Venous smooth muscle tone and responsiveness in older adults

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Submitted 19 April 2006; accepted in final form 23 June 2006

Young, Colin N., Michael E. Stillabower, Angela DiSabatino, and William B. Farquhar. Venous smooth muscle tone and responsiveness in older adults. J Appl Physiol 101: 1362–1367, 2006. First published July 13, 2006; doi:10.1152/japplphysiol.00448.2006.—Venous compliance is lower in older adults compared with younger adults. It is possible that alterations in venous smooth muscle tone and responsiveness may contribute to the age-related differences in venous compliance. To determine the effects of sympathetic activation [cold pressor test (cold pressor test); rhythmic ischemic handgrip (rhythmic ischemic handgrip)] and endothelium-independent decreases in smooth muscle tone [sublingual nitroglycerin (nitroglycerin)] on venous compliance in young and older adults, forearm and calf venous compliance was measured in 12 young (22 ± 1 yr) and 12 old (65 ± 1 yr) supine subjects using venous occlusion plethysmography. Venous compliance was assessed at baseline, during the cold pressor test and rhythmic ischemic handgrip tests, and after nitroglycerin administration. All pressure-volume relationships were modeled with a quadratic regression equation, and β1 and β2 were used as indexes of venous compliance. A repeated-measures ANOVA was used to determine the effect of the age and trial on venous compliance. Calf regression parameters β1 (0.0639 ± 0.0126 vs. 0.0503 ± 0.0059, young vs. older; P < 0.05) and β2 (−0.00054 ± 0.00011 vs. −0.00041 ± 0.00005, young vs. older; P < 0.05) were significantly less in older adults at baseline. Similarly, forearm regression parameters, β1 and β2, were lower in older adults at baseline. Venous compliance was not affected by the cold pressor test test, rhythmic ischemic handgrip, or sublingual nitroglycerin in either group. Data suggest that forearm and calf venous compliance is lower in older adults compared with young. However, this difference probably cannot be explained by alterations in smooth muscle tone or responsiveness.

The VENOUS SYSTEM OF THE HUMAN body can be viewed as a voluminous reservoir, containing ~70% of the total blood volume at rest (16). Appropriate regulation of venous blood volume is critical during various physiological stressors (i.e., hemorrhage, orthostatic challenges) to maintain cardiovascular homeostasis and blood pressure (16). The venous compliance of the extremities is a major determinant of the amount of blood that may be translocated to the central region, because small changes in peripheral blood volume can greatly impact cardiac filling pressure and subsequently cardiac output (16).

All of the present human literature supports an age-related alteration in calf venous compliance (3, 7, 11, 14, 21). Recently, Monahan et al. (11) demonstrated that calf venous compliance was ~40% lower in older adults (27 vs. 65 yr). Similarly, Fu et al. (3), Hernandez and Franke (7), Olsen and Lanne (14), and Tsutsui et al. (21) all found lower calf compliance in older subjects.

The mechanism(s) causing age-related declines in compliance is not known. Structural alterations, such as venous wall thickening and increases in collagen-to-elastin ratio, similar to what is seen in the arteries, may reduce venous compliance (11). Also, a functional mechanism involving chronic increases in smooth muscle tone may cause decreases in compliance. However, acutely altering smooth muscle tone in relation to limb venous compliance has not been investigated in older populations.

Therefore, the purpose of this study was to address the contribution of smooth muscle tone and responsiveness to age-related differences in venous compliance. Consistent with previous studies (11, 14), we hypothesized that older adults would have lower calf venous compliance than young adults. We sought to extend these findings to the forearm and therefore hypothesized that older adults would have lower forearm venous compliance. We also hypothesized that a functional mechanism, as opposed to a structural mechanism, was responsible for the previously reported age differences. Therefore, we assessed venous compliance after administration of sublingual nitroglycerin to produce endothelium-independent decreases in venous smooth muscle tone. Our hypothesis was that older adults would demonstrate a greater increase in limb venous compliance during this perturbation. If this hypothesis were proven to be true, it would suggest that the previously reported age differences are of a functional (i.e., increased basal smooth muscle tone) rather than structural (i.e., increased collagen-to-elastin ratio) nature.

A secondary aim of this study was to assess venous compliance during nonbaroreflex, sympathoexcitatory maneuvers (rhythmic ischemic handgrip and cold pressor test). Although sympathetic activation via rhythmic ischemic handgrip exercise and cold pressor test (which presumably increases smooth muscle tone) has been shown to have no effect on venous compliance in young populations, (2, 6, 12), we also wanted to determine the effects of sympathoexcitatory maneuvers in an older population.

MATERIALS AND METHODS

Subjects

Twelve healthy young subjects (7 men and 5 women) between the ages of 18 and 30 yr and 12 healthy older subjects (7 men and 5 women) between the ages of 60 and 70 yr completed the protocol. Exclusion criteria included presence or history of cardiovascular, pulmonary or metabolic disease, hypertension (blood pressure ≥140/90 mmHg), neurological disease, cancer, tobacco use, a recent change in body weight, use of cardioactive medications, abnormal resting or exercise electrocardiogram, and obesity (body mass index.

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to the dominant arm and leg, and both limbs were positioned slightly above heart level to promote venous drainage.

**Protocol**

After instrumentation, subjects rested in the supine position for 30 min. After 30 min of rest, 2 min of resting heart rate, arterial pressure, and respiration data were obtained.

**Baseline venous compliance 1.** Venous compliance was determined using the method of Halliwill et al. (6). Venous collecting cuff pressure was applied at 60 mmHg and held constant for 8 min. After this 8-min period, collecting cuff pressure was reduced at a rate of 1 mmHg/s from 60 to 0 mmHg.

**Sympathoexcitation and changes in smooth muscle tone.** To determine the effects of changes in smooth muscle tone, venous compliance was assessed during three trials (rhythmic ischemic handgrip, cold pressor stimulus, and sublingual nitroglycerin administration). All trials were separated by 15 min of preceding rest and were randomized. In a first trial, venous collecting cuff pressure was applied at 60 mmHg for 8 min. During the inflation period, the subject performed rhythmic ischemic handgrip (dominant arm) for a period of 3 min (60% of their individual maximal voluntary contraction at a rate of 40 contractions/min). Ischemia was then induced by inflating a blood pressure cuff around the upper arm to ~200 mmHg for a period of 2 min. The collecting cuff pressure was reduced at a rate of 1 mmHg/s to 0 mmHg during the second minute of ischemia. The subject’s maximal voluntary contraction was determined during the 15-min rest period before the trial. During a second trial, collecting cuff pressure was applied at 60 mmHg for 8 min and then released at a rate of 1 mmHg/s to 0 mmHg. During the last minute of the inflation period and during the 1-min deflation period, the subject immersed his or her uninstrumented foot in a bucket of ice water (cold pressor test). Both rhythmic ischemic handgrip and cold pressor testing cause large increases in sympathetic outflow in humans (18, 19). Additionally, pilot data demonstrated that this specific handgrip protocol increased muscle sympathetic nerve activity (via direct recording from the peroneal nerve) ~110% in one subject.

In a third trial, collecting cuff pressure was applied at 60 mmHg for 8 min. Midway through the inflation period, 0.3 mg of sublingual nitroglycerin (NitroQuick, Ethex, St. Louis, MO) was administered. The collecting cuff pressure was reduced at a rate of 1 mmHg/s to 0 mmHg 4 min after the administration of the nitroglycerin tablet when peak concentrations of nitric oxide occur (13).

**Baseline venous compliance 2.** After baseline 1, rhythmic ischemic handgrip, cold pressor testing, and sublingual nitroglycerin administration, a second baseline venous compliance assessment was performed. This baseline condition was the same as the first baseline measurement and was preceded by 15 min of quiet rest.

**Data Analysis**

All data were recorded to a computer (WinDaq, Dataq Instruments) for later analysis. Pressure-volume (P-V) curves were generated from the P-V data points between pressures of 60 and 10 mmHg. Because the relationship between directly measured venous pressure and cuff pressure is less reliable below 10 mmHg, these data were not included in the P-V and compliance curves (6). A quadratic regression was applied to model the data (SigmaPlot software) and the regression parameters $\beta_1$ and $\beta_2$ were then used as an estimate of compliance. The following equations were used (6):

$$\Delta \text{LimV} = \beta_0 + \beta_1 \cdot (\text{cuff pressure}) + \beta_2 \cdot (\text{cuff pressure})^2$$

$$\text{Compliance} = \beta_1 + \beta_2 \cdot (\text{cuff pressure})$$

These data were then graphed to create pressure-dependent compliance lines.
The P-V curves were analyzed using a repeated-measures ANOVA. The dependent variables in the ANOVA model comparing the P-V relationship in men and women were the β parameters, and the independent variables were the effect of the group, the effect of the trial, and the limb measurement (calf and forearm). If main effects were detected, simple main effects were examined and post hoc analysis with a Tukey correction was performed.

Subject characteristics were compared with an unpaired t-test. Venous collecting cuff pressure and intravenous pressure were compared using linear regression during the deflation period of baseline 1 in each subject for pressures from 60 to 10 mmHg. The slopes of the linear regression were compared against the line of identity using a univariate t-test. Univariate correlations were performed between certain variables of interest and a calculated basal compliance value at 30 mmHg (β_1 + 2β_2 at 30 mmHg). The significance level for statistical analysis was set at P < 0.05. All data are presented as means ± SE.

RESULTS

Subject Characteristics

The physical characteristics of all participants are summarized in Table 1. There were no group differences in height, weight, body mass index, resting heart rate, and resting systolic and diastolic blood pressure. There was no difference in estimated VO_2peak (P = 0.13). There was a trend for body fat percent to be lower in the younger adults (P = 0.057). As desired, the groups were significantly different with respect to age (P < 0.05).

Intravenous Pressure

The slope of the relationship between collecting cuff pressure and intravenous pressure was 0.98 ± 0.09 (P > 0.05 vs. the line of identity) in the young and 1.03 ± 0.07 (P > 0.05 vs. the line of identity) in the older subjects during cuff deflation. The correlation coefficient (r) was 0.998 ± 0.000 (P < 0.05) and 0.999 ± 0.000 (P < 0.05) in the young and older subjects, respectively. There were no differences in the slopes and r values between groups (P > 0.05). Therefore, it was determined that collecting cuff pressure could be used as a surrogate of intravenous pressure for further analysis.

Baseline Venous Compliance

Table 2 presents the baseline calf quadratic regression parameters in the young and older groups. The regression parameter β_0 was not different between the two groups (P > 0.05). Calf venous compliance was significantly lower in the older group, reflected by the differences in β_1 and β_2 (both P < 0.01). Similar to the calf, the baseline forearm β_1 and β_2 parameters (Table 3) were significantly different between groups (both P < 0.01), indicating a lower degree of venous compliance in the older subjects. Forearm β_0 was also significantly lower (P < 0.05) in the older group. This age difference is displayed in the P-V and pressure-compliance tracings in Fig. 1. P-V curves in the calf and forearm were steeper in the younger subjects, indicating a higher degree of venous compliance than for the older subjects. We also found that within each group the forearm venous compliance was significantly higher than the calf venous compliance (Table 3). There were no differences between baseline 1 and baseline 2 measurements in either limb in both groups (Tables 2 and 3).

Sympathoexcitation and Changes in Smooth Muscle Tone

Heart rate was not significantly different during any trial in either group (P > 0.05). Systolic blood pressure was significantly higher during the cold pressor and rhythmic ischemic handgrip handgrip trials compared with baseline 1 in the young (cold pressor test: 165 ± 9 mmHg, rhythmic ischemic handgrip trials compared with baseline 1 in the young (cold pressor test: 165 ± 9 mmHg).

Table 1. Selected subject characteristics

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<th>Variable</th>
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<td>Body mass index, kg/m²</td>
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<td>Estimated VO_2peak, ml·kg⁻¹·min⁻¹</td>
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<td>Resting heart rate, beats/min</td>
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Values are means ± SE; n = 12 subjects for both groups. Body fat %, n = 10 in older group. VO_2peak, peak oxygen consumption. *P < 0.01 vs. young group.

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<td>Baseline 2</td>
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| Olderen                       |       |       |
| Baseline 1                    |       |       |
| Cold pressor                  |       |       |
| Sublingual nitroglycerin      |       |       |
| Baseline 2                    |       |       |

Values are means ± SE. ΔLimb volume = β₀ + β₁ × cuff pressure + β₂ × (cuff pressure)². *P < 0.05 vs. same trial in young group. †P < 0.01 vs. same trial in calf within group.
grip: 161 ± 8 vs. 124 ± 5 mmHg; P < 0.01) and older groups (cold pressor test: 156 ± 8 mmHg, rhythmic ischemic handgrip: 153 ± 6 vs. 135 ± 5 mmHg; P < 0.01). The blood pressure response during the sublingual nitroglycerin administration was not different from baseline in either group (young: 132 ± 6 vs. 124 ± 5 mmHg; P > 0.05, older: 142 ± 3 vs. 135 ± 5 mmHg; P > 0.05). Similarly, diastolic blood pressure was higher during the cold pressor test and rhythmic ischemic handgrip trials compared with baseline 1 during nitroglycerin administration (young: 71 ± 3 vs. 64 ± 4 mmHg, P > 0.05; older: 72 ± 2 vs 67 ± 3 mmHg, P > 0.05). There were no differences in the heart rate and blood pressure response between groups during all trials (P > 0.05).

Cold pressor testing, rhythmic ischemic handgrip, and sublingual nitroglycerin did not alter the slope of the calf P-V relationship in either group (Table 2, Fig. 2). Calf pressure-volume curves were shifted slightly downward (lower calf volume) during the cold pressor test and rhythmic ischemic handgrip ($\beta_0$, $P = 0.09$; Table 2). There were no differences in the response between groups. Similarly, there were no differences in forearm venous compliance, compared with baseline, during the perturbations in either group ($P > 0.05$; Table 3). Forearm $\beta_0$ was not different between trials in either group ($P = 0.18$).

Correlations Between Variables of Interest and Venous Compliance at 30 mmHg

There were no significant correlations between height, weight, body mass index, resting heart rate, resting systolic and diastolic blood pressure, and venous compliance at 30 mmHg ($P > 0.05$ for all). Additionally, there were no relationships between body fat percent, lean muscle mass, and lean muscle mass in the dominant leg and venous compliance at 30 mmHg ($P > 0.05$ for all). There was no significant correlation between venous compliance at 30 mmHg and $\text{VO}_2$ peak ($r = 0.110$, $P = 0.627$). No significant correlations were present when examining the groups independently.

DISCUSSION

The primary findings from the present study are 1) calf and forearm venous compliance is lower in older adults at rest, 2) forearm venous compliance is higher in young and older adults compared with calf venous compliance, 3) endothelium-independent decreases in smooth muscle tone and nonbaroreflex, sympatoexcitatory maneuvers do not alter limb venous compliance in either group. Overall, these findings suggest that the age-related decline in limb venous compliance cannot be attributed to a functional mechanism.

The age-related differences in calf venous compliance in the present study are consistent with other reported findings (3, 7, 11, 14, 21). We have also extended the previous findings and found lower venous compliance in the forearm of older subjects. To the best of our knowledge, this is the first demonstration of age-related differences in venous compliance in the upper extremity, using the methodology of Halliwill et al. (6). The present results also demonstrate a lower degree of venous compliance in the calf compared with the forearm in both young and older subjects. Previous findings addressing this issue have reported conflicting results, with some reporting no differences in limb venous compliance, (2, 15) a higher degree of venous compliance in the calf, (6, 9), or a higher degree of compliance in the upper venous system (21). The reason for these contradictory findings is not known. However, when
viewing the present results from the perspective of orthostatic tolerance, lower venous compliance in the calf would appear to be beneficial. That is, with lower venous compliance, there is less potential for excessive venous pooling.

Earlier investigations have reported no effect of non-baroreflex-mediated sympathoexcitation on limb venous compliance in young populations (2, 6, 12). We have also found no change in venous compliance during rhythmic ischemic handgrip and cold pressor testing in young, as well as older, adults. Consistent with previous findings (2, 6, 12), $\beta_0$ was shifted slightly downward in the calf, suggesting an alteration in the capacitance response of the limb. However, the slope of the pressure-volume relationship remained unchanged. Interestingly, $\beta_0$ remained unchanged during sympathetic activation in the forearm. This would suggest that the unstrained volume in the lower extremity is more easily shifted during sympathetic stimuli than the upper extremity.

As others have previously pointed out, $\beta_0$ can be a complicated variable to interpret, because it may be affected by time-dependent volume shifts that may have occurred during the trial measurements (6). In the present study, it is probably not the case that $\beta_0$ was affected by time-dependent volume shifts during the trial because the baseline measurements were not different. Additionally, any effect of volume shift and hysteresis would have been limited by the short occlusion time employed (6). However, sympathoexcitation will also cause increases in vasoconstriction, which could mimic the effects of active vasoconstriction (16), causing a downward shift in $\beta_0$. It is thought that the effects of vasoconstriction impact the small postcapillary venules more than the large conduit veins (16). Although measurements of venous compliance using venous occlusion plethysmography are mainly obtained from the large vessels of the limb (therefore minimal effect of vasoconstriction on the measured venous parameter would be expected), we cannot rule out the contribution of vasoconstriction on any change in $\beta_0$ that was observed.

Additionally, contrary to our hypotheses, sublingual nitroglycerin did not alter limb venous compliance in either group. If the age-related differences in venous compliance were due to functional changes in venous smooth muscle tone, the slope of the P-V relationship would have been expected to increase in the older adults. We would also have expected for the P-V relationship to be shifted upward to a higher volume (capacity response), because of the venodilatory effect of nitroglycerin. Using different techniques, others have reported an increase in capacitance (compliance was not assessed) of the forearm during nitroglycerin administration in the forearm of young and older individuals (4, 5, 17, 20). It may be that much higher doses of nitroglycerin are needed to demonstrate a response using this methodology. For example, doses up to 0.8 mg have been used in the previous studies (4, 5, 20). Regardless of any changes in the capacitance of the limb following sublingual nitroglycerin administration, we did not detect any changes in the venous compliance of the limb. Therefore, it would appear that the age-related differences in venous compliance that have been documented are probably due to structural alterations in the venous wall.

A relationship between fitness and venous compliance has been previously documented by several authors (7, 11). Monahan et al. (11) found that regular endurance exercise was associated with increased calf venous compliance in both young and older men. Similarly, Hernandez and Franke (7) found a tendency for a relationship between $V_{O2\text{peak}}$ and venous compliance (7). Interestingly, in the present study, we did not find a statistically significant correlation between $V_{O2\text{peak}}$ and venous compliance at 30 mmHg. The calf venous compliance values we observed appear to be slightly lower when examining young and older participants in the previous studies with comparable fitness levels. However, the lower calf venous compliance measures we obtained would not explain the lack of a correlation between $V_{O2\text{peak}}$ and venous compliance at 30 mmHg. Perhaps it is not surprising that there was a
lack of a correlation between these two values in this data set, because of the clustering of VO2peak data points. Prior work has examined this relationship over a wider range of maximal oxygen values, allowing the relationship to be explored more fully (7, 11).

Limitations

Previous studies have demonstrated a sex difference in venous compliance in young adults (7, 10, 12). Although the primary aim of the present paper was to address age-related differences in baseline venous compliance and the effect of the various perturbations on venous compliance, it is possible that sex differences could have impacted the results of the present study. As previously mentioned, others have found no effect of nonbaroreflex sympathoexcitatory maneuvers on venous compliance in groups of young men and women (2, 6), as well as only young men (12).

In conclusion, the present study provides support for an age-related decline in limb venous compliance. Additionally, limb venous compliance does not appear to be altered by age-related decline in limb venous compliance. Additionally, the mechanism underlying the age-related differences in limb venous compliance is probably not of a functional nature and warrants further investigation.

ACKNOWLEDGMENTS

The authors thank the subjects for participation in this study. The authors also thank the staff of the Christiana Care Cardiovascular Research, Dr. Christopher Knight for data analysis help, and Megan Wenner and Erin Delaney for assistance.

GRANTS

This work was supported by National Institute on Aging Grant R03 AG-23836-01.

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