Sex differences with aging in nutritive skeletal muscle blood flow: impact of exercise training, nitric oxide, and α-adrenergic-mediated mechanisms

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The incidence of cardiovascular disease increases progressively with advancing age (38). Changes in vascular structure and function are major hallmarks of cardiovascular disease development that are often coincident with advanced age (37). Increased arterial stiffness and decreased vascular and endothelial function may manifest in depressed peripheral blood flow, which has been suggested to be mechanistically linked to the pathogenesis of the metabolic syndrome: a condition that increases in prevalence with advancing age (40). Specifically, depressed nutritive blood flow in the peripheral vasculature may induce adverse metabolic consequences, such as decreased cellular oxygen delivery, decreased glucose disposal, and impaired clearance of metabolic by-products.

One potential mechanism by which peripheral nutritive blood flow may be impaired is through decreased nitric oxide (NO) bioavailability, manifesting in endothelial dysfunction. Endothelial function has been found to be impaired in the lower limb of aged individuals (17, 42, 50, 52). The sympathetic nervous system also exerts control over peripheral blood flow through α-adrenergic-mediated vasoconstriction. Indeed, muscle sympathetic nerve activity (MSNA) has consistently been found to be elevated with advanced age in human populations (8, 19, 21). However, the degree to which increased sympathetic activity with aging results in altered nutritive blood flow in humans is unknown. Furthermore, sex differences in MSNA and the influence of MSNA on peripheral vascular resistance between young men and women (20) suggest that vascular responses to changes in sympathetic activity with age may not be equivalent between men and women.

Despite findings of decreased endothelial function and increased MSNA, resting blood flow in the forearm has been found to be unaltered by aging (6, 47). However, resting blood flow through the femoral artery has been found to be significantly attenuated with advanced age (7, 9, 44, 47). These findings highlight important limb-to-limb discrepancies, which are physiologically relevant as the resistance arteriole network of the lower body is the most prominent site of peripheral vascular resistance. Human studies utilizing cannulation of the femoral artery suggest that reduced lower limb blood flow...
observed in aging is associated with enhanced MSNA (7), but not with endothelial dysfunction (47). These studies have been invaluable to our understanding of blood flow regulation in aging; however, blood flow through the femoral artery supplies blood to many muscles of the leg, adipose tissue, skin, bone, and other nonmuscle tissues. Local, nutritive blood flow is the portion of the blood flow through a tissue that supplies nutrients and hormones to, and removes metabolites from, a tissue (3, 32). Nutritive blood flow is not only affected by arterial flow, arterial pressure, and capillary density, but is also influenced by tissue tortuosity and capillary permeability. The influence of NO on capillary permeability has important implications for both insulin action and blood pressure regulation (2, 18), highlighting the importance of investigating nutritive blood flow in the aged population.

There are many beneficial effects of habitual aerobic physical activity on the vasculature that may help preserve arterial health in advanced age (57). Age-associated decrements in femoral artery blood flow are not present in aged endurance-trained individuals (47). Similarly, it has recently been demonstrated that microvascular blood flow is preserved in various lower limb muscle beds of highly physically active aged men (55). Therefore, the aims of this study were 1) to determine if resting nutritive blood flow in the leg is impaired with healthy aging, 2) to determine if NO-mediated signaling or α-adrenergic-mediated vasoconstriction differentially regulate nutritive muscle blood flow in aged compared with young individuals, 3) to determine if there are any sex differences in the regulation of blood flow with aging, and 4) to determine if short-term (7 day) or long-term (8 wk) aerobic exercise training alleviates any age-associated alterations in blood flow regulation.

METHODS

Subjects. Young individuals (men: n = 8; women: n = 8) age 19–30 yr and aged individuals (men: n = 8; women: n = 8) age 56–76 yr volunteered to participate in the study. Subject characteristics are listed in Table 1. All subjects were sedentary prior to initiation of the study, as defined by participation in no more than 20 min of strenuous physical activity per week. All subjects were healthy nonsmokers, with no history of cardiopulmonary disease. Individuals taking nonsteroidal anti-inflammatory agents, serotonin reuptake inhibitors, or medications for hypercholesterolemia, hypertension, insulin resistance, or non-insulin-dependent diabetes mellitus were excluded. Aged women were not on hormone replacement therapy. Young women were studied in the follicular phase of the menstrual cycle. Subjects were instructed to avoid ingestion of antioxidant or other dietary supplements for 2 wk prior to microdialysis studies. All procedures were approved by the University and Medical Center Institutional Review Board of East Carolina University, and all subjects provided written, informed consent.

Metabolic parameters. Height was measured with a stadiometer to the nearest millimeter. Body mass was measured with a digital scale to the nearest 0.05 kg. Body mass index (BMI) was calculated as body mass in kilograms divided by height in meters squared (kg/m²). Body density was determined via hydrostatic weighing. Residual volume was measured by oxygen dilution (65). Body fat percentage was determined from body density based upon the two-compartment model (59). Blood pressure was determined with an automated blood pressure cuff placed on the right arm. Following microdialysis probe insertion, blood samples were drawn from an antecubital vein. Blood samples were allowed to clot and were centrifuged at 1,500 rpm for 10 min. Serum samples were stored at −80°C until analysis of glucose, insulin, follicle stimulating hormone (FSH), and 17-β estradiol, or 4°C until analysis of lipids within 24 h of sample collection. Serum insulin, FSH, and 17-β estradiol concentrations were determined with an immunoassay (Access Immunoassay System; Beckman Coulter, Fullerton, CA). Serum glucose concentration was determined by an oxidative reaction assay (YSI model 2300 Stat Plus, Yellow Springs Instrument, Yellow Springs, OH). The homeostatic model of insulin resistance (HOMA-IR) was calculated from fasting blood glucose and insulin concentrations as described (41). Serum total cholesterol triglycerides, high-density lipoprotein-cholesterol (HDL-C), and triglycerides were analyzed by Laboratory Corporation of America. Low-density lipoprotein-cholesterol (LDL-C) was calculated using the Friedewald equation (15).

Exercise testing and training. Maximal aerobic capacity (Vo2peak) was measured via open-circuit spirometry (True Max 2400, Parvo

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Young Men</th>
<th>Aged Men</th>
<th>Young Women</th>
<th>Aged Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25.5 ± 1.4</td>
<td>64.3 ± 2.3*</td>
<td>23.8 ± 1.0</td>
<td>65.4 ± 3.0*</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.83 ± 0.02</td>
<td>179.3 ± 1.9</td>
<td>1.66 ± 0.02†</td>
<td>1.55 ± 0.03*†</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>98.3 ± 5.7</td>
<td>85.6 ± 3.8</td>
<td>62.6 ± 2.9†</td>
<td>59.9 ± 4.1†</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29.1 ± 1.4</td>
<td>26.6 ± 1.0</td>
<td>22.7 ± 1.2</td>
<td>24.9 ± 1.4</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>23.6 ± 2.2</td>
<td>25.4 ± 2.2</td>
<td>24.5 ± 1.7</td>
<td>30.0 ± 0.6</td>
</tr>
<tr>
<td>Vo2peak, ml·kg⁻¹·min⁻¹</td>
<td>35.8 ± 2.4</td>
<td>25.9 ± 2.0*</td>
<td>35.4 ± 2.1</td>
<td>28.7 ± 2.9</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>94.0 ± 1.6</td>
<td>99.8 ± 6.5</td>
<td>88.8 ± 2.9</td>
<td>90.4 ± 2.9</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td>7.26 ± 1.60</td>
<td>10.9 ± 4.05</td>
<td>5.04 ± 0.56</td>
<td>5.06 ± 0.97</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.71 ± 0.40</td>
<td>2.92 ± 1.20</td>
<td>1.11 ± 0.14</td>
<td>1.15 ± 0.23</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>170 ± 7.4</td>
<td>211 ± 21</td>
<td>152 ± 7.9</td>
<td>197 ± 9.4*</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>143 ± 22.3</td>
<td>147 ± 27.9</td>
<td>77 ± 15.2</td>
<td>115 ± 16.2</td>
</tr>
<tr>
<td>HDL-C, mg/dl</td>
<td>40.4 ± 2.1</td>
<td>50.3 ± 5.0</td>
<td>56.6 ± 6.2†</td>
<td>67.2 ± 5.4†</td>
</tr>
<tr>
<td>LDL-C, mg/dl</td>
<td>101 ± 5.0</td>
<td>131 ± 16.6</td>
<td>80 ± 11.4</td>
<td>107 ± 9.9</td>
</tr>
<tr>
<td>TG/HDL</td>
<td>3.75 ± 0.71</td>
<td>3.20 ± 0.77</td>
<td>1.59 ± 0.56</td>
<td>1.82 ± 0.40</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>2.58 ± 0.38</td>
<td>2.77 ± 0.38</td>
<td>1.59 ± 0.41†</td>
<td>1.67 ± 0.41†</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>119 ± 1.5</td>
<td>128 ± 3.6</td>
<td>105 ± 1.9†</td>
<td>121 ± 4.5*</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>81 ± 2.4</td>
<td>73 ± 3.1</td>
<td>68 ± 2.5†</td>
<td>69 ± 2.1</td>
</tr>
<tr>
<td>FSH, mIU/l</td>
<td>3.85 ± 0.59</td>
<td>7.66 ± 2.09</td>
<td>5.08 ± 0.86</td>
<td>72.70 ± 13.6†</td>
</tr>
<tr>
<td>17-β Estradiol, pg/ml</td>
<td>37.9 ± 3.0</td>
<td>52.7 ± 4.3</td>
<td>63.1 ± 11.7</td>
<td>24.0 ± 1.6†</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index; Vo2peak, maximal aerobic capacity; HOMA-IR, homeostatic model of insulin resistance; TG, triglycerides; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; BP, blood pressure; FSH, follicle stimulating hormone. *Significant aging effect within sex (P < 0.05). †Significant sex effect within age group (P < 0.05).
Medics, Salt Lake City, UT) with an electronically braked cycle ergometer (Lode, Excalibur Sport, Groningen, the Netherlands). The test began with a 5-min warm-up period at 50 W for the aged group or 125 W for the young group, following which workload was increased 20 W for the aged group or 25 W for the young group every 2 min until volitional fatigue (15). All subjects then participated in seven consecutive days of exercise training, consisting of 60 min of cycle ergometer exercise performed at a workload that elicited a heart rate equivalent to 65% of \( \dot{V}O_{2\text{peak}} \) as determined by the initial exercise test. Seven days of exercise was chosen for the short-term training intervention to elicit training adaptations without changes in capillarity or body composition that may confound the results of longer-term training. Following the initial 7 days, subjects in the aged group continued to exercise 4 days/wk for the subsequent 7 wk. Exercise workload was routinely increased throughout the training program to maintain an exercise heart rate equivalent to 65% of \( \dot{V}O_{2\text{peak}} \). The maximal aerobic capacity test was repeated following the initial 7 days of exercise in the young and aged groups, and following the 8 wk of exercise training in the aged group.

**Microdialysis.** All subjects were studied in the morning following an overnight fast. Four microdialysis probes with a 20-kDa membrane cut-off (CMA 20 Elite, CMA Microdialysis, Solna, Sweden) were inserted into the vastus lateralis, at least 20 mm apart, under sterile techniques and local anesthesia (1% lidocaine HCl) as previously described (35). Probes were perfused with the control solution, consisting of 10 mmol/l ethanol added to 0.9% saline, with microinfusion pumps (CMA 107, CMA/Microdialysis) at a flow rate of 2.0 l/min. Probes were perfused for 60 min to allow for equilibration and recovery from trauma induced by probe insertion (see Fig. 1 for schematic of the perfusion protocol). Following the 60-min equilibration period, two 20-min samples were collected from each probe in 150 l polyethylene collection vials, which were capped and stored at 4°C for analysis of ethanol (within 48 h) for basal blood flow determination. The basal blood flows calculated for each of the four probes were averaged for analysis of basal blood flow. The perfusion media was then changed in three of the probes, where the following pharmacological agents were added to the control solution: probe 2) 13.2 mmol/l N\(^6\)-monomethyl-L-arginine citrate (L-NMMA), a NOS inhibitor; probe 3) 165 mmol/l acetylcholine (ACh); probe 4) 1 mmol/l norepinephrine, an \( \alpha \)-adrenoceptor agonist. Probes were perfused for 20 min to allow for equilibration, and two 20-min samples were collected over the subsequent 40 min. The perfusion media was then changed on two of the probes, where the following pharmacological agents were added to the control solution: in probe 3) 100 mmol/l sodium nitroprusside (SNP), an NO donor; in probe 4) 1 mmol/l phentolamine, an \( \alpha \)-adrenoceptor blocker. Probes were perfused for 20 min to allow for equilibration, and two 20-min samples were collected over the subsequent 40 min. Changes in blood flow upon pharmacological stimulation were expressed as change from control within the same probe. Microdialysis testing was conducted pretraining, following 7 days of exercise, and following 8 wk of exercise in the aged group. Posttraining studies were conducted 1 day following the final exercise bout.

**NOS activity analysis**. Skeletal muscle biopsy samples were obtained from the vastus lateralis using a percutaneous needle muscle biopsy technique (11). Biopsy samples were obtained during the first equilibration phase of microdialysis testing, from the contralateral leg from which microdialysis probes were inserted. Biopsy samples were frozen in liquid nitrogen and stored at −80°C until analysis. Frozen muscle samples were homogenized on ice with a ground glass homogenizer in homogenization buffer (15 l buffer per mg sample; Cayman Chemical, Ann Arbor, MI) containing 25 mmol/l Tris-HCl (pH = 7.4), 1 mmol/l EDTA, and 1 mmol/l EGTA. Homogenate was centrifuged at 10,000 g for 15 min at 4°C, and the supernatant was collected. Total protein content was assessed in triplicate by bicinechonic acid assay kits (Pierce Biotechnology, Rockford, IL) with bovine serum albumin as the standard. NOS activity was assessed in triplicate with a commercial NOS activity assay kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer’s instructions. This assay is based on the conversion of \([^3H]arginine to[^3H]citrulline.\) iNOS activity was assessed in triplicate utilizing the NOS activity assay kit, with each reaction conducted in the presence of 14 mmol/l 1400W hydrochloride (Cayman Chemical), a selective iNOS inhibitor. NOS activity is expressed in radioactive disintegrations per minute (dpm) normalized to the protein content of each sample. iNOS activity was calculated as NOS activity (unstimulated – NOS activity \( \times 100\)).

**Statistical analysis.** Data were presented as means ± SE. Statistical analyses were performed with GraphPad Prism (Version 6, San Diego, CA), with an alpha level of 0.05. To determine effects of aging and short-term exercise training on subject characteristics, basal blood flow, changes in blood flow to pharmacological stimulation, and NOS activity of both sexes were grouped together and statistical differences were determined by a two-way repeated-measures ANOVA (age group × 7-day training) with Sidak’s multiple comparisons post hoc analysis. To determine effects of sex and age for subject characteristics, basal blood flow, changes in blood flow to pharmacological stimulation, and NOS activity, subjects were separated into four groups (young men, aged men, young women, and aged women), and statistical differences were determined by a two-way repeated-measures ANOVA (sex × training) was performed including pretraining, 7-day, and 8-wk time points, where statistical differences were determined by Sidak’s multiple comparisons post hoc analysis.

**RESULTS**

**Subject characteristics.** Subject characteristics are listed in Table 1. Aged individuals were ~40 yr older than young individuals. There were no significant age or sex differences in...
body fat percentage, although there was a trend of increased body fat percentage of aged women compared with young women \((P = 0.065)\). There were no significant age-group differences for fasting serum glucose \((P = 0.337)\) or insulin \((P = 0.407)\) concentration, or HOMA-IR \((P = 0.329)\). Total cholesterol \((P = 0.007)\) and LDL-C \((P = 0.030)\) were elevated in aged compared with young individuals, although there were no differences when age groups were compared within a sex (total cholesterol: \(P = 0.159\) aged vs. young men, \(P = 0.257\) aged vs. young women; LDL-C: \(P = 0.239\) men vs. young men, \(P = 0.536\) aged vs. young women). However, there was a trend for increased HDL-C in aged compared with young individuals \((P = 0.077)\). There were no age-group differences for triglycerides \((P = 0.507)\), triglyceride:HDL-C ratio \((P = 0.749)\), or LDL-C: HDL-C ratio \((P = 0.702)\). There was no difference in FSH in aged compared with young men \((P = 0.087)\), while there was a large elevation in FSH in aged vs. young women \((P < 0.001)\). 17-β Estradiol was decreased in aged vs. young women \((P = 0.003)\) and aged women vs. aged men \((P = 0.046)\).

There were no exercise training-induced changes in body weight. \(\text{VO}_{2}\text{peak}\) increased following seven consecutive days of exercise training in the young women \((P = 0.044)\); however, \(\text{VO}_{2}\text{peak}\) did not change in young men \((P = 0.312)\), aged men \((P = 0.999)\), or aged women \((P = 0.975)\). There were no significant changes in blood pressure induced by exercise training in either age group. There were no significant exercise training-induced changes in serum glucose or insulin concentration in either age group, although there was a trend \((P = 0.074)\) for a reduction in insulin following 8 wk of exercise training in aged individuals. There were no exercise-induced changes in FSH or estrogen in any group, although there was a trend for increased estrogen in the young women following 7 days of exercise \((63.1 \pm 11.7\) vs. \(103 \pm 24\) pg/ml; \(P = 0.075)\).

**NUTRITIVE MUSCLE BLOOD FLOW IN AGING**

**Fig. 2.** Effect of aging, sex, and exercise training on nutritive blood flow. Microvascular nutritive blood flow assessed in the vastus lateralis of young and aged men \((n = 8\) per age group) and women \((n = 8\) per age group) before exercise training (Pre), and following seven consecutive days (7 day), or eight wk (8 wk; aged only) of aerobic exercise training. Seven days of exercise increased microvascular blood flow only in the young men. \(*P < 0.05\) vs. pretraining. \(†\)Significant difference between aged women and young women within each time point \((P < 0.05)\).

There was a significant increase in basal nutritive blood flow following 7 days of exercise training in the young \((P = 0.019)\) that was not observed in the aged \((P = 0.610)\). Post hoc analysis indicates that the increase in nutritive blood flow was due to an increase in young men \((P = 0.005)\) rather than young women \((P = 0.798)\). Eight weeks of exercise training did not change basal nutritive blood flow in aged men \((P = 0.955)\) or aged women \((P = 0.185)\).

**NO-mediated blood flow regulation.** The contribution of NO to basal nutritive blood flow was assessed by perfusion of the NOS inhibitor l-NMMA through the microdialysis probe (Fig. 3A). There were no significant pretraining differences between age groups in nutritive blood flow response to l-NMMA \((P = 0.997)\), nor were there any significant differences associated with exercise training (young: \(P = 0.918\); aged: \(P = 0.918)\). Microvascular endothelial function was assessed with ACh perfusion (Fig. 3B). There were no significant pretraining age-group differences \((P = 0.345)\) or training-induced differences in ACh-stimulated blood flow (young: \(P = 0.776\); aged: \(P = 0.168)\). Endothelium-independent dilation was assessed with SNP perfusion (Fig. 3C). There were no significant pretraining age-group differences \((P = 0.923)\) or training-induced differences for SNP-stimulated blood flow (young: \(P = 0.574\); aged: \(P = 0.574)\). Eight weeks of exercise did not significantly alter the effect of l-NMMA (men: \(P = 0.653\); women: \(P = 0.543)\), ACh (men: \(P = 0.633\); women: \(P = 0.161)\), or SNP (men: \(P = 0.825\); women: \(P = 0.216)\) on blood flow in the aged group (Fig. 3). There were no significant sex differences or sex effects on the training response to l-NMMA, ACh, or SNP.

**α-Adrenergic-mediated blood flow regulation.** α-Adrenergic-mediated regulation of blood flow was assessed by perfusion of norepinephrine (Fig. 4A) and phentolamine (Fig. 4B) through the microdialysis probe. There were no significant pretraining age differences in men \((P = 0.997)\) or women \((P = 0.692)\), nor any training-induced differences in the blood flow response to norepinephrine (young men: \(P = 0.964\); aged men: \(P = 0.515\); young women: \(P = 0.915\); aged women: \(P = 0.946)\). When all men and all women were grouped together, there was a significant sex effect \((P = 0.033)\), indicating a greater vasoconstrictor effect of norepinephrine perfusion on nutritive blood flow in men than women. There were no pretraining age-group differences in the blood flow response to α-adrenergic antagonism with phentolamine in men \((P = 0.341)\) or women \((P = 0.743)\). However, 7 days of exercise training produced a significant reduction in the vasodilatory effect of phentolamine on blood flow in young men \((P = 0.048)\) that was not observed in aged men \((P = 0.827)\), young women \((P = 0.733)\), or aged women \((P = 0.919)\). Eight weeks of exercise in the aged individuals did not significantly alter the effect of norepinephrine (aged men: \(P = 0.642\); aged women: \(P = 0.326\) or phentolamine (aged men: \(P = 0.714\); aged women: \(P = 0.887)\) on microvascular blood flow.

**Skeletal muscle total NOS and iNOS activity.** Analysis of skeletal muscle total NOS activity (Fig. 5A) revealed no significant pretraining differences between age groups (men: \(P = 0.998\); women: \(P = 0.773)\). Likewise, there were no significant pretraining age-group differences in iNOS activity (Fig. 5B; men: \(P = 0.604\); women: \(P = 0.525)\). There was a significant main effect of 7 days of exercise training across both age groups for increased total NOS activity \((P = 0.046)\) and iNOS.
activity ($P = 0.015$). Upon sex analysis of training effects, there was a significant increase in NOS activity in young men following 7 days of exercise training ($P = 0.028$). However, it is likely that some of the increase in NOS activity is derived from iNOS, as mean iNOS increased 197% in young men, despite lack of statistical significance ($P = 0.250$). Seven days of exercise training also elicited an increase in iNOS activity in aged women ($P = 0.046$). Eight weeks of exercise training of aged individuals did not increase total NOS activity (aged men: $P = 0.738$; aged women: $P = 0.691$) or iNOS activity (aged men: $P = 0.055$; aged women: $P = 0.963$) from pretraining levels, although there was a trend for increased iNOS activity in aged men.

**DISCUSSION**

The data from this study demonstrate that resting nutritive blood flow in the vastus lateralis is not altered in a group of healthy aged men relative to young men but is attenuated in a group of healthy aged women relative to young women. There were no apparent age-associated deficiencies in endothelial function, NOS activity, or the contribution of NO to nutritive blood flow, and there was no apparent alteration in the contribution of $\alpha$-adrenergic-mediated vasoconstriction to nutritive muscle blood flow with aging. Seven consecutive days of aerobic exercise augmented resting nutritive blood flow in young men, but not young women or aged individuals. These differences appear to be due to an exercise training-induced reduction in $\alpha$-adrenergic-mediated vasoconstriction that occurs only in the young men. Eight weeks of exercise training was not sufficient to augment resting nutritive blood flow in the aged. This is the first study to investigate nutritive blood flow in healthy, sedentary aged men and women, which indicates...
In contrast to the men, nutritive blood flow was attenuated in aged women compared with young women. These results are in line with our previous findings (24). Credeur et al. (4) have recently observed no difference in femoral artery blood flow between young and postmenopausal women; however, Moreau et al. (44) have previously found that femoral artery blood flow is impaired in postmenopausal women compared with young women: a finding that remained when normalizing to leg fat-free mass. Interestingly, they also investigated a group of postmenopausal women taking hormone replacement therapy (HRT). HRT partially reversed the impairment in femoral artery blood flow, and completely restored blood flow compared with young women when normalized to leg fat-free mass (44). Serum estrogen concentrations were depressed in the aged women relative to young women studied in the follicular phase of the menstrual cycle in the present study. These findings implicate a potential protective role of estrogen on maintenance of microvascular blood flow. The effect of estrogen on the vasculature are well described (51). Estrogen has previously been found to directly induce vasodilation via NO (5). Given that there were no differences between young and aged women in NOS activity or ACh-stimulated nutritive blood flow in the present study, it is unlikely that the age-associated decrement in nutritive blood flow was mediated strictly by an NO-mediated mechanism. Aside from the NO pathway, estrogen may have other vasodilatory effects such as promotion of prostacyclin production, inhibition of endothelin-1 production, and inhibition of Ca\(^{2+}\) influx into vascular smooth muscle cells (51). In addition, estrogen may stimulate \(\beta\)-adrenergic-mediated vasodilation (12) and may influence vascular reactivity via the Rho kinase and protein kinase C pathways (39). Investigation of these potential effects of estrogen on nutritive blood flow was outside of the scope of this study. Interestingly, serum estrogen concentrations of aged men were increased compared with aged women, which may have played a role in the maintenance of nutritive blood flow in aged men.

**NO-mediated blood flow regulation.** Impaired NO-mediated vasodilation in the lower limb of aged human subjects has been demonstrated in several studies (17, 42, 45, 50, 52, 53, 63). Vascular conductance of the lower limb upon passive leg movement is attenuated in aged men relative to young men (17, 42). Mortensen et al. (45) recently coupled the passive leg movement technique with femoral artery L-NMMA infusion to demonstrate that the impaired passive leg movement-induced vascular conductance with advanced age is indeed NO-dependent. In addition, flow-mediated dilation of the popliteal artery is impaired in aged men (50) and women (52, 53). In contrast to these reports, aged men have demonstrated preserved lower limb endothelial function assessed by femoral artery infusion of ACh (47) or flow-mediated dilation of the deep and superficial femoral arteries (68). The present study demonstrates a preserved ACh-stimulated vasodilatory response and an equivalent vasodilatory response to an NO donor (SNP) in aged compared with young individuals, indicating preservation of endothelial function with coincident preserved NOS activity levels. Additionally, NOS inhibition with L-NMMA had similar effects on nutritive blood flow between age groups, which is consistent with the previous observation of similar total resting hindlimb vascular conductance and responses to NOS inhibition between young and old rats (28). Collectively, the

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**Fig. 5.** Effect of aging, sex, and exercise training on total nitric oxide synthase (NOS) and inducible NOS (iNOS) activity. Activity of total NOS (A) and iNOS (B) in skeletal muscle homogenates from the vastus lateralis of young and aged men (n = 8 per age group) and women (n = 8 per age group) before exercise training (Pre), and following seven consecutive days (7 day), or 8 wk (aged only) of aerobic exercise training. *Significant effect of exercise training (P < 0.05). †Young women significantly different from young men following 7 days of exercise training (P < 0.05).

that a healthy aging-associated decrement in nutritive blood flow is specific to women.

**Lower limb blood flow with aging.** Previous investigations indicate that total lower limb blood flow through the femoral artery is attenuated in aged men relative to young men (7, 9, 45). In contrast, the results of the present study suggest that nutritive blood flow in the vastus lateralis is not different between healthy aged and young men. Donato et al. (10) found that total lower limb blood flow was depressed 26% in aged compared with young men, but was the same when expressed as blood flow per kilogram of quadriceps muscle mass. Together, these findings suggest that aging alone does not impair muscle nutritive blood flow in healthy aged men. Loss of muscle mass is well documented with aging. Thus loss of skeletal muscle could contribute substantially to the decrement in total limb blood flow observed in aged men, as thigh muscle mass correlates with resting leg blood flow (14). In addition, total limb blood flow incorporates blood flow to skin, bone, adipose tissue, connective tissues, and all muscle beds, comprising muscles of predominantly oxidative, glycolytic, or mixed fiber type composition. Considering all of the organs that determine total limb blood flow may be critical, as blood flow to bone in the lower limb has been found to be attenuated in aged human subjects (36).
results from the present study suggest that NO-mediated nutritive blood flow is not impaired in healthy aged men or women.

**α-Adrenergic-mediated blood flow regulation.** MSNA has consistently been found to be elevated with advanced aging in human populations (8, 19, 21). One manifestation of enhanced MSNA is a chronically elevated α-adrenergic-mediated vasoconstrictor state (58). Indeed, α-adrenergic blockade with phentolamine restores the attenuated leg blood flow in aged individuals, although a vasodilatory effect of similar magnitude was observed in the young (9). Despite enhanced MSNA in the aged, further sympathetic stimulation tends to have a reduced vasoconstrictor effect in aged compared with young individuals (53, 67). Parker et al. (53) observed reduced sympathetic vasoconstrictor activity upon a vasodilatory stimulus of the popliteal artery in aged vs. young women, while Wray et al. (67) showed that aged men vasoconstrict less than young men upon α1-agonism of the femoral artery. No preexercise training age-associated alterations of α-adrenergic-mediated nutritive muscle blood flow were observed in the present study. Norepinephrine perfusion appeared to increase blood flow in aged women, which is counterintuitive as norepinephrine is a vasoconstrictor. However, postmenopausal women have previously demonstrated an increase in blood flow upon varying concentrations of norepinephrine infusion; thus this finding is not without precedent (19). Women have previously been found to have a reduced α-adrenergic receptor sensitivity to norepinephrine (33). Accordingly, norepinephrine had a greater vasoconstrictor effect in men than women in this study when all ages were grouped together. Previous studies suggest that lower limb blood flow is influenced by MSNA in men more than women (29, 70). This study provides evidence that men have a greater vasoconstrictor response to norepinephrine in the skeletal muscle microvasculature than women, suggesting that nutritive skeletal muscle blood flow is under increased α-adrenergic control in men than women.

**Exercise training.** Cross-sectional studies reveal that aged endurance athletes do not exhibit age-associated lower limb endothelial dysfunction (47, 55). Given that microvascular endothelial function was not impaired in the vastus lateralis of the aged subjects, it is not surprising that exercise training did not further augment endothelial function in these individuals. Likewise, it was not surprising that endothelium-independent dilation was not affected by exercise training, as the majority of studies demonstrate that SNP-stimulated vasodilation is not altered by exercise training (6, 34, 47). In contrast to the young men, nutritive blood flow did not increase following exercise training in the young women or aged men or women. Although young men demonstrated an increase in total NO activity following 7 days of exercise, it appears that much of that effect is derived by iNOS. In addition, Ach-stimulated nutritive blood flow did not increase in young men following 7 days of exercise. Thus it is unlikely that the increase in nutritive blood flow in young men is due to an NO-mediated mechanism. More likely, however, is a differential α-adrenergic response to exercise training between young men and the remaining groups. The vasodilatory effect of α-blockade with phentolamine is significantly reduced in young men following training, while there was no change in the effectiveness of phentolamine in response to training in the women or aged men. Collectively, these findings suggest that exercise training induces an alleviation of α-adrenergic-mediated vasconstriction in young men that is not induced in women or aged men. A differential α-adrenergic response to exercise between young and aged men is in line with previous studies that demonstrate exercise training decreases plasma norepinephrine levels concomitant with decreased peripheral vascular resistance in young men, while older endurance athletes actually demonstrate increased MSNA relative to older sedentary individuals (30, 43, 49). Interestingly, 12 wk of exercise training has been shown to increase sympathetic-mediated tone of the femoral artery in aged individuals (62). In addition, prior studies indicate that vascular tone is regulated by sympathetic activity in men more than women (20, 19, 21). Thus it is conceivable that exercise could induce a decrement in sympathetic activity in the young that specifically affects vascular tone regulation in young men.

**iNOS activity.** Elevated skeletal muscle iNOS expression has recently been reported to be a contributing factor to advanced age-associated insulin resistance (54). Aging was not associated with elevated iNOS activity in the present study, although the aged individuals did not demonstrate any impairment in glucose or insulin homeostasis. Interestingly, iNOS activity increased following 7 days of exercise training in aged women. Aerobic exercise training has previously been shown to up-regulate iNOS expression in the aorta (69), cardiac muscle (1), and skeletal muscle (16). Although iNOS activity is associated with inflammation and generally deemed deleterious (31), exercise-induced iNOS has been shown to yield beneficial effects such as cardioprotection against ischemia-reperfusion injury (1) and suppression of neutrophil apoptosis (61). In addition, iNOS inhibition blunts the exercise training-induced suppression of α1-adrenergic mediated vasoconstriction in young rats (69). Thus we cannot be certain that the observed effect of exercise on α-adrenergic control of nutritive blood flow in young men is not mediated by an iNOS-mediated mechanism.

**Experimental considerations.** There were several limitations to the present study. We chose to study a metabolically healthy aged group. As these aged subjects had comparable metabolic profiles and body composition to the younger group, and were healthy enough to not require chronic medication, the results of this study may not be reflective of the aged population as a whole. Even though all subjects were sedentary at the onset of the study, by recruiting subjects for an 8-wk exercise intervention we may have artificially selected an exceptionally healthy aged cohort, as all subjects were willing to participate in such an intensive intervention. Thus we cannot extend our conclusions to an aged population with the metabolic syndrome or a metabolically healthy elderly population over the age of 80. Additionally, with a sample size of eight in each group and multiple training time points, potential significant differences could have been masked by low statistical power and the necessity of multiple comparisons analysis. For example, nutritive blood flow was the lowest in aged women, where there was a nonsignificant increase following 8 wk of exercise. Power analyses indicate that a doubled sample size is required to achieve statistical significance in this instance. Furthermore, we studied the vastus lateralis alone; therefore, our results are only reflective of a mixed fiber type muscle bed and do not rule out the possibility that vascular abnormalities exist in highly oxidative muscle beds of healthy aged individuals, as has been demonstrated in rodent models (46, 66). As norepinephrine and phentolamine are nonselective α-adrenergic agonists and an-
tagonists, respectively, as these results do not preclude a possible differential effect of aging on α1- or α2-adrenergic-mediated blood flow regulation. In addition, nonselective α-adrenergic blockade may induce β-mediated vasodilation (56); thus potential differences in α-mediated blood flow regulation may be masked by potential differences in β-mediated vasodilation due to our failure to inhibit β-receptors during α-receptor stimulation or antagonism.

Conclusions. In conclusion, this study demonstrated that the impairment in resting nutritive blood flow in the vastus lateralis associated with advanced age is specific to women. These findings suggest that in the absence of the metabolic syndrome, the stimulus of aging is not alone sufficient to induce disturbances of nutritive blood flow in men. There were no pretraining age-associated impairments in NO-mediated or α-adrenergic-mediated nutritive blood flow regulation in any age or sex group. Thus the decrement in nutritive blood flow in aged women cannot be ascribed to NO-mediated or α-adrenergic-mediated mechanisms. However, the inability of the aged men to upregulate nutritive blood flow following exercise training, as demonstrated in young men, is suggestive of an age-specific inability to reduce sympathetic-mediated vascular tone with exercise training.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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