Ultrasound assessment of popliteal vein compliance during a short deflation protocol

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Young CN, Prasad RY, Fullenkamp AM, Stillbower ME, Farquhar WB, Edwards DG. Ultrasound assessment of popliteal vein compliance during a short deflation protocol. J Appl Physiol 104: 1374–1380, 2008. First published February 28, 2008; doi:10.1152/japplphysiol.00825.2007.—The purpose of the present study was to determine whether ultrasound is a useful tool to measure the venous characteristics of the lower extremity during a standard venous collecting cuff deflation protocol. To accomplish this, lower extremity pressure-volume relationships were measured in eight (25 ± 1 yr) supine subjects. Popliteal vein CSA was assessed by using high-resolution ultrasound, while calf volume changes were simultaneously assessed by using venous occlusion plethysmography (VOP). Pressure-CSA and pressure-volume relationships were assessed at baseline, during the cold pressor (CP) test, and following sublingual nitroglycerin (NTG) administration. Relationships were modeled with a quadratic regression equation, and β1 and β2 were used as indexes of venous compliance. Popliteal vein regression parameters β1 (8.485 ± 2.616 vs. 7.638 ± 2.664, baseline vs. CP; 8.485 ± 2.616 vs. 7.734 ± 3.076, baseline vs. NTG; both P < 0.05) and β2 (−0.0841 ± 0.0241 vs. −0.0793 ± 0.0242, baseline vs. CP; −0.0841 ± 0.0241 vs. −0.0771 ± 0.0280, baseline vs. NTG; both P > 0.05) were not affected by CP or NTG. Similarly, calf regression parameters β1 and β2, obtained with VOP, were not altered during either trial. Intraclass correlations for venous compliance assessed with ultrasound and VOP were 0.92 and 0.97, respectively, indicating acceptable reproducibility. These data suggest that ultrasound is a functional and reproducible tool to assess the venous characteristics of the lower extremity, in addition to VOP. Additionally, popliteal vein and calf compliance were not affected by the CP test or NTG.

The venous circulation serves as a blood reservoir, containing ~70% of the total blood volume at rest. Small changes in venous volume in the capacitance bed, via changes in venous tone, will cause large volume shifts to or from the central circulation (24, 25). Thus the lower limb venous system is an integral part of blood pressure regulation, as redistribution of venous volume will impact right atrial pressure, cardiac filling, and overall cardiovascular homeostasis (24–26).

The common methodology used for measuring the venous pressure-volume relationship in humans has been venous occlusion plethysmography (VOP). Although VOP is a useful noninvasive tool, it has several limitations. First, it measures whole limb volume changes. Therefore, shifts in volume (i.e., capillary filtration) that occur during the measurement will influence the observed results (2, 9). Although the use of a short occlusion time and obtaining venous measurements during venous collecting cuff deflation have shown to be beneficial in limiting the effects of filtration on the measurement (9), there is still the potential for changes in venous volume to be overestimated (2). Additionally, it is known that vasoconstriction can mimic the effects of active venoconstriction (25, 26). Therefore, attempts to measure changes in whole limb venous tone by using VOP (i.e., during sympathoexcitatory maneuvers) may be confounded by changes in limb arterial vascular resistance.

High-resolution ultrasound has recently been suggested as a tool to measure lower limb venous parameters that overcomes the limitations of VOP (2). Performing ultrasound in a large conduit vein of the extremity will minimize the effect of volume shifts, as the majority of filtration is thought to occur in the capillaries and small postcapillary venules. The use of ultrasound to measure changes in venous tone will also help eliminate the confounding effects of alterations in whole limb vasoconstriction by focusing on a single limb conduit vein (2).

de Groot et al. (2) recently utilized a step-wise venous occlusion inflation protocol to measure popliteal vein parameters with high-resolution ultrasound. Although popliteal vein characteristics were able to be appropriately modeled in this previous study, the use of an inflation protocol is limited to measuring venous parameters under resting conditions and cannot be used during maneuvers that perturb the cardiovascular system (i.e., sympathoexcitation and pharmacological administration). Importantly, Halliwill et al. (9) have previously demonstrated that the use of a short deflation protocol eliminates many of the assumptions involved when using VOP. The methodology of Halliwill et al. allows for the generation of the overall pressure-volume relationship and eliminates the assumption that resting venous pressure is equal to zero, as is assumed when a cuff inflation protocol is used. Venous compliance measurements obtained during collecting cuff deflation are also minimally influenced by capillary filtration if a short (4–8 min) occlusion period is employed (9). In addition, this methodology has been shown to be useful during brief sympathoexcitatory maneuvers (4, 9, 21, 34).

Therefore, the primary aim of the present study was to determine whether ultrasound is a useful tool to measure the characteristics of the venous system in humans during a short deflation protocol. We reasoned that the use of a deflation protocol (9), in combination with high-resolution ultrasound...
would allow for the full characterization of the pressure-cross-sectional area (CSA) relationship of the popliteal vein. A secondary aim of this study was to assess the effects of alterations in smooth muscle tone on popliteal vein compliance. Previous experiments have shown no effect of non-baroreflex, sympathoexcitatory maneuvers (cold pressor test and rhythmic handgrip) (4, 9, 21, 34) and endothelium-independent decreases in smooth muscle tone (sublingual nitroglycerin) (34) on limb venous compliance. However, whether these results of whole limb venous compliance are reflective of the characteristics of a single limb conduit vein is unknown. As such, we generated pressure-CSA relationships during a cold pressor test and following sublingual nitroglycerin administration to determine the effects of sympathoexcitation and endothelium-independent decreases in smooth muscle tone on popliteal vein compliance.

MATERIALS AND METHODS

Subjects

Eight recreationally active subjects volunteered for the studies (Table 1). Exclusion criteria included presence or history of cardiovascular, pulmonary, or metabolic disease, hypertension (blood pressure \( \geq 140/90 \text{ mmHg} \)), neurological disease, cancer, tobacco use, a recent change in body weight, use of cardiovascular medications, abnormal resting electrocardiogram, and obesity (BMI \( \geq 30 \text{ kg/m}^2 \)). All of the women had a negative pregnancy test.

Subjects reported to the laboratory at least 4 h postprandial, having refrained from alcoholic and caffeinated beverages and exercise for at least 12 h. Experimental protocols were approved by the Institutional Review Board of the University of Delaware, and all subjects provided written informed consent before participation and were verbally informed of the nature, risks, and benefits of the study.

Instrumentation

Heart rate and blood pressure. A single-lead ECG was recorded throughout the experiment and used to calculate heart rate (Dinamap Dash 2000; GE Medical Systems, Milwaukee, WI). The BNC output connection on the Dinamap Dash 2000 was used to transmit heart rate data to a National Instruments PCI-6221 eight-channel DAQ board (National Instruments, Austin, Texas). The DAQ board was used to synchronously record all analog data at a sample rate of 1 kHz. Automated oscillometric arterial blood pressure was also determined with the Dinamap Dash 2000.

High-resolution ultrasound. Images of the right popliteal vein were obtained from the popliteal space with the use of a 10-MHz linear-phased array ultrasound transducer (SonoSite TITAN, Bothell, WA). The location and image of the popliteal vein was determined, and the transducer was then clamped into place (Flexbar, Islandia, NY) to prevent movement. Popliteal vein images were transmitted from the SonoSite TITAN to a National Instruments IMAQ PCI-1411 image acquisition board by way of an S-video connection. Images were captured by the IMAQ board and saved at a frequency of 20 frames per second. The 20-Hz image acquisition frame rate was determined by limitations in image buffering and SonoSite output frequency. Despite the necessity to limit ultrasound frame rate to 20 Hz, the frame rate was appropriate for time matching the analog and ultrasound data, such that subsequent ultrasound images correspond with every 50th analog data sample.

VOP. VOP was performed in the same leg as ultrasound measurements during all trials. The right leg was positioned slightly above heart level to promote venous drainage. Mercury-in-Silastic strain gauges (EC6, D.E. Hokanson, Bellevue, WA) were electronically calibrated (11) before instrumentation and then were placed around the calf at the point of maximal girth. A venous collecting cuff (24-cm width) was placed around the thigh proximal to the knee and connected to a rapid cuff inflator (E-20, D.E. Hokanson), which was attached to an external air source (AG101, D.E. Hokanson). The VOP data was transmitted to the DAQ board and sampled at a rate of 1 kHz.

Table 1. Selected subject characteristics

| Men/Women | 4/4 |
| Age, yr | 25 ± 1 |
| Height, cm | 172 ± 3 |
| Weight, kg | 70 ± 3 |
| Resting heart rate, beats/min | 55 ± 3 |
| Blood pressure, mmHg | |
| Systolic | 122 ± 3 |
| Diastolic | 65 ± 4 |
| Mean | 84 ± 3 |

Values are means ± SE.

Protocols

Baseline venous compliance. Subjects were instrumented for ultrasound and VOP as described above and were studied supine with the leg elevated slightly above heart level. Resting longitudinal ultrasound images of the popliteal vein were obtained. The methodology described by Halliwill et al. (9) was then used to generate pressure-diameter and pressure-volume relationships. The venous collecting cuff on the thigh was inflated to 60 mmHg for 8 min. Ultrasound images and VOP volumes were recorded for 10 s at the beginning of each minute during the 8-min occlusion period. After this 8-min period, collecting cuff pressure was manually reduced at a rate of 1 mmHg/s from 60 to 0 mmHg. Longitudinal ultrasound images and volume changes from VOP were measured continuously during the deflation period. Arterial blood pressure was obtained at rest, at 3.5 min, and at 7.5 min into the occlusion period.

Effect of cold pressor test and sublingual nitroglycerin on venous compliance. The purpose of these perturbations was to determine the effect of nonbaroreflex-mediated sympathetic activation and endothelium-independent decreases in smooth muscle tone on venous compliance. The two trials were randomized, and 15 min of quiet rest separated each trial.

Baseline venous compliance assessment was performed as described above. During a second trial, the venous collecting cuff was inflated to 60 mmHg for 8 min. During the last minute of the inflation period, the subject immersed his or her hand in ice water. The pressure in the venous collecting cuff was then reduced at 1 mmHg/s to 0 mmHg after the 8-min occlusion. The subject’s hand remained in the ice water during this deflation period. In a third trial, the venous collecting cuff was inflated to 60 mmHg for 8 min and then released at a rate of 1 mmHg/s to 0 mmHg. Midway through the inflation period, 0.4 mg sublingual nitroglycerin (Nitrostat; Pfizer, New York, NY) was administered, and deflation of the venous collecting cuff occurred 4 min after the administration when peak concentrations of nitric oxide occur (22). The sublingual nitroglycerin trial was not performed in one female subject due to a low resting blood pressure (\( \sim 100/60 \text{ mmHg} \)). Longitudinal ultrasound images and VOP calf volume changes were obtained during each minute of the occlusion period in both trials, and ultrasound images and VOP volume changes were determined continuously during the 1-min deflation period. A second baseline trial was performed following the two perturbation trials.

Data Analysis

Custom designed automated edge detection software was developed using National Instruments LabVIEW 8.0. The software was used to determine popliteal vein diameter from leading edge to leading edge. Specifically, the software was designed to allow an
experimenter to identify a venous region of interest (ROI) in an ultrasound image. The software then prompted the experimenter to define an intensity threshold, which was used to identify the venous walls within the selected ROI. Finally, the edge detection settings were saved so that they could be automatically implemented for all of the ultrasound images from a given subject. The edge detection process described above ensures that ROIs and edge thresholds are standardized for each subject.

The 10-s segments (~200 frames) obtained at the beginning of each minute were averaged to generate a single diameter measurement for each minute. The same software was similarly used to determine calf volume changes obtained from VOP during the occlusion period. The popliteal vein diameter and calf volume measurements obtained during venous collecting cuff deflation were binned over 1-mmHg increments. Popliteal vein CSA was calculated from the diameter measurements with the equation $\pi r^2$. Previous studies (2) have demonstrated that the shape of the vein resembles a circle across a range of pressures and that CSA can be accurately calculated from diameter measurements (13). In addition, popliteal vein CSA was expressed as a percentage change from resting measurements, similar to VOP measurements. Pressure-CSA and pressure-volume curves were then generated from the data points between 60 and 10 mmHg during the deflation period. Directly measured venous pressure does not track external collecting cuff pressure well below 10 mmHg (9). Therefore, data points below 10 mmHg were not included in the analysis. CSA measurements obtained from ultrasound images and calf volume changes obtained from VOP were plotted against cuff pressure. A quadratic regression was applied to model the data (SigmaPlot, Chicago, IL). The regression parameters $\beta_1$ and $\beta_2$ were used as an estimate of venous compliance (9). The following equations were used: $f \Delta$popliteal vein CSA or $\Delta$calf volume $= \beta_0 + \beta_1$ (cuff pressure) + $\beta_2$ (cuff pressure)$^2$ and 2) popliteal vein or calf compliance $= \beta_1 + 2 \beta_2$ (cuff pressure). The regression parameter $\beta_0$ was taken as an estimate of the capacitance response for each trial. In addition, as $\beta_0$ is a complex parameter, influenced by numerous factors (9), we further investigated venous capacitance (i.e., CSA or calf volume) at 10-mmHg intervals during cuff deflation.

Statistics

A repeated-measures ANOVA (SPSS 15.0, SPSS Chicago, IL) was used to compare popliteal vein CSA and calf volume during the inflation period to determine when maximal venous filling occurred. Importantly, this statistical calculation was performed to also determine that a plateau in popliteal vein and calf volume was reached before collecting cuff deflation in all subjects. One baseline pressure-CSA and two baseline pressure-volume relationships could not be modeled due to subject movement during collecting cuff deflation. Therefore, the cold pressor and sublingual nitroglycerin trials are modeled due to subject movement during collecting cuff deflation.

The use of high-resolution ultrasound allows for the assessment of single vein compliance in contrast to whole calf compliance as commonly assessed with VOP. Furthermore, using ultrasound during venous collecting cuff deflation allows for the measurement of limb conduit vein compliance during sympathoexcitatory maneuvers or pharmacological administration. By utilizing ultrasound under these conditions, the main findings of this study are as follows: 1) popliteal vein compliance can be reproducibly measured using ultrasound during a short collecting cuff deflation protocol, and 2) popliteal vein and calf compliance were not altered by sympathoexcitation or
during endothelium-independent decreases in smooth muscle tone.

Use of collecting cuff deflation. To date, only one study (2) has utilized ultrasound to effectively examine popliteal vein compliance. Importantly this study performed an inflation-type protocol and, as Halliwill et al. (9) have previously discussed, there are several assumptions associated with this type of methodology: 1) the technique assumes resting venous pressure is equal to zero; 2) inflation protocols deal inadequately with the hysteresis in limb volume during pressure changes; and 3) most inflation methodologies require a significant amount of time, preventing their use in studying venous compliance during perturbations (i.e., sympathoexcitation). Considering the above assumptions, we chose to use a short deflation protocol for the present investigation. In the present study, we have demonstrated that popliteal vein compliance can be effectively measured via the use of ultrasound under resting conditions in young, healthy subjects. Additionally, the short-term reproducibility (intraclass correlation = 0.92) of this methodology is within acceptable limits.

Moreover, we were able to utilize ultrasound to measure the properties of the popliteal vein during sympathetic activation and sublingual nitroglycerin administration. Previous studies (4, 9, 34) utilizing VOP have demonstrated that, during rhythmic ischemic handgrip, whereas whole limb venous capacitance is decreased (i.e., decrease in $b_0$), limb venous compliance is not altered. Similarly, the cold pressor test has been shown to decrease calf venous capacitance (34) while not altering calf venous compliance (21, 34). Therefore, whole limb venous compliance does not appear to be affected by nonbaroreflex-mediated sympathetic activation, whereas limb venous capacitance may be decreased. However, as active vasoconstrict-

Table 2. Regression parameters $b_0$, $b_1$, and $b_2$

<table>
<thead>
<tr>
<th>Trial</th>
<th>$b_0$</th>
<th>$b_1$</th>
<th>$b_2$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ultrasound</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>$-19.29 \pm 12.64$</td>
<td>$8.485 \pm 2.616$</td>
<td>$-0.0841 \pm 0.02419$</td>
<td>0.99</td>
</tr>
<tr>
<td>Cold pressor</td>
<td>$-11.73 \pm 12.81$</td>
<td>$7.638 \pm 2.664$</td>
<td>$-0.0793 \pm 0.02424$</td>
<td>0.98</td>
</tr>
<tr>
<td>Sublingual nitroglycerin</td>
<td>$-18.29 \pm 14.58$</td>
<td>$7.734 \pm 3.076$</td>
<td>$-0.0771 \pm 0.02801$</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>Venous occlusion plethysmography</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>$0.649 \pm 0.024^*$</td>
<td>$0.131 \pm 0.002^*$</td>
<td>$-0.0011 \pm 0.00002^*$</td>
<td>0.99</td>
</tr>
<tr>
<td>Cold pressor</td>
<td>$0.736 \pm 0.025^*$</td>
<td>$0.124 \pm 0.002^*$</td>
<td>$-0.0010 \pm 0.00003^*$</td>
<td>0.99</td>
</tr>
<tr>
<td>Sublingual nitroglycerin</td>
<td>$0.884 \pm 0.021^*$</td>
<td>$0.127 \pm 0.001^*$</td>
<td>$-0.0010 \pm 0.00002^*$</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Values are means ± SE. Change in ($\Delta$) popliteal vein cross-sectional area or $\Delta$calf volume = $b_0 + b_1 \times$ cuff pressure + $b_2 \times (cuff \ pressure)^2$. $r^2$ represents the fit of the quadratic regression for the respective trial. *$P < 0.05$ vs. respective ultrasound trial.
tion can mimic the effects of active venoconstriction (25, 26), it is possible that these previous demonstrations of a decrease in whole limb venous capacitance do not reflect only venoconstriction. In the present study, by focusing on a single vein, we did not see a change in popliteal vein compliance or capacitance during the cold pressor test. This finding would suggest that a large conduit vein of the lower limb does not actively venoconstrict under nonbaroreflex sympathoexcitatory conditions.

Of note, we also did not see a decrease in calf venous capacitance, assessed as \( C_0 \) or calf volume at 10-mmHg increments, during the cold pressor test. Previous work by Monahan and Ray (21) also did not find a decrease in venous capacitance in male subjects during a cold pressor stimulus by subjectively evaluating the shift from a rapid filling response to a slower increase in limb volume during collecting cuff inflation. However, Young et al. (34) demonstrated a decrease in venous capacitance (assessed as \( C_0 \)) in both young and older individuals (34). However, similar to conditions of sympathetic activation, whether these results are characteristic of a single vein is unknown. If sublingual nitroglycerin were to affect popliteal vein compliance and capacitance, the slope of the pressure-diameter relationship would have been expected to increase, and the relationship would have been shifted to a higher volume, respectively, due to the venodilatory effect of nitroglycerin. By focusing on a single limb conduit vein, we did not see any change in popliteal vein compliance or capacitance, suggesting that endothelium-independent decreases in smooth muscle tone do not alter single (or whole limb) vein parameters in young, healthy subjects. The dosage of nitroglycerin (0.4 mg) used in the present study was chosen because it is a common clinical dose. Previous studies (7, 8, 27, 30) utilizing different methodologies of VOP have shown an increase in limb venous capacitance (compliance was not assessed) with higher doses of sublingual nitroglycerin (0.8 mg). It may be that much higher doses of nitroglycerin are necessary to elicit a response, and therefore the present findings in popliteal and calf venous parameters can only be applied to a clinical dose of 0.4 mg.

To date only one other study has used high-resolution ultrasound to examine the compliance of the popliteal vein. In this previous study, de Groot et al. (2) found that popliteal vein and calf venous compliance were significantly related, with the popliteal vein contributing \(~39\%\) to whole limb compliance. In the present study, we did not find a relationship between

![Fig. 2. Pressure-CSA, pressure-volume, and pressure-compliance curves assessed by using ultrasound (A and B) and VOP (C and D) at baseline and during sublingual nitroglycerin administration. Popliteal and calf venous compliance were not affected by sublingual nitroglycerin (\( P > 0.5 \); \( n = 7 \).)]
popliteal and calf venous compliance. It is possible the discrepancies between the present study and the findings of de Groot et al. (2) are due the different methodologies employed (deflation vs. inflation type protocols). In addition, the study of de Groot et al. examined venous compliance in healthy subjects and those with spinal cord injury, allowing for the relationship to be explored across a wide range of values. Therefore, the lack of a correlation in the present data set between popliteal vein and whole limb venous compliance may also be due to the small range of values examined.

Perspectives. The findings from the present study suggest that nonbaroreflex-mediated sympathoexcitation and sublingual nitroglycerin do not alter popliteal vein or whole limb venous compliance. Importantly, the subject population in the present study was comprised of young, healthy adults. Therefore, care should be taken when extrapolating these results to other populations. Interestingly, alterations in limb venous compliance have been documented in numerous conditions in humans including healthy aging (6, 10, 20, 23, 31, 34), endurance training (10, 20), orthostatic intolerance (3, 4, 29), diabetes (1, 27), heart failure (14, 15), multiple system atrophy (17), spinal cord injury (5, 12, 32, 33), and between sexes (10, 16, 18, 19, 21). The findings obtained by using VOP to assess venous parameters in such physiological states are limited in that they provide a global index of overall limb venous function, and whether these changes are reflective of alterations at the level of a single vein is unknown. Thus we would suggest that high-resolution ultrasound may be a useful tool, in conjunction with VOP, to fully characterize venous function in other physiological and pathophysiological conditions. In addition, the use of a deflation protocol allows for perturbations to be performed in order to investigate the underlying mechanisms contributing to alterations in venous compliance.

Limitations. Although the use of ultrasound, in addition to traditional whole limb measures, appears to be a useful methodology to investigate venous function during a deflation protocol, several potential limitations must be considered when the results are interpreted. First, differences in resting whole limb venous compliance have been documented between sexes (18, 19, 21). Importantly, the primary aim of the present paper was to address whether ultrasound is a useful methodology to assess venous function during a deflation protocol, and, as such, grouping men and women together would not have affected our primary finding. However, previous studies (21) have also demonstrated a difference in the calf venous compliance response to baroreflex-mediated sympathoexcitation (lower body negative pressure) between men and women; therefore it is possible that sex differences may have influenced the results of the secondary aim. Additionally, whether sex-related differences in whole limb venous compliance can be extrapolated to a single vein warrants further investigation, as recent studies (16) suggest that other factors, such as capillary filtration, may explain the differences in whole limb venous compliance between sexes. Second, collecting cuff pressure was used as a surrogate of intravascular pressure. Although previous studies have demonstrated that this is a reasonable assumption for the upper limbs (9), whether this finding can be extrapolated to the lower limb remains unknown. Last, our findings in response to sympathoexcitation can only be applied to a nociceptive stimulus as other methodologies, such as exercise pressor reflex and baroreflex-mediated sympathoexcitation, may yield different results.

In conclusion, the present study demonstrates the use of ultrasound, in conjunction with VOP, to measure venous compliance during a brief cuff deflation protocol. In addition, sympathoexcitation or sublingual nitroglycerin administration in young, healthy subjects does not alter popliteal or calf venous compliance.

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