Effects of age on brachial artery myogenic responses in humans

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that flow velocity is an excellent surrogate for volume flow (i.e., diameter does not change), and changes in PP (i.e., changes in VP) do not explain our findings.

METHODS

Subjects

Twelve younger subjects (19–30 yr) and twelve older (61–73 yr) men and women from the Hershey, PA, area and surrounding communities participated in the study. The Institutional Review Board of the Milton S. Hershey Medical Center approved the experimental protocol. Each subject had the purposes and risks of the protocol explained to them before written informed consent was obtained. Groups were matched by gender and body mass index (Table 1). All subjects in both groups were recreationally active, but none participated in high-intensity exercise (>5 days/wk) in the last several months.

All subjects were nonobese and nonsmokers. The older subjects’ forearms were significantly smaller than the younger subjects’ forearms. No subjects had a history or symptoms of cardiac, vascular, pulmonary, metabolic, or neurological disease. Hypertensive individuals (resting blood pressure ≥ 140/90 mmHg) were excluded. No subjects were taking medications that had significant hemodynamic effects except one older subject was on oral contraceptives 1 days before testing. The younger women were tested 19 ± 1 days into their menstrual cycle with three of six being on oral contraceptives. Two of the six older women were on hormone replacement therapy.

Experimental Design

In experiment 1, younger and older subjects (n = 12/group) had MBV measured during changes in transmural pressure using the forearm-pressurized tank. Increases in transmural pressure were elicited by two methods: application of suction (50 mmHg), and on the release of pressure. In another subset of older subjects (2 men and 2 women) returned on a separate day to examine the influence of increasing transmural pressure using the forearm pressurized tank on the venous system (i.e., VP and forearm volume).

Experimental Measurements

Measurement of heart rate and blood pressure. A standard electrocardiogram was used to monitor heart rate (HR). Systolic and diastolic blood pressures were measured using the volume-clamp method (Finapres, Ohmeda, Madison, WI) with mean arterial pressure (MAP) calculated from the Finapres waveform. Before testing, Finapres pressure was confirmed by an automated sphygmomanometer (Dinamap, Critikon, Tampa, FL). HR and MAP were measured continuously and collected online at 100 Hz using a MacLab system (AD Instruments, Castle Hill, Australia).

Measurement of MBV and diameter. Brachial MBV was measured on a beat-by-beat basis using a 4-MHz pulsed wave Doppler ultrasound probe (model 500M Multigon; Yonkers, NY) taped in a fixed position ~8–10 cm proximal to the antecubital fossa with a 45° angle of insonation. Maximal Doppler frequency shift was obtained with manual adjustment of the Doppler probe. MBV was measured continuously and collected online as noted above. Brachial diameter was determined using ultrasound (range of 5–12 MHz; Advanced Technology Laboratories, model HDI 5000CV, Bothell, WA) during positive and negative tank pressure.

Experimental Testing

Experiment 1. MBV was measured using Doppler ultrasound at rest, under pressure (±50 mmHg), and on the release of pressure. In this study, MBV was used as an index of myogenic responsiveness and blood flow. Previous animal studies have demonstrated similar directional patterns in blood flow and vessel diameters with evaluating myogenic responsiveness (1, 5, 19). MBV as an index of blood flow is also a valid assumption as long as the vessel cross-sectional area remains relatively constant (flow = velocity × vessel diameter).

Subjects were instructed to abstain from products containing caffeine and alcohol as well as abstain from any exercise for 24 h before testing. Measurements were carried out in a quiet, dimly lit laboratory at a temperature between 21 and 24°C. Subjects were in a supine position with their nondominant forearm positioned at heart level.

Two neoprene cuffs were fitted around the midaspect of the arm creating a snug nonconstricting seal, and the subject’s lower forearm was placed in an airtight tank. From an external source, negative and positive pressures were directed to an air pressure box. From this pressurized box, a manual switch opened the system to the forearm tank, providing the appropriate pressure within 0.2 s. Likewise, pressure in the tank was released within 0.2 s. Before all conditions, a test trial was performed to ensure that the appropriate pressure was present within the forearm pressure tank. The Doppler probe was then taped into position on the upper forearm, electrocardiogram leads were applied, and the Finapres device was placed on the subject’s opposite arm and positioned at heart level. Subjects rested for ~20 min before data were collected. All subjects were exposed to two pressure conditions (negative and positive) that were examined separately and randomly (17). For the resting paradigm, after a 1-min baseline at ambient air pressure, arm tank pressure was changed to negative or positive pressure for 2 min. A 2-min recovery period followed the release of tank pressure. For each pressure, a minimum of two trials separated by a 5-min resting period was performed, and the responses were averaged. The coefficient of variability for MBV measurements at rest and during an increase or release of pressure for younger and older subjects was 8 ± 1 and 7 ± 1%, respectively.

Experiment 2. Brachial diameter was measured at rest during the application of positive and negative tank pressure and on release of

Table 1. Subject demographic, anthropometric, and resting hemodynamic measurements

<table>
<thead>
<tr>
<th></th>
<th>Young Subjects</th>
<th>Older Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (men/women)</td>
<td>6/6</td>
<td>6/6</td>
</tr>
<tr>
<td>Age, yr</td>
<td>25±1</td>
<td>65±1*</td>
</tr>
<tr>
<td>Height, cm</td>
<td>174±3</td>
<td>170±3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>75±3</td>
<td>71±4</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.7±0.9</td>
<td>24.6±1.0</td>
</tr>
<tr>
<td>Mid forearm circumference, cm</td>
<td>26.6±0.6</td>
<td>24.6±0.7*</td>
</tr>
<tr>
<td>Resting hemodynamics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>56±3</td>
<td>66±2*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>85±2</td>
<td>94±3*</td>
</tr>
<tr>
<td>MBV, cm/s</td>
<td>5.83±0.32</td>
<td>7.44±0.74</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index; MAP, mean arterial pressure; MBV, mean blood velocity. *Significant difference between groups.
Resting MBV measurements were not significantly different between the older and younger subjects. Changes in transmural pressure did not significantly affect HR or MAP in either group.

*Increases in transmural pressure.* When transmural pressure was raised to 50 mmHg of forearm suction, there was an initial transient increase in MBV in both age groups (Fig. 1A). However, the increase in peak MBV from baseline was significantly smaller in the older than in the younger subjects (Δ 8.93 ± 1.37 vs. Δ 12.20 ± 0.81 cm/s; \( P = 0.05 \), Fig. 1B) (Delta = Δ). After 30 s of sustained negative pressure, MBV was lower than baseline in both groups (young: Δ −1.05 ± 0.22 cm/s; old: Δ −1.89 ± 0.35 cm/s; \( P < 0.05 \), Fig. 1A). Older subjects had significantly greater reductions in ΔMBV responses from baseline compared with the younger group at 30 and 120 s (\( P < 0.05 \)). Release of +50 mmHg of external pressure was also used to raise transmural pressure by 50 mmHg (from ambient −50 mmHg to ambient; Fig. 1C). With the release of +50 mmHg, a transient rise in MBV was noted in both groups, and again this response compared with baseline MBV was significantly less in the elderly subjects (Δ12.43 ± 1.16 vs. Δ17.97 ± 2.01 cm/s; \( P < 0.05 \), Fig. 1D). After peak flows, MBV returned toward baseline values quickly (within 30 s) in both groups (Fig. 1, C and D). As described previously, abrupt increases in transmural pressure cause a rise in flow that is followed by a two-phase dynamic fall in flow (vasoconstriction): the initial phase is “rapid” and the second phase is “less rapid” (Fig. 2A) (17). On the release of 50 mmHg of positive pressure, older subjects compared with younger subjects had significantly reduced “rapid” and “less rapid” vasoconstrictor responses (rapid: −1.88 ± 0.17 vs. −2.90 ± 0.28 cm·s\(^{-1}\)·s\(^{-1}\); less rapid: −0.11 ± 0.02 vs. −0.18 ± 0.02 cm·s\(^{-1}\)·s\(^{-1}\); \( P < 0.05 \), Fig. 2B). These aging differences were maintained when the rates of MBV fall were corrected for changes in MBV pressure as previously described in experiment 1. The scanhead probe was secured over the brachial artery just above the neoprene cuff. Multiple luminal-intima measurements (3 to 5) were performed and averaged during diastole for each pressure condition. In our laboratory, within-day variability for diameter measurements at rest and during changes in forearm task pressure is 1.9 ± 0.7%.

Experiment 3. VP was measured by the placement of a 20-gauge catheter inserted retrogradely into a deep forearm vein of the arm placed into the tank. VP changes were measured as transmural pressure was increased (change to −50 mmHg and release of +50 mmHg) as previously described in experiment 1. PP was calculated from VP (corrected to be additive) and MAP (i.e., PP = −VP + MAP). In addition, forearm volume was measured by strain-gauge plethysmography (Hokanson EC-4 Plethysmograph, Hokanson). A mercury-filled strain gauge was placed around the forearm at the point of largest circumference and calibrated (29). MBV was simultaneously measured during these additional studies.

**Data Analysis**

The following variables were measured on a beat-by-beat basis: HR, MAP, and MBV. Trials for each pressure condition were averaged for each individual. ΔMBVs were calculated from the MBV baseline means or trough. For MBV slope analysis, we used MBV derivative estimates as previously described by Lott et al. (17). Unpaired and paired \( t \)-tests were used to examine the effects of transmural pressure on the measured variables between and within the two different age groups. Data are presented as means ± SE, and level of significance was \( P \leq 0.05 \).

**RESULTS**

**Experiment 1: MBV Responses to Changes in Transmural Pressure**

Older subjects compared with the younger subjects had significantly higher resting HR and MAP values (Table 1).
magnitude. When transmural pressure was raised by applying 
−50 mmHg of suction, we did not observe an effect of aging on the rapid and less rapid dynamic phases (Fig. 2C).

Reductions in transmural pressure. With the change to +50 mmHg, older and younger subject’s MBV fell to a similar nadir (Fig. 3A). The rate of rise from the nadir (dilation) tended to be greater in the older subjects (1.61 ± 0.29 vs. 0.96 ± 0.21 cm·s⁻¹·s⁻¹; P = 0.08; Fig. 3B). In addition, the older subjects had significantly greater changes in MBV from the trough to the peak value (Δ7.71 ± 1.32 vs. Δ4.38 ± 0.71 cm·s⁻¹·s⁻¹; P < 0.05, Fig. 3C). During sustained reductions in transmural pressure, older subjects’ MBV approached baseline values within 30 s, whereas younger subjects’ MBV remained below baseline values. With the second method of transiently lowering transmural pressure i.e., releasing of negative pressure back to ambient pressure, no significant difference in the rate of velocity increase was noted in the two groups [old: 1.84 ± 0.28 vs. young: 2.31 ± 0.20 cm·s⁻¹·s⁻¹; not significant (NS), Fig. 3, D and E]. Increases in MBV from trough velocity to the following peak velocity were not different in the two groups (old: Δ13.91 ± 2.13 vs. young: Δ15.96 ± 1.46; NS; Fig. 3F). After peak flows, MBV returned toward baseline quickly in both groups (Fig. 3F).

Experiment 2: Diameter Responses to Changes in Transmural Pressure

Older subjects (n = 4) demonstrated no significant change in resting brachial diameter (0.44 ± 0.04 cm) between baseline, peak MBV (~2 s), and 30 s after change in pressure or release of pressure. There was no significant increase in brachial artery diameter with increases in transmural pressure. These results are similar to previous findings in young subjects (17).

Experiment 3: Effects of Increasing Transmural Pressure on the Venous System

The VP responses to increasing transmural pressure in the older group (n = 4) were similar to the younger subjects as reported in a previous study (17). An increase in transmural pressure led to a rapid rise in VP (corrected to be additive) and PP. However, the fall in MBV occurred as VP and PP remained relatively constant. Forearm volume changed very little with increases in transmural pressure (i.e., during negative pressure, 1.7 ± 0.8, 1.7 ± 0.8, and 1.6 ± 0.9% at 2, 5, and 30 s, respectively). Thus neither perfusion gradient nor changes in forearm volume explain the velocity responses observed with increasing transmural pressure in the older subjects.

DISCUSSION

In these studies, we examined the effects of age on brachial artery MBV responses to altered transmural pressure. The main findings of this study were that compared with younger subjects, old subjects exhibited 1) attenuated peak MBV responses to abrupt increases in transmural pressure, 2) enhanced steady-state vasoconstrictor responses to sustained increases in transmural pressure, 3) attenuated dynamic “rapid” rates of fall in MBV (vasoconstrictor) responses after a transient increase in transmural pressure, and 4) enhanced vasodilator response after sustained reductions in transmural pressure.

Study Findings

When transmural pressure was raised, a rapid transient increase in MBV in both age groups was observed. This rapid increase in arterial inflow appears to be due to the compliant veins lowering their internal pressure quickly to create a suction effect as indicated by our findings (Fig. 4) and suggested by others (1, 12). Increasing transmural pressure with the release of positive pressure back to ambient resulted in a greater change in the magnitude of flow velocity compared with the application of suction. This finding would suggest that the magnitude of the acute response to a given rise in transmural pressure is dependent on the level of pressure seen before the transmural pressure changes. The magnitude of the peak MBV responses was attenuated in the older subjects compared with the younger subjects. The mechanisms underlying this difference are unclear; however, one possible explanation is that the reduced magnitude of change may be due to increased vessel stiffness and reduced vascular compliance associated with aging (10, 18, 31).

Interestingly, older subjects were able to vasoconstrict slightly more than younger subjects when steady-state responses were examined under sustained increases in transmural pressure. Our findings are different from those reported using mouse mesenteric and rat skeletal muscle arterioles. These
design differences (ramping pressures vs. abrupt studies may be due to the type of study (in vitro vs. in vivo), reasons for the differences between our results and animal reports which used pressure ramping paradigms suggest that aging attenuates myogenic vasoconstriction when transmural pressure is raised by a given amount (20, 21, 25). Possible reasons for the differences between our results and animal studies may be due to the type of study (in vitro vs. in vivo), design differences (ramping pressures vs. abrupt ±50 mmHg), as well as species and vessel differences. Further investigations in humans using a ramping design with a range of pressures may be necessary to further evaluate this issue.

After the transient increase in MBV due to increasing transmural pressure, the acute reduction in MBV (vasoconstriction) was characterized by two dynamic phases: "rapid" and "less rapid" (17). In this study, we observed that both phases of vasoconstriction were attenuated in the older subjects when transmural pressure was raised by withdrawing positive pressure. During steady states of increasing transmural pressure, aging has been associated with an increase in the set point, which is the pressure needed to elicit the myogenic response (13). Whether aging affects the set point for the dynamic phases of the myogenic response is unknown.

To our knowledge, this is the first human study to examine the effects of aging on the dilator response to a reduction in transmural pressure. With the lowering of intraluminal pressure, aging has been associated with altered diameter responses (20). In the present report, the rate of rise of MBV after a reduction in transmural pressure (+50 mmHg) occurred quickly in both age groups. The speed of this response makes it unlikely to have been due to a metabolic effect (15). Under sustained reductions in transmural pressure, older subject’s responses exhibited an enhanced vasodilating effect with MBV returning toward baseline. This is contrasted with the younger subject’s MBV responses, which remained below baseline values during a sustained reduction in transmural pressure. Additional experiments will be necessary to further examine this issue.

The resting blood vessel diameters in older subjects were similar as reported in younger subjects (17). Previous studies have shown aging increases (32) or does not change (2) resting brachial diameter. In our study, older subjects’ vessel diameter like the young subjects (17) did not significantly change in response to changes in transmural pressure. The lack of change in brachial or femoral diameters in humans has also been observed during exercise and tilt (24, 28). Since diameters did not change with the abrupt changes in transmural pressure, MBV was used as an index of the myogenic response. The lack of conduit diameter changes does not imply that there were no changes in vessel diameter in other vessels within the forearm. In fact, since the myogenic response is likely to occur in smaller vessels (i.e., arterioles), it is likely that resistance arteries in the forearm underwent diameter changes that led to the increase in brachial artery flow velocity.

When forearm is exposed to negative pressure, the effect of suction transcends similarly through arterial and venous systems. To exclude that VP changes could explain our observations, we performed additional experiments to examine the temporal relationships between VP and MBV in four older subjects. We demonstrated that the MBV decreased as VP and PP remained relatively steady. A similar response was demonstrated in a previous study in younger subjects (17). Thus changes in VP and PP cannot explain the differences observed in MBV. Last, the use of the arm-pressurized tank could also have altered interstitial fluid pressure. We cannot exclude this possibility. However, forearm volumes remained fairly constant during increases in transmural pressure. Thus, unless a rise in transmural pressure evoked marked redistribution of volume, we doubt that a change in interstitial fluid pressure could explain our results.

**Potential Mechanisms Explaining the Aging Effect**

There are several potential mechanisms that may help explain the aging effect on the myogenic response. First, it is possible that voltage-gated calcium channel activity increases with age. Increases in transmural pressure have been associated with an influx of calcium through L-type voltage-gated calcium channels (6). In a recent animal study using heart myocytes, the number of active calcium channels as well as a delay of inactivation of these channels increases with aging (16). Thus aging may alter smooth muscle L-type calcium channels in a similar way. This could explain the reduced rate of dynamic constriction as well as the enhanced steady-state level of vasoconstriction seen in the present report. Another potential explanation for the enhanced vasoconstriction associated with aging may be that aging impairs the endothelium’s release of vasodilators opposing the myogenic stimulus to vasoconstrict. Although the myogenic response can occur independent of the endothelium (8, 30) it is clear that endothelial substances (nitric oxide and endothelial relaxing factors) are important modulators of the myogenic response (7, 22, 26). The blunted dynamic phases of the myogenic response in the aged may also be due to structural changes associated with aging that make it more stiff and less likely to initially vasodilate (10, 18, 31). Last, vascular wall composition and structure change with aging, and this effect in turn may alter pressure-sensing mechanisms within the vessel (11, 13, 18, 20).

**Potential Limitations to the Study**

There are a number of limitations to the present study. First, we cannot entirely exclude effects of metabolic products on
limb flow (1, 19). However, as mentioned earlier, the time course of the rapid and less rapid vasoconstriction phases make metabolic dilatation unlikely. Although the study’s design did not measure the endothelial contribution to the myogenic response, we conclude that the time course of changes in flow velocity is likely to be too rapid to be influenced by endothelial processes. Forearm flow studies suggest that the peak endothelial effects are seen ~1 min after the stimulus for endothelial dilatation is initiated (4). Last, we cannot exclude the influence of asymptomatic cardiovascular disease in the elderly group.

Physiological Significance

The attenuation of the dynamic phases of vasoconstriction to the abrupt increases in transmural pressure may predispose the aged to orthostatic intolerance. However, further studies on the lower limbs will need to examine this mechanism. Enhanced steady-state vasoconstriction during sustained increases in transmural pressure as demonstrated in the elderly may help explain the high incidence of hypertension seen in the elderly (9). Finally, that enhanced vasodilatation seen as transmural pressure is reduced may be a compensatory mechanism that helps counteract the reduced level of limb flow seen with moderate to heavy exercise in the aged (23).

In conclusion, aging leads to impaired acute dynamic phases of rapid vasoconstriction but enhances steady-state vasoconstriction and vasodilator responses to sustained increases and decreases in forearm transmural pressure, respectively. These findings have implications for local blood flow regulation in older adults.

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