Age and regional specificity of peak limb vascular conductance in women

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Ridout, Samuel J., Beth A. Parker, and David N. Proctor. Age and regional specificity of peak limb vascular conductance in women. J Appl Physiol 99: 2067–2074, 2005.—First published August 18, 2005; doi:10.1152/japplphysiol.00825.2005.—The influence of age on limb vasodilator capacity in women is unclear. The objectives of this study were to characterize and compare age-associated changes in forearm and calf peak vascular conductance (VCpeak, a functional index of arterial structure) in women and to identify physiological characteristics predictive of variation in limb-specific VCpeak. Peak conductance (plethysmographic flow/mean arterial pressure), VCpeak of the forearm (forearm VCpeak), and calf (calf VCpeak) after 10 min of arterial occlusion were measured in 58 healthy, normally active women aged 21–79 yr. Aerobic capacity (cycle peak oxygen uptake), arterial health (pulse-wave velocity, ankle-brachial index), total cholesterol, limb-specific tissue composition (dual-energy X-ray absorptiometry), and isometric strength (handgrip, plantar flexion) were also assessed. The relative decline in calf VCpeak with age (−6.8% per decade, $P < 0.001$) was greater than the forearm (−4.4% per decade, $P = 0.004$), in contrast to results previously reported for men (forearm decline > calf decline). Limb VCpeak per kilogram muscle declined with age in the calf (−6.0% per decade; $P = 0.002$), but not the forearm ($P = 0.12$). Age, cholesterol, and regional tissue composition were significant predictors of peak conductance in both limbs; however, age was a stronger predictor of peak conductance in the calf. These results suggest that healthy aging is associated with a linear decline in limb vasodilator capacity in women, but the magnitude of this effect is region specific. Further research will be required to determine whether the decline in lower extremity vasodilator capacity with age explains diminished exercising leg vasodilation in older women.

Aging; gender; muscle blood flow; vasodilation; reactive hyperemia; venous occlusion plethysmography

AGE-ASSOCIATED ALTERATIONS in the ability to increase limb muscle blood flow both in response to exercise and during reactive hyperemia (RH) have recently been identified and could have important implications for cardiovascular regulation and physical function in our rapidly expanding older population (18, 29, 31). Recently, our laboratory reported significant age-associated reductions in leg blood flow and vascular conductance during submaximal leg exercise in women (29). Additionally, leg blood flow and vascular conductance were 29 and 38% lower, respectively, in the older women compared with the younger women during peak exercise (30). Older women also display diminished levels of systemic vascular conductance (i.e., elevated peripheral vascular resistance) during exercise compared with either younger women or older men (8, 11, 26, 29). These results suggest that women experience dramatic reductions in exercise-induced peripheral vasodilation with advancing age. Understanding the determinants of impaired peripheral vasodilation with advancing age is particularly important for women as they experience more functional impairment and cardiovascular morbidity in old age than men (10, 23).

Reductions in systemic and exercising leg vasodilation with advancing age in women could reflect, in part, structural changes in the arterial vasculature of the lower extremity that limit the extent of vasodilation (29, 30). Direct assessment of arterial structure in humans is difficult. However, peak dilatory responses (peak flow and vascular conductance) immediately after limb occlusion have been widely used as functional indexes of arterial size (vascular density, volume, and/or geometry) (20, 21, 28, 33, 34, 36, 41). Using a 10-min period of limb occlusion, our laboratory recently confirmed age-dependent declines in calf and forearm reactive hyperemia and vascular conductance in men (31). To the best of our knowledge only two studies have directly examined the influence of age on limb reactive hyperemia in women. Lind and colleagues (19, 35) reported an inverse relationship ($r = −0.56; P < 0.01$) between age and peak forearm hyperemia after 3 min of cuff occlusion in women aged 20 to 69. Martin et al. (21) reported that maximal calf hyperemia and vascular conductance were significantly lower ($P < 0.05$) in older (60–71 yr) compared with younger (20–35 yr) sedentary women. Two additional studies of peak reactive hyperemia in the leg (19, 27, 34) compared peak conductance values in samples of older and younger subject groups, with disparate results. Olive et al. (27) found that peak conductance was lower in older subjects, whereas Rueckert and Hanson (34) did not find an age group difference. These findings may have been influenced by the fact that the investigators combined data from women and men. Taken together, these studies indicate that there may be a decline in limb dilator capacity with age in women, but a comprehensive examination of this issue has not been conducted.

Because most of the studies of peak limb vasodilation compared two discrete age ranges, it is not known whether age-related changes in limb flow capacity or peak conductance in women are linear or exponential. This is important because several studies indicate nonlinear age-associated declines in endothelium-dependent dilator responses in women. Specifically, previous studies of endothelium-dependent dilator responses in the forearm indicate preservation until menopause followed by a steep decline (3, 37, 39). Finally, most of the available data on peak vasodilator capacity and age in women are based on the leg whereas most of the available data on endothelial dilator responses are based on the forearm. Thus it...
would be helpful to know whether age-associated reductions in the peak flow and conductance within both limbs are similar or whether there is regional specificity in this response, as our laboratory has previously reported in men (31). This regional specificity has not been explored in women.

With this information as a background, the present study utilized a standardized reactive hyperemia test (10 min of cuff occlusion proximal to the elbow and knee) in healthy, normally active women across the adult age range to characterize the age-associated changes in peak limb blood flow and vascular conductance in the forearm and calf. We hypothesized that age-associated declines in peak hyperemia and vascular conductance would be greater in the calf than the forearm of healthy women owing to previous reports of greater age-associated reductions in muscle mass (16) and strength (15) of the lower vs. upper extremity of normally active women. In addition, we sought to determine whether fitness, muscle mass, or other physiological characteristics mediate age-associated changes in peak limb vascular conductance in women.

METHODS

Subjects. Fifty-eight women between the ages of 21 and 79 yr completed this study. All subjects, with one exception (71-yr-old with overt chronic diseases as evaluated by medical history questionnaire, and hemoglobin ≥ 11 g/dl, total cholesterol ≤ 140/90 mmHg), were normotensive (seated resting blood pressure ≤ 140/90 mmHg). Subjects were free of overt chronic diseases as evaluated by medical history questionnaire, a physical examination, resting ECG, and blood chemistry (i.e., hemoglobin ≥ 11 g/dl, total cholesterol ≤ 6.2 mmol/l). Subjects were nonsmokers and abstained from caffeine, aspirin, or ibuprofen, and herbal supplements for at least 12 h before limb blood flow testing. No subjects were taking medications that had significant hemodynamic effects, but two women (age 64 and 79 yr) did take baby aspirin on a regular basis. Subjects gave their written, informed consent to participate. This study was approved by the Office for Research Protections and the Institutional Review Board at The Pennsylvania State University.

Occupational and physical activity history. Most subjects under 30 yr of age were college students. The majority of the intermediate (30–65 yr) age group were either employed (primarily desk jobs) or homemakers caring for children. The older (>65) age groups were predominantly retired. No subjects reported a current physically demanding job or, if retired, one within the past decade. Subjects completed a questionnaire to provide information about the type and frequency of their recreational activities and exercise, including participation in aerobic and/or strength exercises during the past 6 mo. Subjects ranged from completely sedentary to moderately active with no chronically trained endurance athletes enrolled in the study. The percentage of women reporting some weekly aerobic exercise activity (walking, biking, aerobic machines, etc.) during the preceding 6 mo decreased from ~88% in the younger subjects (20–39 yr) to ~55% in the older groups, averaging 2–4 days per week. Resistance exercises were reported in 17 of the 47 subjects over age 30 yr. The frequency of this activity averaged two to four times a week, and subjects universally reported a light to moderate intensity. The youngest subjects (<30 yr) reported no resistance training activity.

Other than the peak oxygen uptake (V\textsubscript{O\textsubscript{2}}\textsuperscript{peak}) test, all protocols were performed in the first 7 days of the menstrual cycle for all premenopausal subjects. This was done to control for cycle phase and therefore estrogen and progesterone levels, as they have been shown to influence hemodynamic measurements (12, 17, 43). All protocols and measurement techniques have been previously described in a recent publication by our laboratory (31). However, a brief explanation of these will follow.

\( V\textsubscript{O\textsubscript{2}}\text{peak} \) test. Subjects pedaled an electronically braked cycle ergometer (Lode) to maximal exertion to screen for cardiovascular abnormalities and to determine \( V\textsubscript{O\textsubscript{2}}\text{peak} \), as described previously (32). No subject exhibited evidence of ECG or blood pressure abnormalities during the test that prohibited them from participating in the subsequent protocols. All subjects except two (with values of 1.11 and 1.12) had a peak respiratory exchange ratio >1.20 and achieved an average of 99.7% of their age-predicted maximum heart rate (40). Only four subjects (ages 21, 25, 27, 32 yr) had \( V\textsubscript{O\textsubscript{2}}\text{peak} \) values that exceeded 85% of their age-adjusted norms (42), consistent with the fact that subjects were nonathletes who ranged from sedentary to moderately active.

Body composition. Total and regional body composition was estimated using dual-energy X-ray absorptiometry (DXA; model QDR 4500W, Hologic, Waltham, MA). Bone-free lean mass (index of muscle mass) and percent fat for the forearm and calf of each subject’s nondominant limbs were calculated with region-of-interest software (version 9.80D, Hologic). All DXA scans were performed and analyzed by the same manufacturer-trained operator who also performed weekly quality control calibrations.

Strength testing. Isometric strength of the nondominant hand was measured using a Jamar handgrip dynamometer (Sammons Preston, AbilityOne). The highest of three maximal efforts, each separated by a rest period of 1–2 min, was used as an index of maximal forearm strength. Isometric strength of the plantar flexors of the corresponding leg was measured by using an isokinetic dynamometer (Multi-Joint System 3 Pro, Biodex Medical Systems) at 0°/s. During measurements the subjects were fixed in place with chest, waist, and thigh straps, and the knee joint was fixed at 180°. The rotational axis of the ankle was set at 90° by using a goniometer. The highest of three maximal efforts, each separated by a 1-min rest period, was used as an index of maximal calf strength.

Measurement of ankle-brachial index and arterial pulse wave velocity. Ankle-brachial index (ABI) and pulse wave velocity (PWV) were measured after 10 min of quiet supine rest by using the VP2000 vascular profiling machine (Colin Medical) as previously described (6). Day-to-day variability (coefficient of variation, %) was assessed in a subset of subjects (n = 45; 22–79 yr) on two separate visits (~28 days apart) and averaged 3.0 and 3.8 for ABI and brachial-to-ankle PWV, respectively.

Venous occlusion plethysmography. Forearm and calf blood flows (ml·100 ml·1·min\(^{-1}\)) of the nondominant limbs were measured via a Hokanson venous occlusion plethysmography system (14). Limb blood flow studies were conducted in a cool, temperature-controlled room (20–22°C) to minimize the contribution of skin blood flow. Subjects were placed in a reclined position (i.e., torso ~45° from horizontal) on a padded chair with the nondominant forearm and calf elevated 10–20 cm above heart level as necessary to facilitate venous drainage. Mercury-in-Silastic strain gauges were placed around the widest portion of the forearm and calf and calibrated electronically. Venous occlusion cuffs placed around the upper arm and thigh were rapidly inflated to 45 mmHg every 15 s (7 s inflate, 8 s release) during flow measurements. Wrist and ankle cuffs were used to occlude blood flow to the hand and foot, respectively, during all flow measurements.

Measurement of peak limb vascular conductance. Plethysmographic measurements of forearm and calf blood flow were obtained during rest and RH (after 10-min arterial occlusion). To account for any potential residual effects from the hyperemia in the first extremity studied, the order of the limbs occluded (forearm or calf first) was randomized for each subject on each visit. Subjects rested quietly for ~10 min before baseline measurements. To familiarize the subject with the reactive hyperemia test and to check strain gauge performance, a 2-min arterial occlusion, followed by cuff release, was performed. After a 5-min recovery period subjects were instructed to raise and contract their forearm or calf muscles to evacuate the venous vasculature. During this contraction the occlusion pressure was applied (80–100 mmHg suprasystolic). Subjects then relaxed the limb.
for the remainder of the 10-min occlusion period. Reactive hyperemic flow was measured within 3 s after cuff release and every 15 s thereafter. Peak limb blood flow occurred within the first two or three measurements (30–45 s) for all subjects and declined rapidly thereafter.

Blood flows were calculated from the slope of the ascending, linear portion of the plethysmographic response curve by using the line-fitting option in the Hokanson analysis software package (NIVP3) and manual adjustment (to exclude cuff inflation artifact) by a single investigator blinded to the age of the subject. Systolic and diastolic blood pressures were measured by auscultation of the brachial artery of the contralateral arm. Forearm and calf vascular conductance were estimated as limb blood flow divided by the corresponding brachial mean arterial pressure (MAP; diastolic blood pressure + 1/3 of the pulse pressure).

Rationale for using the 10-min vascular occlusion protocol. The rationale for selecting this stimulus to elicit an approximate peak vasodilation and therefore vascular conductance has been thoroughly described previously (31). The 10-min passive occlusion protocol used in this study has been shown to yield equally high levels of peak calf conductance compared with ischemic contractions to fatigue both in younger and older subjects (28, 34).

Reproducibility. Test-retest reproducibility was established for the measurement of peak conductance in both the forearm and calf by performing a second study visit on some subjects (n = 48 forearm; n = 45 calf). The time between study visits varied from 2 days to 2 mo. Paired t-tests indicated that there were no significant mean differences (all P ≥ 0.63) between peak limb blood flow or peak conductance responses measured during the two study visits. However, test-retest differences of ±20% for peak flow and conductance were noted in a substantial number of subjects, resulting in an overall coefficient of variation of ~11% for both limbs. When peak conductance from visit 1 was plotted against visit 2, there was no evidence of systematic variation from the line of identity (P = 0.77 forearm, 0.81 calf). Still, we decided to define “peak” flow and vascular conductance as the highest individual value achieved on the two visits. The use of the highest individual response is a widely used and valid approach in studies evaluating peak physiological capacity. Importantly, the overall relations between age and peak conductance in the forearm and calf were nearly identical for the first and second study visits: forearm conductance = 0.416 – 0.002 × age (visit 1) and 0.438 – 0.002 × age (visit 2); calf conductance = 0.548 – 0.004 × age (visit 1) and 0.521 – 0.003 × age (visit 2).

Statistical analysis. Data grouped by decade (subject characteristics in Table 1) were compared by using an unbalanced one-way ANOVA. Bonferroni’s post hoc test was used to determine the specific age decades that were significantly different from the youngest subjects (i.e., 20- to 29-yr-old group). Line fitting of limb-specific hemodynamic variables vs. age (Fig. 1) was performed by simple linear regression. Comparison of slope differences between the forearm and calf were assessed with an F-test.

Pearson correlations were calculated to assess the relation between peak limb conductance and other measured variables in the entire sample of subjects (Table 2). Forward stepwise multiple regression (P < 0.15 to enter) was then performed to determine which combination of variables explained the most variance in forearm and calf peak conductance (Table 3) with age entered in both models. Each model was checked for the existence of influential data points. These points did not influence the variance of any models (forearm and calf), and so these data were retained in the regression analyses.

All data are presented as means ± SE. Statistical significance was accepted at P < 0.05. Minitab version 14 was used for all statistical analyses.

RESULTS

Subject characteristics. When compared with the 20–29 yr age group, there were no significant differences in weight between the decades tested (Table 1). Only the 60–69 yr age group exhibited a significant increase in body mass index. A significant increase in total body fat percent was noted in the 50–59 and 60–69 yr age groups. There was an age-related increase in total cholesterol that became statistically significant in the 50–59 yr age group and thereafter. When compared with the 20–29 yr, the 70–79 yr group did exhibit a modest increase in supine resting systolic blood pressure; the same is true for the diastolic pressure of the 60–69 yr group. Brachial-to-ankle PWV showed a progressive increase with age, which became significant by the fifth decade (40–49 yr old). Cycle $\dot{V}O_{2peak}$ declined an average of ~7.5% per decade.

Limb characteristics. Unlike a prior experiment performed in men using an identical protocol (31), age-associated declines were not observed for limb circumference, muscle mass, or planter flexor strength. There was a nearly significant decrease in forearm muscle mass with advancing age (P = 0.058). Neither forearm (P = 0.282) nor calf (P = 0.681) percent fat exhibited a significant age-associated change.

Relation between age and limb blood flow. Peak reactive hyperemic flow decreased with advancing age in both limbs (Fig. 1). The relative decline in peak blood flow was greater in Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Decade</th>
<th>20–29 yr</th>
<th>30–39 yr</th>
<th>40–49 yr</th>
<th>50–59 yr</th>
<th>60–69 yr</th>
<th>70–79 yr</th>
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<td>Age, yr</td>
<td>12</td>
<td>9</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Height, cm</td>
<td>164±1.6</td>
<td>161.6±1.9</td>
<td>166.4±2.1</td>
<td>164.7±2.2</td>
<td>161.0±1.9</td>
<td>162.7±2.0</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>63.7±2.6</td>
<td>59.2±2.8</td>
<td>64.3±3.4</td>
<td>65.2±2.4</td>
<td>67.3±2.7</td>
<td>62.0±3.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.3±0.64</td>
<td>23.0±1.2</td>
<td>23.1±0.75</td>
<td>24.0±0.74</td>
<td>26.0±1.1</td>
<td>23.4±1.0</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>29.8±1.2</td>
<td>29.7±2.4</td>
<td>30.1±1.8</td>
<td>33.4±9</td>
<td>37.1±5.8</td>
<td>32.4±1.9</td>
</tr>
<tr>
<td>Cholesterol, mmol/l</td>
<td>3.91±0.19</td>
<td>4.07±0.24</td>
<td>4.24±0.25</td>
<td>5.11±0.17*</td>
<td>5.44±0.16*</td>
<td>5.29±0.25*</td>
</tr>
<tr>
<td>SBPrest, mmHg</td>
<td>114.7±3.5</td>
<td>112.0±3.1</td>
<td>111.1±4.0</td>
<td>114.4±4.2</td>
<td>119.4±3.9</td>
<td>133.8±1.9*</td>
</tr>
<tr>
<td>DBPrest, mmHg</td>
<td>70.3±1.9</td>
<td>72.3±1.9</td>
<td>70.2±2.7</td>
<td>71.7±2.9</td>
<td>78.0±2.3</td>
<td>78.3±3.5</td>
</tr>
<tr>
<td>MAPrest, mmHg</td>
<td>80.0±2.3</td>
<td>83.3±2.6</td>
<td>87.2±3.6</td>
<td>89.8±3.3</td>
<td>88.6±3.4</td>
<td>97.6±2.8*</td>
</tr>
<tr>
<td>MAPpeak, mmHg</td>
<td>80.6±3.0</td>
<td>78.8±1.9</td>
<td>85.3±3.3</td>
<td>86.3±3.2</td>
<td>90.0±2.0</td>
<td>93.7±4.8</td>
</tr>
<tr>
<td>PWV, cm/s</td>
<td>1,081±37</td>
<td>1,134±27</td>
<td>1,186±17</td>
<td>1,244±58</td>
<td>1,523±60*</td>
<td>1,871±22*</td>
</tr>
<tr>
<td>ABI</td>
<td>1.09±0.03</td>
<td>1.09±0.02</td>
<td>1.09±0.02</td>
<td>1.15±0.02</td>
<td>1.17±0.02</td>
<td>1.14±0.02</td>
</tr>
<tr>
<td>$\dot{V}O_{2peak}$, ml·kg⁻¹·min⁻¹</td>
<td>32.2±1.9</td>
<td>30.1±1.9</td>
<td>26.5±9.9</td>
<td>24.4±1.1*</td>
<td>19.2±2.7*</td>
<td>18.1±4.0*</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index; SBPrest, resting systolic blood pressure; DBPrest, resting diastolic blood pressure; MAPrest, mean arterial pressure during forearm or calf peak conductance measurement; $\dot{V}O_{2peak}$, oxygen consumption during peak upright leg cycle ergometry; PWV, brachial-to-ankle pulse wave velocity; ABI, ankle-brachial index. *P < 0.05 vs. 20–29 yr decade.
the calf (−5.3% per decade, \( P < 0.001 \)) than the forearm (−3.0% per decade, \( P = 0.022 \)).(Fig. 1).

Relation between age and peak limb conductance. Postischemic conductance declined as a linear function of increasing age in both the upper and lower extremity (Fig. 1). The decline in peak forearm conductance was −4.4% per decade (\( P = 0.002 \)), and the decline in peak calf conductance was −6.8% per decade (\( P < 0.001 \)); the difference in these two rates of decline in peak conductance of the upper and lower extremity was found to be significant (\( P = 0.032 \)). During the calf RH test there was no statistically significant age-associated increase in the peak MAP response (Table 1). During the forearm RH test the oldest group did exhibit a significant increase in the peak MAP response compared with the youngest age group (Table 1).

When peak vascular conductance was normalized per kilogram of limb muscle mass (ml blood flow·100 ml tissue⁻¹·min⁻¹·mmHg⁻¹·kg muscle⁻¹), the age-associated decline was abolished in the forearm (\( P = 0.274 \)) and only slightly attenuated in the calf (−6.0% per decade, \( P = 0.002 \)).

Predictors of limb-specific peak vascular conductance. Table 2 shows the univariate correlations between age, peak limb conductance, and selected physical characteristics. Age was significantly correlated with VO₂peak, PWV, percent body fat, and total cholesterol, but not with any of the limb-specific variables except for grip strength. Forward stepwise linear regression analysis was then performed on potential predictors of peak conductance in the forearm and calf. The variables entered were age, cholesterol, VO₂peak, PWV, and limb-spe-

Fig. 1. Peak blood flow (top), peak vascular conductance (middle), and peak conductance normalized for limb-specific muscle mass (bottom) of the forearm (A) and calf (B) plotted as a function of age.
cific measures of isometric strength, muscle mass, and percent fat. Limb circumference was not included in these models because of its strong association with limb muscle mass (Table 2) and because plethysmographic flows (and therefore peak conductance) are already normalized to limb circumference.

Table 3 shows the simple and stepwise multiple regression models describing the variance in peak conductance of the forearm and calf. Chronological age, by itself, was a statistically significant predictor of peak conductance in both limbs (both \( P < 0.01 \)). In the forearm, age was the strongest overall predictor of peak conductance. Total cholesterol and forearm muscle mass were the second and third factors to enter the equation, improving \( R^2 \) from 0.18 to 0.25. In the calf, age was the strongest overall predictor of peak conductance. Total cholesterol and calf percent fat also entered the model, significantly improving the model \( R^2 \) for the calf from 0.38 to 0.53. \( V_0_2\text{peak} \) did not enter the multiple regression models for peak forearm or calf conductance unless age was excluded.

Table 3. Regression analysis of peak vascular conductance of the forearm and calf

<table>
<thead>
<tr>
<th>Limb Dependent Variable</th>
<th>Predictors</th>
<th>Cumulative ( R^2 )</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forearm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak conductance</td>
<td>Age</td>
<td>18.2%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Peak conductance</td>
<td>Age</td>
<td>18.2%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Cholesterol</td>
<td>21.6%</td>
<td>0.145</td>
</tr>
<tr>
<td></td>
<td>Regional muscle mass</td>
<td>24.9%</td>
<td>0.149</td>
</tr>
<tr>
<td>Calf</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak conductance</td>
<td>Age</td>
<td>37.6%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peak conductance</td>
<td>Age</td>
<td>37.6%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Cholesterol</td>
<td>49.9%</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Regional %fat</td>
<td>53.1%</td>
<td>0.082</td>
</tr>
</tbody>
</table>

Simple regression (vs. age alone) and multiple regression (forward stepwise) models for peak conductance of the forearm and calf.

**DISCUSSION**

The principal new findings of the present investigation are as follows. First, peak vascular conductance of the forearm and calf are linearly and inversely related to age in healthy normally active women. The magnitude of decline in peak conductance with age is limb specific, with a steeper rate of decline in the calf (−6.8% per decade) compared with the forearm (−4.4% per decade). Second, normalization of peak conductance to limb-specific muscle mass abolishes the age-associated decline in the forearm but has little impact in the calf. Third, chronological age, total serum cholesterol, and limb-specific tissue composition are significant predictors of peak conductance in both limbs; however, age is a stronger predictor of peak conductance in the calf. Collectively, these findings indicate that age-associated declines in skeletal muscle vasodilator capacity are greater in the leg than in the forearm of normally active women.

**Age and forearm vasodilator capacity in women.** Although several studies have examined endothelium-dependent vasodilator responses in the forearm as a function of age in women (3, 9, 35, 37, 39), very few studies have actually reported the responses to a standardized reactive hyperemia test (i.e., passive arterial occlusion) in healthy women across the adult age range. Lind and colleagues (19, 35) reported a linear decline in peak forearm reactive hyperemia with age in “apparently healthy” women who were not taking any form of estrogen therapy (\( n = 30; 20–69 \) yr). However, several smokers and hypertensive subjects were included in the study sample, and there was no control for menstrual cycle phase in the premenopausal women. Therefore, to the best of our knowledge, the present study is the first to document the effects of age on peak forearm reactive hyperemia and vascular conductance in healthy women in a controlled menstrual cycle phase. Our results indicate modest but significant overall declines in peak
forearm blood flow (−3.0% per decade) and conductance (−4.4% per decade) with age. The slopes of these relationships were linear and did not differ between our pre- and postmenopausal women. This latter finding suggests that the peak dilatory response to 10 min of passive arterial occlusion, a measure of structural changes in the resistance vasculature, is relatively insensitive to menopausal status.

When peak forearm conductance was normalized to an index of regional muscle mass (DXA lean tissue mass), the age-associated reduction was abolished ($P = 0.27$). This finding suggests that the age-associated decline in peak conductance in the forearm is secondary to the loss of muscle.

Age and calf vasodilator capacity in women. To our knowledge, only one previously published study has directly compared reactive hyperemic responses in the legs of younger and older groups of women. In that study, Martin et al. (21) reported significantly lower reactive hyperemic responses to maximal ischemic lower leg (calf) exercise in sedentary older (60–71 yr) women compared with younger (20–35 yr) controls. The age group difference in maximal calf conductance they reported is comparable to that observed over a similar age range (~40 yr) in the present study (~25–30% lower in the older women), even though the stimulus for calf vasodilation differed between the Martin et al. study (standing, ischemic toe/heel raises to fatigue) and the present study (supine, passive arterial occlusion for 10 min). One noteworthy difference between studies, however, is the blood pressure response observed when peak hyperemia was measured; the ischemic exercise model utilized by Martin et al. resulted in significant elevations in MAP that were markedly higher (>15 mmHg higher) in their older compared with younger women. This makes it difficult to determine the extent to which reduced responses in their older subjects reflect the effects of augmented arterial perfusion pressure and sympathoexcitation vs. the intrinsic dilator capacity of the local resistance vessels. By contrast, the peak MAP response to 10 min of passive calf occlusion in the present study did not differ significantly between the younger (20–29 yr) and older groups, providing more direct evidence for diminished vasodilator responsiveness in the calf musculature of sedentary older women as a function of age. However, it is important to note that the peak MAP response in our 70–79 yr old group did exhibit a trend toward a significant elevation compared with the 20- to 29-yr-old group ($P = 0.07$).

Is there a primary effect of age on limb vasodilator capacity in women? Age was the strongest predictor of peak conductance in the forearm and calf, explaining 18 and 38% of the variance, respectively, in these women. Total cholesterol and limb-specific tissue composition improved the model $r^2$ in both limbs (Table 1). However, when age was excluded from the multiple regression models, $\dot{V}O_2$ peak was the only variable that entered (explaining 8 and 29% of the variance, respectively). Because $\dot{V}O_2$ peak and cholesterol are strongly influenced by age in women (Table 2), it is difficult to determine whether aging is exerting a primary or secondary effect on limb vasodilator capacity in the present study. To address this question, we examined conductance changes within a $\dot{V}O_2$ peak-matched (24.3 ± 0.4 ml·kg$^{-1}$·min$^{-1}$) sample of our subjects (22–71 yr; n = 26). Controlling for fitness in this way abolished the age-associated decline in peak conductance of the forearm but not for the calf. The same results were obtained when we controlled for cholesterol, i.e., a highly significant decline in peak calf conductance with age, but not in the forearm (data not shown). Collectively, these results suggest that chronological age is a stronger predictor of vasodilator capacity of the leg than of the forearm in healthy normally active women. The decline in peak calf conductance with age in women is not simply due to the age-related loss of muscle mass (i.e., sarcopenia) because there is a persistent reduction in peak conductance with age when normalized to the muscle mass of the calf (~6.0% per decade, $P = 0.002$).

Gender differences in peak limb vasodilator capacity with age. The age-associated reductions in peak calf blood flow and conductance observed in the present sample of women are approximately twofold greater than those seen in our previous study of men (~3% decline per decade). It is interesting to note that Coggan et al. (5) reported gender differences of a similar magnitude for capillary-to-fiber ratios of gastrocnemius muscle biopsies taken from older and younger sedentary humans (~40% age difference in women; ~20% in men). Taken together, these results support the premise that peak conductance during reactive hyperemia reflects structural changes in the skeletal muscle vasculature (21, 28, 33, 34).

Greater age-associated reductions in calf vasodilator capacity and vascular density in women vs. those seen in men could result from greater reductions in lower body physical activity in women vs. men with advancing age. We cannot directly address this possibility because of the cross-sectional design of
the present study and the fact that we did not assess the frequency or duration of physical activity (past or present) in our male cohort (31). However, there is some evidence that the prevalence of regular aerobic activities (primarily lower body) differed between women and men across the age range studied, i.e., the percentage of women reporting some weekly aerobic exercise activity (walking, biking, aerobic machines, etc.) during the preceding 6 mo decreased from ~88% in our younger subjects (20–39 yr) to ~55% in the older groups whereas these activities tended to become more prevalent in our older (compared to younger) men. Thus reduced prevalence of aerobic activity participation in older women (4) could explain their greater reductions in calf vasodilator capacity with advancing age than men. The impact of gender differences in physical activity on peak calf conductance, however, does not appear to be mediated through changes in aerobic fitness because $V\dot{O}_2$ peak declined similarly with age in the overall samples of women (~7.5% per decade) and men (~7.7% per decade) and 2) the association between peak calf conductance and $V\dot{O}_2$ peak was not markedly different in women vs. men (Table 2).

Declines in forearm vasodilator capacity with age may also be gender dependent, as suggested by the smaller reductions in women (~4.4% per decade) compared with men (~6.6% per decade). In fact, all forearm characteristics we studied (grip strength, DXA lean tissue mass, etc.) underwent less rapid change with age in women vs. men, consistent with previous studies in the literature (1, 15, 16). The smaller declines in forearm strength, muscle mass, and vasodilator capacity in women could reflect their lower absolute values, in the youngest age group, compared with the men (i.e., baseline effect). This is best illustrated in Fig. 2A, which includes a direct comparison of peak forearm conductance between men and women for each decade. This baseline effect is probably more reflective of inherent gender differences in muscle size and vascularity (5, 16) than gender differences in the prevalence of upper body strength training in our younger subjects (20–39 yr, 21% in women vs. 40% in men) because this form of training does not normally lead to augmented limb dilator capacity (2, 13).

Experimental considerations. Although the vast majority of women in the present study were normotensive, there was an increase in baseline resting blood pressure in the oldest age groups. Additionally, there was a greater peak MAP response in the oldest women (70–79 yr) during the forearm RH test and a nonsignificant trend toward a greater peak MAP response during the calf RH test (Table 1). These elevated MAP responses could lead to underestimates of the reductions in limb vasodilator capacity in older women.

Circulating estrogen can influence both vascular structure and function (3, 21, 22). Because none of the women in this study were taking any form of hormone replacement therapy, it cannot be determined whether the differences in limb vasodilator capacity observed across age or between women and men were due to age- or gender-related differences in circulating estrogen levels. However, as discussed above, the slopes of the age vs. peak conductance relationship for the forearm and calf were linear and did not differ between our pre- and postmenopausal women. Moreover, gender differences in peak conductance were present well before (age 20–40 yr in the forearm) and after (age 60 and beyond in the calf) the menopausal transition (Fig. 2). Finally, because of the region-specific nature of these results, it is unlikely that changes in a circulating (i.e., systemic) factor would exert differential effects in the arms and legs. Consequently, we do not think the natural loss of estrogen in women is playing a major role in our results. Whether hormone replacement might modify pressure dependent adaptations in the upper and lower extremities of older adults (24, 25) because of upright posture and/or habitual limb-specific activity is unknown.

Finally, although structural changes in the limb resistance vessels are the most likely explanation for age-associated declines in peak limb conductance in women, it is possible that changes in responsiveness to vasodilators may also have been involved. Specifically, there is evidence that aging alters forearm vasodilation in response to prostaglandin inhibition (38) and that prostaglandins may modulate peak RH responses in human limbs (7). Age-related declines in nitric oxide-mediated vasodilation, however, are less likely to contribute to the observed changes in the present study because the role of nitric oxide in mediating the peak reactive hyperemic response is thought to be minimal (7).

In conclusion, these findings support our original hypothesis that there is a greater decline in vasodilator capacity of the calf than of the forearm with advancing age in women. This regional specificity is directionally opposite that seen in men (i.e., greater decline in peak forearm conductance vs. the calf) across the same age range when using identical techniques. Region-specific declines in forearm and calf vasodilator capacity with aging in healthy women and men could reflect gender differences in upper and lower body physical activities with advancing age. However, much of the decline in peak limb conductance with age (especially in the calf) remains unexplained. Further research will be required to determine whether the decline in lower extremity vasodilator capacity with age reported here explains diminished exercising leg hyperemia and vascular conductance in older women.

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