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VeCIN and Vu and a rise in VEROUT during HUT are documented. P < 0.01. This is the first study in which a reproducible reduction in heart cannot store blood, venous return and cardiac output must by the venous system (9). An alteration in its capacity has capacity; autonomic system

The venous system plays an important role in hemodynamic control. About two-thirds of the total blood volume is captured by the venous system (9). An alteration in its capacity has profound effects on venous return and cardiac output. As the heart cannot store blood, venous return and cardiac output must be equal at any time. With increasing blood supply to the heart, the (stretched) ventricles increase their force of contraction (Starling’s law), leading to a (transient) larger stroke volume and, therefore, cardiac output (9, 27, 31, 43).

Women with prepregnant low plasma volume are prone to develop vascular complications in pregnancy (2). Those women are unable to augment stroke volume during physical activity (3), and an extra volume load cannot be accommodated in their venous compartment (1), leading to a state of relative venous overfill. These observations suggest reduced venous dimensions and, therefore, a reduced venous storage capacity.

Orthostatic stress testing is a valuable tool to measure the adjustments, and the buffering capacity, of the venous compartment. Positive head-up tilt (HUT) induces an initial decrease in venous return, thereby reducing cardiac preload. The decrease in venous return will normally be counterbalanced by sympathetic stimulation. The venous system is highly sensitive to sympathetic activation (6, 15, 31). Sympathetic vasoconstriction, reducing venous unstressed volume (Vu), but also decreasing venous compliance, will raise venous pressure and thus right atrial pressure, filling, and cardiac output (6, 31, 43). Determination of these presumed venous responses during HUT requires fast methods, but these have not been established.

The aims of this study were to assess 1) responses of the venous system to orthostatic stress and 2) their reproducibility. We repeatedly assessed forearm pressure-volume (P-V) curves (venous occlusion plethysmography) during inflow (VeCIN) and outflow [venous emptying rate (VEROUT)]. VeCIN was defined as the ratio of the slope of the volume-time curve and pressure-time curve, with direct intravenous pressure measurement. VEROUT was determined using the derivative of a quadratic regression model using cuff pressure. We defined Vu as the (stretched) ventricles increase their force of contraction (Starling’s law), leading to a (transient) larger stroke volume and, therefore, cardiac output (6, 31, 43).

Subjects
Eight healthy nonpregnant women participated in the study after being fully informed of the risks associated with the procedures and signing informed consent. All participants were recruited by advertisement. None of them took any medication. This study was approved by the Institutional Review Board (CMO no. 2006/111).

All participants were studied on day 5 ± 2 of the follicular phase to prevent hormonal influence. Participants were requested not to smoke or drink alcohol after 8 PM on the day before the measurements. Participants were tested in four sessions on 2 successive days. Sessions were 1.5 h after a light breakfast and 1.5 h after a light lunch.

Experimental Protocol
Subjects were positioned on a comfortable mattress on a tilt table. The forearm was positioned at the heart level to ensure adequate venous emptying during the deflation period. In addition, the arm was stabilized to minimize muscular activity. Environmental conditions were kept constant. Room temperature was kept at 26°C, and distractions due to light and sound were minimalized (11).

Participants remained supine for 10 min; thereafter, orthostatic stress was imposed by passively changing the body posture from 20° head-down tilt (−20°) to 60° HUT (+60°), each time with increasing steps of 20° at 8-min intervals.

Measurements
At each rotational step, venous adjustments and autonomic function were assessed at steady state. Hemodynamic changes occur immediate

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after rotation to realize a new hemodynamic steady state. Previous experiments in our laboratory have shown that a period of <60 s is needed to reach stability after a postural change. Therefore, in the present study, we decided to exclude the data of the first minute after postural change. Head-down tilt was performed to test the response with maximized venous return (38). As Zaidi et al. (49) demonstrated that little additional effect is expected with increasing tilt angles beyond 60°, we evaluated orthostatic stress through HUT until +60°.

Venous adjustments. Venous adjustments were measured by strain-gauge venous occlusion plethysmography with direct intravenous pressure measurements. An intravenous catheter was inserted in an antecubital vein and connected to a pressure transducer system at atrial height. Changes in forearm volume were measured by a mercury in Silastic strain gauge at 5 cm distal to the antecubital crease. Changes in limb volume were expressed in milliliters per deciliter of limb tissue. A venous collecting cuff was placed 5 cm proximal to the antecubital crease. The pressure cuff was connected to a rapid cuff inflator (Hokanson E20) to ensure rapid and accurate filling and deflation of the cuff. Data signals were recorded with a computer system, at a sampling rate of 100 Hz, and stored for further analysis (MIDAC, Biomedical Engineering Department, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands). Pressure-volume curves were assessed in two different sessions of venous inflow (VeCIN) or venous outflow (VEROUT). Before the tilt test, randomization determined in which order the VeCIN and VEROUT measurements would be applied at each rotational step.

INFLOW. Cuff pressure was gradually increased from 0 to 40 mmHg in 60 s. Changes in forearm volume and intravenous pressure were recorded. VeCIN was defined as the ratio of the slope of volume-time curve and slope of the pressure-time curve as follows:

\[ VeCIN = \frac{\Delta \text{volume}}{\Delta \text{time}} \times \frac{\text{pressure}}{\Delta \text{time}} \]

Only the data of the linear part of this relationship were used, until the increase rate of intravenous pressure and forearm volume diminished.

OUTFLOW. The method used was a modification of the method described by Halliwill et al. (10). Cuff pressure was immediately set at 40 mmHg (instead of 60 mmHg). We chose this modification because using a cuff pressure of 60 mmHg would be inappropriate for the obstetric field. Capillary leakage is increased in pregnancy. This might induce more extravasation in pregnant women, affecting Starling forces by increasing tissue pressure. The effect is intensified when using a higher cuff pressure, as diastolic blood pressure is usually lower in (non)pregnant women. Cuff pressure was kept at this pressure for 4 min. Subsequently, the cuff was deflated to 0 mmHg in 1 min. Pressure-volume curves were generated during deflation of the cuff. The change in forearm volume was recorded, and intravenous pressure was assumed to be equal to cuff pressure. Data obtained at a cuff pressure below 10 mmHg were excluded. Pressure-volume curves were compared by means of the following quadratic regression model:

\[ \Delta \text{volume} = \beta_0 + (\beta_1 \times \text{cuff pressure}) + (\beta_2 \times \text{cuff pressure})^2 \]

where \( \beta_0, \beta_1, \) and \( \beta_2 \) are regression parameters. VEROUT was defined as the derivate of the pressure-volume curve as follows:

\[ \text{VEROUT} = \beta_1 + (2 \times \beta_2 \times \text{cuff pressure}) \]

To be able to compare values within and between sessions, an (arbitrary) cuff pressure of 20 mmHg was used (10). For both methods, we considered pressure-volume regression lines with an explained variation \( r^2 > 0.9 \) to be representative of original data. Only those were included for further analysis. VeCIN was defined as the volume when transmural pressure is equal to 0 mmHg (6, 9). We determined VeCIN during each rotational step for both methods. During VeCIN, the volume-pressure curve was generated using a linear model of both the volume-time curve and pressure-time curve. The \( y \)-intercept of this constructed pressure-volume curve was used to determine \( V_p \). For the VEROUT method, the averaged volume-pressure curve was generated using the quadratic regression model. In this model, regression parameter \( \beta_0 \) was defined to represent \( V_p \) (10).

Autonomic function. Fluctuations in heart rate and arterial blood pressure (ABP) were measured continuously using a finger ABP-monitoring device attached to the third digit of the right hand at a sampling rate of 100 Hz (Finometer, Finapres) to determine sympathetic activity, parasympathetic activity, and baroreflex sensitivity (BRS). We derived relative brachial pressure from the finger arterial pressure by the application of waveform filtering and level correction.

We quantified autonomic activity and BRS by the spectral analysis technique (18). Recordings were subdivided into data segments of 100 s, overlapping for 50%, and resampled at 5.12 Hz. Each segment was then analyzed with a fast Fourier transformation that searched for rhythmic fluctuations in systolic blood pressure (SBP) and pulse interval (PI) with a frequency range between 0 and 2.56 Hz. The amplitude of each fluctuation determined the power at each frequency. Subsequently, SBP and PI powers were expressed as a function of the frequency. Vascular sympathetic activity was defined as the natural logarithm of the power of the low-frequency (LF) component of the variations in SBP, and the ratio of absolute LF and high-frequency powers of the PI was assumed to represent the cardiac autonomic balance between the sympathetic and vagal system. BRS, which provides information about the changes in heart rate (output) in response to fluctuations in SBP (input), was defined as the (LF) transfer gain from SBP to PI (expressed in ms/mmHg).

Statistics

Data are presented as median (interquartile ranges) unless otherwise stated. Differences between venous responses during inflow and outflow, heart rate, vascular sympathetic activity, cardiac autonomic balance, and BRS during HUT were analyzed by the Wilcoxon signed-ranks test for paired values. The pooled coefficient of variation (CV) and coefficient of reproducibility (CR) were calculated per method. CV was determined by the pooled ratio of each individual SD and median value of VeC or VER, respectively. CR at each rotational step was determined using the following formula:

\[ CR = 2 \sqrt{2} \times \frac{\sqrt{\sum (r_i - 1)^2 \text{SD}_i^2}}{\sum (r_i - 1)} \]

in which \( r_i \) is the number of measurements in each participant (generally 4 measurements in the present study) and SDi is each individual SD (4).

Nonparametric Spearman’s rho analysis was used to test correlations between variables.

RESULTS

We included eight women with a median age of 25 (21–26) yr and a median body mass index of 23 (19–24) kg/m². SBP was 114 (110–123) mmHg, and diastolic blood pressure was 60 (58–66) mmHg. None of the measurements during both VeCIN and VEROUT were excluded due to low \( r^2 \) of the pressure-volume regression line.

During the orthostatic stress test, heart rate and vascular sympathetic activity increased and BRS decreased (Fig. 1). In the supine position, VeCIN was 0.066 (0.043–0.089) ml•dl⁻¹•mmHg⁻¹ and VEROUT was 0.052 (0.040–0.059) ml•dl⁻¹•mmHg⁻¹ \( (P < 0.01) \). As shown in Fig. 2, VeCIN decreased by -35%, -45%, and -53% at 20, 40, and 60° HUT, respectively (all \( P < 0.05) \). In contrast, during VEROUT, the effect was reversed. VEROUT was +23%, +30%, and +60% higher compared with the supine position (all \( P < 0.01) \).
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0.05). At −20° head-down tilt, VER\textsubscript{OUT} was 43% lower compared with the supine position, but VeC\textsubscript{IN} remained unchanged.

We calculated the CR and CV of both methods to assess reproducibility (Table 1). In the supine position, the CR of VeC\textsubscript{IN} was higher compared with VER\textsubscript{OUT} (0.068 vs. 0.050 ml\textcdot dl\textsuperscript{-1}\textcdot mmHg\textsuperscript{-1}) but lower during the tilt test at 20, 40, and 60° HUT. The CV of the VeC\textsubscript{IN} method varied between 19% and 25%, and the CV of the VER\textsubscript{OUT} method varied between 12% and 21%.

As sympathetic venoconstriction reduces \( V_v \) to compensate for the decline in venous return, we determined the change in \( V_v \) both during VeC\textsubscript{IN} and VER\textsubscript{OUT}. Figures 3 and 4 show the constructed averaged volume-pressure curves during VeC\textsubscript{IN} (using a linear model) and VER\textsubscript{OUT} (using a quadratic regression model), respectively. Using the pressure-volume curve during VeC\textsubscript{IN}, a decrease in the \( y \)-intercept during HUT was observed. In the supine position, \( V_v \) was 0.01 (−0.3 to 0.6) ml/dl. It decreased to −0.5 (−0.2 to −0.9) ml/dl at +60° HUT (\( P = 0.045 \)).

VER was determined using a quadratic regression model. The three parameters \( \beta_0, \beta_1, \) and \( \beta_2 \) decreased with tilt (all \( P < 0.01 \)). The HUT-induced decreases in \( \beta_0, \beta_1, \) and \( \beta_2 \) resulted in a downward shift of the pressure-volume curve and an upward shift of the VER-pressure curve, which are shown in Fig. 4. Parameter \( \beta_0 \) was assumed as a representative of \( V_v \). It decreased from 1.1 (0.8−1.3) ml/dl at the supine position to −1.9 (−2.8 to −1.3) ml/dl at +60° HUT (\( P < 0.01 \)).

Correlation analysis showed that the change in VeC\textsubscript{IN} assessed during inflow and VER\textsubscript{OUT} related to the change in vascular sympathetic activity measured at the different tilt angles (\( r = −0.36, P < 0.01, \) and \( r = 0.48, P < 0.01; \) Fig. 5).

DISCUSSION

The aim of the present study was to measure venous responses during stepwise-inflicted orthostatic stress. We assessed pressure-volume curves during inflow and outflow of the forearm veins at different tilt angles. Theoretically, HUT induces an initial decrease in venous return, which is compensated by a decrease in venous capacitance through a decrease in \( V_v \) and VeC (6, 31, 43). To our knowledge, this is the first study in which these venous alterations, and their reproducibility, during HUT are documented.

We observed a reproducible decrease in VeC and \( V_v \) during HUT and an increase in VER. Both observations relate, at least partly, to changes in vascular sympathetic activity. Veins are highly sensitive to sympathetic stimulation (6, 31). Karim et al. (15) reported 50% of the maximum response (at 20 Hz) of the capacitance vessels at only 1-Hz sympathetic stimulation, in contrast to 10% of the maximum response of the resistance vessels. Sympathetic stimulation realizes an increase in stressed volume and a decrease in VeC (6). Many studies have observed a downward shift of the pressure-volume curve, which implicates a decrease in \( V_v \), but unaffected the slope of the curve (and thus a similar VeC) during sympathetic stimulation (6, 9, 10, 31). We observed both a decrease in \( V_v \) during inflow and outflow and a decrease in the slope of the pressure-volume curve during venous inflow. In contrast to exclusively applying sympathetic stimulation by \( \alpha \)-adrenergic agonists, cold pressure, isometric handgrip, or mental arithmetic stress, as used in most studies (5, 10, 21, 33, 35, 36), during HUT, both an increase in vascular sympathetic activity and baroreflex activation and a decrease in parasympathetic activity occurs. Studies on the venous response at lower body negative pressure (LBNP) and carotid sinus baroreflex activation have suggested sympathetic stimulation of the veins via...
both cardiopulmonary low-pressure and high-pressure receptors (35, 37, 41, 42, 50). We speculate that the cooperative activation of these two types of receptors during HUT might contribute to the decrease in $V_a$ and venous compliance, to counterbalance the reduction in cardiac preload during HUT. Monahan et al. (21) performed venous outflow measurements at the calf and observed, in contrast to our findings, no change in the response to LBNP in women. Nonetheless, both venous capacitance and $V_u$, as indicated by the $y$-intercept of the pressure-volume curve, decreased, suggesting activation of the venous contractile system. Compared with our study, there are some methodological differences. First, in the Monahan et al. study, measurements were performed at the right calf while LBNP was applied to the left leg and pelvic region. During this procedure, women more than men tend to pool more blood in the pelvic region (47), so this procedure may have affected calf venous outflow. Second, the researchers used a cuff pressure of 60 mmHg for 8 min, which might be supradiastolic in (young) women, promoting fluid extravasation and, with it, tissue pressure. For this reason, in the present study, a cuff pressure of 40 mmHg for 4 min was used. Finally, as the menstrual phase may affect plasma volume, all participants in our study were, in contrast to the Monahan et al. study, evaluated during the midfollicular phase.

Caution needs to be taken when interpreting the relation between spectral results and venous functioning. Autonomic function, as assessed by spectral analysis, is based on fluctuations in SBP and heart rate. Although arterial and venous function is coupled and act in concert with each other (24), our sympathetic data are, predominantly, a measure of arterial and cardiac sympathetic control and cannot be translated to a measure of sympathetic activity on veins. Dysfunction at any site of the baroreflex loop interferes with sympathetic modulation of venous function. Using a control group with impaired autonomic activation, such as spinal cord-injured subjects, would have contributed to the quantification of the role of the sympathetic system in the changes in VeC. Subjects with paraplegia are reported to exhibit blunted changes in VeC during head-down tilt (45), suggesting a possible role for diminished sympathetic modulation of VeC. However, concomitant features in this group of patients, such as assumed venous atrophy, reductions in muscle mass, and the effects of inactivity and compensatory mechanisms to ascertain cardiac hemodynamics, complicates to discern the role of the sympathetic nervous system in venous function (7, 13, 23, 46). Alternatively, venous sympathetic activity can be impaired by $\alpha$-adrenoceptor antagonists and ganglionic blockers (23), which might be used in future research to study the contribution of the sympathetic system to venous function during HUT.

We determined autonomic function with the use of spectral analysis, which is a noninvasive, well-validated technique and frequently used during HUT (18,28–30). Although this method has limitations (25,39), in this study, we used the changes in sympathetic activity to correlate with venous responses. It has been reported that the changes in blood pressure variability results correspond well with the changes in muscle sympathetic nerve activity (22). In addition, it has good reproducibility both in the resting supine position and at higher tilt angles (12,16,26) and, therefore, might be valid to present.

For the inflow method, we used the first part of the pressure-volume curve, in which a linear relationship between intravenous pressure and forearm volume is present, resulting in one value for VeC. The pressure-volume relationship in the vein is dependent on tissue pressure and, therefore, on the used cuff pressure (10,14,17,32). Using the first (linear) part of the pressure-volume relationship can adequately represent VeC, especially when using intrave-

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**Table 1. Reproducibility of VeC during head-up tilt**

<table>
<thead>
<tr>
<th>Head-Up Tilt, °</th>
<th>VeCIN</th>
<th>VEROUT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (interquartile range), ml·dl⁻¹·mmHg⁻¹</td>
<td>CR, ml·dl⁻¹·mmHg⁻¹</td>
</tr>
<tr>
<td>−20</td>
<td>0.06 (0.05-0.09)</td>
<td>0.08</td>
</tr>
<tr>
<td>0</td>
<td>0.07 (0.04-0.09)</td>
<td>0.07</td>
</tr>
<tr>
<td>20</td>
<td>0.04 (0.04-0.08)*</td>
<td>0.06</td>
</tr>
<tr>
<td>40</td>
<td>0.04 (0.03-0.06)*</td>
<td>0.04</td>
</tr>
<tr>
<td>60</td>
<td>0.03 (0.02-0.04)*</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>0.03 (0.03-0.03)*</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>0.05 (0.04-0.06)</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>0.07 (0.06-0.07)*</td>
<td>0.07</td>
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<td></td>
<td>0.07 (0.06-0.09)*</td>
<td>0.08</td>
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<tr>
<td></td>
<td>0.09 (0.07-0.10)*</td>
<td>0.08</td>
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</tbody>
</table>

VeCIN, venous compliance (VeC) measured during inflow; VEROUT, venous emptying rate measured during outflow. *P < 0.05 compared with the supine position.

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**Fig. 3.** Model of the pressure-volume curve during venous inflow (means and SE) at −20° head-down tilt (●) and +60° HUT (○). Unstressed volume (VeC; $y$-intercept) was lower at each rotational step compared with the supine position.
venous pressure measurements, and makes comparisons between the different situations possible (32). VeC is generally measured in the calf. In the present study, we choose to perform measurements at the forearm to prevent influencing factors during HUT. First, activation of the calf muscles is a natural response to gravitational forces. Passive tilting, using a tilt table with a footstep, as used in this study, can minimize muscle activation compared with the response to standing. Although calf muscle activation only minimally affects volume expansion (44), plethysmographic measures would be less reproducible. Second, loss of (intravascular) plasma volume occurs during HUT due to increased fluid filtration through capillary walls, which is also influenced by the duration of the orthostatic stimulus (20, 40, 48). This effect might be more pronounced in the lower part than upper part of the body during HUT. In addition, stress relaxation might play a role. Stress relaxation refers to the characteristic of the vessel wall of progressive delayed stretching in response to an increase in volume, returning pressure back to normal. This phenomenon occurs in minutes to hours (9). Measuring VeC at the forearm, which is placed at heart level in a stable position, will minimize muscle contraction, prevent capillary filtration, and diminish the contribution of stress relaxation. However, active distribution of venous volume, by reducing VeC in the nonsplanchnic part of the venous system, accounts for only 25% of the total blood transfer (34) and the legs taking a larger part than the arms. In the present study, we intended to qualify this response, but one must realize that the splanchnic veins are thought to play the most important role in restoring venous return as these vessels contain the most venous blood (6).

VeC, measured during inflow of the vein, can be assessed in 1 min. This short duration makes this method appropriate for measurements in which brief maneuvers are allocated, as in orthostatic stress testing. Additionally, due to its slowly increasing and finally low cuff pressure (8), it is a reliable method to measure VeC in those with low blood pressure, as in this study.

In conclusion, HUT reproducibly decreases VeC and V_a and increases VER and sympathetic activity. The extent of changes in sympathetic activity relate to the magnitude of venous adjustments made during HUT.
REFERENCES


