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## Effects of gender and hypovolemia on sympathetic neural responses to orthostatic stress

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**Fu, Qi, Sarah Witkowski, Kazunobu Okazaki, and Benjamin D. Levine.** Effects of gender and hypovolemia on sympathetic neural responses to orthostatic stress. *Am J Physiol Regul Integr Comp Physiol* 289: R109–R116, 2005. First published March 10, 2005; doi:10.1152/ajpregu.00013.2005.—We tested the hypothesis that women have blunted sympathetic neural responses to orthostatic stress compared with men, which may be elicited under hypovolemic conditions. Muscle sympathetic nerve activity (MSNA) and hemodynamics were measured in eight healthy young women and seven men in supine position and during 6 min of 60° head-up tilt (HUT) under normovolemic and hypovolemic conditions (randomly), with ~4-wk interval. Acute hypovolemia was produced by diuretic (furosemide) administration ~2 h before testing. Orthostatic tolerance was determined by progressive lower body negative pressure to presyncope. We found that furosemide produced an ~13% reduction in plasma volume, causing a similar increase in supine MSNA in men and women (mean  $\pm$  SD of  $5 \pm 7$  vs.  $6 \pm 5$  bursts/min;  $P = 0.895$ ). MSNA increased during HUT and was greater in the hypovolemic than in the normovolemic condition ( $32 \pm 6$  bursts/min in normovolemia vs.  $44 \pm 15$  bursts/min in hypovolemia in men,  $P = 0.055$ ;  $35 \pm 9$  vs.  $45 \pm 8$  bursts/min in women,  $P < 0.001$ ); these responses were not different between the genders (gender effect:  $P = 0.832$  and  $0.814$  in normovolemia and hypovolemia, respectively). Total peripheral resistance increased proportionately with increases in MSNA during HUT; these responses were similar between the genders. However, systolic blood pressure was lower, whereas diastolic blood pressure was similar in women compared with men during HUT, which was associated with a smaller stroke volume or stroke index. Orthostatic tolerance was lower in women, especially under hypovolemic conditions. These results indicate that men and women have comparable sympathetic neural responses during orthostatic stress under normovolemic and hypovolemic conditions. The lower orthostatic tolerance in women is predominantly because of a smaller stroke volume, presumably due to less cardiac filling during orthostasis, especially under hypovolemic conditions, which may overwhelm the vasomotor reserve available for vasoconstriction or precipitate neurally mediated sympathetic withdrawal and syncope.

muscle sympathetic nerve activity; vascular resistance; arterial pressure; head-up tilt

WOMEN, PRIMARILY YOUNG WOMEN, have a greater incidence of orthostatic intolerance than men (10, 33), and this difference is especially dramatic after spaceflight (9, 45) or bed rest (6), in which hypovolemia and “cardiovascular deconditioning” occur. However, the underlying mechanisms remain unclear. It is likely that certain gender-specific factors such as differences in some hormonal levels, which may affect the neurohumoral regulation of arterial pressure, or physical characteristics such

as a smaller and less “distensible” heart (10) may influence orthostatic blood pressure (BP) control.

Results regarding gender differences in sympathetic neural responsiveness to orthostatic challenges are few but controversial. Similar (10, 12) or attenuated (1, 3, 45) adrenergic responses during orthostatic stress have been reported in healthy women compared with men. There is only one study showing lower muscle sympathetic nerve activity (MSNA) responses, when expressed as average amplitude per burst, in healthy young women vs. men during a graded head-up tilt (HUT). However, both MSNA burst frequency (bursts per minute) and burst incidence (bursts per 100 heartbeats) were not different between the genders; moreover, peripheral vascular resistance responses did not differ between men and women in this study (35). Thus evidence for the conclusion that women have a lower sympathetic neural response than men is not definitive.

Our group (10) recently demonstrated that the high incidence of orthostatic intolerance in young women is associated with decreased cardiac filling rather than a reduced responsiveness of vascular resistance during orthostatic challenges under normovolemic conditions. This study indicated that human vasoconstrictor capability is comparable in men and women but more likely to be overwhelmed in women because of their smaller and functionally stiffer hearts. However, it is unclear whether this is also the case under hypovolemic conditions. Circulating blood volume has a profound effect on arterial pressure during orthostatic stress in humans (20, 24, 25). Individuals with reduced vascular volumes exhibit subnormal cardiac filling pressure, and the capacity to buffer orthostatic reductions in central blood volume is limited (11, 20, 22, 32). Women are more susceptible to orthostatic intolerance compared with men and appear especially so when they are dehydrated. It may be possible that sympathetic neural and vascular resistance responses during orthostatic challenges are attenuated in women, particularly when they are hypovolemic (45).

The present study was performed to test the hypothesis that women have blunted vasomotor sympathetic responses to orthostatic stress compared with men, which may be elicited under hypovolemic conditions. To accomplish this objective, we measured MSNA, plasma catecholamines, and hemodynamics in healthy young women and men in the supine position and during acute 60° HUT under both normovolemic and hypovolemic conditions and compared the responses between the genders. Additionally, to determine the maximal orthostatic

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tolerance, progressive lower body negative pressure (LBNP) to presyncope was applied in all subjects under both conditions.

## METHODS

### Subjects

Eight healthy young women and seven men matched for age and race were recruited. All were normotensive individuals. No subject smoked, used recreational drugs, or had medical problems. None was an endurance-trained athlete (21). No woman was pregnant during the study. All had regular menstrual periods of ~28 days and did not take oral contraceptives (19, 29). The subjects were screened with a careful medical history, physical examination, and ECG. Individuals with a history of fainting or neurally mediated syncope were excluded. All subjects were informed of the purpose and procedures used in the study and gave their written, informed consent to a protocol approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center and Presbyterian Hospital of Dallas. A summary of the descriptive data for the subjects in both groups is presented in Table 1.

### Measurements

**Heart rate and BP.** Heart rate (HR) was monitored from lead II of the ECG (Hewlett-Packard), and beat-to-beat arterial pressure was derived by finger photoplethysmography (Finapres, Ohmeda). Cuff BP was measured by electrophygmomanometry (model 4240, Suntech), with a microphone placed over the brachial artery to detect Korotkoff sounds. Respiratory excursions were detected by a nasal cannula.

**Cardiac output.** Cardiac output (CO) was measured with the acetylene rebreathing technique (39). CO is calculated from the disappearance rate of acetylene in expired air, measured with a mass spectrometer (model MGA1100, Marquette), after adequate mixing in the lung was confirmed by a stable helium concentration. This method has been validated in our laboratory against standard invasive techniques, including thermodilution and direct Fick during orthostatic stress with a typical error (expressed as coefficient of variation) of 4–5% (Table 2).

**MSNA.** Multiunit recordings of postganglionic MSNA were obtained with tungsten microelectrodes inserted into muscle fascicles of a peroneal nerve (40). Briefly, a recording electrode was placed in the peroneal nerve at the popliteal fossa, and a reference electrode was placed subcutaneously 2–3 cm from the recording electrode. The nerve signals were amplified (total gain: 70,000–160,000), band-pass filtered (700–2,000 Hz), full-wave rectified, and integrated with a resistance-capacitance circuit (time constant of 0.1 s). Criteria for adequate MSNA recording included 1) pulse synchrony, 2) facilitation during the hypotensive phase of the Valsalva maneuver and suppression during the hypertensive overshoot after release, 3) increases in response to breath holding, and 4) insensitivity to emotional stimuli, i.e., loud noise (40, 42).

Table 1. Subject characteristics

| Variables                          | Men<br>(n = 7) | Women<br>(n = 8) | t Value | DF | P Value |
|------------------------------------|----------------|------------------|---------|----|---------|
| Age, yr                            | 27.6±5.3       | 27.9±6.2         | -0.101  | 13 | 0.921   |
| Height, cm                         | 178.4±6.1      | 169.0±11.3       | 1.981   | 13 | 0.069   |
| Weight, kg                         | 71.1±8.5       | 63.1±10.2        | 1.646   | 13 | 0.124   |
| Body surface area, m <sup>2</sup>  | 1.88±0.13      | 1.72±0.17        | 1.947   | 13 | 0.073   |
| Body mass index, kg/m <sup>2</sup> | 22.3±2.4       | 21.9±3.7         | 0.138   | 13 | 0.892   |
| Hematocrit, %                      | 42.1±2.9       | 36.2±3.4         | 3.513   | 12 | 0.004   |

Values are means ± SD. DF, degrees of freedom. Comparisons between men and women were made using unpaired *t*-tests.

Table 2. Comparison of cardiac output determined with thermodilution, direct Fick, and the acetylene rebreathing methods

| Method                | Posture     |               |
|-----------------------|-------------|---------------|
|                       | Supine rest | Standing rest |
| Thermodilution        | 7.19±1.17   | 4.78±0.92     |
| Direct Fick           | 6.36±1.63   | 4.53±0.90     |
| Acetylene rebreathing | 7.20±1.01   | 4.97±1.01     |

Values (in l/min) are means ± SD; n = 14 healthy young men and women. Statistical comparisons were made using two-way ANOVA (method and posture). No significant differences were observed among methods in the same posture. Typical error (SD of differences divided by  $\sqrt{2}$ , expressed as coefficient of variation) was generally 4–5% between all methods in both body positions.

**Blood samples.** Blood samples were drawn from an intravenous catheter placed in the antecubital vein. Plasma norepinephrine and epinephrine concentrations were measured by an independent laboratory using high-precision liquid chromatography according to standardized procedures (31). Hematocrit (Hct) was determined with a microcentrifuge. The percent change in plasma volume (PV) with administration of a diuretic (furosemide) in the hypovolemic condition was estimated from the Hct according to the method previously described by Van Beaumont (41).

### Acute Hypovolemia

PV was reduced with the administration of 20 mg of furosemide (iv). This dosage was chosen because it induced a reduction in PV of 7–14% after administration for ~2 h, equivalent to the loss of PV observed after 2 wk of head-down bed rest (17, 32). An oral potassium supplement of 20 meq was given before the injection of furosemide. After injection, urine was collected to quantify the volume and estimate the magnitude of diuresis after administration of furosemide, and arm BP was measured every 15 min. About 2 h later, the following protocols were performed.

### Protocols

The experiment was carried out in the morning  $\geq 2$  h after a light breakfast and  $\geq 12$  h after the last caffeinated or alcoholic beverage in a quiet, environmentally controlled laboratory with an ambient temperature of ~25°C. The same protocol was performed under both normovolemic and hypovolemic conditions (in random order), with ~4 wk interval; therefore, although the menstrual cycle phase was not required to be the same for all females, each individual female subject was in the same approximate phase of her own individual menstrual cycle for both studies, which was confirmed verbally.

**60° HUT.** After  $\geq 30$  min of quiet rest in the supine position, baseline data were collected for 6 min. The subject was then tilted passively to a 60° HUT for 6 min. A belt was placed across the subject's waist to make sure he or she would not fall. The subject supported the body weight by standing on a plate at the end of the tilt bed on one leg, allowing the other leg to be relaxed for microneurography. After that, the subject was returned to the supine position for recovery. During all procedures, HR, BP, respiration waves, and MSNA were recorded continuously. CO was measured, and blood samples were taken in the supine position and at the 6th min of tilting. After completion of this protocol, the microneurography electrodes and the intravenous catheter were removed to avoid any influences on the measurement of maximal orthostatic tolerance.

**Maximal orthostatic tolerance test.** After a sufficient recovery ( $\geq 20$  min), the subject was placed in a Plexiglas LBNP tank sealed at the iliac crest level in the supine position. Suction was provided by a vacuum pump and controlled with a variable autotransformer, cali-

brated against a mercury manometer. After  $\geq 30$  min of quiet rest, baseline measurements, including HR, BP, respiration, and CO, were repeated to confirm a return to the hemodynamic steady state. Maximal orthostatic tolerance was determined by using progressive LBNP to presyncope in all subjects. LBNP was begun at  $-15$  mmHg for 5 min and then increased to  $-30$  and  $-40$  mmHg for 5 min each, followed by an increase in LBNP by  $-10$  mmHg every 3 min until presyncope was achieved. Presyncope was defined as a decrease in systolic BP (SBP) to  $<80$  mmHg; a decrease in SBP to  $<90$  mmHg associated with symptoms of lightheadedness, nausea, or diaphoresis; or progressive symptoms of presyncope accompanied by a request from the subject to discontinue the test (22). A true hypotensive endpoint was reached in all subjects in this study. The recovery lasted for 5 min. A cumulative stress index (CSI) was calculated by adding the product of negative pressure and duration at each level of LBNP and was used as a continuous measure of orthostatic tolerance.

#### Hemodynamic Calculations

Stroke volume (SV) was calculated from CO, and HR measured during rebreathing. Both SV and CO were normalized to the body surface area as stroke index (SI) and cardiac index (CI). Total peripheral resistance (TPR) was calculated as the quotient of mean arterial pressure and CO and multiplied by 80 (expressed as dynes per second per  $\text{cm}^5$ ). Mean arterial pressure was calculated as  $[(\text{SBP} - \text{DBP})/3] + \text{DBP}$ , where SBP and DBP are cuff systolic and diastolic BP measured during rebreathing.

#### Data Analyses

Sympathetic bursts were identified by a computer program (5) and then were confirmed by an experienced microneurographer. The number of bursts per minute (burst frequency), the number of bursts per 100 heartbeats (burst incidence), and the sum of the integrated burst area per minute (total activity) were used as quantitative indexes. Because the amplitude of bursts of sympathetic activity depends critically on electrode position, whereas determinations of burst frequency are stable between recording sessions (38), total activity was normalized to the resting supine value to allow comparisons between normovolemic and hypovolemic conditions. Therefore, the supine baseline recording was assigned a value of 100%, and subsequent changes of total activity were expressed as percentages of this baseline value. MSNA, BP measured by Finapres, and HR were averaged for 6 min of resting supine baseline. Data were collected from the 3rd to 5th min during HUT and were averaged for 3 min.

#### Statistical Analyses

Data are expressed as means  $\pm$  SD. Subject characteristics and comparisons at baseline between the groups were made by using unpaired *t*-tests. Comparisons of sympathetic and hemodynamic variables in the supine position and during HUT in men and women in the normovolemic or hypovolemic condition were analyzed using two-way ANOVA, with Bonferroni method post hoc for multiple comparisons. The relationship between MSNA and SV, as well as SI in the supine position and during HUT under both normovolemic and hypovolemic conditions, was determined for each subject by least squares linear regression analysis, and the slopes between the genders were compared by unpaired *t*-tests. A second-order polynomial regression analysis was also performed, which improved the  $r^2$  by  $\sim 4\%$ . Although this improvement was statistically significant, the physiological significance was minor, and, in the interests of parsimony, we report the correlation coefficient and the slope for the linear regression. Comparisons of the CSI within and between the groups under both conditions were made by paired and unpaired *t*-tests, respectively. All statistical analyses were performed with a personal computer-based analysis program (SigmaStat, SPSS). A *P* value of  $<0.05$  was considered statistically significant.

## RESULTS

### Physical Characteristics

The two groups did not differ in age, height, weight, body surface area, and body mass index, but Hct was lower in women than in men (Table 1). Supine resting HR, SBP, and DBP were not different between the groups ( $P = 0.723$ ,  $0.694$ , and  $0.306$ , respectively; Table 3). SV was smaller in women compared with men ( $P = 0.038$ ), whereas SI was not different between the genders ( $P = 0.143$ ; Table 3). CO was lower in women than in men ( $P = 0.029$ ), whereas CI did not differ between the genders ( $P = 0.097$ ; Table 3). Supine resting TPR was not different between the gender groups ( $P = 0.182$ ; Table 3).

Figure 1 displays original tracings of MSNA of one representative male and female subject. Supine resting MSNA burst frequency ( $t = 1.311$  with 13 degrees of freedom,  $P = 0.212$ ; Fig. 2A) and burst incidence ( $22 \pm 10$  bursts/100 heartbeats in men vs.  $15 \pm 8$  bursts/100 heartbeats in women,  $t = 1.479$  with 13 degrees of freedom,  $P = 0.163$ ) were not different between the genders. Supine plasma norepinephrine concentration was similar in both groups ( $t = -0.553$  with 12 degrees of freedom,  $P = 0.590$ ; Fig. 3A).

### Acute Hypovolemia and Supine Resting Values

Furosemide induced a similar diuresis of urine volume ( $1.6 \pm 0.6$  liters in men vs.  $1.4 \pm 0.3$  liters in women,  $P = 0.357$ ) and a similar increase in Hct ( $3.7 \pm 1.4$  vs.  $3.1 \pm 0.7\%$ ,  $P = 0.347$ ), resulting in a similar reduction in PV ( $13.9 \pm 4.9$  vs.  $12.5 \pm 2.5\%$ ,  $P = 0.490$ ) in both genders. Supine resting MSNA burst frequency increased in men and women ( $P =$

Table 3. Hemodynamic responses to HUT in men and women under normovolemic and hypovolemic conditions

| Variable  | Men ( $n = 7$ )  |                  | Women ( $n = 8$ ) |                   |
|---|------------------|------------------|-------------------|-------------------|
|   | Supine           | 60° HUT          | Supine            | 60° HUT           |
| <i>Normovolemia</i>                                     |                  |                  |                   |                   |
| SBP, mmHg   | 116 $\pm$ 5      | 129 $\pm$ 19*    | 105 $\pm$ 14      | 113 $\pm$ 11†     |
| DBP, mmHg   | 60 $\pm$ 5       | 78 $\pm$ 13*     | 62 $\pm$ 6        | 71 $\pm$ 6*       |
| HR, beats/min   | 64 $\pm$ 3       | 92 $\pm$ 3*      | 67 $\pm$ 17       | 91 $\pm$ 20*      |
| SV, ml  | 110 $\pm$ 24     | 75 $\pm$ 34*     | 88 $\pm$ 20†      | 59 $\pm$ 20*      |
| SI, ml/m <sup>2</sup>                                   | 59 $\pm$ 11      | 40 $\pm$ 19*     | 51 $\pm$ 8        | 34 $\pm$ 8*       |
| CO, l/min   | 8.47 $\pm$ 1.88  | 7.56 $\pm$ 2.94  | 6.41 $\pm$ 1.36†  | 5.58 $\pm$ 1.22†  |
| CI, l $\cdot$ min <sup>-1</sup> $\cdot$ m <sup>-2</sup> | 4.50 $\pm$ 0.90  | 4.02 $\pm$ 1.53  | 3.74 $\pm$ 0.76   | 3.57 $\pm$ 0.93   |
| TPR, dyn $\cdot$ cm <sup>-5</sup>                       | 772 $\pm$ 161    | 1115 $\pm$ 349*  | 999 $\pm$ 252     | 1190 $\pm$ 356*   |
| <i>Hypovolemia</i>                                      |                  |                  |                   |                   |
| SBP, mmHg   | 116 $\pm$ 8      | 128 $\pm$ 24     | 107 $\pm$ 6       | 110 $\pm$ 11†     |
| DBP, mmHg   | 62 $\pm$ 3       | 77 $\pm$ 11*     | 63 $\pm$ 8        | 69 $\pm$ 8*       |
| HR, beats/min   | 64 $\pm$ 5       | 104 $\pm$ 13*‡   | 72 $\pm$ 17       | 109 $\pm$ 25*‡    |
| SV, ml  | 87 $\pm$ 16§     | 57 $\pm$ 16*‡    | 66 $\pm$ 17†‡     | 41 $\pm$ 11*†‡    |
| SI, ml/m <sup>2</sup>                                   | 46 $\pm$ 8§      | 30 $\pm$ 8*      | 38 $\pm$ 8†‡      | 23 $\pm$ 6*†‡     |
| CO, l/min   | 6.12 $\pm$ 0.87‡ | 6.08 $\pm$ 0.87  | 5.03 $\pm$ 1.39†‡ | 4.78 $\pm$ 1.92†‡ |
| CI, l $\cdot$ min <sup>-1</sup> $\cdot$ m <sup>-2</sup> | 3.27 $\pm$ 0.48‡ | 3.24 $\pm$ 0.34  | 2.91 $\pm$ 0.68‡  | 2.71 $\pm$ 0.91‡  |
| TPR, dyn $\cdot$ cm <sup>-5</sup>                       | 1,071 $\pm$ 140‡ | 1,245 $\pm$ 143* | 1,353 $\pm$ 518‡  | 1,504 $\pm$ 498‡  |

Values are mean  $\pm$  SD. HUT, head-up tilt; SBP, systolic blood pressure measured by Finapres; DBP, diastolic blood pressure measured by Finapres; HR, heart rate; SV, stroke volume; SI, stroke index; CO, cardiac output; CI, cardiac index; TPR, total peripheral resistance. \* $P < 0.05$  compared with the supine position within the group in the same normovolemic or hypovolemic condition; † $P < 0.05$  compared with the men in the same body position under the same condition; ‡ $P < 0.05$  compared with the normovolemic condition in the same position within the group.



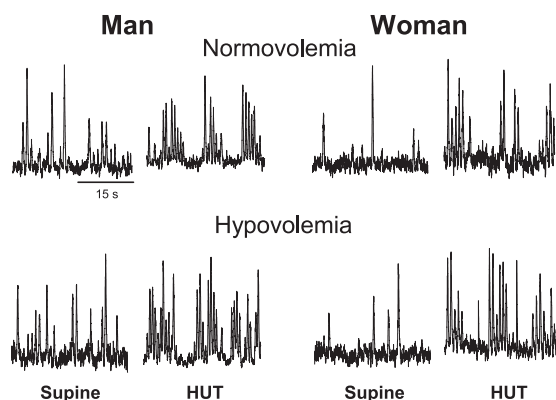


Fig. 1. Original tracings of muscle sympathetic nerve activity (MSNA) from one representative male and female subject in the supine position and at 60° head-up tilt (HUT) under both normovolemic and hypovolemic conditions.

0.05 and 0.019; Fig. 2A), and the changes in burst frequency were not different between the genders ( $5 \pm 7$  bursts/min in men vs.  $6 \pm 5$  bursts/min in women,  $t = -0.134$  with 13 degrees of freedom,  $P = 0.895$ ). In addition, the changes in MSNA burst incidence were not different between the genders ( $9 \pm 12$  bursts/100 heartbeats in men vs.  $7 \pm 7$  bursts/100 heartbeats in women,  $t = 0.416$  with 13 degrees of freedom,  $P = 0.684$ ).

Supine resting HR did not change in men ( $P = 0.779$ ) but increased in women ( $P = 0.036$ ; Table 3). SBP and DBP remained unchanged in both groups (Table 3). Supine resting SV, SI, CO, and CI decreased in both genders (all  $P < 0.05$ ; Table 3), whereas SV was smaller in women than in men under the hypovolemic condition ( $t = 2.414$  with 13 degrees of freedom,  $P = 0.031$ ; Table 3). TPR increased in both groups ( $P = 0.013$  and  $0.003$  for males and females; Table 3), and the increments in TPR were not different between the genders ( $298 \pm 89$  dyn·s·cm<sup>-5</sup> in men vs.  $354 \pm 365$  dyn·s·cm<sup>-5</sup> in women;  $t = -0.396$  with 13 degrees of freedom,  $P = 0.698$ ).

#### Hemodynamic Responses to 60° HUT

HR increased in all subjects during HUT ( $P < 0.05$ ) and was greater in the hypovolemic condition than in the normovolemic condition in both groups (both  $P < 0.05$ ; Table 3), and these responses were not different between the genders (gender effect,  $P = 0.894$  and  $0.397$  in normovolemia and hypovolemia). However, SBP was lower in women than in men during HUT under both normovolemic and hypovolemic conditions ( $P = 0.027$  and  $0.020$ ; Table 3). DBP increased during HUT in men and women under both conditions, and these responses were not different between the groups (gender effect,  $P = 0.486$  and  $0.356$  in normovolemia and hypovolemia; Table 3). Respiratory rate did not change during upright tilt in all subjects.

SV decreased in all subjects during HUT (all  $P < 0.05$ ), and it was smaller in the hypovolemic condition than in the normovolemic condition in both groups ( $P = 0.017$  in men and  $0.002$  in women; Table 3). SV was smaller in women than in men during HUT under the hypovolemic condition ( $P = 0.026$ ; Table 3). SI also decreased during HUT and was smaller in women than in men in the hypovolemic condition ( $P = 0.044$ ; Table 3). CO and CI were lower during HUT in women in the

hypovolemic condition than in the normovolemic condition (both  $P < 0.001$ ; Table 3).

TPR increased during HUT under normovolemic condition ( $P = 0.006$  and  $0.023$  for men and women; Table 3) but did not change under hypovolemic condition in both groups ( $P = 0.061$  and  $0.163$ ; Table 3). TPR was not different between the genders during HUT under both conditions (gender effect:  $P = 0.623$  in normovolemia and  $0.153$  in hypovolemia); in addition, the increase in TPR from supine to tilt did not differ between the genders ( $342 \pm 217$  in men vs.  $243 \pm 129$  dyn·s·cm<sup>-5</sup> in women in normovolemia,  $P = 0.319$ ;  $175 \pm 201$  vs.  $205 \pm 342$  dyn·s·cm<sup>-5</sup> in hypovolemia,  $P = 0.840$ ).

#### MSNA Responses to 60° HUT

MSNA burst frequency increased in all subjects during HUT ( $P < 0.05$ ; Fig. 2A) and was greater in the hypovolemic condition than in the normovolemic condition in both groups ( $P = 0.018$  in men and  $<0.001$  in women); these responses were not different between the genders (gender effect,  $F = 0.0468$  with 1 degree of freedom,  $P = 0.832$  in normovolemia;  $F = 0.0573$ ,  $P = 0.814$  in hypovolemia). MSNA burst incidence also increased in all subjects during tilt ( $P < 0.05$ ), and these responses were similar between the genders ( $35 \pm 7$  in men vs.  $38 \pm 10$  bursts/100 heartbeats in women in normovolemia,  $P = 0.383$ ;  $43 \pm 17$  vs.  $41 \pm 6$  bursts/100 heartbeats in hypovolemia,  $P = 0.862$ ). Normalized total activity increased similarly during HUT in both groups under both conditions (gender effect,  $P = 0.207$  in normovolemia, and

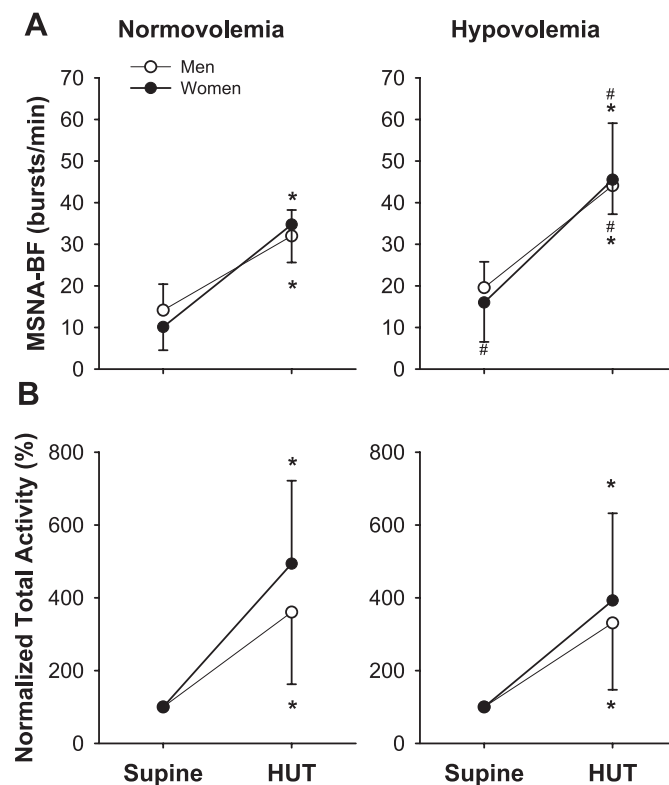


Fig. 2. MSNA burst frequency (MSNA-BF; A) and normalized total activity (B) in response to 60° HUT in men and women under normovolemic and hypovolemic conditions. Values are mean  $\pm$  SD. \* $P < 0.05$  compared with the supine baseline in the same condition. # $P < 0.05$  compared with the normovolemic condition in the same position within the group.

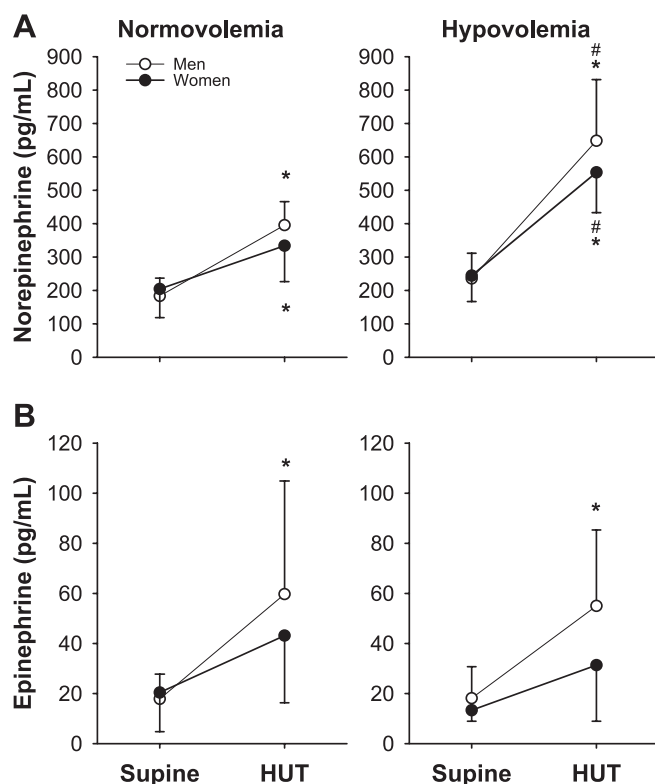


Fig. 3. Plasma norepinephrine (A) and epinephrine (B) concentrations in men and women in the normovolemic and hypovolemic HUT in response to 60° HUT in men and women in the normovolemic and hypovolemic condition. Values are mean  $\pm$  SD. \* $P < 0.05$  compared with the supine baseline in the same condition. # $P < 0.05$  compared with the normovolemic condition in the same position within the group.

$P = 0.454$  in hypovolemia; Fig. 2B). Plasma norepinephrine increased during HUT and was greater in the hypovolemic condition than in the normovolemic condition in both groups (both  $P < 0.001$ ), and these responses were not different between the genders (gender effect:  $F = 0.142$  with 1 degree of freedom,  $P = 0.712$  in normovolemia, and  $F = 1.570$ ,  $P = 0.236$  in hypovolemia; Fig. 3A). However, plasma epinephrine increased during HUT only in men but not in women under both conditions ( $P = 0.006$  in men and  $0.097$  in women under normovolemic condition;  $P = 0.001$  and  $0.329$  under hypovolemic condition; Fig. 3B).

To compare the interplay between the stimulus and response during orthostatic stress in both genders, we plotted MSNA burst frequency as functions of SV and SI in the supine position and during HUT under both normovolemic and hypovolemic conditions, since MSNA has been well demonstrated to be directly and inversely related to the changes in SV or SI during orthostatic challenges (4, 23). The correlation coefficient for the relationship between MSNA and SV or SI was not different between the genders ( $r^2$ ,  $0.930 \pm 0.073$  in men vs.  $0.894 \pm 0.167$  in women;  $t = 0.527$  with 13 degrees of freedom,  $P = 0.607$ ). Moreover, the slope relating MSNA and SV/SI was not different between the groups ( $-0.466 \pm 0.233$  in men vs.  $-0.605 \pm 0.240$  bursts $\cdot$ min $^{-1}\cdot$ ml $^{-1}$  in women,  $P = 0.275$ ;  $-0.943 \pm 0.401$  in men vs.  $-1.211 \pm 0.228$  bursts $\cdot$ min $^{-1}\cdot$ ml $^{-1}\cdot$ m $^{-2}$  in women,  $P = 0.156$ ). The average slopes of MSNA responses to SV and SI are displayed in Fig. 4.

### Maximal Orthostatic Tolerance

Consistent with all previous findings, maximal orthostatic tolerance was lower in women than in men under normovolemic conditions (CSI,  $696 \pm 102$  in women vs.  $968 \pm 238$  mmHg $\cdot$ min in men,  $P = 0.017$ ). Acute hypovolemia resulted in a decrease in orthostatic tolerance in women ( $P = 0.041$ ) but not in men ( $P = 0.263$ ). Maximal orthostatic tolerance was much lower in women than in men under hypovolemic conditions ( $478 \pm 263$  vs.  $910 \pm 209$  mmHg $\cdot$ min,  $P = 0.007$ ).

### DISCUSSION

The new findings from this study are that 1) men and women have similar vasomotor sympathetic (MSNA and plasma norepinephrine concentration) and vasoconstrictor (TPR and DBP) responses during orthostatic stress, not only under normovolemic conditions but also under hypovolemic conditions; and 2) SBP was lower in women than in men, predominantly because of a smaller SV during orthostatic challenges under both conditions. These results suggest that the lower orthostatic tolerance in women, especially under hypovolemic conditions, is not derived from a blunted sympathetic neural responsiveness but from the smaller SV presumably due to less cardiac filling during orthostasis, which may overwhelm the vasomotor reserve available for vasoconstriction or precipitate neurally mediated sympathetic withdrawal and syncope.

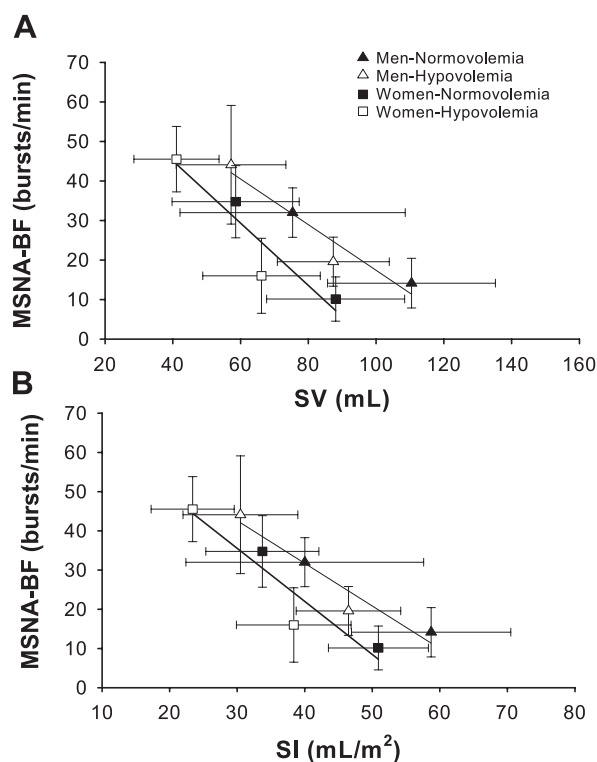


Fig. 4. Relationships between MSNA-BF and stroke volume (SV; A) and stroke index (SI; B) in the supine position and during HUT in men and women under both normovolemic and hypovolemic conditions. Linear regressions are calculated from mean values. For SV vs. MSNA-BF (A), linear equation for women is  $y = -0.786x + 76.514$  ( $r^2 = 0.875$ ) and for men  $y = -0.576x + 75.080$  ( $r^2 = 0.929$ ). For SI vs. MSNA-BF (B), the linear equation for women is  $y = -1.349x + 75.973$  ( $r^2 = 0.881$ ) and for men  $y = -1.087x + 75.200$  ( $r^2 = 0.930$ ).

### *Gender and Sympathetic Neural Control of Orthostasis*

Vasomotor sympathetic neural control plays an important role in maintaining hemodynamic homeostasis during orthostatic challenges in humans through an increase in sympathetic nerve activity and thereby an increase in peripheral vascular resistance (18, 36, 44). When this compensatory mechanism fails or is overwhelmed, arterial pressure will drop and syncope may occur (11, 14, 36, 44).

Results regarding gender differences in sympathetic neural control of orthostasis are few and controversial and are almost all from the measurements of plasma norepinephrine concentrations (1, 10, 12, 26, 45). However, the plasma norepinephrine concentration provides only a crude index of overall sympathetic nerve activity in normal humans under a wide variety of stressful conditions (2, 13, 16). In addition, the circulating norepinephrine level is not only dependent on release from adrenergic nerve endings but also dependent on its removal from the circulation (8). We found in the present study that the correlation coefficient for the relationship between plasma norepinephrine concentration and MSNA burst frequency during changes in posture under both normovolemic and hypovolemic conditions in all subjects was relatively low ( $r^2 = 0.432$ ), confirming the relative imprecision of the plasma norepinephrine concentration as an index of sympathetic activation. Therefore, the results obtained from the plasma measurements need to be verified by direct intraneural measurements of sympathetic activity, namely, the microneurographic technique. This technique permits a close look at the timing of sympathetic activation or inactivation unimpeded by the much slower events at the effector sites of a target organ (40, 43). There is only one study that used microneurography to compare gender differences in MSNA responses during orthostatic challenges under normovolemic conditions (35). The authors found that MSNA burst frequency and burst incidence increased similarly in men and women during HUT; however, average amplitude per burst increased only in men but not in women. On the other hand, the increases in TPR during tilting were not different between the genders. Our results obtained during HUT under normovolemic conditions are consistent with these findings, except that we did not compare the average amplitude per burst between the groups because the amplitudes of bursts of sympathetic activity depend critically on electrode position (38). Rather, we expressed burst area over time as total activity and normalized it to the supine resting value to allow comparisons between the groups during upright tilt, since it has been demonstrated clearly that total activity, but not the average amplitude per burst, is closely and linearly correlated to norepinephrine spillover in healthy humans (16); moreover, quantifying MSNA burst area using frequency domain analysis can be used not only for evaluation of intraindividual variations but also for interindividual comparisons (37). Therefore, our method of quantifying total activity was likely to be related more closely to the actual neural signal and norepinephrine release than to the average burst size alone.

We extended our study by comparing sympathetic neural responses during orthostatic stress in both genders under hypovolemic conditions. Similar to the results obtained in the normovolemic condition, MSNA burst frequency, burst incidence, and normalized total activity all increased similarly in men and women during HUT in the hypovolemic condition.

Moreover, the increases in plasma norepinephrine concentration and TPR were not different between the genders during HUT. These results are consistent with our previous report in an entirely different group of subjects (10), showing similar peripheral vascular resistance and plasma norepinephrine responses during progressive LBNP to presyncope in both genders. Together, we interpret these data to suggest that healthy young men and women as groups have comparable sympathetic neural control and vascular resistance responses during orthostatic stress. This notion was further supported by the observation that the slopes relating MSNA response and SV, as well as SI in the supine position and during HUT, were similar in men and women under both conditions in the present study. Based on these findings, we would reason that, although individual variability in sympathetic neural and vasoconstrictor reserve may be an important determinant of variability in orthostatic tolerance among individuals (11), it is not likely to be the primary mechanism of gender differences.

### *Regulation of Arterial Pressure During Orthostasis in Women*

Despite comparable neural responses, women in this study clearly had lower orthostatic tolerance than men, particularly when hypovolemic. One clear hemodynamic difference between the genders identified in the present study was that SBP was significantly lower in women than in men during HUT under both normovolemic and hypovolemic conditions. The lower SBP in women was predominantly because of a smaller SV, due presumably to a decreased cardiac filling, particularly under hypovolemic conditions when vascular volume was decreased and the capacity to buffer orthostatic reductions in central blood volume was limited. The smaller PV or total blood volume of women may not be the entire explanation because our previous study (10) showed that the difference in PV or total blood volume between the genders disappeared when normalized to body weight. Although recent studies have shown that limb venous compliance is less in women than in men and does not fluctuate across the menstrual cycle (27, 30), differences in PV could be compounded by the fact that capacitance vessel compliance in the pelvic area varies between the genders, leading to differences in blood pooling in the pelvic region and reducing cardiac preload in women more than in men during orthostatic stress (46). These factors, combined with a smaller and less "distensible" heart in women, may be the predominant mechanisms of decreased cardiac filling and SV during orthostatic stress.

Indeed, previous studies have demonstrated that gender-specific factors do affect left ventricular chamber size and function. For example, women have a smaller left ventricular chamber, which may be related to a higher systolic elastance but a lower diastolic compliance (7, 15, 34). It is possible that the smaller and less distensible left ventricle in women may increase their sensitivity to fluid shifts and dehydration. We found previously that women had steeper maximal slopes of the Starling curves compared with men (10), resulting in a greater reduction in cardiac filling during orthostatic stress. Although SBP was lower in women during orthostatic stress in the present study, this low SBP did not account directly for their low maximal orthostatic tolerance, since SBP was stable at a lower level until sudden hemodynamic collapse. Rather,



the cardiac mechanics and Frank-Starling relationship may be important mechanisms underlying the gender differences in orthostatic tolerance, possibly making women more prone to cardiac afferent stimulation than men during orthostatic challenges.

In contrast to SBP, we observed that DBP increased similarly in men and women during HUT, not only in the normovolemic condition but also in the hypovolemic condition. The similar increase in DBP during tilting was consistent with the similar increases in MSNA, plasma norepinephrine, and TPR in both genders. These observations provide strong evidence that women and men have comparable adrenergic and vasoconstrictor responses during orthostatic challenges. Ultimately, orthostatic hypotension and presyncope occurred in all subjects because this vasoconstrictor reserve was overwhelmed by impaired cardiac filling or hypovolemic "shock." The latter may also have contributed to reflex sympathetic withdrawal as the final common pathway to cardiogenic syncope.

#### Study Limitations

There are two limitations in this study. First, the number of subjects was small. We only examined eight women and seven men. This work was highly laborious, and obtaining an adequate sympathetic recording in subjects on two occasions within ~4 wk limited the total number of subjects. Hence, we present the exact *P* values as much as possible in our report, as recommended for physiological studies with relatively small subject numbers (47). However, it should be emphasized that the number of subjects required for hypothesis testing was determined from clear published data from our group (10) and others (35). Thus, despite the small number of subjects, the study was well powered to make most of the hypothesized comparisons, with power >0.80–0.90 for virtually all comparisons between postures (supine and tilt) and hydration levels (normovolemia vs. hypovolemia). Although for some between-group comparisons power was lower than 0.80, the primary reason for this is that the mean responses were very similar; in some cases, such as for the change in MSNA burst frequency, the response for women was actually greater than for men. Therefore, the chance of a type II error with the actual sympathetic response for women being lower than men is exceedingly low.

Second, we did not control the menstrual cycle in female subjects in the present study. Minson et al. (28) found that the hormonal fluctuations that occur during the normal menstrual cycle may alter sympathetic outflow but not the transduction of sympathetic activity into vascular resistance during pharmacological changes in BP and during handgrip exercise. Although the menstrual cycle phase was not required to be the same for all females in our study, each individual female subject was in the same phase of her menstrual cycle for both studies, which minimized the influence of the menstrual cycle on sympathetic neural responses. Furthermore, many of the differences between the groups observed in the present study were similar to gender differences found in a previous investigation when the menstrual cycle was well controlled (3).

In summary, the present study demonstrates that vasomotor sympathetic neural and vascular resistance responses during orthostatic stress are quite comparable in healthy young men and women under both normovolemic and hypovolemic con-

ditions. We found no evidence that women have a blunted sympathetic neural control during orthostatic stress. Therefore, although individual variability in vasomotor sympathetic neural and vasoconstrictor reserve may be an important determinant of variability in orthostatic tolerance, it is not likely to be the mechanism of gender differences. The key difference between men and women in this study was a smaller SV presumably due to a smaller cardiac filling in the upright position, particularly during hypovolemic conditions.

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