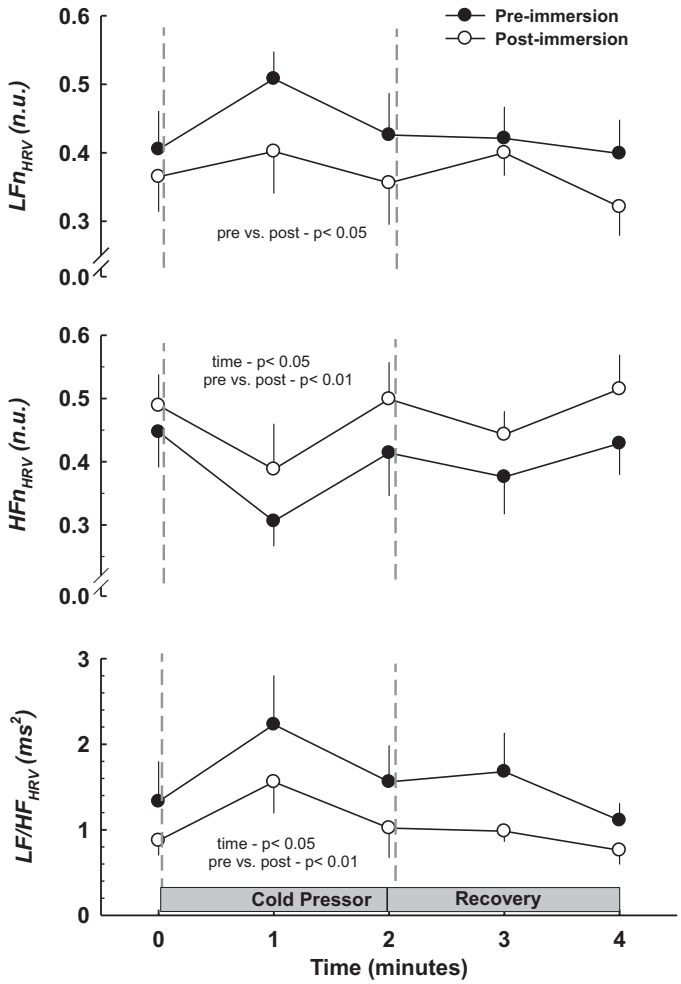

Fig. 3. Heart rate variability responses during cold pressor test before and after a 6-h water immersion. See *Glossary* for abbreviations. Values are group means ± SE. Statistical comparisons are noted on each plot. Postimmersion LF/HF_{HRV} and LFn_{HRV} were lower and HFn_{HRV} was higher than preimmersion values throughout cold pressor and recovery.

compared with the respective baselines. The handgrip-induced increase in $\dot{Q}$ was significantly lower after than before WI.

HRV, BPV, and ApEn. RMSSD progressively decreased from baseline throughout handgrip, returned to baseline during PEMI, and was not different from baseline during recovery. Post-WI RMSSD during the 2nd min of PEMI rebounded significantly higher than baseline RMSSD and returned to basal levels during recovery. Compared with baseline, total HRV significantly decreased during handgrip, and post-WI total HRV was similar to pre-WI values. LF_{HRV} significantly decreased during handgrip but was similar to baseline during PEMI and recovery. LF_{HRV} was significantly lower than pre-WI LF_{HRV} during handgrip baseline, PEMI, and recovery, but not during handgrip exercise. Whereas pre-WI HF_{HRV} significantly decreased during handgrip but returned to baseline during PEMI and recovery, post-WI HF_{HRV} did not decrease from baseline but significantly increased during PEMI and returned to baseline during recovery. A significant increase from baseline in HFn_{HRV} during PEMI was also observed after, but not before, WI. No significant changes in LFn_{HRV} during any stage of pre-WI handgrip testing were noted, but LFn_{HRV} significantly increased from baseline during the late phase of handgrip and was significantly lower than pre-WI values during PEMI. The LF-to-HF_{HRV} ratio increased during the latter half of handgrip compared with baseline, and pre- and post-WI values were not substantially different. Total BPV did not change during the beginning of handgrip exercise but increased significantly from baseline by the end of handgrip. LF_{BPV} followed a pattern similar to total BPV, but HF_{BPV} increased by the end of handgrip only in the pre-WI trial. ApEn decreased during handgrip but returned to baseline during PEMI and recovery. This trend did not change significantly after WI.

BRS. In both conditions, BRS measured using the sequence technique decreased during handgrip, fully recovered during PEMI, and remained at baseline during recovery. The reduction in transfer function gain (GainLF) during handgrip was similar before and after WI.

70° Head-Up-Tilt

Orthostatic tolerance (estimated by the orthostatic index and $+\Delta HR_{max}$ during HUT) was reduced following WI. The $-\Delta SBP_{max}$ and $-\Delta DBP_{max}$ during HUT were also significantly larger following WI. There was a trend toward reduced HUT time following WI, but the reduction was not significant ($P = 0.092$). Although all subjects completed the 15-min HUT before WI without symptoms, three subjects became presyncopal after 6.9, 10.4, and 9.4 min of post-WI HUT, respectively. Hemodynamic and PSD HRV measurements before, during, and after HUT are presented in Figs. 6 and 7. BRS, BPV, and time-domain HRV measures are shown in Table 5.

Hemodynamic measurements. As expected, HR increased and SBP and SV decreased from baseline during HUT. The cardioacceleration and drop in SBP and SV were larger after than before WI. DBP and FBF were significantly lower and FVR was higher after than before WI, and all were significantly different from their respective post-WI baselines by the end of HUT.

HRV, BPV, and ApEn. Before and after WI, RMSSD, total HRV, LF_{HRV}, HF_{HRV}, and HFn_{HRV} decreased by the first 5 min of HUT, remained reduced throughout HUT, and returned to baseline levels during recovery. LF/HF_{HRV} did not increase during tilt before WI but did increase from baseline following WI, and a significant pre/post-WI × time interaction was noted for LFn_{HRV}, where LFn_{HRV} increased from baseline during HUT more after than before WI. The cardiac SNS component estimated by PDM analysis increased more during HUT after than before WI. The SNS/PNS activity increased by the late portion of HUT, but this response was not affected by WI. Total BPV in the time and frequency domains did not change before WI but was significantly increased throughout HUT after WI. This pattern was seen in both frequency domain components, LF_{BPV} and HF_{BPV}. ApEn did not change during pre-WI HUT but was significantly reduced during post-WI HUT.

BRS. BRS measured using the sequence technique decreased significantly during the beginning of HUT, plateaued through the rest of HUT, and returned to baseline values during recovery. GainLF followed a similar pattern during HUT, but post-WI values were significantly lower than pre-WI values.

Table 3. *Autonomic responses to cold pressor test before and after 6 h of WI*

	Time					*P* Value		
	Cold pressor			Recovery		Main effect		Interaction‡
Variable	Baseline	*Minute 1*	*Minute 2*	*Minute 1*	*Minute 2*	Pre/post	Time	Pre/post × time
SDNN, ms								
Pre	76.8 ± 12.9	60.4 ± 8.04	67.9 ± 12.4	102.0 ± 14.2	76.2 ± 11.9	0.392	**0.004**	0.730
Post	72.2 ± 14.7	75.9 ± 10.9	77.8 ± 16.8	98.1 ± 14.7	82.8 ± 15.7			
RMSSD, ms								
Pre	85.0 ± 18.1	50.2 ± 9.3	75.1 ± 19.5	101.0 ± 18.0	82.4 ± 15.9	0.201	**0.018**	0.836
Post	86.1 ± 19.3	71.1 ± 16.9	90.1 ± 25.3	99.0 ± 17.3	94.5 ± 20.9			
Nseq, AU								
Pre	14.4 ± 6.5		12.0 ± 4.4		13.9 ± 5.1	0.531	0.362	0.937
Post	13.0 ± 5.6		10.5 ± 3.1		13.7 ± 5.3			
BRS, ms/mmHg								
Pre	25.3 ± 4.0		21.5 ± 5.1		22.5 ± 3.4	0.900	0.932	0.422
Post	23.8 ± 5.0		27.2 ± 5.0		23.1 ± 3.6			
Transfer gain, LF, ms/mmHg								
Pre	18.2 ± 3.1	13.0 ± 2.0	12.5 ± 1.8	18.3 ± 4.0	20.5 ± 4.2	0.298	**0.002**	0.943
Post	15.2 ± 3.1	12.7 ± 2.8	11.5 ± 2.0	18.2 ± 2.8	17.9 ± 2.7			
ApEn, AU								
Pre	0.95 ± 0.02		0.91 ± 0.03		0.94 ± 0.03	0.468	**0.010**	0.467
Post	0.95 ± 0.04		0.86 ± 0.02*		0.94 ± 0.03			
SBP-SD, mmHg								
Pre	3.98 ± 0.43	4.72 ± 0.62	3.96 ± 0.65	4.89 ± 0.68	3.68 ± 0.39	0.678	**<0.001**	0.534
Post	3.85 ± 0.40	5.69 ± 0.70*	3.92 ± 0.42	4.71 ± 0.67	3.58 ± 0.40			
Total BPV, $mmHg^2$								
Pre	12.7 ± 2.42	15.1 ± 3.69	17.1 ± 5.91	18.2 ± 3.92	11.6 ± 2.26	0.543	**0.048**	0.724
Post	11.1 ± 1.92	18.2 ± 3.20	13.1 ± 2.53	16.8 ± 3.80	10.6 ± 2.14			
LF_{BPV}, $mmHg^2$								
Pre	6.28 ± 1.68	6.37 ± 1.42	8.94 ± 3.14	8.74 ± 1.60	6.19 ± 1.34	0.124	0.210	0.789
Post	4.76 ± 1.31	6.52 ± 1.36	5.66 ± 1.23	7.87 ± 2.19	5.07 ± 1.22			
HF_{BPV}, $mmHg^2$								
Pre	1.68 ± 0.29	2.84 ± 0.89	5.43 ± 2.40*	3.35 ± 0.94	1.62 ± 0.22	0.799	**0.002**	0.936
Post	2.07 ± 0.27	2.73 ± 0.59	4.71 ± 1.31	3.84 ± 0.81	2.28 ± 0.47			

Values are mean ± SE for pre- and post-WI variables. Nseq, number of sequences; see *Glossary* for other abbreviations. Boldface values indicate statistical significance. *$P < 0.05$ vs. baseline. ApEn during CP was significantly reduced following WI. ‡By 2-way (pre/post × time) repeated-measures ANOVA.

DISCUSSION

The current study is the first to demonstrate that the sympathetic and parasympathetic arms of the autonomic nervous system are differentially activated/depressed during multiple physiological stressors following a 6-h WI. Specifically, compared with pre-WI responses, post-WI responses during cold pressor and PEMI favored a lower HR, secondary to increased cardiac parasympathetic activation and decreased sympathetic activation. In contrast, higher HR, secondary to decreased cardiac parasympathetic activation and increased sympathetic activation, was favored during HUT after WI. An additional new finding is that post-WI ApEn is reduced during cold pressor and HUT compared with pre-WI responses.

In agreement with previous reports of ≥6 h of WI (4, 31, 46) where PV was measured using Evans blue dye or changes in Hb and Hct, PV in the present study was significantly reduced by 11.3% 1 h after WI. The reduced PV is not unexpected, given the large volume of urine excreted and body weight lost in the present study, measurements that are also in agreement with previous studies (4, 46). Our observations that aldosterone, ANP, AVP, and NE were unchanged 1 h after WI support earlier findings (10, 12, 57) but differ from a recent study by Mourot et al. (46). The discrepancies may be related to differences among studies in activity during WI, hydration status, and timing of blood sampling.

The reductions in resting supine SBP, SV, and CBF and increase in CVR in our study suggest an increase in peripheral sympathetic activation in response to PV contraction (32). Subjects in the present study also did not experience a change in resting HRV following WI, which suggests that cardiac autonomic compensation may not be necessary for individuals in the supine position with mild hypovolemia (16). Chouchou et al. (7) noted increased parasympathetic activity during a recreational SCUBA dive followed by increased sympathetic activation ~10 min after the dive. However, since "basal" HR in that study was ~94 beats/min, true baseline comparisons may have been lacking (7). Although few resting parameters were affected, numerous changes in hemodynamic and autonomic responses to the three stressors were apparent after the WI.

Cold Pressor

The cold pressor test has been used clinically and experimentally to assess autonomic cardiac control (45) and sympathetic neural control of muscle, splanchnic, and renal vasculature in humans (8, 62). Typical responses include transient increase in HR and $\dot{Q}$ within the first 30–60 s followed by augmentation of MSNA and BP that is sustained until termination of the test. Although the cold pressor test augments central sympathetic activation independently of the baroreflex, baroreflex control of MSNA and HR is still active. Pre-WI results from the current study are in accord with characteristic cold pressor responses documented in other studies (8, 17, 62). Since mean SVR values decreased over the 1st min, the initial

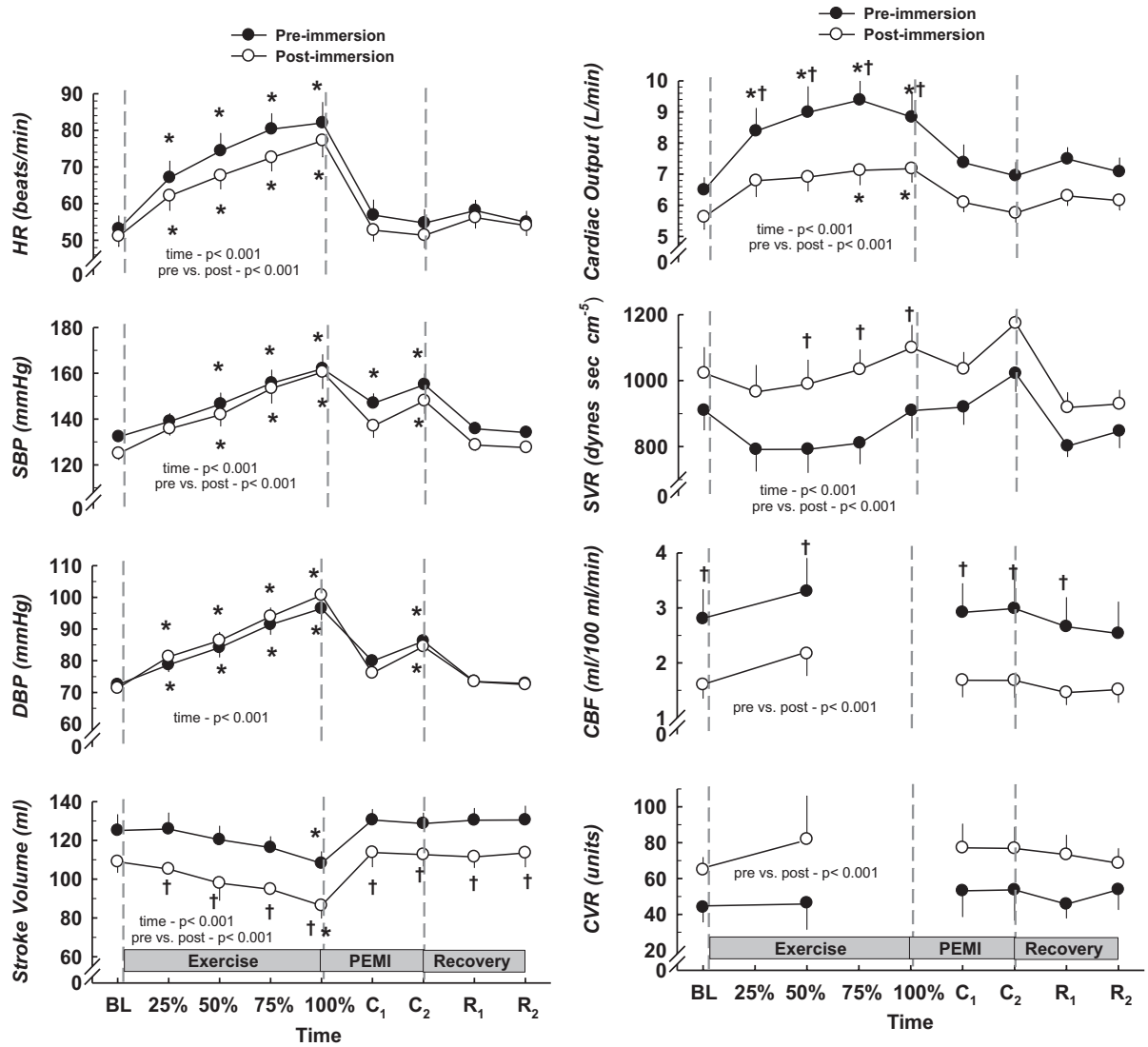

Fig. 4. Hemodynamic responses at baseline (BL) and during static handgrip exercise, postexercise muscle ischemia (PEMI), and recovery (R) before and after a 6-h water immersion. *x*-Axis during exercise corresponds to the percentage of time to fatigue. C1 and C2, *minutes 1* and *2* of PEMI; R1 and R2, *minutes 1* and *2* of recovery; see *Glossary* for other abbreviations. Values are group means ± SE. Statistical comparisons are noted on each plot. *$P < 0.05$ vs. baseline. †$P < 0.05$ vs. preimmersion. Compared with preimmersion values, postimmersion HR, SBP, stroke volume, cardiac output, and CBF were significantly reduced and SVR and CVR were increased across phases of handgrip testing.

increase in BP originated primarily from an increase in $\dot{Q}$, mostly driven by tachycardia. The continued rise in BP over the 2nd min resulted from a shift toward increasing SVR and an attenuated contribution of $\dot{Q}$. Our observation that CVR did not increase, despite the expected increase in MSNA and NE spillover (30, 61, 62), is in agreement with a previous study (30) showing dissociation between NE spillover and limb vascular responses during cold pressor. In the current study, no changes in any of the time- or frequency-domain HRV measures during cold pressor or recovery were observed, perhaps due to heterogeneous cardiac reactivity noted in previous studies (45, 61).

Our data suggest that peripheral vasoconstrictor responses to hypovolemia were intact following WI (i.e., increased post- vs. pre-WI CVR throughout cold pressor) (4). Interestingly, although baseline HR and $\dot{Q}$ values before and after WI were similar, the post-WI cold pressor-induced increases in HR and $\dot{Q}$ were blunted relative to pre-WI changes. Since SBP after WI was reduced relative to SBP before WI (although ΔSBP during cold pressor was preserved) and baroreflex sensitivity was unaltered, it is unlikely that the reduced HR was baroreflex-mediated. Ligtenberg et al. (36) showed that the HR response to cold pressor was preserved during simulated hypovolemia. Thus our results suggest that WI, and not just the resulting hypovolemia, may directly affect cardiac autonomic function. The HRV results following WI are also consistent with this notion. Compared with pre-WI cold pressor responses, post-WI cold pressor LF/HF_{HRV} and LFn_{HRV} were reduced, and HFn_{HRV} was augmented.

The hemodynamic and autonomic response patterns to cold pressor after WI, including decreased ApEn, seem to indicate a pattern of cardiac autonomic coactivation and reduced complexity (45, 61). Using detrended fluctuation analysis (DFA), Mourot et al. (45) measured the short-term fractal scaling

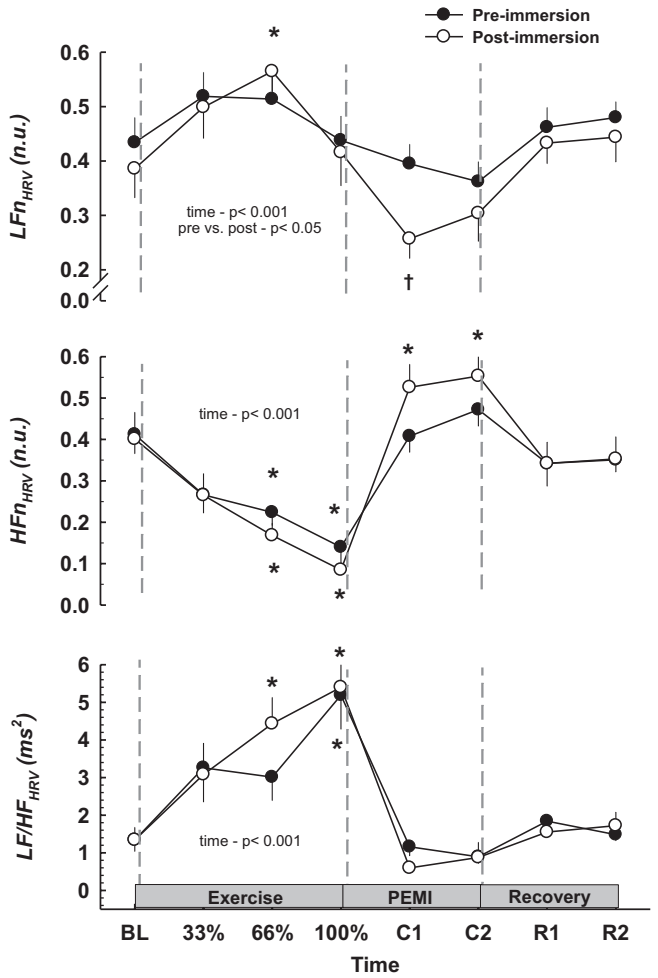

Fig. 5. HRV responses at baseline and during static handgrip exercise, PEMI, and recovery before and after a 6-h water immersion. *x*-Axis during exercise corresponds to percentage of time to fatigue. See Fig. 4 legend and *Glossary* for abbreviations. Values are group means ± SE. Statistical comparisons are noted on each plot. *$P < 0.05$ vs. baseline. †$P < 0.05$ vs. preimmersion. After water immersion, responses during PEMI favored increased cardiac parasympathetic activation (HFn_{HRV}) and decreased sympathetic activation (LFn_{HRV}).

exponent (α_1) during cold pressor in two groups of healthy individuals who showed distinct α_1 and HRV patterns to cold stimulation. Although baseline hemodynamic and autonomic data were similar between the two groups, the first group responded to cold pressor with a pattern of reciprocal interplay (elevated α_1, HR, LF/HF_{HRV}, and LFn_{HRV} and reduced HFn_{HRV}), and the second responded with a pattern of cardiac autonomic coactivation (reduced α_1, LF/HF_{HRV}, and LFn_{HRV}, elevated HFn_{HRV}, and elevated, followed by reduced, HR). The current study was not designed to look at interindividual variability, but on average the responses show patterns of reciprocal interplay before WI and a shift more toward coactivation after WI. Previous reports suggest that cardiac autonomic coactivation may lead to a more rigid and less adaptable system (45, 61). Future studies should include DFA, direct measures of sympathetic nerve activity, or regional measures of NE spillover to further assess autonomic activation to cold pressor following WI.

Handgrip

During static exercise, the increase in HR is controlled primarily by central command and the mechanoreflex, whereas BP is regulated by central command along with mechano- and metaboreflexes, and MSNA is controlled predominantly by the metaboreflex (53). In addition to changes in stimuli and end-organ responses, alterations can occur at a number of points along the muscle mechano- and metaboreflex arcs (e.g., in afferent response, central integration, or efferent signal) and in central command. In healthy subjects, the rise in BP, particularly at the onset of exercise, is attributed mostly to an increase in $\dot{Q}$, with no change or minimal increase in systemic and peripheral vascular resistance (27, 59). Cardiac autonomic balance shifts in favor of higher LF/HF_{HRV} (28, 59). Most (9, 28, 37, 59), but not all (27), studies have shown a reduction in the sensitivity of baroreflex modulation of the sinus node during static exercise followed by a return of BRS to control levels during PEMI and recovery (28).

Pre-WI measurements during static handgrip and PEMI in the present study agree with the characteristic responses (21, 28, 59, 63). Similar to post-WI cold pressor responses, peripheral vasoconstriction was accentuated throughout handgrip and PEMI, while SV and $\dot{Q}$ were reduced from pre-WI values throughout the test. BP increased during handgrip before and after WI. However, the increase before WI resulted solely from an increase in $\dot{Q}$, while that following WI was caused by a combined increase in $\dot{Q}$ and SVR.

Central command. Immediately at the onset of exercise, central command modulates parasympathetic and sympathetic efferent activity to the heart and vasculature (53). Although we have no quantitative measures in this study, the blunted cardiovascular responses during handgrip are consistent with the concept that central command may have been reduced following WI. A study of dry immersion supports this idea: although peripheral contraction responses to tests of muscle function were largely intact after dry immersion, central motor drive was reduced (33). Since central motor and cardiovascular command interact, the reduction in motor command likely extends to autonomic circuits as well (35, 67).

Muscle mechanoreflex. Mechanosensitive afferents, primarily group III and some group IV fibers, respond to stimuli such as stretch, contraction, and pressure (41). The mechanoreflex increases HR mainly through cardiac vagal inhibition (19) but may also augment sympathetic activation (40). Because the sensitivity of muscle afferents is inversely proportional to interstitial fluid volume (15, 40), reductions in PV and interstitial fluid that occur with bed rest, spaceflight, and WI (10, 50) may desensitize the mechanoreceptors. Our results are consistent with mechanoreflex impairment, although an impaired response cannot be assessed from our data.

Muscle metaboreflex. The metaboreflex, which elicits increases in MSNA and arterial pressure (53), is isolated during PEMI when mechanical stimulation and central command influences are absent. There were no apparent differences in hemodynamic responses to isolated metaboreceptor stimulation before and after WI; however, HRV measures indicated reduced sympathetic and increased parasympathetic cardiac dynamics following WI. Recent reports (14, 28) have suggested that cardiac parasympathetic tone may mask sympathetically mediated tachycardia during PEMI. Whether a shift

Table 4. *Autonomic responses to handgrip exercise and PEMI before and after 6 h of WI*

Variable	Time				*P* Value		
					Main effect		Interaction‡
	Baseline	Handgrip	PEMI	Recovery	Pre/post	Time	Pre/Post × time
SDNN, ms							
Pre	95.3 ± 17.7	53.5 ± 7.7*	97.2 ± 15.0	87.6 ± 16.5	0.873	**<0.001**	0.972
Post	82.8 ± 18.9	56.0 ± 7.1	99.6 ± 17.9	84.2 ± 10.6			
RMSSD, ms							
Pre	102.0 ± 22.6	26.1 ± 6.6*	108.0 ± 19.4	83.9 ± 16.4	0.958	**<0.001**	0.539
Post	79.2 ± 16.0	19.5 ± 3.8*	126.0 ± 27.5*	82.2 ± 14.5			
Nseq, AU							
Pre	17.5 ± 4.0	12.5 ± 2.5*	8.4 ± 2.9	15.7 ± 3.6	0.093	**0.002**	0.850
Post	13.7 ± 4.3	11.5 ± 2.7	7.0 ± 2.3	11.9 ± 3.4			
BRS, ms/mmHg							
Pre	23.2 ± 3.6	12.0 ± 2.1*	20.3 ± 2.6	24.2 ± 3.8	0.418	**<0.001**	0.613
Post	24.3 ± 4.7	11.2 ± 1.8*	25.3 ± 3.7	24.3 ± 3.8			
Transfer gain, LF, ms/mmHg							
Pre	16.0 ± 3.2	5.5 ± 0.8*	16.9 ± 3.3	17.0 ± 2.9	0.492	**<0.001**	0.800
Post	14.4 ± 2.2	4.5 ± 1.0*	19.5 ± 3.4	19.7 ± 3.4			
ApEn, AU							
Pre	0.94 ± 0.04	0.80 ± 0.02*	0.91 ± 0.03	0.93 ± 0.04	0.446	**<0.001**	0.924
Post	0.95 ± 0.03	0.80 ± 0.03*	0.92 ± 0.03	0.96 ± 0.03			
SBP-SD, mmHg							
Pre	4.76 ± 0.55	6.78 ± 1.45	3.89 ± 0.38	4.78 ± 0.51	0.104	**<0.001**	0.960
Post	4.00 ± 0.34	6.72 ± 0.99*	3.44 ± 0.34	3.73 ± 0.38			
Total BPV, $mmHg^2$							
Pre	19.2 ± 3.7	47.8 ± 21.6*	13.4 ± 2.7	19.4 ± 3.6	0.163	**<0.001**	0.995
Post	12.5 ± 2.1	39.0 ± 10.3*	10.1 ± 1.7	12.4 ± 2.7			
LF_{BPV}, $mmHg^2$							
Pre	9.96 ± 1.69	22.90 ± 8.07*	7.18 ± 1.61	10.20 ± 1.89	0.114	**<0.001**	0.995
Post	6.08 ± 1.65	21.70 ± 6.45*	4.53 ± 0.84	5.72 ± 1.01			
HF_{BPV}, $mmHg^2$							
Pre	4.03 ± 1.25	13.90 ± 9.72*	3.70 ± 1.05	2.90 ± 0.83	0.291	**0.019**	0.920
Post	2.87 ± 0.83	7.85 ± 2.89	3.29 ± 0.92	3.99 ± 1.44			

Values are means ± SE. See *Glossary* for abbreviations. Boldface values indicate statistical significance. *$P < 0.05$ vs. baseline. RMSSD, an indicator of parasympathetic activation, significantly increased during PEMI following WI. ‡By 2-way (pre/post × time) repeated-measures ANOVA.

toward increased parasympathetic activation is required to return HR to baseline levels following WI is unclear but is plausible. Iellamo et al. (28) suggested that a vagally mediated baroreflex mechanism was responsible for the return of HR to basal levels even with sympathetic competition. Therefore, it is possible with hypovolemia and increased peripheral sympathetic activation that greater vagal activation is needed to control HR. Moreover, if arterial baroreflex responses to post-WI hypovolemia maintain BP at the same exercise set point as before WI, there may be no evidence of the degree of baroreceptor involvement.

Head-up Tilt

Assumption of upright posture leads to a translocation of 300–500 ml of blood from the chest to the dependent regions, leading to a reduction in venous return and SV. To counteract the reduction in SV and to maintain BP and cerebral perfusion, the baroreflex reduces vagal activity and increases sympathetic activation, contributing to tachycardia and arterial vasoconstriction (2). In healthy individuals, typical responses early in HUT include a 10–20 beat/min increase in HR and small or insignificant changes in BP.

Reduced orthostatic tolerance has been observed following spaceflight (5), bed rest (50), and WI (29, 58), all of which reduce gravitational loading. The decreased loading results in fluid shifts, diuresis, hypovolemia, and cardiovascular and neuroendocrine adjustments. The post-WI increase (compared with pre-WI) in the orthostatic index (+79%) and $+\Delta HR_{max}$ (+47%), both indicative of reduced orthostatic tolerance, are comparable to previous studies with ≥6 h of WI (29, 58). Moreover, although all subjects successfully completed the 15-min HUT before WI, three subjects experienced symptoms of nausea, pallor, and lipothymia and became presyncopal after 6.9, 10.4, and 9.4 min of post-WI HUT, respectively. These results indicate that WI alters autonomic and cardiovascular responses to HUT and that the compensatory responses are not always adequate to avert hypotension.

Several mechanisms, including *1*) reduced blood volume, *2*) modified cardiac function, *3*) modulation of autonomic function, *4*) altered baroreflex function, and *5*) altered vascular smooth muscle tone and responsiveness, may have contributed to the altered post-WI HUT responses.

An excessive reduction in blood volume and postural SV, even in the face of increased constriction, may contribute to reduced orthostatic tolerance. Typical regulatory patterns to compensate for the 11% reduction in PV (and resulting drop in SV) in our study and the 10–15% decrease reported from other studies (4, 5, 31, 46, 50) include increased sympathetic activation and release of NE, marked tachycardia, and vasoconstriction. However, although hypovolemia may be a predominant contributing factor to orthostatic intolerance following WI, bed rest, or spaceflight, the reduction in PV does not fully

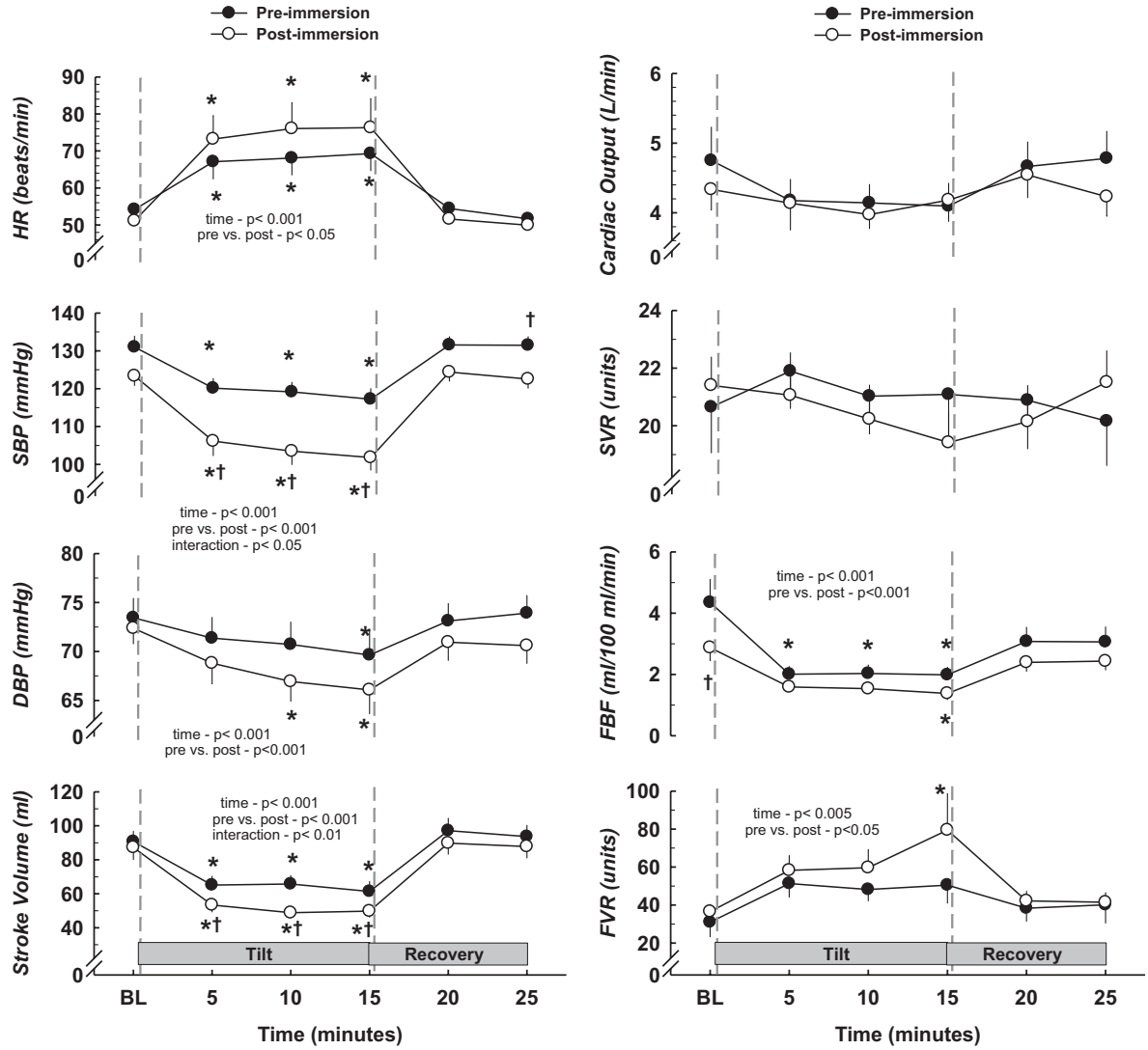

Fig. 6. Hemodynamic responses during 70° head-up tilt testing before and after a 6-h water immersion. See *Glossary* for abbreviations. Values are group means ± SE. Statistical comparisons are noted on each plot. *$P < 0.05$ vs. baseline. †$P < 0.05$ vs. preimmersion. Cardioacceleration and drop in SBP and stroke volume were larger after than before immersion. DBP and FBF were significantly lower and FVR was higher after than before immersion, and all were significantly different from their respective postimmersion baselines by the end of head-up tilt.

explain the altered orthostatic responses, since maintenance or restoration of PV does not ameliorate orthostatic intolerance (64). During physiological stressors such as cold pressor or handgrip exercise, cardiac autonomic activation may be dissociated from muscle, splanchnic, or other regional autonomic responses. Unlike these stressors, HUT elicits a generalized increase in sympathetic neural traffic. During HUT, the increases in LFn_{HRV} and LF_{BPV} are known to mirror the increase in peroneal MSNA (18). As expected, the pre-WI HRV and BPV measures in this study were significantly augmented or tended to increase during HUT, and these changes were significantly greater after WI, consistent with results following spaceflight (34), bed rest (50), and a 3-day dry immersion (29). The PDM analysis also confirmed greater sympathetic activation following WI. Whereas before WI, cardiac SNS activity significantly increased by the latter half of the HUT, post-WI SNS activity was augmented during the early and late portions of HUT. These results, together with the increase in time- and frequency-domain measures of LF_{BPV}, indirect markers of sympathetic vasomotor control (18), indicate that the sympathetic responses to orthostatic stress were enhanced after WI. However, the facts that SVR was no greater after than before WI and that hypotension was clearly evident in some subjects suggest that the global autonomic orthostatic compensation was inadequate.

Although few studies have examined ApEn during orthostatic stress, Goldberger et al. (20) showed that reduced ApEn may be linked to reduced tolerance to lower body negative pressure following bed rest. The current study extends that concept by showing that ApEn, while similar before and after WI during HUT baseline and recovery, was markedly reduced during HUT after a single 6-h WI. The lower ApEn may be ascribed to the simplification of HR dynamics that occurs with sympathetic activation and vagal withdrawal, thus leading to a less adaptable system.

In agreement with previous reports of reduced carotid-cardiac BRS following spaceflight (5), the reduced GainLF

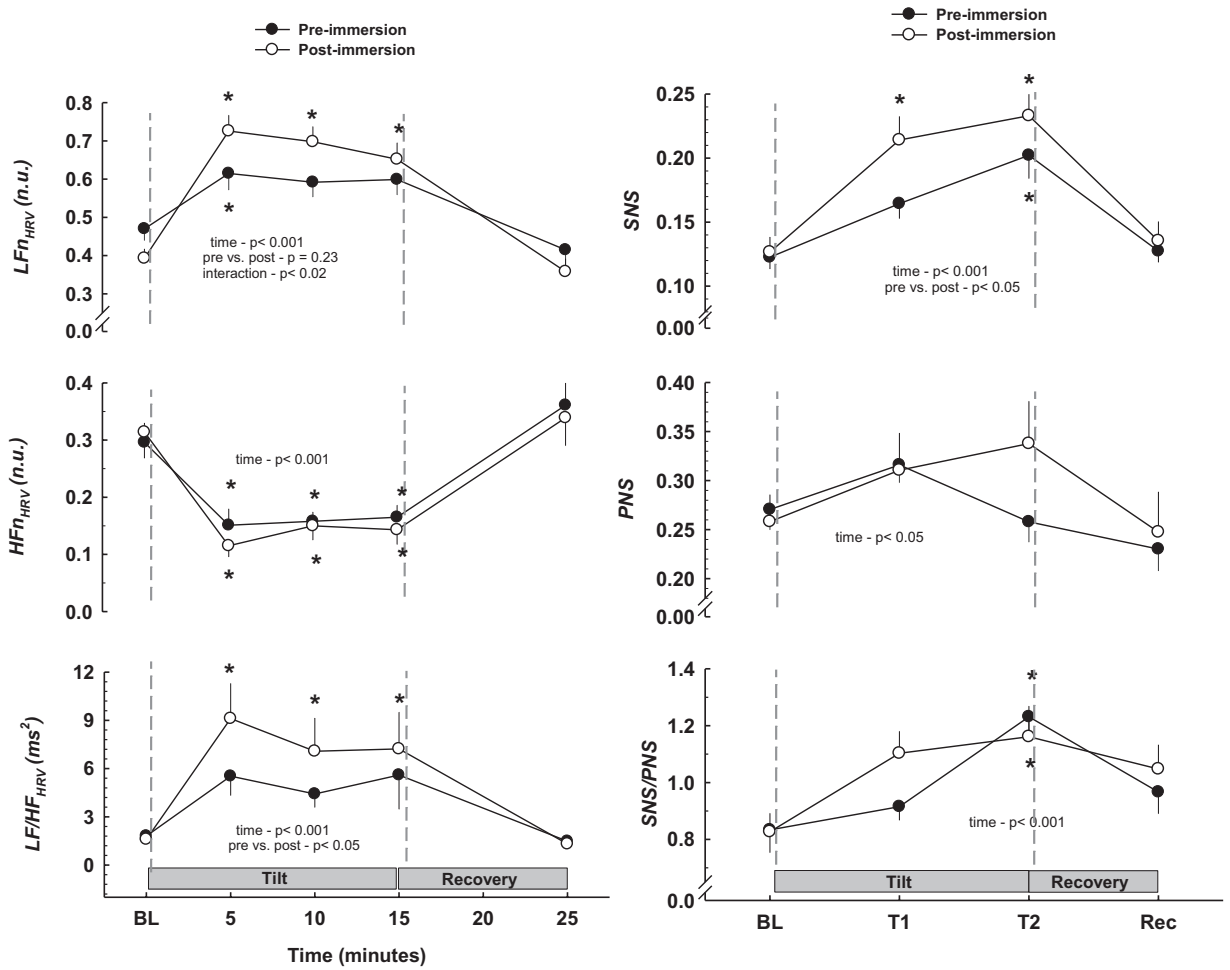

Fig. 7. HRV responses during 70° head-up tilt testing before and after a 6-h water immersion. SNS, sympathetic nervous system activity; PNS, parasympathetic nervous system activity; T1, early tilt; T2, late tilt. Values are group means ± SE. Statistical comparisons are noted on each plot. *$P < 0.05$ vs. baseline. Cardiac sympathoexcitation (LFn_{HRV}, LF/HF_{HRV}, and SNS) increased more after than before immersion.

during HUT after WI likely reflects a deterioration of BRS. This change is also confirmed in a recent report from our group using more sophisticated causal parametric approaches (13). In that report, BRS was reduced more during the late portion of HUT following WI than before WI, and BRS was further reduced following multiple 6-h WIs. Moreover, the causal coherence increased significantly before WI and not significantly after WI, indicating a reduction in baroreflex coupling (13).

Recent reports agree that sympathetic control of the muscle vasculature remains intact following bed rest (50), spaceflight (34), and 3 days of dry immersion (29), but vascular responsiveness to sympathetic discharge may be blunted (29). Although MSNA and vascular responsiveness were not measured in the current study, the reduction in FBF and increases in FVR and LF_{BPV} during HUT, coupled with peripheral vasoconstriction following a 6-h WI in a previous study (4), suggest that peripheral vasoconstrictor mechanisms are intact following WI. However, sympathetic activation and constriction in other vascular beds (e.g., splanchnic) may be impaired following WI. Although FVR increased in our study, reduced constriction elsewhere may have contributed to the attenuated or even absent rise in SVR (5, 47).

In summary, mechanisms underlying the post-WI reduction in orthostatic tolerance are likely to be multifactorial. The primary candidates may include diminished SV from PV contraction and reduced cardiac filling, direct and/or indirect changes in regional autonomic control, changes in BRS, and inadequate vasoconstriction in compliant vascular beds. The total integrated response may also be compromised as a result of a loss of the link between cardiac, vascular, and sympathetic components (13, 18).

Experimental Considerations

Several factors should be considered when interpreting the results of this study. *1*) HRV, which is an indirect measure of cardiac sympathetic and parasympathetic activity, may not always accurately indicate sympathetic activation in other regions. Future studies should include more direct measures such as MSNA or NE spillover. *2*) Breathing pattern was not strictly controlled, nor was respiratory frequency measured. Instead, subjects were encouraged to breathe normally. Since changes in breathing patterns may affect respiratory-related HR oscillations, apparent changes in autonomic balance during the stressors may have been influenced by respiratory adjust-

Table 5. *Autonomic responses to 70° HUT testing before and after 6 h of WI*

	Time					*P* Value		
	HUT					Main effect		Interaction‡
Variable	Baseline	5 min	10 min	15 min	Recovery	Pre/post	Time	Pre/post × time
SDNN, ms								
Pre	101.0 ± 15.2	61.1 ± 4.6*	61.8 ± 5.4*	60.7 ± 4.9*	104.0 ± 13.2	0.509	**<0.001**	0.987
Post	101.0 ± 16.2	53.8 ± 5.6*	56.6 ± 5.4*	59.7 ± 9.1*	102.0 ± 18.1			
RMSSD, ms								
Pre	98.3 ± 19.0	37.9 ± 4.5*	38.9 ± 4.6*	39.1 ± 4.5*	116.0 ± 20.9	0.360	**<0.001**	0.987
Post	97.6 ± 20.6	29.9 ± 5.4*	33.0 ± 4.2*	36.2 ± 8.3*	105.0 ± 22.0			
Nseq, AU								
Pre	24.3 ± 7.2	46.4 ± 9.7*	46.7 ± 9.7*	46.8 ± 9.7*	17.6 ± 5.7	0.251	**<0.001**	0.294
Post	17.9 ± 3.9	65.0 ± 6.6*	55.4 ± 8.0*	51.2 ± 7.3*	17.1 ± 4.6			
BRS, ms/mmHg								
Pre	28.1 ± 4.6	12.7 ± 1.8*	13.2 ± 2.0*	13.4 ± 2.6*	28.8 ± 5.4	0.181	**<0.001**	0.669
Post	30.7 ± 5.5	9.9 ± 2.3*	10.2 ± 1.5*	9.4 ± 1.6*	25.1 ± 3.5			
Transfer gain, LF, ms/mmHg								
Pre	22.6 ± 5.3	10.7 ± 1.3*	10.3 ± 1.2*	10.5 ± 1.2*	23.0 ± 4.7	**0.008**	**<0.001**	0.716
Post	17.4 ± 2.6	8.7 ± 1.7*	9.8 ± 1.2*	8.0 ± 1.1*	16.6 ± 2.5			
ApEn, AU								
Pre	1.11 ± 0.04	0.99 ± 0.05	1.01 ± 0.05	0.97 ± 0.07	1.13 ± 0.04	**<0.001**	**<0.001**	**0.044**
Post	1.10 ± 0.03	0.82 ± 0.06*	0.77 ± 0.08*†	0.81 ± 0.08*	1.11 ± 0.03			
SBP-SD, mmHg								
Pre	4.40 ± 0.21	5.07 ± 0.78	4.99 ± 0.52	5.27 ± 0.54	4.36 ± 0.26	0.111	**<0.001**	0.208
Post	3.83 ± 0.28	6.24 ± 0.97*	6.12 ± 1.04*	6.47 ± 1.05*	4.10 ± 0.31			
Total BPV, $mmHg^2$								
Pre	16.7 ± 1.7	27.7 ± 10.4	24.5 ± 6.0	26.9 ± 6.7	16.5 ± 1.9	0.070	**0.002**	0.369
Post	12.9 ± 1.9	40.9 ± 14.1*	40.1 ± 14.6*	44.1 ± 15.8*	14.9 ± 2.1			
LF_{BPV}, $mmHg^2$								
Pre	8.12 ± 1.26	21.00 ± 8.42	17.50 ± 4.85	19.00 ± 5.36	7.58 ± 1.37	**0.022**	**<0.001**	0.336
Post	6.04 ± 1.13	31.10 ± 10.84*	28.90 ± 10.67*	32.60 ± 11.80*	6.41 ± 1.23			
HF_{BPV}, $mmHg^2$								
Pre	1.84 ± 0.27	3.10 ± 1.06	3.51 ± 1.09	3.61 ± 1.16	1.75 ± 0.27	**0.033**	**<0.001**	0.410
Post	1.70 ± 0.19	4.50 ± 1.23	6.00 ± 2.00*	5.46 ± 1.64*	1.73 ± 0.30			

Values are means ± SE. See *Glossary* for abbreviations. Boldface values indicate statistical significance. *$P < 0.05$ vs. baseline. †$P < 0.05$ vs. pre-WI. Although all variables changed during HUT, postimmersion transfer gain and ApEn were significantly lower and LF_{BPV} and HF_{BPV} were significantly higher than pre-WI HUT values. A significant interaction for ApEn signifies a greater change (reduction) with time in ApEn during post-WI than pre-WI HUT. ‡By 2-way (pre/post × time) repeated-measures ANOVA.

ments. However, previous studies (28, 45) have shown that influences of respiratory modulation on HRV during cold pressor or static exercise are minimal. Moreover, the influence of respiratory modulation on baroreflex gain was minimized by considering only the LF band. *3*) Instead of randomizing the order of stressors, a serialized order, employed in previously published studies (17, 34), was chosen to minimize the impact of any order effect on pre- and post-WI comparisons. The schedule allowed sufficient recovery from one stressor to the next to minimize carryover effects. The fact that baseline and recovery data were not different from one stressor to the next suggests that this strategy was appropriate. *4*) The current study included only healthy young men. Whether there are any important age- or sex-related differences in response to WI requires additional studies.

Conclusions

This study provides the first evidence that the sympathetic and parasympathetic arms of the autonomic nervous system are differentially activated during multiple physiological stressors following a 6-h WI. Although peripheral sympathetic responses may have remained intact, responses during cold pressor and PEMI after WI favored a lower HR, together with increased cardiac parasympathetic activation and decreased sympathetic activation, than those before WI. In contrast, during HUT after WI, higher HR, together with decreased cardiac parasympathetic activation and increased sympathetic activation, was favored. An additional new finding is that post-WI ApEn is reduced relative to pre-WI ApEn during cold pressor and HUT. The integrated findings of this study suggest that WI alters autonomic control, and cardiovascular and autonomic adjustments to HUT orthostasis are insufficient to adequately maintain BP.

Although many studies over the last 30 years have clarified the physiology of diving and immersion, the understanding of postimmersion responses to stressors has been limited. Effects of a single WI, including reduced orthostatic tolerance and altered autonomic and cardiovascular control during stressors, are important to the millions of divers in the commercial, military, and recreational diving communities who may execute extended-duration dives. Through further research, clinicians should become aware of effects of hypertension medications, antidiuretics, and tricyclic antidepressants, among others, on normal compensatory responses to WI. Future studies should parse out the effects of dehydration from other effects of immersion on responses to physiological stressors. Since divers also use breathing gases other than air (e.g., 100% O_2 and trimix) during dives, the effect of different gases on postimmersion physiological responses should also be examined.

APPENDIX

orthostatic index

$$= \frac{RR_s, h}{RR_s, v} \times \frac{RR_d, h}{RR_d, v} \times \frac{fv}{fh} \times \sqrt{S^2RR_s + S^2RR_d + S^2f}$$

where RR is blood pressure (mmHg), s represents systolic, d represents diastolic, f is pulse rate (beats/min), S^2 is square of the standard deviation of the denoted parameter, h is horizontal position, and v is vertical position.

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DISCLAIMER

The opinions and assertions contained herein are those of the authors, not to be construed as official or reflecting the views of the Department of the Navy or the US Government.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.P.F. and B.E.S. are responsible for conception and design of the research; J.P.F., E.E.S., and B.E.S. performed the experiments; J.P.F., E.E.S., K.H.C., L.F., and B.E.S. analyzed the data; J.P.F., K.H.C., L.F., and B.E.S. interpreted the results of the experiments; J.P.F., K.H.C., and L.F. prepared the figures; J.P.F. drafted the manuscript; J.P.F., E.E.S., K.H.C., L.F., and B.E.S. edited and revised the manuscript; J.P.F., E.E.S., K.H.C., L.F., and B.E.S. approved the final version of the manuscript.

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