Effects of aging and exercise training on skeletal muscle blood flow and resistance artery morphology

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Submitted 21 August 2012; accepted in final form 1 October 2012

Effects of aging and exercise training on skeletal muscle blood flow and resistance artery morphology. J Appl Physiol 113: 1699–1708, 2012. First published October 4, 2012; doi:10.1152/japplphysiol.01025.2012.—With old age, blood flow to the high-oxidative red skeletal muscle is reduced and blood flow to the low-oxidative white muscle is elevated during exercise. Changes in the number of feed arteries perforating the muscle are thought to contribute to this altered hyperemic response during exercise. We tested the hypothesis that exercise training would ameliorate age-related differences in blood flow during exercise and feed artery structure in skeletal muscle. Young (6-7 mo old, n = 36) and old (24 mo old, n = 25) male Fischer 344 rats were divided into young sedentary (Sed), old Sed, young exercise-trained (ET), and old ET groups, where training consisted of 10-12 wk of treadmill exercise. In Sed and ET rats, blood flow to the red and white portions of the gastrocnemius muscle (GastRed and GastWhite) and the number and luminal cross-sectional area (CSA) of all feed arteries perforating the muscle were measured at rest and during exercise. In the old ET group, blood flow was greater to GastRed (264 ± 13 and 195 ± 9 ml/min·100 g−1 in old ET and old Sed, respectively) and lower to GastWhite (78 ± 5 and 120 ± 6 ml/min·100 g−1 in old ET and old Sed, respectively) than in the old Sed group. There was no difference in the number of feed arteries between the old ET and old Sed group, although the CSA of feed arteries from old ET rats was larger. In young ET rats, there was an increase in the number of feed arteries perforating the muscle. Exercise training mitigated old age-associated differences in blood flow during exercise within gastrocnemius muscle. However, training-induced adaptations in resistance artery morphology differed between young (increase in feed artery number) and old (increase in artery CSA) animals. The altered blood flow pattern induced by exercise training with old age would improve the local matching of O2 delivery to consumption within the skeletal muscle. Aging; skeletal muscle; blood flow; oxygen delivery; resistance artery perfusion of the high-oxidative red portion (GastRed) and overperfusion of the low-oxidative white portion (GastWhite) in old rats during exercise (44) due, in part, to old age-associated arterial rarefaction (8) and a diminished nitric oxide (NO) contribution to vasomotor control in highly oxidative muscle during exercise (28). Therefore, aging disrupts the normally tight coupling between O2 delivery and the oxidative capacity of muscle during exercise. The altered blood flow pattern during exercise cannot be explained by differences in relative workloads (44) but is likely due to fiber type-specific alterations in vascular function (40, 41) and changes in resistance artery geometry (8) that occur with advancing age.

Exercise training induces peripheral vascular alterations that result in enhanced blood flow capacity due, in part, to intrinsic changes in the vascular endothelium (13, 17, 62–64). Furthermore, in young animals, endurance training alters the perfusion patterns among and within skeletal muscle during exercise (2), with high-oxidative motor units receiving a higher blood flow and low-oxidative muscles receiving a lower blood flow than in sedentary (i.e., untrained) rats. Given the similarities of skeletal muscle blood flow patterns during exercise in the sedentary vs. trained condition to that of the old vs. young, it is possible that exercise training normalizes exercising blood flow distribution in skeletal muscle with old age. In aged humans, endurance training enhances aerobic capacity (3, 9) and is associated with a substantial increase in exercising leg peak blood flow (4). Furthermore, endurance exercise training improves the matching of O2 delivery to O2 utilization in young (27) and aged subjects (38, 43). Whether this improved matching is due to functional vascular adaptations or involves structural alterations of resistance arteries is unknown.

Therefore, the purpose of this investigation was to test the hypotheses that 1) the pattern of blood flow distribution for greater O2 delivery to low-oxidative muscle and lower perfusion of high-oxidative muscle with old age can be reversed with chronic aerobic exercise training and 2) endurance exercise training can increase the number of feed arteries perforating the gastrocnemius muscle, counteracting the arterial rarefaction observed with old age (8). To test these hypotheses, two studies were undertaken in young and old, sedentary and endurance-trained animals. The first study, which measured blood flow at rest and during exercise in the different portions of the gastrocnemius muscle, i.e., high-oxidative red (GastRed: 51% type I, 35% type IIA, and 13% type IIX fibers), mixed (Gastmixed: 3% type I, 6% type IIA, 34% type IIX, and 57% type IIB fibers), and low-oxidative white (GastWhite: 8% type IIX and 92% type IIB fibers) portions (14), demonstrated the less precise matching of O2 delivery to muscle oxidative

AGING IS ASSOCIATED WITH a decline in maximal aerobic and exercise capacity (32, 46, 49, 54), resulting in a reduced capability for regular physical activity, which increases the risk of chronic disease. Age-associated alterations in exercising muscle blood flow and the resultant mismatching of O2 delivery to O2 consumption (6, 7, 11, 26) represent a key mechanism for this age-related functional decline. During submaximal exercise, blood flow to the working limbs is reduced in old vs. young subjects (4, 20, 37, 55, 67) and distribution of blood flow is altered among and within skeletal muscles (44). For example, in the gastrocnemius muscle, there is relative under...
capacity with old age during exercise (44). The second study quantified the number and cross-sectional area (CSA) of all feed arteries perforating Gast\textsubscript{Red}, Gast\textsubscript{Mixed}, and Gast\textsubscript{White}.

**METHODS**

All procedures were approved by the Institutional Animal Care and Use Committees at West Virginia University, Texas A & M University, and the University of Florida. All methods complied fully with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (revised 1996). Male Fischer 344 rats were obtained at 4–5 and 22 mo of age to represent young (n = 36) and old (n = 25) animals, respectively. The rats were housed individually at 23°C and maintained on a 12:12-h light-dark cycle, with rat chow and water provided ad libitum. This strain was chosen because cardiovascular function decreases with age without the development of hypertension (33).

*Endurance exercise training.* Young and old rats were randomly assigned to a sedentary (Sed) control group [young Sed (n = 20) and old Sed (n = 14)] and an exercise-trained (ET) group [young ET (n = 16) and old ET (n = 11)]. ET rats were habituated to treadmill exercise, during which each rat walked on a motor-driven treadmill at 15 m/min (0° incline) 5 min/day for 3 days. After the habituation period, the incline was raised to 15° for the duration of the training period while the 15 m/min speed was maintained. During the first 5 wk of training, the time of exercise was increased by 10 min/wk, until 60-min duration was reached by the 6th week. The ET rats continued to exercise 5 days/wk for 60 min/day for the remainder of the 10- to 12-wk training period.

*Study 1: blood flow at rest and during exercise.* Blood flow to the gastrocnemius muscle in young Sed (n = 13), young ET (n = 9), old Sed (n = 7), and old ET (n = 5) animals at rest and during exercise was determined using the radionuclide-tagged microsphere technique, as previously described (12, 34, 44). Prior to the surgical procedure, Sed animals were familiarized with treadmill running. During the familiarization period (<2 wk), animals exercised 10 min/day at 15 m/min at a 15° incline two to three times per week. At ≥24 h after the last exercise bout during the familiarization or training period, animals were anesthetized with 2.5% isoflurane-balance O\textsubscript{2}, and a catheter (Silastic, Dow Corning; 0.6 mm ID, 1.0 mm OD) was advanced past the mammary arteries into the ascending aorta via the right carotid artery. This catheter was used to infuse radiolabeled microspheres for tissue blood flow measurements and to monitor mean arterial pressure. The carotid catheter was externalized at the base of the neck and secured to the skin between the shoulder blades. A second polyurethane catheter (Braintree Scientific; 0.36 mm ID, 0.84 mm OD) was implanted in the caudal tail artery and externalized at the tail. This catheter was used to obtain a reference blood sample, which serves as an artificial organ for calculating tissue flows. After closure of the incisions, the animals were given ≥4 h to recover, as previous studies demonstrated that circulatory dynamics, regional blood flow, arterial blood gases, and acid-base status are stable in the awake rat 1–6 h after gas anesthesia (24).

After the recovery period, the rat was placed on the treadmill, and the tail artery catheter was connected to a 1-ml plastic syringe that was connected to an infusion-withdrawal pump (model 907, Harvard, Cambridge, MA), and the carotid artery catheter was connected to a blood pressure transducer (model MLT844, ADInstruments, Colorado Springs, CO). Exercise was initiated (15 m/min) at a 0° incline to ensure that all animals ran continuously during the testing period without changing gait patterns (e.g., stopping and starting running on the treadmill). After 4 min of total exercise time, blood withdrawal from the caudal artery at 0.25 ml/min was begun. The right carotid artery catheter was disconnected from the pressure transducer, and a distinct radiolabeled (\textsuperscript{46}Sc, \textsuperscript{113}Sn, \textsuperscript{57}Co, or \textsuperscript{85}Sr) microsphere (15 μm diameter; DuPont/NEN, Boston, MA) was infused (~2.5 × 10\textsuperscript{5} in number) into the ascending aorta, which was flushed with warmed saline to ensure clearance of the beads. Blood withdrawal from the caudal artery continued for 45 s after microsphere infusion. After a 30-min recovery period, a second microsphere infusion was performed in conscious, standing animals following the procedures described above. This strategy was utilized to minimize the preexercise anticipatory response (1) and facilitates an accurate “resting” measurement. After the microsphere infusion, animals were euthanized with pentobarbital sodium (>100 mg/kg ip). The gastrocnemius muscle and kidneys were removed, and the gastrocnemius muscle was sectioned into the Gast\textsubscript{Red}, Gast\textsubscript{Mixed}, and Gast\textsubscript{White} portions (14). The radioactivity level of the tissues was determined by a gamma scintillation counter (Cobra II Auto Gamma Counter, Packard, Downers Grove, IL) set to record the peak energy activity of each isotope for 1–5 min. Total blood flow to each tissue was calculated using the reference sample method (34, 44) and expressed in milliliters per minute per 100 g of tissue. To account for possible changes in perfusion due to alterations in arterial pressure, vascular conductance was calculated (i.e., blood flow/mean arterial pressure) and expressed in milliliters per minute per 100 g of tissue per mmHg. Only data for animals that displayed adequate mixing of the microspheres, as verified by a <20% difference in blood flow between the right and left kidneys, were analyzed and reported.

*Study 2: vascular morphology.* The number of feed arteries perforating the gastrocnemius muscle and the vessel CSA (i.e., πr\textsuperscript{2}, where r is radius) were determined in young Sed (n = 7), young ET (n = 7), old Sed (n = 7), and old ET (n = 6) animals. After the animals were euthanized with pentobarbital sodium (>100 mg/kg ip), the plantaris-gastrocnemius muscle group was carefully dissected free and placed in a 4°C filtered Ca\textsuperscript{2+}-free physiological saline solution buffer (in mM: 147 NaCl, 4.7 KCl, 1.2 NaH\textsubscript{2}PO\textsubscript{4}, 1.17 MgSO\textsubscript{4}, 5.0 glucose, 2.0 pyruvate, and 3.0 MOPS, pH 7.4). The feed arteries perforating the Gast\textsubscript{Red}, Gast\textsubscript{Mixed}, and Gast\textsubscript{White} were identified and isolated using a stereomicroscope, removed from the muscle, and transferred to a Lucite chamber. Subsequently, both ends of the feed artery were cannulated with glass micropipettes filled with the filtered Ca\textsuperscript{2+}-free physiological saline solution and secured to the pipettes with 11-0 ophthalmic suture. After cannulation, each vessel in the tissue chamber was transferred to the stage of an inverted microscope (model IX70, Olympus) coupled to a video camera (model BP310, Panasonic) and video-capture software (Microcirculation Research Institute, Texas A & M University). Intraluminal pressure in the isolated artery was set at 75 cmH\textsubscript{2}O, and 10\textsuperscript{−4} M sodium nitroprusside was added to the vessel chamber to prevent development of spontaneous tone and ensure maximal dilation.Leaks were detected by pressurizing the vessel and then closing the valves to the reservoirs and verifying that intraluminal diameter remained constant. Vessels free of leaks equilibrated for ≥1 h at 37°C, with the bathing solution replaced every 20 min during this period, before maximal diameter was measured for calculation of CSA.

*Muscle oxidative enzyme activity.* Citrate synthase, a mitochondrial enzyme and marker of muscle oxidative potential, was measured in duplicate from Gast\textsubscript{White} muscle homogenates, according to the method of Srere (65). Citrate synthase activity, expressed as micro- moles per minute per gram wet weight, was measured spectrophotometrically using a Spectramax M5 microplate (Molecular Devices, Sunnyvale, CA) in 300-μl aliquots at 30°C to determine the efficacy of the training protocol.

*Statistical analysis.* One-way ANOVA was used to determine differences in the number and CSA of feed arteries, body and muscle mass, and citrate synthase activity. A two-way ANOVA with repeated-measures design was used to compare within-group (resting and exercising) and between-group (young vs. old and Sed vs ET) differences in arterial pressure, blood flow, and vascular conductance. When a significant F ratio was demonstrated, a Student-Newman-Keuls post hoc test was performed to determine differences between mean values. Where established literature values clearly justified a directional hypothesis [i.e., augmented flow to Gast\textsubscript{Red} and dimin-
ished flow to \( \text{GastWhite} \) after training (2), a one-tailed test was used. All values are means ± SE. \( P < 0.05 \) was required for significance.

RESULTS

Body mass, muscle mass, citrate synthase activity, and arterial pressure. Body mass, gastrocnemius mass, gastrocnemius-to-body weight ratio, and mean arterial pressure are described in Table 1. The efficacy of the training program was confirmed with an increase of \( \sim 40\% \) in citrate synthase activity of the \( \text{GastWhite} \) in young and old groups (Table 1).

Blood flow at rest and during exercise. There were no differences in blood flow to any portions of the gastrocnemius muscle among groups at rest (Fig. 1). Exercising hyperemia was lower in the \( \text{GastRed} \) (Fig. 2A) and higher in the \( \text{GastWhite} \) (Fig. 2C) of old Sed than young Sed rats. Vascular conductance during exercise paralleled blood flow (Fig. 3). Blood flow (Fig. 2B) and vascular conductance (Fig. 3B) during exercise were also higher in the \( \text{GastMixed} \) of old Sed than young Sed animals. Exercise training of old rats resulted in an \( \sim 36\% \) increase in blood flow to the \( \text{GastRed} \) and an \( \sim 35\% \) decrease in perfusion to the \( \text{GastWhite} \) (Figs. 2 and 4). In the young group, there was a lower flow (\( \sim 20\% \) decrease) to the \( \text{GastWhite} \) after training. Although there was \( \sim 15\% \) higher absolute exercising blood flow to the \( \text{GastRed} \) in young ET than young Sed animals (Fig. 4), it did not reach statistical significance (\( P = 0.055 \)). Exercise training abolished age-associated differences in blood flow to the \( \text{GastWhite} \) (Fig. 2C). Exercise training did not affect exercising blood flow to the \( \text{GastMixed} \) within age groups (Fig. 4).

Vascular morphology. There were 1.3 fewer total feed arteries perforated the gastrocnemius muscle of old Sed than young Sed rats (Fig. 5), with fewer arteries perforating the highly oxidative \( \text{GastRed} \) and \( \text{GastMixed} \) regions (Fig. 6B). Exercise training increased the total number of feed arteries perforating the gastrocnemius muscle in young ET vs. young Sed rats. Conversely, there was no change in the total number of arteries perforating the gastrocnemius muscle of the old ET rats (Fig. 5), resulting in an increased disparity in the number of resistance arteries between the two age groups.

Average CSA of feed arteries was greater in the \( \text{GastRed} \) and \( \text{GastWhite} \) in old than young Sed rats (Fig. 6, A and C). In old rats, exercise training increased the average CSA of feed arteries from the \( \text{GastRed} \) and \( \text{GastWhite} \) by 20\% and 35\%, respectively, relative to that in old Sed animals (Fig. 6, A and C). In the young group, exercise training did not result in an increase in CSA in arteries perfusing any muscle section, although there was a tendency (\( P = 0.08 \)) for an increase in the \( \text{GastRed} \).

DISCUSSION

With advancing age, there is a redistribution of skeletal muscle blood flow during exercise, such that high-oxidative regions of a muscle (e.g., \( \text{GastRed} \)) are relatively underperfusion and low-oxidative portions (e.g., \( \text{GastWhite} \)) are relatively overperfused vs. younger animals (44). In addition to alterations in vasomotor function (13, 17, 18, 40, 41, 62–64), an old-age-associated arterial rarefaction in high-oxidative muscles is thought to be a key mechanism for this redistribution of blood flow during exercise (8). Therefore, the primary purpose of this investigation was to determine whether endurance training could mitigate the old age-associated redistribution of skeletal muscle blood flow during exercise. The data demonstrate that exercise training in old animals enhances the matching of \( O_2 \) delivery to muscle oxidative capacity by increasing vascular conductance and blood flow to the \( \text{GastRed} \) and decreasing vascular conductance and perfusion of the \( \text{GastWhite} \) with no change in the \( \text{GastMixed} \) (Figs. 2 and 3). The secondary purpose of this study was to investigate how exercise training affected the number and CSA of feed arteries perforating the gastrocnemius muscle. In young rats, there was an increase in the total number of arteries perforating the gastrocnemius muscle but no change in average CSA. Conversely, with old age, there was no increase in the number of perforating arterial vessels following training but an increase in feed artery CSA (Fig. 5). The training effect in the young rats to increase feed artery number occurred in the \( \text{GastMixed} \), while the training effect to increase CSA in feed arteries from the old rats occurred in the \( \text{GastRed} \) and \( \text{GastWhite} \).

Muscle blood flow with aging and exercise training. Skeletal muscle blood flow capacity was reduced with old age (20, 37, 56, 67). During submaximal exercise, leg blood flow is lower in sedentary older adults than younger individuals (4, 52). Even when whole limb blood flow during submaximal exercise is similar between young and old rats, the distribution profile within and among muscles is affected by age. Specifically, Musch et al. (44) demonstrated a significant blood flow reduction in high-oxidative muscles and muscle sections concomitant with an increased flow in the low-oxidative glycolytic muscles during submaximal exercise. Therefore, the available evidence demonstrates not only a reduction in limb blood flow during exercise in older adults but also an uncoupling of the

Table 1. Mean body mass, muscle characteristics, white gastrocnemius muscle citrate synthase activity, and mean arterial pressure responses of young and old sedentary and exercise-trained rats

<table>
<thead>
<tr>
<th></th>
<th>Sedentary (Young n = 20)</th>
<th>Sedentary (Old n = 14)</th>
<th>Exercise-Trained (Young n = 16)</th>
<th>Exercise-Trained (Old n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>374 ± 9</td>
<td>436 ± 7*</td>
<td>354 ± 3†</td>
<td>385 ± 6‡</td>
</tr>
<tr>
<td>Gastrocnemius wt, g</td>
<td>1.55 ± 0.06</td>
<td>1.52 ± 0.05</td>
<td>1.60 ± 0.04†</td>
<td>1.59 ± 0.03†</td>
</tr>
<tr>
<td>Gastrocnemius wt-to-body wt ratio, mg/g</td>
<td>4.16 ± 0.20</td>
<td>3.50 ± 0.15*</td>
<td>4.53 ± 0.14†</td>
<td>4.14 ± 0.13*†</td>
</tr>
<tr>
<td>Citrate synthase activity, μmol·min⁻¹·g wet wt⁻¹</td>
<td>15.8 ± 1.5</td>
<td>15.0 ± 1.4</td>
<td>22.5 ± 1.5†</td>
<td>22.3 ± 1.6†</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>135 ± 4</td>
<td>129 ± 5</td>
<td>138 ± 4</td>
<td>125 ± 6*</td>
</tr>
<tr>
<td>Exercise</td>
<td>156 ± 5†</td>
<td>149 ± 4‡</td>
<td>159 ± 4†</td>
<td>148 ± 3††</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \) number of rats. MAP, mean arterial pressure. \( * P < 0.05 \) vs. corresponding young group. \( † P < 0.05 \) vs. age-matched sedentary group. \( ‡ P < 0.05 \) vs. rest.
typically precise matching of O₂ delivery to O₂ requirements in different sections of the working muscle, both of which would contribute to premature fatigue in the aged individual.

After exercise training, there is a substantial increase in submaximal leg blood flow in older men (4), even though exercising flow is still lower in endurance-trained older than younger men (56, 67). In the latter studies, although muscle blood flow was still lower in older than younger subjects posttraining, there is likely a more precise matching of O₂ delivery to O₂ requirements in the active muscle, as fractional extraction is increased in the older trained vs. sedentary subjects (39, 56). In the current study, after endurance training, there was an ~36% increase in exercising blood flow to the Gastₚ (predominantly type I and IIA fibers) in the old group (Fig. 4). Furthermore, there was a significant reduction in exercising blood flow to the Gastₚ after training in the old group (Fig. 4). Although our data do not reveal the precise mechanism(s) for the altered blood flow pattern after exercise training, it is likely due to a combination of alterations in vascular function (see below) and fiber recruitment patterns. With respect to the latter, the slightly higher relative work intensity in the old Sed group [as discussed by Musch et al. (44)] would theoretically result in a higher flow to the Gastₚ and Gastₚ on the

![Fig. 1. Resting blood flow in red gastrocnemius (Gastₚ, A), mixed gastrocnemius (Gastₚ, B), and white gastrocnemius (Gastₚ, C) of young and old sedentary (Sed) and exercise-trained (ET) rats.](image1)

![Fig. 2. Blood flow measured during steady state of exercise in Gastₚ (A), Gastₚ (B), and Gastₚ (C) of young and old Sed and ET rats. *P < 0.05 vs. Sed of corresponding age group. †P < 0.05 vs. young group in the same condition. #P = 0.055 vs. young Sed.](image2)
basis of the higher relative work rate (34). However, there is
decreased flow to the Gast\textsubscript{Red} and a substantial increase in
flow to the Gast\textsubscript{White} with aging during exercise relative to
the younger counterparts. Therefore, in the sedentary con-
dition with old age, a diminished blood flow to the Gast\textsubscript{Red}
during exercise may force a greater reliance on the recruit-
mment of muscle fibers from the Gast\textsubscript{White} to sustain locomo-
tion. However, after exercise training, the distribution pat-
tern of blood flow during exercise is similar with age. This
suggests that the elevated exercising blood flow to the
Gast\textsubscript{Red} with old age after training, through alterations in
vascular function and/or structure, may result in a better
matching of \textit{O}_2 delivery to \textit{O}_2 consumption in this muscle
section and diminish the need to recruit the Gast\textsubscript{White} after
training (which is supported through the reduced exercising
flow to this section in old ET vs. old Sed; Fig. 2C).

**Functional and structural vascular modifications with age
and exercise training.** Aging is associated with decrements in
skeletal muscle endothelial function (13, 19, 31, 41, 61, 63)
that can be improved with aerobic exercise training (22, 38,
47, 60, 62–64, 66). These training-induced adaptations in
endothelial function with old age are due, in part, to an
increased NO bioavailability (27, 62–64). Therefore, func-
tional alterations in vasomotor regulation after exercise
training appear to favor vasodilation vs. vasoconstriction. In
the old group, these functional changes and the increase in
resistance artery CSA (Fig. 6) likely contributed to the
increased vascular conductance and hyperemia that occur in
high-oxidative muscle such as the Gast\textsubscript{Red} (Figs. 2A and 3A)
after training. Conversely, in the Gast\textsubscript{White}, the hyperemic
response indicates an enhanced constriction of the resistance
vasculature with age after exercise training. However, the
literature does not support an upregulation of vasoconstric-
tor pathways in this muscle, as endurance training does not
increase \textit{\alpha}-adrenergic-induced (17), endothelin-1-induced
(18), or angiotensin II-induced (48) vasoconstriction. How-
ever, similar to previous findings in the coronary circulation
(42), we have found an enhanced myogenic vasoconstriction
in the Gast\textsubscript{White} resistance arteries after training in old rats
(unpublished observation), which, along with a putative
reduced recruitment of type IIB fibers, could contribute to
the reduced hyperemic response during exercise.

In old rats, there is a structural remodeling of the resistance
vasculature (e.g., arterial rarefaction) in skeletal muscle (8). It
appears that the old age-related decrements in physical activity
and, thus, attenuated muscular activation and blood flow con-
tribute to this vascular remodeling (8). Specifically, a reduced
physical activity with old age would diminish the daily hyper-
emic response (and associated shear stress) in resistance arter-
ies and metabolite production in skeletal muscle. As endothe-
lium-derived NO is a major mediator of vascular remodeling

**Fig. 3.** Vascular conductance calculated during steady state of exercise in
Gast\textsubscript{Red} (A), Gast\textsubscript{Mixed} (B), and Gast\textsubscript{White} (C) of young and old Sed and ET rats.

<table>
<thead>
<tr>
<th></th>
<th>Young SED</th>
<th>Young ET</th>
<th>Old SED</th>
<th>Old ET</th>
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<tbody>
<tr>
<td>Gast\textsubscript{Red}</td>
<td>2.5</td>
<td>2.0</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Gast\textsubscript{Mixed}</td>
<td>1.0</td>
<td>0.5</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Gast\textsubscript{White}</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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*P < 0.05 vs. Sed of corresponding age group; †P < 0.05 vs. young group in
the same condition. #P = 0.055 vs. young Sed.

**Fig. 4.** Percent differences in exercising muscle blood flow between Sed and
ET young and old groups. Percent difference was calculated as follows:

\[
\text{Percent Difference} = \left( \frac{\text{blood flow ET} - \text{blood flow Sed}}{\text{blood flow Sed}} \right) \times 100
\]
a reduction in NO bioavailability with old age may destabilize vascular networks in oxidative muscle (53) and preferentially increase flow through one network and decreases flow through another. Over time, this would likely lead to a recession of some networks and an increase in luminal diameter of others. Exercise is one of the most powerful stimulants of capillary growth (i.e., angiogenesis) in active tissue, and if the exercise stimulus is chronic, an increase in the number of arteries (i.e., arteriogenesis) can occur (68). Therefore, we hypothesized that chronic exercise training and the subsequent increased NO bioavailability (22, 62, 63), together with similar angiogenic potential with old age (25, 57), would increase the number of arteries perforating skeletal muscle in the old group. Contrary to our hypothesis, there were no increases in the number of feed arteries in the old group (Fig. 5), whereas there was an increase in the young group. However, the old animals demonstrated an increase in luminal CSA after exercise training in the GastRed and GastWhite, whereas the young group exhibited a trend (P = 0.08) for a greater CSA in the GastRed with no change in the GastWhite (Fig. 6).

It is unclear why there are differential training-induced adaptations with age, but it may be related to differences in relative exercise intensity. According to Musch et al. (44), the heavier body mass of the old group would have resulted in these animals exercising at ~10–20% higher relative intensity than the young group. With this higher relative intensity of exercise and fewer perforating arteries, the blood flow rate through the arteries would be greater in the old group, which is a likely contributing mechanism to the enhanced CSA observed with age and exercise training.

Skeletal muscle blood flow pattern with aging and exercise training. In the current study, young and old groups demonstrated an elevated flow to the GastRed and a lower flow to the GastWhite during exercise after endurance training (Fig. 4), suggesting a training effect in both muscles. Although citrate synthase activity in the GastWhite from young and old groups was enhanced after training (Table 1), we did not measure citrate synthase activity of the GastRed. However, a similar training intensity enhances citrate synthase activity in the GastRed from young and aged animals (45), and the GastRed shows a robust hyperemia during exercise (Fig. 2A), which provides further evidence of recruitment and training in the GastRed of young and aged animals with this training protocol.

A paradox exists, in that the GastRed and GastWhite demonstrated significant increases in CSA after training with old age (Fig. 6, A and C), whereas the GastRed demonstrated an enhanced hyperemia and the GastWhite demonstrated a reduced hyperemia. On the basis of Poiseuille’s law, a small change in vessel radius results in a large alteration in flow through a vessel or tube, if it is assumed that pressure gradient, vessel length, and blood viscosity are unaltered. Therefore, in the GastRed, even if there were no functional alterations in vasomotor responsiveness, an increase in feed artery radius of ~7.5% via greater vasodilation or a structural expansion would result in an ~35% increase in blood flow through a given vessel. Given that exercising blood flow in the GastRed from the old group after training was ~35% greater, with an average radius increase of ~10% in the feed arteries, the changes in vascular morphology could fully account for the increased flow. However, with aging in the GastWhite, a similar increase in feed artery radius was observed after training but was associated with a reduction in exercising blood flow vs. the sedentary condition. Therefore, changes in feed artery CSA after exercise training with age can be a poor predictor of skeletal muscle blood flow at these low intensities of exercise. Indeed, Laughlin and Roseguini (36) stated that training-induced increases in blood flow capacity are not mediated solely by structural adaptations, a conclusion that is supported by the findings from the current study. Previous studies demonstrated an old age-related endothelial dysfunction in skeletal muscle feed arteries (70, 71) and arterioles (13, 41) that would expectly affect total muscle blood flow and intramuscular blood flow distribution, respectively (69). Therefore, the enhanced endothelial function in these vascular branches with aging after exercise training (63, 64, 66) likely works in concert with structural alterations to improve muscle perfusion during exercise. Whether similar alterations in large, conduit artery structure and function with aging and exercise...
training (15, 16, 23) affect downstream muscle perfusion during exercise remains to be determined.

Ramifications of altered muscle blood flow with age after exercise training. Within skeletal muscle, there must be a coordinated balance in the rates of $O_2$ delivery to $O_2$ consumption, as an imbalance between these two variables will promote fatigue (50, 51). In skeletal muscle, with advancing age, there are significant impairments in capillary hemodynamics (e.g., decreased lineal density of capillaries supporting red blood cell flow) at rest (59) and during exercise (10). These altered
capillary hemodynamics with old age contribute to an O$_2$ delivery-O$_2$ consumption mismatch at rest (6, 38), in response to an increased metabolic demand (6, 11, 21), as well as during the recovery from exercise (29). This O$_2$ delivery-O$_2$ consumption mismatch with old age, due, in part, to a blunted rate of vasodilation of upstream resistance arteries (5, 30), would reduce the functional capacity to perform daily activities (e.g., climbing stairs) in older adults. After exercise training with old age, there was a significant increase in blood flow to the GastRed, which, considering that this fiber type demonstrates the highest oxidative capacity of the hindlimb skeletal muscles (14), indicates a better matching of O$_2$ delivery to the metabolic characteristics of the muscle at these running speeds. Indeed, it has been demonstrated that there is a more precise matching of O$_2$ delivery to O$_2$ requirements after exercise training with old age (38, 43), which is likely a key mechanism for the increased exercise capacity in the aged individual after training.

Conclusions. The current study examined the effects of aging and exercising training on resting and exercising blood flow to muscle sections composed of high-oxidative (GastRed), mixed (GastMixed), and low-oxidative glycolytic (GastWhite) fiber types. Similar to the findings of Musch et al. (44) in untrained animals, we observed a reduced flow to the GastRed and an increased flow to the GastWhite in the old vs. young Sed group during exercise. This pattern of muscle perfusion was reversed after endurance training; i.e., there was a greater exercise hyperemia to the GastRed and a lower flow to the GastWhite of old rats. Unlike young animals, in old animals, exercise training did not result in an increase in the number of feed arteries to the muscle. Rather, there was an increase in feed artery CSA after training in the old group that did not occur in young rats. These findings suggest that training-induced increases in resistance artery CSA in old rats may contribute to the elevation of vascular conductance and blood flow capacity in the high-oxidative muscles, whereas the decrease in vascular conductance and exercise hyperemia in the low-oxidative muscles is not the result of structural vascular adaptations but is likely due to changes in the functional vasomotor properties of the resistance vasculature [enhanced vasodilatory responsiveness (63, 64) and myogenic autoregulation (unpublished observations)] and altered fiber type recruitment patterns. Collectively, structural and functional changes in the aging resistance vasculature that allow for a more precise matching of O$_2$ delivery to muscle oxidative capacity appear to be a key mechanism for the increased exercise capacity in aged individuals after exercise training.

REFERENCES


AUTHOR CONTRIBUTIONS

B.J.B., J.M.M.-D., and M.D.D. are responsible for conception and design of the research; B.J.B., M.W.R., J.N.S., J.M.D., R.T.D., and D.J.M. performed the experiments; B.J.B. analyzed the data; B.J.B., M.W.R., J.N.S., and M.D.D. wrote the results of the experiments; B.J.B. prepared the figures; B.J.B. and M.D.D. drafted the manuscript; B.J.B., M.W.R., J.N.S., J.M.D., R.T.D., D.J.M., J.M.M.-D., and M.D.D. edited and revised the manuscript; B.J.B., J.N.S., J.M.D., R.T.D., D.J.M., J.M.M.-D., and M.D.D. approved the final version of the manuscript.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

GRANTS

This study was supported, in part, by National Aeronautics and Space Administration Grants NAG2-1340 and NCC2-1166 (M. D. Delp), National Institutes of Health Grants AG-3137 (B. J. Behnke) and R01 HL-077224 (J. M. Muller-Delp), Florida Biomedical Research Program Grant 1BN-02 (B. J. Behnke), and the Jane Adams Edmonds Endowed Doctoral Fellowship (J. M. Dominguez 2nd) and LaPradd Fellowship (D. J. McCullough and R. T. Davis 3rd) from the Department of Applied Physiology and Kinesiology at the University of Florida.


