Tolerance to central hypovolemia: the influence of oscillations in arterial pressure and cerebral blood velocity

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Rickards CA, Ryan KL, Cooke WH, Convertino VA. Tolerance to central hypovolemia: the influence of oscillations in arterial pressure and cerebral blood velocity. J Appl Physiol 111: 1048–1058, 2011.—Higher oscillations of cerebral blood velocity and arterial pressure (AP) induced by breathing with inspiratory resistance are associated with delayed onset of symptoms and increased tolerance to central hypovolemia. We tested the hypothesis that subjects with high tolerance (HT) to central hypovolemia would display higher endogenous oscillations of cerebral blood velocity and AP at presyncope compared with subjects with low tolerance (LT). One-hundred thirty-five subjects were exposed to progressive lower body negative pressure (LBNP) until the presence of presyncopal symptoms. Subjects were classified as HT if they completed at least the -60-mmHg level of LBNP (93 subjects; LBNP time, 1,880 ± 259 s) and LT if they did not complete this level (42 subjects; LBNP time, 1,277 ± 199 s). Middle cerebral artery velocity (MCAv) was measured by transcranial Doppler, and AP was measured at the finger by plethysmography. Mean MCAv and mean arterial pressure (MAP) decreased progressively from baseline to presyncope for both LT and HT subjects (P < 0.001). However, low frequency (0.04–0.15 Hz) oscillations of mean MCAv and MAP were higher at presyncope in HT subjects compared with LT subjects (MCAv: HT, 7.2 ± 0.7 vs. LT, 5.3 ± 0.6 (cm/s)2, P = 0.075; MAP: HT, 15.3 ± 1.4 vs. 7.9 ± 1.2 mmHg2, P < 0.001). Consistent with our previous findings using inspiratory resistance, high oscillations of mean MCAv and MAP are associated with HT to central hypovolemia.

lower body negative pressure; hemorrhage; hypovolemia; high tolerance; low tolerance

HEALTHY HUMANS EXHIBIT A CONTINUUM of tolerance to progressive central hypovolemia induced by lower body negative pressure (LBNP; Refs. 10–11, 26, 37). High tolerance (HT) to LBNP has been attributed to a number of factors, including elevated release of vasoactive hormones (11, 26), enhanced compensatory tachycardia (10, 26) and vasoconstriction (11), and protection of central blood volume (i.e., cardiac output) and cerebral blood velocity (37). In recent studies (52–53), we showed that improved tolerance to central hypovolemia was associated with increases in low frequency (LF) and high frequency (HF) oscillations of middle cerebral artery velocity (MCAv) and arterial pressure (AP) when subjects breathed with inspiratory resistance via an inspiratory threshold device (ITD). We proposed that respiration was predominantly responsible for the increase in HF oscillations, and sympathetic nerve activity was driving LF oscillations (53), although a centrally mediated mechanism could also be an important contributing factor (33). While we were able to investigate the relationship between LBNP tolerance and oscillations induced by an exogenous device (i.e., an ITD), the potential relationship between endogenously occurring oscillations (i.e., respiration or reflex mediated) and tolerance to central hypovolemia has not been investigated.

This oscillatory response to hypovolemia has been extensively reported. In 1951 Guyton and Harris (27) observed a distinctive oscillatory pattern in AP following a 25% hemorrhage in dogs, which has been quantified in other animal models of hemorrhage (48–49), and more recently, in humans exposed to actual (72) and simulated hemorrhage via LBNP (58, 66, 70). Traditionally, however, increased hemodynamic variability has been associated with imminent syncope during orthostatic stress (18, 40) and reduced tolerance to LBNP (70). Conversely, Lewis et al. (38) proposed that the pulsatile pattern of cerebral blood flow observed in hemorrhaging sheep was a protective mechanism to maintain cerebral perfusion, despite the progressive reduction in absolute cerebral blood flow. This contention is supported by Zhang and Levine (68), who have suggested that pulsatile AP and cerebral blood flow may protet cerebral perfusion via flow-mediated mechanisms (e.g., via release of nitric oxide, or inhibition of endothelin), which we propose may actually protect against syncpe.

If tolerance to hypovolemia is dependent on the generation of pulsatile AP and MCAv, we hypothesized that subjects with HT to progressive reductions in central blood volume would display higher endogenous oscillations in AP and MCAv compared with subjects with low tolerance (LT).

METHODS

Subjects and ethical approval. One-hundred and thirty-five (52 female, 83 male) healthy, normotensive, nonsmoking subjects volunteered to participate in this study (age: 28 ± 8 yr; height: 174 ± 11 cm; weight: 76 ± 15 kg; means ± SD), conducted at the US Army Institute of Surgical Research (Fort Sam Houston, TX). Data from these subjects are also reported in a related study by Convertino et al. (8). This study was conducted under a protocol reviewed and approved by the Institutional Review Board of the Brooke Army Medical Center (Fort Sam Houston, TX) and in accordance with the approved protocol. A complete medical history and physical examination were obtained on each of the potential subjects before being approved for testing. In addition, female subjects underwent a urine pregnancy test within 24 h before experimentation and were excluded if pregnant. Subjects were instructed to maintain their normal sleep pattern and refrain from exercise, alcohol, and stimulants such as caffeine and other nonprescription drugs 24 h before testing to reduce their potential acute effects on cardiovascular responsiveness. During a familiarization session that preceded each experiment, subjects received a verbal briefing and a written description of all procedures and risks associated with the experiments and were made familiar with the risks associated with the experiments and were made familiar.

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with the laboratory, the protocol, and procedures. Each subject gave written informed consent to participate in the study.

Study design. LBNP was used as an experimental tool to reduce central blood volume in humans (i.e., simulation of hemorrhage; Ref. 14). Application of negative pressure to the lower body (below the iliac crest) results in the distribution of blood away from the upper body (head and heart) to the abdomen and lower extremities, eliciting controlled, experimentally induced hypovolemia.

All subjects were instrumented for the noninvasive, continuous measurement of heart rate (HR) via a standard ECG, and beat-to-beat AP via infrared finger plethysmography with a Finometer blood pressure monitor (TNO-TPD Biomedical Instrumentation, Amsterdam, The Netherlands). An appropriately sized Finometer blood pressure cuff was placed on the middle finger of the left hand, which, in turn, was laid at heart level. End tidal CO₂ (ETCO₂) was measured on a breath-by-breath basis as subjects breathed through a facemask connected to a capnograph (BCI CapnOcheck Plus; Smiths Medical, Waukesha, WI). Cerebral blood velocity was recorded from the right middle cerebral artery (MCA) using a 2-MHz Doppler probe (EZ-Dop; DWL Elektronische Systeme, Sipplingen, Germany), positioned at a constant angle over the temporal window, located above the zygomatic arch. The transcranial Doppler technique for measuring cerebral blood velocity has previously been described in detail (1, 46).

Each subject underwent exposure to a LBNP protocol designed to test his or her tolerance to experimentally induced hypotensive hypovolemia. The LBNP protocol consisted of a 5-min controlled rest period followed by 5 min of chamber decompression at −15, −30, −45, and −60 mmHg and additional increments of −10 mmHg every 5 min until the onset of cardiovascular collapse or the completion of 5 min at −100 mmHg. Cardiovascular collapse was defined by one or a combination of the following criteria: 1) sudden bradycardia; 2) a precipitous fall in systolic arterial pressure (SAP) >15 mmHg; 3) progressive diminution of SAP below 70 mmHg; or 4) voluntary subject termination due to the onset of subjective presyncopal symptoms such as grey-out, sweating, nausea, dizziness, or general discomfort.

A subset of 40 subjects wore a facemask and breathed through a two-way valve (T-Shape Two-Way Non-Rebreathing Valve; Hans-Rudolph, Shawnee, KS) connected to a metabolic cart (TrueOne 2400; Parvo Medics, Sandy, UT), where respiration rate, tidal volume, and minute ventilation (Ve) were recorded on a breath-by-breath basis. For these subjects, ETCO₂ was also recorded via the capnograph, as described above.

Data analysis. Continuous, beat-to-beat ECG, Finometer and Doppler, and beat-to-beat ETCO₂ recordings were sampled at 500 Hz and recorded directly to a computer-based data acquisition software package (WinDAQ; Datqaq Instruments, Akron, OH, USA) and then transferred to data analysis software (WinCPRS; Absolute Aliens, Turku, Finland). R waves generated from the ECG signal were detected and marked at their occurrence in time. SAP and diastolic arterial pressure (DAP) and diastolic and systolic cerebral blood velocities were subsequently marked from the Finometer and Doppler tracings. With the use of the AP waveform as an input, stroke volume (SV) was estimated on a beat-by-beat basis using the pulse contour method outlined previously (32). Mean arterial pressure (MAP) and mean MCAv were automatically calculated as the area under the AP and cerebral blood velocity waveforms via the WinCPRS software.

All time and frequency domain variables were calculated from the final 3 min of each completed level of LBNP. For the final LBNP level, the last 1 min of data was used for all time domain variables, and the last 3 min of data were used for all frequency domain variables. In the cases where there was less than the required time available during the final level of LBNP, the 1-min and 3-min data crossed into the previous LBNP level.

Oscillatory patterns of arterial blood pressures and cerebral blood velocities were determined with fast Fourier power spectral analysis. Data were made equidistant by interpolating linearly and resampling at 5 Hz. Data were then passed through a low-pass impulse response filter with a cutoff frequency of 0.5 Hz. Three-minute data sets were fast Fourier transformed with a Hanning window to obtain power spectra. Spectral power was expressed as the integrated area within the LF (0.04–0.15 Hz) and HF (0.15–0.4 Hz) ranges.

We calculated the coherence between MAP and mean MCAv, and between R-R intervals (RRI) and SAP, by dividing the cross-spectral densities of the two signals by the product of the individual autospectra. At the LF where signals are coherent (i.e., $\approx 0.5$), transfer function magnitudes among MAP and mean MCAv represent a frequency dependence of dynamic cerebral autoregulation (23, 69), and transfer function magnitudes between RRI and SAP represent vagnally mediated arterial-cardiac baroreflex gain (15). Transfer functions were considered valid and averaged at the LF only when coherence values were $\approx 0.5$.

As there is a continuum of tolerance to central hypovolemia, subjects were not able to complete the same number of LBNP levels. Subjects were classified as HT if they completed at least the −60 mmHg level of LBNP, and LT if they did not complete this level. Physiological responses were compared between the HT and LT subjects at each level of LBNP up to −60 mmHg; the maximum level of LBNP common between groups. To ensure physiological responses were calculated using the same length of data in HT and LT subjects at the −60 mmHg time point, the final 1 min (time domain) or 3 min (frequency domain) of data were assessed for each subject. In addition, to compare the time of cardiovascular collapse across all subjects, regardless of their LBNP tolerance, the final 1 min (PS 1-min) of time domain data or final 3 min (PS 3-min) of frequency domain data before presyncope were assessed. To determine whether presyncope values of oscillations represent the maximal response, values at presyncope (maximal LBNP, "max") were compared with values from the preceding nonoverlapping LBNP level ("submax").

Unpaired t-tests were used to compare the subject demographic data between the HT and LT groups. For all data up to −60 mmHg, a two-way (tolerance and LBNP level) ANOVA for repeated measures was used for comparison of all physiological variables, followed by Tukey post hoc tests. Unpaired t-tests were used to compare HT and LT subjects at the PS 1-min and PS 3-min time points. Unless otherwise stated, all data are presented as mean ± SE, and exact P values are presented for all comparisons.

RESULTS

LBNP tolerance. The LBNP protocol was terminated at −30 mmHg LBNP for 4 subjects, −45 mmHg LBNP for 6 subjects, at −60 mmHg LBNP for 32 subjects, −70 mmHg LBNP for 37 subjects, −80 mmHg LBNP for 39 subjects, −90 mmHg LBNP for 15 subjects, and −100 mmHg for 2 subjects. As such, 93 subjects were classified as HT and 42 subjects were classified as LT. Table 1 compares the baseline characteristics of each group.

Cardiovascular responses to LBNP. SV decreased progressively from baseline at each level of LBNP in both the HT and LT groups ($P = 0.003$) with greater decreases in LT subjects ($P = 0.05$; Fig. 1A). By the final minute of LBNP (PS 1-min), however, SV had decreased by 43 ± 2% in LT subjects compared with 58 ± 1% in HT subjects ($P < 0.001$; Table 1). Compensatory increases in HR were elicited by these reductions in SV in both groups (Fig. 1B), and by the final minute of LBNP (PS 1-min), HR was higher ($P < 0.001$) in the HT subjects (118 ± 2 beats/min) compared with the LT subjects (94 ± 3 beats/min; Table 1).

MAP was maintained at baseline levels until −60 mmHg in HT subjects, while in LT subjects MAP fell below baseline from −30 mmHg and was lower compared with the HT group.
at −30, −45, and −60 mmHg LBNP (P ≤ 0.07; Fig. 1C). By presyncope, however, MAP had fallen to similar levels between the HT and LT subjects (P = 0.27; Table 1). In contrast, mean MCAv began to fall below baseline values from −30 mmHg LBNP in both HT and LT groups (P < 0.001) and was lower in the LT group at −60 mmHg LBNP (P = 0.03; Fig. 1D); at presyncope, however, mean MCAv was lower in the HT group (P = 0.08; Table 1).

HF oscillations in MAP and mean MCAv increased progressively for both HT and LT groups during LBNP and were statistically indistinguishable between groups (P ≥ 0.10), except for MAP HF at presyncope, which was higher in the HT group (P = 0.02). In the HT group, LF oscillations in MAP increased above baseline values at −45 and −60 mmHg LBNP (P < 0.001), and mean MCAv LF increased above baseline at −60 mmHg; both MAP LF and mean MCAv LF were higher than the LT group at −60 mmHg (P ≤ 0.001) and at presyncope (P ≤ 0.08; Fig. 2). By comparison, in the LT group, LF oscillations in mean MCAv did not change from baseline during LBNP (P ≥ 0.80), and MAP LF oscillations increased above baseline at −30 and −45 mmHg (P ≤ 0.05) but fell back to baseline levels by −60 mmHg (P = 0.15; Fig. 2). Mean MCAv LF oscillations decreased (P = 0.01) between submax and max (i.e., presyncope) in the HT group but were statistically indistinguishable in the LT group (P = 0.35); MAP LF oscillations were also statistically indistinguishable between submax and max for both HT and LT subjects (P ≥ 0.17).

Representative tracings of respiration (via the ETCO₂ waveform) and oscillations in MAP and mean MCAv in one HT and

| Table 1. Demographics for subjects with HT and LT to LBNP at baseline and presyncope |
|---------------------------------|------------------|--------------------|
|                                 | HT               | LT                |
| n                               | 93               | 42                |
| LBNP tolerance time, s          | 1,880 ± 27       | 1,277 ± 31        |
| Sex, female                     | 32 (34%)         | 20 (48%)          |
| Sex, male                       | 61 (66%)         | 22 (52%)          |
| Age, yr                         | 29 ± 9           | 27 ± 7            |
| Height, cm                      | 174 ± 10         | 172 ± 12          |
| Weight, kg                      | 77 ± 14          | 75 ± 18           |
| Baseline HR, beats/min          | 64 ± 1           | 67 ± 1            |
| Baseline MAP, mmHg              | 97 ± 1           | 99 ± 1            |
| Baseline SV, ml                 | 99 ± 2           | 97 ± 4            |
| Baseline mean MCAv, cm/s        | 71 ± 2           | 71 ± 3            |
| Presyncopal HR, beats/min       | 118 ± 2          | 94 ± 3            |
| Presyncopal MAP, mmHg           | 80 ± 1           | 77 ± 1            |
| Presyncopal SV, %change         | −58 ± 1          | −43 ± 2           |
| Presyncopal mean MCAv, cm/s     | 49 ± 1           | 53 ± 3            |

Data are means ± SD for age, height, and weight, and means ± SE for all other data. HT, high tolerance; LT, low tolerance; HR, heart rate; MAP, mean arterial pressure; SV, stroke volume; MCAv, middle cerebral artery velocity. The term “presynopal” refers to the final 1-min values at maximum lower body negative pressure (LBNP) tolerance.

Fig. 1. Stroke volume (SV; A), heart rate (HR; B), mean arterial pressure (MAP; C), and mean middle cerebral artery velocity (MCAv; D) responses to progressive lower body negative pressure (LBNP) up to −60 mmHg in high tolerant (HT) and low tolerant (LT) subjects. *P ≤ 0.07, between HT and LT.
one LT subject are shown in Fig. 3. These tracings are recorded from the final 3 min before presyncope; both subjects are breathing in the HF range (peak respiration frequency: HT, 0.28 Hz; LT, 0.22 Hz), but LF oscillations in both MAP and mean MCAv are higher in the HT subject than in the LT subject (MAP LF: 39.8 vs. 3.6 mmHg²; mean MCAv LF: 5.1 vs. 1.6 (cm/s)²).

Coherence between MAP and mean MCAv only increased in the HT group during LBNP and was higher than the LT group at 45 and 60 mmHg LBNP and at presyncope (P = 0.001; Fig. 4, A and B). Transfer function between MAP and mean MCAv decreased progressively from 30 mmHg LBNP in both HT and LT groups, and there were no differences between groups, except at presyncope (HT: 0.68 ± 0.03 vs. LT: 0.81 ± 0.05 cm·s⁻¹·mmHg⁻¹; P = 0.03; Fig. 4, C and D).

Similarly, coherence between SAP and RRI increased during LBNP in the HT group and was higher than the LT group by −60 mmHg LBNP and at presyncope (P ≤ 0.07; Fig. 5, A and B). Baroreflex gain, indicated by the transfer function between SAP and RRI, decreased from −15 mmHg in both HT and LT groups and was only different between groups at presyncope (HT: 3.0 ± 0.3 vs. LT: 7.5 ± 1.0 ms·mmHg⁻¹; P < 0.001; Fig. 5, C and D).

Respiratory response to LBNP. ETCO₂ decreased progressively in both HT and LT groups and was not statistically distinguishable between groups at any level of LBNP (baseline: HT, 5.8 ± 0.1 vs. LT, 5.8 ± 0.1%; 60 mmHg LBNP: HT, 5.0 ± 0.1 vs. LT, 4.8 ± 0.2%; P ≥ 0.23), except for presyncope (HT, 4.2 ± 0.2 vs. LT, 4.9 ± 0.1%; P = 0.001). Respiration rate was similar between HT and LT groups throughout LBNP (baseline: HT, 14 ± 0.4 vs. LT, 15 ± 0.6 breaths/min; 60 mmHg LBNP: HT, 14 ± 0.4 vs. LT, 13 ± 0.6 breaths/min; P = 0.32), except at presyncope where it was marginally higher in the HT group (15 ± 0.5 vs. 13 ± 0.5 breaths/min; P = 0.09).

In the subset of 40 subjects breathing through the metabolic cart during LBNP, 24 were classified as HT and 16 were LT. The respiratory parameters measured during LBNP in these subjects are shown in Table 2. Consistent with the respiration rate measured from the ETCO₂ signal, HT subjects were breathing ~2 breaths/min faster than LT subjects at presyncope, although this was not statistically distinguishable (P = 0.18). Both HT and LT subjects demonstrated increases in V̇E and V̇E at −60 mmHg LBNP, but there were no differences between groups at each level of LBNP, including presyncope.

**DISCUSSION**

In this study, we characterized a number of important differences in the physiological responses between subjects exhibiting HT and LT to central hypovolemia. Specifically, our data supported the hypothesis that subjects with HT to reduced central blood volume would exhibit higher oscillations in AP and cerebral blood velocity compared with LT subjects. At the
same level of LBNP (−60 mmHg). LF oscillations in MAP and mean MCAv were 76% and 62% higher in HT subjects than LT subjects, and these differences persisted at presyncope. While LBNP-induced increases in LF AP (2, 58, 66, 70) and cerebral blood velocity oscillations (70) have been previously reported, to our knowledge, our current data are the first to associate increases in oscillations with HT to central hypovolemia and, conversely, attenuated oscillations with LT. Contrary to our observations, this increase in hemodynamic variability has often been termed “instability” (40, 58) and has traditionally been associated with physiological dysfunction and increased susceptibility to syncope during orthostasis, including LBNP (18, 40, 59, 70). The findings of our study highlight a group of individuals who are at the greatest risk of early cardiovascular collapse with central hypovolemia, but who do not demonstrate increases in hemodynamic variability, even at presyncope.

**Hemodynamic oscillations and tolerance to LBNP.** At −60 mmHg (the last common level of LBNP between groups), LT subjects had greater reductions in SV, MAP, and mean MCAv, all associated with impending cardiovascular collapse. HT subjects, however, were characterized by being able to tolerate greater reductions in central blood volume (i.e., SV) and cerebral blood flow (i.e., mean MCAv), as indicated by lower presyncope values. We propose that the oscillatory nature of AP and cerebral blood velocity contributed to this increase in tolerance. Our laboratory began investigating the potential protective nature of AP and cerebral blood velocity oscillations following completion of a study investigating the effect of inspiratory resistance breathing on tolerance to LBNP. While AP and cardiac output were protected when breathing through an ITD resulting in prolonged LBNP tolerance (9, 53, 56), mean MCAv decreased to the same level as during the control experiment, despite a delay in the onset of presyncope symptoms (53). Assessment of the oscillatory patterns of AP and cerebral blood velocity, however, revealed an almost threefold increase in MAP and mean MCAv LF oscillatory power with ITD breathing compared with the sham trial (53), suggesting that the pulsatile pattern of AP and cerebral blood velocity confers a protective benefit. As outlined in prior studies (52, 55, 68), pulsatile flow may increase shear stress on cerebral vessels, eliciting release of vasodilators and increasing flow and oxygen delivery to cerebral tissue, thus protecting against the reduction in AP and cerebral perfusion pressure induced by hypovolemia, including LBNP (68) and hemorrhage (38). While the differences in oscillatory power between HT and LT groups in the current study are not as striking as those induced with ITD breathing, these endogenous oscillations in HT subjects appear to have a similar protective effect during significant central hypovolemia. This protective effect is further highlighted by the observation that LF oscillations in mean MCAv are reduced at presyncope (“max”) compared with the

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**Fig. 3.** Representative tracings of end-tidal CO2 (ETCO2), MAP, and mean MCAv in 1 HT subject (A) and 1 LT subject (B) during the final 3 min of presyncope limited LBNP. Note that respiration rate measured from the ETCO2 waveform was −14.3 breaths/min (0.24 Hz) for the HT subject and −13.3 breaths/min (0.22 Hz) for the LT subject.
submaximal time point in HT subjects, coincident with the onset of hypotension and/or symptoms. By comparison, in LT subjects MCAv LF oscillations did not change from baseline throughout LBNP (Fig. 2) and were also not different between the submaximal and maximal time points, potentially contributing to the LT of these subjects.

Mechanisms for increased oscillations. Mayer originally described spontaneous oscillations in AP occurring at frequencies below respiration in conscious humans (i.e., centered ~0.1 Hz; Ref. 33). The underlying mechanism responsible for Mayer waves is controversial, and currently unresolved, with two competing theories; the central oscillator theory (34, 51) and the baroreflex theory (15, 27). The details of each theory, however, are the subject of numerous reviews (e.g., Ref. 33) and beyond the scope of this study. Instead, this discussion will focus on potential mechanisms underlying the differences in oscillatory responses between HT and LT groups.

Baroreflex control. Decreased vagal cardiac baroreflex sensitivity induced by administration of antimuscarinic agents (atropine or glycopyrrolate) has been associated with an increase in AP variability during LBNP (66), suggesting that the baroreflex has a buffering effect on AP fluctuations; this finding is corroborated in both healthy (62) and diseased (59) subject populations. Indeed, the greater reduction in cardiac baroreflex sensitivity in the HT group at presyncope may contribute to the increase in MAP oscillations at this time point. At ~60 mmHg LBNP, however, when oscillations were significantly higher in HT subjects, cardiac baroreflex sensitivity was statistically indistinguishable between HT and LT groups.

While greater attenuation of the vagally mediated baroreflex does not account for this increase in AP oscillations in HT subjects at ~60 mmHg LBNP, it is likely that these oscillations may reflect control by a sympathetically mediated baroreflex response. The reduction in central blood volume with LBNP (13, 30) or head-up tilt (12, 22, 35) elicits a progressive increase in muscle sympathetic nerve activity (MSNA), which is characterized by increases in MSNA LF oscillations (12–13, 22, 35). In a subset of 20 subjects (selected from the 135 used in this study) with direct measures of MSNA, we found a strong amalgamated correlation between SAP LF and MSNA LF oscillations in HT subjects ($R^2 = 0.97; n = 15$) compared with a poor correlation in LT subjects ($R^2 = 0.51; n = 5$; unpublished observations), despite increases in absolute MSNA in both groups (57). These data suggest there is stronger coupling between fluctuations in MSNA and AP in HT subjects during LBNP than in LT subjects, potentially accounting for the higher AP LF oscillations in the HT group. The protective effect of MSNA-mediated AP oscillations is supported by data from Kamiya et al. (35), who showed a persistent elevation in LF oscillations of MSNA and AP in “nonsyncopal” subjects during head-up tilt, while “syncopal” subjects exhibited marked reductions in these oscillations. Collectively, these observations support the concept that sympathetically

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**Fig. 4.** Coherence (COH; A and B) and transfer function (TF; C and D) between MAP and mean MCAv in HT and LT subjects. A and C: during progressive LBNP up to ~60 mmHg. B and D: at presyncope. *$P \leq 0.07$, between HT and LT.
mediated, rather than vagally mediated, baroreflex control of AP oscillations is a fundamental mechanism underlying effective compensation to reductions in central blood volume.

Cerebral regulation. The relationship between cerebral regulation and cerebral oscillations is more complicated. Analogous to the association between decreased baroreflex sensitivity and AP variability, the concept of attenuated cerebral autoregulation observed during LBNP has been advanced and linked to the increase in variability of cerebral blood velocity (70). Zhang et al. (70) reported an increase in the transfer function gain between MAP and mean MCAv with LBNP up to −60 mmHg, which they suggested accounts for the observed increase in cerebral blood velocity variability and reduction in cerebral blood velocity, both contributing to the onset of presyncope. In the current study, the MAP-mean MCAv gain decreased by a similar magnitude in both HT and LT groups throughout LBNP to −60 mmHg and was only different between groups at presyncope (i.e., lower in HT group). With the use of the traditional definition of reduced transfer function gain between cerebral blood velocity and MAP (23), these observations suggest that central hypovolemia elicits an improvement in cerebral autoregulation, regardless of

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

![Graph D](image4)

Fig. 5. COH (A and B) and TF (C and D) between systolic arterial pressure (SAP) and R-R intervals (RRI) in HT and LT subjects. A and C: during progressive LBNP up to −60 mmHg; B and D: at presyncope. *P < 0.001, between HT and LT.

Table 2. Respiratory responses during presyncopal LBNP in HT and LT subjects

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<th>Respiratory Parameter/Group</th>
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<th>30</th>
<th>45</th>
<th>60</th>
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<td>14 ± 0.9</td>
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<td>14 ± 0.9</td>
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<td>HT</td>
<td>647 ± 35</td>
<td>665 ± 48</td>
<td>712 ± 65</td>
<td>738 ± 67</td>
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<tr>
<td>LT</td>
<td>647 ± 62</td>
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<td>743 ± 99</td>
<td>826 ± 189</td>
<td>1,029 ± 288*</td>
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<td>8.6 ± 0.3</td>
<td>8.8 ± 0.3</td>
<td>8.8 ± 0.5</td>
<td>9.2 ± 0.5</td>
<td>9.9 ± 0.6†</td>
<td>12.5 ± 0.9</td>
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<td>9.4 ± 0.5</td>
<td>9.5 ± 0.8</td>
<td>11.1 ± 1.9*</td>
<td>11.0 ± 1.3</td>
</tr>
</tbody>
</table>

Data are means ± SE; 24 HT subjects; 16 LT subjects; n = 10 for LT −60 mmHg, as remaining 6 subjects did not make it to this level of LBNP. RR, respiration rate; VT, tidal volume; VtE, minute ventilation. Volumes are in BTPS. *P ≤ 0.05, within tolerance group across LBNP; †P = 0.06, within tolerance group across LBNP.
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tolerance, which, in turn, cannot account for the increased oscillations in HT subjects.

However, when the operating MAP is considered, assessment of MAP-mean MCAv transfer gain has to be interpreted very differently. While MAP-mean MCAv gain between HT and LT groups at ~60 mmHg LBNP is identical, MAP for the LT group is 76 and 93 mmHg for the HT group, i.e., the magnitude of MAP transferring to mean MCAv in HT subjects is equivalent to LT subjects, despite the fact that HT subjects have higher MAP and are at submaximal levels of LBNP. While this response could be interpreted as an impairment of cerebral regulation, it was observed in our HT subjects so it does not appear to impact the ability to tolerate higher levels of central hypovolemia and may be contributing to the higher mean MCAv oscillations.

Similarly, the linear coupling between oscillations of MAP and mean MCAv (i.e., coherence) increased progressively in the HT group but did not change in the LT group. While it is clear that increased MAP-mean MCAv coherence reflects greater linear dependence of cerebral oscillations on AP oscillations, the interpretation of higher coherence is somewhat contentious. Some investigators use coherence only as a threshold criterion for subsequent assessment of transfer function gain and phase (i.e., if coherence is ≥0.5; Refs. 29, 41, 47), while others have also used coherence as an independent indicator of cerebral regulatory capacity (16, 23–24, 50, 69–71). Using the latter approach, if high coherence is interpreted to reflect reduced capacity for cerebral autoregulation and compromised cerebral perfusion, then we would expect LT subjects to have a higher MAP-mean MCAv coherence than HT subjects when challenged with similar reductions in central volume. Instead, because we found that subjects with HT to central hypovolemia had higher coherence and transfer function gain at a higher operating MAP than those with LT, our results challenge the traditional interpretation that high coherence is reflective of impaired cerebral autoregulation or compromised cerebral perfusion. Indeed, as HT subjects were able to tolerate greater reductions of central volume and cerebral blood flow, it appears as though the pulsatile pattern of AP and cerebral blood velocity and subsequent increased MAP-mean MCAv coherence may actually improve cerebral perfusion and protect subjects against the onset of presyncope. These findings are consistent with a recent study by Romero et al. (54) who showed that an increase in MCAv oscillations and MAP-mean MCAv coherence during the combined hypovolemic stressors of dehydration and head-up tilt protected subjects from presyncope, despite lower absolute MCAv compared with the euhydrated condition. Collectively, these data support the notion that the pattern of cerebral blood flow is potentially of greater importance than absolute perfusion and the coupled oscillatory pattern of AP and MCAv may be associated with improvements in the delivery of oxygen to the cerebral tissues.

Recent evidence suggests that the increased coherence between both SAP and RRI and between MAP and mean MCAv observed in the current study is most likely due to the increase in the magnitude of the “input” signal i.e., oscillations in AP. With the use of either repeated squat-stand maneuvers (5, 67) or oscillatory LBNP (61) within the LF range, a number of studies have demonstrated very high LF coherence (≥0.8) in SAP-RRI and MAP-mean MCAv. We propose that as the level of central hypovolemia progresses, the increase in LF oscillations of MSNA in HT subjects drives the oscillations in AP and subsequent oscillations in cerebral blood velocity, as occurs with repeated squat-stand or oscillatory LBNP maneuvers, accounting for the higher coherence between MAP and mean MCAv.

**Influence of respiration.** At ~0.1 Hz, Mayer waves occur below the respiratory frequency of most conscious humans (6 breaths/min). However, LF oscillations in the current study were assessed between 0.04 and 0.15 Hz, equating to breathing frequencies of 2.4–9.0 breaths/min. In our studies of tolerance to central hypovolemia, we do not control respiratory rate or volume as reflex alterations in breathing dynamics stimulated by baroreflex unloading (60) may serve as a protective mechanism against impending syncope during LBNP (7, 30) and actual hemorrhage (19). As such, it is possible that differences in breathing frequencies between tolerance groups could account for the increase in LF oscillations in the HT subjects. While data presented in Table 2 demonstrate that the average rate and depth of breathing were similar between HT and LT groups throughout LBNP, there was a small proportion of subjects who breathed at respiratory rates of ≤9 breaths/min, i.e., ≤0.15 Hz (13% of HT and 16% of LT subjects at ~60 mmHg; and 12% of HT and 7% of LT subjects at presyncope). As there were equivalent proportions of HT and LT subjects breathing within the LF frequency band, however, it appears unlikely that breathing rate is responsible for the higher LF oscillations in MAP and mean MCAv in HT subjects. In further support of the limited role of respiration on LF oscillations in AP or cerebral blood velocity are studies using paced breathing protocols, where breathing frequency is outside the LF range. Under conditions of progressively increasing head up tilt angle (12), head-up tilt with dehydration (54), and LBNP (3), LF oscillations in MSNA, AP, and MCAv increase despite respiration remaining fixed at frequencies ≥0.2 Hz. These data indicate that factors other than respiration (e.g., oscillatory sympathetic drive and/or central command) are contributing to the observed increase in LF oscillations.

**Methodological considerations.** Assessment of transfer function gain requires that the input and output signals are linear and relatively “stationary.” As we assessed the transfer function gain between RRI and SAP and between MAP and mean MCAv during the final 3 min before presyncope, the data may not have conformed to these requirements. However, coherence between these two pairs of signals was consistently >0.5 throughout LBNP and at presyncope, suggesting the condition of linearity was achieved (69). Secondly, the responses of SAP-RRI TF and Mean MCAv-MAP TF conform to a predictable trajectory, even at presyncope. As the data do not diverge wildly at presyncope from previous levels of LBNP, it does not appear as though the issue of nonstationarity is having an impact on the data that were used in this analysis.

A second limitation of transfer function analysis, recently highlighted in a review by Willie et al. (65), is that it does not differentiate between increasing and decreasing changes in AP on cerebral blood velocity and, as such, does not consider where on the regulatory curve the gain values are positioned. However, as we were only comparing transfer function gain and coherence as AP was progressively decreasing, the effect of this limitation is attenuated; although as previously indicated in this discussion, knowledge of relative APs between tolerance groups was essential for accurate interpretation of transfer.
function gain values and should always be considered when reporting these results.

This study was not designed to specifically assess the effect of sex or blood volume on tolerance to LBNP. As such, we did not intentionally request specific information from female subjects regarding the timing of their menstrual cycle in relation to the time of the study nor did we request specific details about whether they were taking oral contraceptive medications, both of which can impact cardiovascular responses to hypovolemia (4, 20–21). While there is evidence to suggest that females are more orthostatically intolerant than males (6, 17, 45, 64), we found no significant difference in sex ratios between HT and LT groups. Moreover, the range of tolerance between males and females was very similar (males: 670–2516 vs. females: 809–2,019 s), and a male actually had the lowest tolerance (670 s; LT subject depicted in Fig. 4).

There is some contention about whether blood volume status influences tolerance to LBNP. In studies where blood volume is experimentally modified, LBNP tolerance is reduced in volume-depleted subjects (39) and improved in subjects with restored blood volume [e.g., following heat stress (36)]. In a multifactorial analysis, blood volume was also positively correlated (albeit only moderately, r = 0.58) with LBNP tolerance (44). Conversely, both Convertino and Sather (11) and Greenleaf et al. (26) assessed blood volume in cohorts of male subjects exposed to presyncopal LBNP and found that baseline blood volume was identical between HT and LT subjects. Thus, although acute reductions in blood volume induced in any individual reduces his or her LBNP tolerance, in subjects with “normal” hydration status, differences in blood volume are an unlikely explanation for the difference in tolerance between HT and LT subjects. Furthermore, while females have lower total blood volume compared with males (6), the equal ratios of males vs. females between the HT and LT groups without differences in baseline stroke volume (Table 1) provide further evidence that blood volume was probably not influencing the differential responses between these groups.

Finally, in the absence of measurements to describe the physical fitness of our subjects, we cannot dismiss the potential influence of fitness on the outcome of tolerance in our subjects. However, such a notion is not supported by previous work (11) that demonstrated no difference in fitness between HT and LT subjects.

Perspectives

A recent study by Lucas et al. (43) highlighted the misconception of static cerebral autoregulation as a plateau region of cerebral blood flow for a given range of AP. By inducing systematic and progressive increases and decreases in AP in healthy human subjects, these investigators elegantly demonstrated that mean MCAv changes by 0.82% for every 1-mmHg change in MAP, clearly challenging the cerebral autoregulation paradigm (43). As indicated by these investigators (42–43) and in a related letter to the editor (31), despite the initial challenge against the concept of cerebral autoregulation in 1983 (28), this theory and associated terminology are still accepted doctrine in original research publications and textbooks.

Our findings also challenge some of the traditional concepts of cerebral autoregulation, as outlined in the above discussion. The use of transfer function analysis for assessment of “dynamic cerebral autoregulation” is now commonplace within the physiology literature. However, it is clear that the term “autoregulation” in this context is also a misnomer. Even in studies where AP is maintained within the so-called “autoregulatory range,” cerebral blood velocity decreases and calculations of coherence and transfer function all suggest a dynamic interplay between AP and cerebral blood flow that, theoretically, should be minimized if cerebral autoregulation is intact. Since tolerance to progressive central hypovolemia is associated with protection of cerebral perfusion as indicated by delayed onset of presyncopal symptoms (25, 37, 63), we anticipated that traditional indexes of intact cerebral autoregulation would be exhibited in HT subjects (i.e., lower mean MCAv-MAP coherence and transfer function). Against expectations, protection of cerebral perfusion in HT subjects was associated with tighter coupling (i.e., increased coherence) between increases in LF oscillations of AP and mean MCAv and transfer of MAP to mean MCAv at higher operating MAPs; this is despite a traditional interpretation that higher coherence reflects “impaired cerebral autoregulation.” We propose that this response is not impaired cerebral autoregulation but an alteration in the “dynamic control of cerebral perfusion” that, in this case, appears to be protective as evidenced by the delay in the onset of presyncopal symptoms and an associated increase in tolerance to central hypovolemia.

Conclusions

The primary novel finding of this study is that subjects with HT to central hypovolemia exhibit increased variability in AP and cerebral blood velocity that is associated with the ability to tolerate greater reductions in central volume and cerebral blood velocity. The delay in the onset of presyncopal symptoms and associated increase in tolerance to hypovolemia in HT subjects indicates that the pulsatile pattern and increased synchronicity of AP and cerebral blood velocity protect cerebral perfusion and defend against the onset of syncpe.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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