Aerobic Exercise Improves Microvascular Function in Older Adults

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ABSTRACT

HURLEY, D. M., E. R. WILLIAMS, J. M. CROSS, B. R. RIEDINGER, R. A. MEYER, G. S. ABELA, and J. M. SLADE. Aerobic Exercise Improves Microvascular Function in Older Adults. *Med. Sci. Sports Exerc.*, Vol. 51, No. 4, pp. 773–781, 2019. Microvascular function is reduced with age, disease, and inactivity. Exercise is well known to improve vascular health and has the potential to improve microvascular function in aging and disease. **Purpose**: The study aimed to assess changes in peripheral microvascular function in sedentary older adults after aerobic exercise training. **Methods**: Twenty-three sedentary older adults (67 ± 5 yr, body mass index = 29 ± 5, mean ± SD) successfully completed a randomized 12-wk graded treadmill walking intervention. The exercise group (EX) performed 40 min of uphill walking 4 d·wk^{-1} at 70% heart rate reserve. The control group (CON) maintained a sedentary lifestyle for 12 wk. Blood oxygen level–dependent (BOLD) responses of the soleus measured by magnetic resonance imaging were used to evaluate microvascular function; brief (1 s) maximal plantarflexion contractions were performed. Separately, blood flow in the popliteal artery was measured by ultrasound after brief contraction. Phosphorus magnetic resonance spectroscopy of the calf was used to examine muscle oxidative capacity, and whole-body peak oxygen consumption ($\dot{V}O_{2peak}$) was used to confirm training-induced cardiorespiratory adaptations. **Results**: Peak postcontraction BOLD response increased by 33% in EX (PRE, 3.3% ± 1.0%; POST, 4.4% ± 1.4%) compared with CON (PRE, 3.0% ± 1.3%; POST, 3.2% ± 1.5%), $P < 0.05$. EX with hypertension tended to show a blunted peak BOLD increase ($n = 6$, 15%) compared with EX normotensive ($n = 7$, 50%), $P = 0.056$. Peak postcontraction blood flow increased by 39% in EX (PRE, 217 ± 88 mL·min^{-1}; POST, 302 ± 167 mL·min^{-1}) compared with CON (PRE, 188 ± 54 mL·min^{-1}; POST, 184 ± 44 mL·min^{-1}), $P < 0.05$. EX muscle oxidative capacity (k_{PCr}) improved by 40% (PRE, 1.60 ± 0.57 min^{-1}; POST, 2.25 ± 0.80 min^{-1}) compared with CON (PRE, 1.69 ± 0.28 min^{-1}; POST, 1.76 ± 0.52 min^{-1}), $P < 0.05$. $\dot{V}O_{2peak}$ increased by 9% for EX (PRE, 19.0 ± 3.1 mL·kg^{-1}·min^{-1}; POST, 20.8 ± 2.9 mL·kg^{-1}·min^{-1}) compared with a 7% loss in CON (PRE, 21.9 ± 3.6 mL·kg^{-1}·min^{-1}; POST, 20.4 ± 3.5 mL·kg^{-1}·min^{-1}), $P < 0.05$. **Conclusion**: Moderate aerobic exercise significantly improved microvascular function of the leg in older adults. **Key Words:** HEMODYNAMIC RESPONSE, MUSCLE BOLD, FUNCTIONAL MRI AGING, HYPERTENSION

Microvascular function is critical in the control of blood flow and tissue perfusion (1,2). Furthermore, microvascular function is important because the increase in blood flow during the initial phase of exercise is controlled by the rapid vasodilation of the terminal arterioles within the microcirculation (3,4). Microvascular function decreases with age (5–7) and is further reduced with peripheral arterial disease (8) and diabetic peripheral neuropathy (9,10). Age-related vascular decrements have been attributed to local vasodilatory mechanisms, a reduced ability to modulate vasoconstriction, and an elevated sympathetic response (11). These factors contribute to a decrease in muscle efficiency, via a blunted blood flow response to the active skeletal muscles (12,13). This can lead to an imbalance in the matching of oxygen delivery to meet the contracting skeletal muscle demand leading to exercise intolerance (14). Consequently, interventions that attenuate these decrements may hold significant value.

Although difficult to assess noninvasively, several innovative methods have emerged as ways to quantify and assess microvascular function. One technique is functional magnetic resonance imaging (MRI) using blood oxygen level–dependent (BOLD) imaging in skeletal muscle (15). Muscle BOLD responses are sensitive to vasoactive substances (16) and are decreased with age (6), vascular disease (17), and obesity (18). The muscle BOLD response is quantitatively explained by increases in skeletal muscle blood volume and blood oxygenation and, thus, can reflect muscle perfusion as a measure to evaluate small vessel function (19).

Endurance exercise training has been shown to mitigate many age-related changes in vascular function (20), including

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improving arterial compliance (21) and capillary density (22). Microvascular function is also increased in young and old endurance trained adults (6,23) compared with sedentary counterparts. Further, a recent study found that habitual endurance training offsets the age-related decline of contraction-induced rapid onset vasodilation in both upper and lower extremities (24). Thus, single contraction-induced muscle BOLD and rapid onset vasodilation may prove highly useful in evaluating changes in small vessel function, as they are both sensitive to differences in physical activity. Coupled with these novel techniques, these data suggest that exercise training can enhance small vessel function, even in older populations.

Therefore, the purpose of this study was to evaluate the effects of aerobic exercise on small vessel function in the lower limb of older adults. We hypothesized that an aerobic endurance intervention would improve the microvascular function and small vessel vasodilation of older adults when compared with a control group as evidenced by a significant increase in the postcontractile peak muscle BOLD response and large artery hyperemia.

MATERIALS AND METHODS

Thirty sedentary older adults (60–80 yr old) were recruited from the Lansing Community. Sedentary was defined as exercising two or fewer days per week, for 30 $min \cdot d^{-1}$ or less over the last 6 months. Exclusion criteria for all subjects included heart disease, peripheral artery disease, stroke, type 1 diabetes, insulin use, and cigarette smoking. Subjects with any ferrous implants or with metal implants in the leg or knee were also excluded. The pretesting included four visits: a familiarization visit, a treadmill stress test with oxygen consumption ($\dot{V}O_{2peak}$), MRI for postcontractile peak BOLD response, and ultrasound for postcontractile peak blood flow response. In addition, serum blood lipids and glucose were measured after an overnight fast (≥8 h) to characterize the recruited sample. Blood serum was analyzed through Sparrow Laboratories using standard enzyme procedures with spectrophotometry (glucose and lipid panel) and chemiluminescence (insulin). After the pretesting, subjects were equally randomized into a sedentary control group (CON) or exercise group (EX). After 12 wk of intervention, posttesting was completed. All participants provided written informed consent before participation. This research was approved by the Human Research Protection Program at Michigan State University.

Familiarization visit. An initial laboratory visit was done to assess baseline characteristics and to familiarize participants with the treadmill and ankle ergometer. Height, weight, resting heart rate, and blood pressure were measured. Supine blood pressure was measured in the brachial artery using a standard arm blood pressure cuff and a sphygmomanometer. To determine the ankle–brachial index (ABI), supine blood pressures were measured in the brachial, posterior tibial, and dorsalis pedis arteries with a Dopplex D900 handheld Doppler ultrasound. ABI was calculated (ankle artery pressure average/brachial artery pressure) for both right and left sides to rule out peripheral artery disease (ABI ≤ 0.90 in either leg).

Next, the subject was familiarized with the treadmill. The subject was instructed to walk at a comfortable pace to establish a baseline speed for the stress test with $\dot{V}O_2$. The subject was also familiarized with the mask for respiratory gas analysis and with the BORG RPE 6–20 scale. Last, the subject lay supine on an MRI table with the dominant leg placed through an MRI extremity coil and the dominant foot placed into a custom-built ankle ergometer. Velcro straps were placed over the midfoot and the forefoot. In addition, a 14-inch-wide GE Velcro security strap was placed around the torso and hips to minimize movement of the body away from the footplate. Ankle plantarflexion (PF) was done with the ankle fixed at 115°. Several (5–10) maximal voluntary isometric contractions (MVC) lasting 3–4 s were completed with verbal cuing. The subject then performed a short practice test of single brief PF MVC (1 s in duration) with visual cues and real-time force feedback on a projected computer screen. The subject also performed repeated maximal pushing to simulate the exercise for the ^{31}P MRS protocol (described below).

Stress test with $\dot{V}O_2$. On a separate visit, subjects performed an exercise stress test. Subjects abstained from caffeine 12 h before testing and were encouraged to eat a light breakfast. All medications were taken as prescribed. A modified Balke protocol on a treadmill was used to measure peak whole-body oxygen consumption using respiratory gas analysis (Parvo Medics TrueOne 2400, Sandy, UT). Subjects walked at a self-selected pace, and the treadmill grade was increased every 2 min until exhaustion. Blood pressure, heart rate, and RPE were recorded at each stage. Oxygen consumption ($mL \cdot min^{-1}$) was averaged every 30 s to determine $\dot{V}O_{2peak}$. Heart rate and rhythm were measured with 12-lead EKG. The test was terminated when subjects reached their maximal exertion or were advised to stop by the cardiology team. The subject was stopped if unsteady, had inappropriate blood pressure responses, or if the EKG showed severe abnormalities consistent with guidelines established by the American College of Cardiology and the American Heart Association (25).

MRI visits. On a separate day, subjects had an MRI after an overnight fast (≥8 h); the testing was done in the morning, and each subject was tested at approximately the same hour of the day for pre- and posttesting, ±90 min. Subjects were asked to refrain from aspirin and pain medications 12 h before testing and to avoid exercise for 24 h before testing. Subjects taking blood pressure medications took them as normally prescribed. Other medications taken in the morning, including aspirin, were postponed until the completion of the MRI.

MRI images and spectra (MRS) of the lower leg were acquired at 3-T GE Excite system (GE Healthcare, Milwaukee, MI). Subjects lay supine on the MRI table. The subject's leg was placed in an eight-channel quadrature transmit/receive extremity coil, and the foot was secured in the ankle ergometer as described in the familiarization visit. Support

padding was placed around the leg to reduce motion. Anatomical images (axial fast spin echo, repetition time [TR]/echo time [TE] = 600/12.4 ms, 10 mm slice, 5 mm separation, 256 × 192 matrix, field of view = 16) were acquired to quantify muscle volume and to locate the imaging slice for BOLD MRI. After higher order shimming, the subject performed PF MVC during BOLD MRI; an echo planar BOLD sequence (gradient recalled echo, TR/TE = 1000/40, 64 × 64 matrix, field of view = 16) of the slice with the largest calf area was used to evaluate microvascular function. Five 1-s PF MVC were done with each contraction separated by 120 s. The cues for the timing of contractions ("rest," "ready," and "push") were displayed to the subject on the face of the magnet bore. Each subject had a minimum of 20 min of supine rest before the target BOLD scans. Scans were repeated when subjects had significant motion or low force during scanning. We have previously shown that peak soleus BOLD response has good test reliability in older adults (intraclass correlation coefficient = 0.95, coefficient of variation = 8.6%) (6).

MRI peak BOLD magnitude, time to peak (TTP) BOLD, and half recovery time from peak BOLD were measured in the soleus during the single contraction protocol. The peak BOLD postcontractile response was normalized to baseline for each contraction. TTP BOLD was determined as the time between the start of contraction and the peak BOLD response. The BOLD responses that corresponded with the highest force (±85% MVC) and highest BOLD responses without subject motion were averaged together, representing three to four postcontractile responses for each subject. Subjects were excluded if venous occlusion and subsequent filling occurred during BOLD MRI as venous filling grossly enhances BOLD responses (6,26). Fat-free muscle volume of the posterior leg was measured starting with the first slice containing the proximal tibia and the final slice containing the medial malleolus using Winvessel custom software.

^{31}P MRS was used to examine muscle oxidative capacity of the calf. An elliptical ^{31}P MRS 12-cm surface coil was placed directly below the largest region of the calf to acquire chemical spectra during 30 s of rest, 30 s of PF exercise, and 5 min of recovery. The coil was positioned under the largest area of the calf, visualized by a vial of water located in the center of the coil. During the 30-s exercise, two rapid MVC were done every 3 s for a total of 20 PF contractions. Single shot spectra were acquired throughout the protocol (51.7 MHz, 1024 complex points, sweep width 2500 Hz, 60° pulse width at coil center, TR = 3 s). Data were analyzed with jMRUI software (version 3.0) using the AMARES fitting algorithm. The phosphocreatine (PCr) recovery time constant was determined by fitting data from each time point (3-s time resolution) to a monoexponential recovery. The following equation was used:

$$Y(t) = Y(\mathrm{Bs1}) + \mathrm{Amp} \times \left(1 - e^{k_{\mathrm{PCr}}t}\right).$$

Y represents PCr at any time (t), Bsl is the baseline PCr value, Amp is the amplitude change in PCr, and k_{PCr} is the recovery rate constant. For pH, two free induction decays were first summed and then analyzed using the chemical shift of inorganic phosphate relative to PCr.

Ultrasound visits. On a separate day, popliteal artery blood flow during the single contraction protocol was measured with ultrasound. The testing restrictions were consistent with the MRI visit. Subjects lay supine on a laboratory table with their foot secured to the ergometer. There was an adjustable gap in the table to allow the ultrasound probe to be placed in the popliteal fossa. Subjects performed four to five practice MVC contractions and practiced the timing of the 1-s MVC with the visual cue. A duplex Doppler ultrasound scanner (Logiq P6, GE Healthcare) was used to acquire ultrasound data of the popliteal artery at rest and after single, isolated muscle contractions of the calf. A 9-MHz linear probe was placed on the popliteal artery ~2 cm proximal to the split into the tibial arteries. B-mode was used to acquire resting vessel diameter during diastole. During pulse wave recording, the insonation angle was set at ≤60°. Several contraction attempts were made until five suitable trials were acquired. Each contraction was separated by at least 2 min. The contractions were repeated if the force was low or if the ultrasound probe was not maintained in position after the MVC. Subjects lay supine for a minimum of 20 min before ultrasound data were recorded.

Blood velocity during the three contractions with the highest peak blood velocity and highest force was included in the final analysis. The average resting velocity was measured in the five to eight cardiac cycles immediately before each contraction. Postcontractile blood velocity was apparent starting ~1–2 s after the contraction, when the calf muscle returned to a relaxed position. The average velocity was measured over the cardiac cycle (time averaged mean) for each cardiac waveform for up to 90 s after MVC using the GE Logic P6 software. Flow was calculated as follows: velocity × $60(\pi(d/2)^2)$, where d = diameter; velocity was recorded in milliliters per second. Resting vessel diameter was used in all calculations of blood flow. The peak postcontractile flow increase is reported as the peak flow minus the resting flow. Absolute flow ($\mathrm{mL \cdot min^{-1}}$) and flow relative to calf muscle volume ($\mathrm{mL \cdot min^{-1}}$ per 100 mL) are reported. Resting heart rate was determined from the blood velocity waveforms and was used to evaluate the influence of the exercise intervention.

Muscle force. A strain-gauge force transducer (Interface, model SSM-EV-250, Scottsdale, AZ) was mounted to the underside of the footplate, and force was digitized (DATAQ Instruments, model DI-195B, Akron, OH), sampled at 120 Hz, and recorded on a personal computer. MVC force was quantified during the highest 500 ms. Force during BOLD MRI and ultrasound was quantified over the highest 500 ms and is expressed relative to MVC as BOLD responses are influenced by relative effort (27). The force during ^{31}P acquisition was measured as the peak force because of the short time available to complete two pushes every 3 s; force is reported as the average peak force over the 20 contractions and is expressed as percent MVC.

Exercise intervention. The EX group completed 12 wk of supervised graded treadmill walking. The training program

used a set protocol to progressively increase walking intensity according to the subject's heart rate reserve (HRR). HRR was calculated from the HR_{max} observed during the exercise stress test. A Polar® heart rate monitor was worn during exercise. The training began by walking for 30 min at 50% HRR in week 1. The time and target HRR progressed over the first 3 wk to meet the target of 40 min of walking at 70% HRR at the start of week 4. During weeks 4–12, the target HRR remained at 70% HRR; this target was primarily met by increasing the treadmill grade to maintain 70% HRR. Each session began with 5 min of walking at a self-selected speed and grade. The training was done 4 $d \cdot wk^{-1}$. The CON group was asked to maintain a sedentary lifestyle.

Statistics. Baseline subject characteristics were compared between groups with independent *t*-tests. All other outcomes were analyzed with between group repeated-measures ANOVA. Group data are reported as mean ± SD unless noted otherwise. Normality was tested with the Shapiro–Wilk test. Pearson's *r* was used to examine correlations between peak BOLD and relative peak flow responses. Significance was set at $P < 0.05$. Data were analyzed using IBM SPSS version 24.

RESULTS

The final group of participants included 13 EX and 10 CON (Table 1). Three subjects dropped from the study due to hip pain (EX = 1), relocation (CON = 1), and unrelated surgery (CON = 1). Four subjects are not included in the analysis because of MRI data that were confounded by venous occlusion (EX = 1, CON = 2) or involuntary muscle motion during BOLD MRI and ultrasound (CON = 1). The participant characteristics are shown in Table 1. There were no differences in body mass index (BMI), blood pressure, blood parameters, or age between the groups. ABI was above 1.00 for all subjects. Type 2 diabetes (EX = 1, CON = 1) and controlled hypertension (EX = 6, CON = 5) were present. Daily medication use included statins (EX = 6, CON = 5), aspirin (EX = 4, CON = 4), hydrochlorothiazide (EX = 1, CON = 4), ACE inhibitor (EX = 2, CON = 1), ARB (EX = 3) (CON = 1), and calcium channel blockers (EX = 1, CON = 2). Medication, including dosage, did not change during the study.

TABLE 1. Background characteristics at baseline.

Characteristic	CON (*n* = 10)	EX (*n* = 13)
Age (yr)	66 ± 4	67 ± 6
Male (*n*)	2	2
BMI	28 ± 3	29 ± 6
Systolic BP (mm Hg)	132 ± 10	130 ± 16
Diastolic BP (mm Hg)	77 ± 6	74 ± 8
BP medication	50%	42%
Glucose ($mg \cdot dL^{-1}$)	92 ± 6	92 ± 14
Total cholesterol ($mg \cdot dL^{-1}$)	183 ± 28	199 ± 42
LDL ($mg \cdot dL^{-1}$)	101 ± 21	119 ± 30
HDL ($mg \cdot dL^{-1}$)	59 ± 15	56 ± 17
Triglycerides ($mg \cdot dL^{-1}$)	118 ± 28	120 ± 50
Insulin (μIU)	8.8 ± 6.9	6.4 ± 3.4
$\dot{V}O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	21.5 ± 3.6	20.0 ± 4.1

Values are presented as mean ± SD.

EX completed 12 wk of training with 94% attendance. The average treadmill speed and grade at week 4 were 3.1 ± 0.4 MPH and 5.4% ± 2.0% grade, respectively, and progressed to 3.2 ± 0.5 MPH and 8.5% ± 4.1%, respectively, by week 12. The average weekly target HRR was met (target HR = 125 ± 10 bpm, actual HR = 125 ± 10 bpm). At baseline (PRE) muscle oxidative capacity was not different between the groups (Fig. 1). There was a significant interaction for muscle oxidative capacity, $F(1,20) = 7.691$, $P = 0.012$ (Fig. 1); the PCr resynthesis rate constant improved by 40% in EX. There were no differences in muscle pH or force between the groups or across time, but there was a significant effect of time on PCr hydrolysis, $F(1,20) = 5.195$, $P = 0.034$ (Table 2). Muscle oxidative capacity was not determined in one EX subject during posttesting because of a technical failure. There were no differences in initial $\dot{V}O_{2peak}$ between the groups (Table 1). There was a significant interaction for $\dot{V}O_{2peak}$ with the intervention, $F(1,15) = 10.214$, $P = 0.006$ (Fig. 1); EX showed a 9% increase, and CON had a 7% decline. $\dot{V}O_{2peak}$ measures were only repeated for posttesting in a subgroup because of scheduling difficulties (EX = 10, CON = 6). There were no group differences in other variables associated with peak $\dot{V}O_2$ testing, e.g., RPE, maximal HR, and RER (Table 2). There was a significant interaction for body weight, $F(1,20) = 5.363$, $P = 0.031$. EX lost approximately 1 kg (PRE = 79.4 ± 15.6 kg; POST = 78.5 ± 14.9 kg), and CON gained just under 1 kg (PRE = 76.5 ± 9.4 kg; POST = 77.2 ± 9.0 kg). Muscle volume and MVC force were not different between groups or across time (Table 2). There was an outlier for force (CON), but this subject was an outlier in all force measures; the force did not change over time, and therefore the subject remained in the analysis. Exercise training had a significant effect on resting heart rate: EX, PRE = 66 ± 7 bpm, POST = 63 ± 8 bpm versus CON, PRE = 67 ± 8 bpm, POST = 71 ± 9 bpm, $F(1,21) = 11.915$, $P = 0.002$.

BOLD responses are shown in Figure 2. Baseline (PRE) values for peak contractile BOLD response were similar between groups. There was a significant interaction between groups for peak BOLD response, $F(1,21) = 4.738$, $P = 0.041$; the average peak microvascular BOLD response increased by 33% for EX. There was a significant interaction for TTP BOLD response, $F(1,21) = 5.095$, $P = 0.035$, and BOLD response half recovery time, $F(1,21) = 6.956$, $P = 0.015$. The TTP was 25% faster after training, and time to recovery was ~20% faster for EX. BOLD force averaged ~94% of MVC and was not different between groups or across time (see Table 2).

Popliteal artery blood flow responses are shown in Figure 3 and include the 23 subjects with BOLD outcomes. At baseline (PRE), the peak contraction-mediated flow increase was not different between groups. There was a significant interaction for peak flow increase relative to muscle volume ($mL \cdot min^{-1}$ per 100 mL), $F(1,21) = 6.541$, $P = 0.018$; the peak postcontractile flow improved by ~35% for EX. The change was also significant when expressed as absolute change in milliliters per minute: EX (PRE, 217 ± 88 $mL \cdot min^{-1}$; POST,

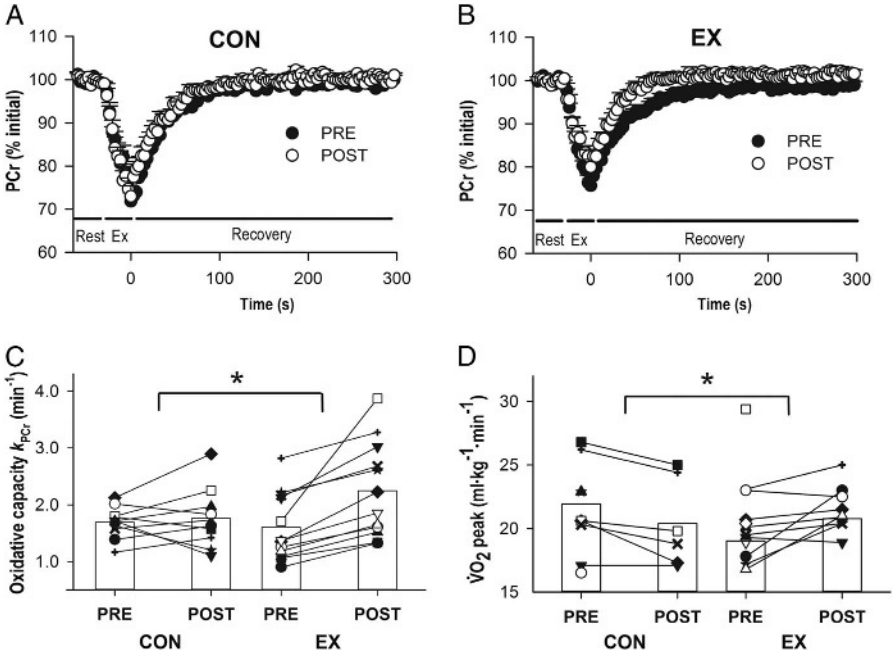

FIGURE 1—Muscle oxidative capacity and $\dot{V}O_{2peak}$ changes with exercise training. PCr changes measured from the calf are shown at rest (30 s), during dynamic PF (30 s), and during recovery (300 s) (A, B; mean ± SE). The recovery rate (k_{PCr}) reflecting the muscle oxidative capacity is shown for individuals (*lines*) and groups (*open bars*) before (PRE) and after (POST) the training period (C). Peak whole-body oxygen consumption, $\dot{V}O_{2peak}$, is shown for individuals (*lines*) and groups (*open bars*) before (PRE) and after (POST) the training period (D). For D, group bars include only subjects with pre- and posttesting. CON, control; EX, exercise. *$P < 0.05$ for the interaction between groups.

302 ± 167 mL·min^{-1}) compared with CON (PRE, 188 ± 54 mL·min^{-1}; POST, 184 ± 44 mL·min^{-1}), $F(1,21) = 8.198$, $P = 0.009$. These changes were significant if all subjects with flow testing were included ($n = 27$, data not shown). Force during flow acquisition averaged ~92% of MVC and was not significantly different across time or between groups (Table 2). The peak BOLD response was moderately correlated with relative peak blood flow when evaluating the group as a whole (PRE, $r = 0.49$, $P = 0.017$; POST, $r = 0.58$, $P < 0.001$; $n = 23$). When evaluating the groups separately at each time point, the correlation reached significance for EX POST ($r = 0.57$, $P = 0.044$, $n = 13$).

A *post hoc* analysis was done on EX subjects who were normotensive (EX-N, no history of hypertension, $n = 7$) versus hypertensive (EX-H, diagnosis of hypertension, $n = 6$). These EX subgroups were similar in BMI and age, and each had one male. Baseline systolic blood pressure (141 ± 12 mm Hg) and mean arterial pressure (99 ± 4 mm Hg) were significantly elevated in EX-H compared with EX-N (121 ± 14 and 87 ± 9 mm Hg), $P \leq 0.019$. In addition to blood pressure medication, EX-H had a higher prevalence of statin use (EX-H = 4/6 vs EX-N = 2/7). The baseline peak BOLD response was not different between EX-H and EX-N (Fig. 4). This was also true when including all subjects (CON and EX) with baseline data (data not shown). EX-H tended to have a blunted increase in peak BOLD response (15% improvement) compared with EX-N (50% improvement) (Fig. 4), $F(1,11) = 4.461$, $P = 0.058$. There were no significant differences in

TABLE 2. $\dot{V}O_{2peak}$, ^{31}P MRS, and muscle measures.

	CON		EX	
	Pre	Post	Pre	Post
$\dot{V}O_{2peak}$	($n = 6$)		($n = 10$)	
RPE	18.8 ± 1.9	18.6 ± 1.9	17.8 ± 1.9	18.6 ± 1.4
HR_{max} (bpm)	164 ± 17	161 ± 11	150 ± 17	151 ± 18
RER	1.09 ± 0.05	1.10 ± 0.06	1.06 ± 0.06	1.06 ± 0.05
^{31}P MRS	($n = 10$)		($n = 12$)	
pH rest	7.03 ± 0.03	7.02 ± 0.02	7.03 ± 0.02	7.04 ± 0.02
pH end exercise	7.05 ± 0.04	7.04 ± 0.03	7.05 ± 0.02	7.05 ± 0.02
pH minimum	6.96 ± 0.05	6.97 ± 0.03	6.99 ± 0.03	6.99 ± 0.02
PCr minimum (%)*	72.4 ± 9.4	73.5 ± 7.8	75.6 ± 5.3	78.8 ± 5.3
Muscle volume and force	($n = 10$)		($n = 13$)	
Muscle volume (cm^3)	747 ± 125	757 ± 136	827 ± 174	838 ± 182
MVC (*N*)	301 ± 87	291 ± 78	265 ± 59	270 ± 57
MVC (N·cm^{-3})	0.410 ± 0.127	0.394 ± 0.117	0.332 ± 0.093	0.334 ± 0.089
BOLD (% MVC)	93.7 ± 3.6	94.0 ± 3.8	95.6 ± 2.9	94.3 ± 4.1
Flow (% MVC)	92.0 ± 3.5	90.9 ± 4.6	92.2 ± 4.2	93.2 ± 4.2
^{31}P (% MVC)	91.5 ± 2.0	92.7 ± 4.0	92.5 ± 3.3	92.1 ± 3.5

Values are presented as mean ± SD. *$P < 0.05$ for time effect.

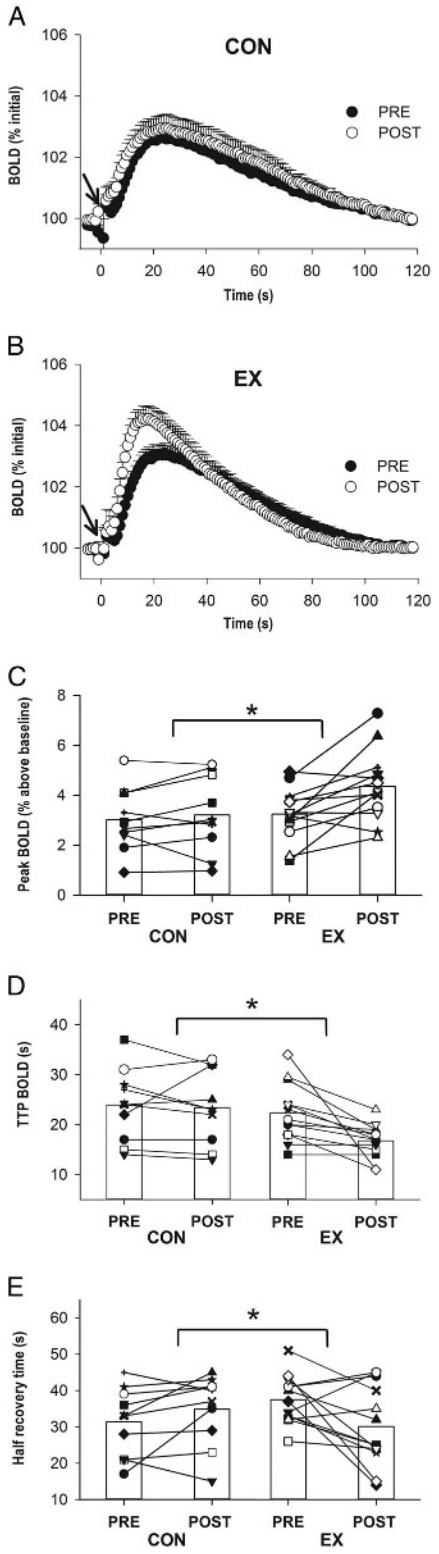

FIGURE 2—The influence of exercise training on muscle BOLD responses. The time course of the BOLD response is shown after 1 s maximal PF (A, B; mean ± SE); the contraction is at time = 0, indicated with an arrow. The measured parameters from the time course are shown for individuals (*lines*) and groups (*open bars*) before (PRE) and after (POST) the training period (C, D, E). The symbols representing each individual are consistent with Figure 1. CON, control; EX, exercise, TTP, time to peak BOLD response. *$P < 0.05$ interaction between the groups.

peak blood flow after contraction (data not shown). Both subgroups had significant increases in muscle oxidative capacity after training: EX-H = 54% ± 16% versus EX-N = 27% ± 4% (mean ± SE).

DISCUSSION

The major finding of the study is that a moderate-intensity aerobic exercise program improved microvascular function in older adults. This finding is consistent with the improved functional vasodilation of arterioles measured in rodents after endurance training (28). It is well established that vascular function decreases with age, including declines in vasodilatory

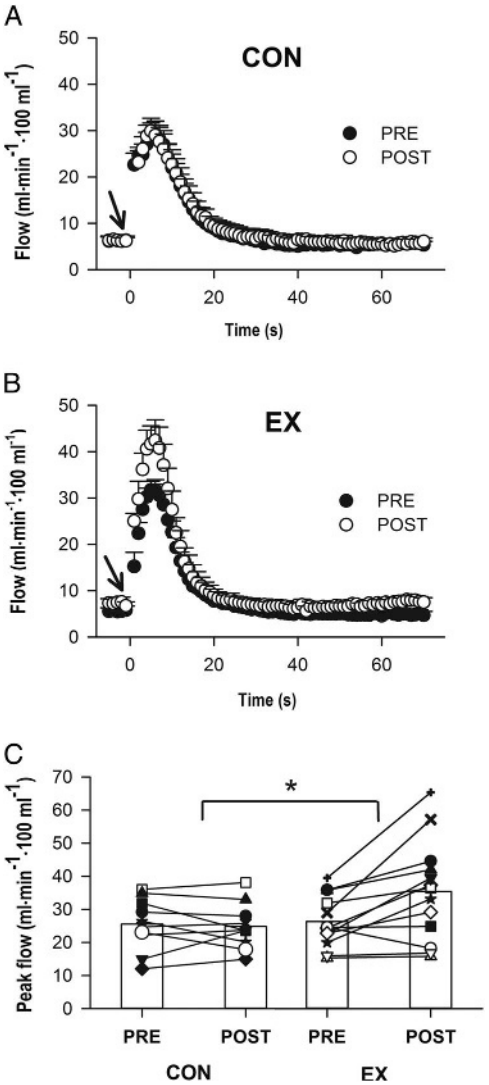

FIGURE 3—The influence of exercise training on popliteal artery blood flow. The top graphs show the time course of blood flow increases after 1 s maximal PF (A, B; mean ± SE); the contraction is done at time = 0, indicated with an arrow. The bottom graph (C) shows the individual (*lines*) and group (*open bars*) peak blood flow responses before (PRE) and after (POST) the training period. The symbols representing each individual are consistent with Figure 1. CON, control; EX, exercise. *$P < 0.05$ for the interaction between groups.

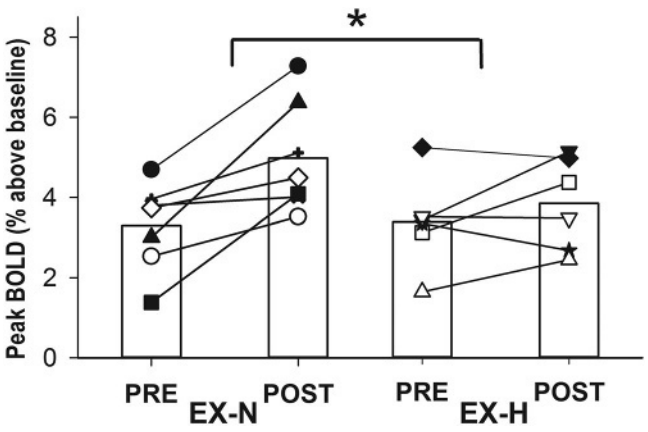

FIGURE 4—The influence of hypertension and exercise training on muscle BOLD responses. The peak BOLD response is shown after 1-s PF for individuals (*lines*) and groups (*open bars*) before (PRE) and after (POST) exercise training. EX-N, exercise group normotensive; EX-H, exercise group hypertensive. *$P = 0.058$ for the interaction between the subgroups.

capacity and rate (29). Chronic endurance exercise training can attenuate many of the age-related changes in vascular function (20,24,30). For example, recent findings show that rapid onset vasodilation in the common femoral artery during submaximal knee extensions is greater in chronically trained older endurance athletes compared with sedentary age-matched counterparts (24). Likewise, both younger and older endurance trained adults have increased muscle BOLD responses compared with sedentary adults (6,23). The current study extends these findings by demonstrating an increase in muscle BOLD MRI responses and peak postcontractile blood flow after 12 wk of endurance exercise training in older adults. While many studies have evaluated group differences in BOLD MRI responses, we believe this is the first to show an improvement in BOLD responses as well as rapid onset vasodilation with an exercise intervention and notably in a population with reduced microvascular function (6). As expected, both measures improved, which is in accordance with the consensus that exercise improves vascular function.

The changes in microvascular function after exercise training are both statistically significant and physiologically relevant. The peak BOLD response POST for EX was similar to the peak BOLD response in young sedentary adults (~4.5% ± 1.4%) (6). In the current study, the peak BOLD response improved by 33%; similarly, young sedentary adults had a 36% greater peak BOLD response in the soleus compared with older sedentary adults (6). The findings in the current study show a 35%–40% improvement in postcontractile popliteal artery peak blood flow after moderate exercise training. In comparison, Hughes et al. (12), reported approximately 50% greater femoral artery postcontractile peak blood flow responses in young untrained adults compared with older untrained adults. In addition, Hughes et al. (24), reported that highly endurance trained older adults ($\dot{V}O_{2peak}$ = 37.5 mL·kg^{-1}·min^{-1}) had approximately 80% greater femoral artery peak blood flow compared with untrained older adults. Overall, the vascular changes reported in the current study represent meaningful improvements especially given the moderate intensity of the exercise training.

Vascular responses after a single brief contraction arise principally from rapid arteriole vasodilation (31,32) reflecting microvascular function. Muscle BOLD responses are an integration of changes in small vessel oxygen saturation and blood volume and reflect a balance of blood flow and oxygen consumption kinetics (19). After endurance exercise, BOLD MRI responses were higher in amplitude and had a faster time course. As predicted from BOLD modeling (19), the faster TTP BOLD response after training is likely a reflection of faster blood flow kinetics. In agreement, the slope of the blood flow response clearly increases after training (Fig. 3). The peak blood flow response was moderately correlated with the peak BOLD response, showing that while related, the two measures provide distinct information as shown in prior data on young adults (19). Furthermore, a divergence of large artery flow and microvascular function has also been reported after repetitive exercise in the arm of diabetics with microvascular disease (10). Consistent with the current study, lower limb initial blood flow kinetics during acute repetitive exercise are improved with short-term endurance training (33). The initial blood flow kinetics during exercise have been shown to be more responsive than steady state flow adaptations with disuse or exercise training (23,33,34). Collectively, these studies support the premise that the initial blood flow response may be the major adaptation to blood flow changes after endurance exercise and sedentary behavior.

As microvascular function is influenced by several factors, the underlying mechanisms to explain the increased vascular responses in the current study may be a reflection of a combination of vascular adaptations. First, capillary density and ultrastructure may explain the increased responses. Data from biopsy studies have shown increases in capillary density (22) and reductions in capillary basement membrane (35) after exercise training. The ability to improve this contributor of microvascular function is considerably meaningful in older adults as low capillary density has been linked to reductions in activities of daily living (8,36). Exercise training also could enhance arteriolar vasodilation through increased bioavailability or sensitivity to vasodilators, including nitric oxide (NO), ATP, or potassium. In support, habitual exercise training blunts age-related declines in NO bioavailability (30) and improves functional sympatholysis (37,38). On the other hand, increasing NO in older adults was not associated with lower extremity blood flow, suggesting other factors are predominating (30). A reduction of vasoconstrictors, such as endothelin-1, may also play a role in improved exercise hyperemia in older adults (39). The underlying mechanisms have yet to be resolved.

This study sample included older adults with health conditions that may influence the primary outcome measures. Although none had a serious prior cardiovascular or cerebral vascular event or peripheral arterial disease, half of the recruited subjects were under pharmacological treatment for hypertension. Our data in this study show that adults with hypertension

did not have reduced baseline microvascular function as has been reported previously in a separate sample of older adults (6). Although the current study was not designed to study the effect of hypertension, older hypertensive adults in the current study appeared to have a blunted BOLD microvascular increase after exercise training compared with the normotensive subgroup. EX-H showed improvements in muscle oxidative capacity, suggesting an adequate exercise stimulus. It is possible that the older hypertensive group in the current study is part of an angiogenic nonresponder population that does not experience increases in capillary density in response to endurance training (40). In contrast to our finding, studies in younger hypertensive adults have shown improvements in capillarization and capillary ultrastructure (41) and improvements in functional sympatholysis (42) after endurance exercise training. Increased peripheral vascular resistance associated with hypertension may have limited the changes in microvascular function. Essential hypertension has been characterized by a reduction in arteriole lumen and media/lumen ratio that contribute to a reduced vasodilator reserve (43). A study focusing on hypertension in a setting where local vascular resistance could be determined would help in understanding (limitations in) microvascular adaptations to endurance training.

The study had limitations related to the recruited sample. The sedentary older adults had elevated BMI and many were treated for high cholesterol and hypertension. It is well known that hypertensive medications influence blood pressure, blood flow, and heart rate. To limit within subject variation, all medications were consistent during testing and training periods. In addition, the medication intake and testing time was held constant for the testing days.

In the present study, we explored the effects of an aerobic exercise regimen on age-related microvascular changes using innovative, noninvasive techniques to quantify microvascular function in the lower leg. Overall, these results suggest that the age-related declines in small vessel function can be improved in the lower limb with a moderately intense aerobic exercise intervention. The study also supports the use of single contraction blood flow and MRI BOLD responses to evaluate changes in microvascular function. Aerobic exercise may also have the capacity to improve microvascular function in other populations, such as patients with diabetic peripheral neuropathy who have reduced peripheral vascular function.

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REFERENCES

1. Hamann JJ, Buckwalter JB, Clifford PS. Vasodilatation is obligatory for contraction-induced hyperaemia in canine skeletal muscle. *J Physiol*. 2004;557(Pt 3):1013–20.
2. Marshall JM, Tandon HC. Direct observations of muscle arterioles and venules following contraction of skeletal muscle fibres in the rat. *J Physiol*. 1984;350:447–59.
3. VanTeeffelen JW, Segal SS. Rapid dilation of arterioles with single contraction of hamster skeletal muscle. *Am J Physiol Heart Circ Physiol*. 2006;290(1):H119–27.
4. Armstrong ML, Dua AK, Murrant CL. Time course of vasodilation at the onset of repetitive skeletal muscle contractions. *Am J Physiol Regul Integr Comp Physiol*. 2007;292(1):R505–15.
5. Schulte AC, Aschwanden M, Bilecen D. Calf muscles at blood oxygen level–dependent MR imaging: aging effects at postocclusive reactive hyperemia. *Radiology*. 2008;247(2):482–9.
6. Tonson A, Noble KE, Meyer RA, Rozman MR, Foley KT, Slade JM. Age reduces microvascular function in the leg independent of physical activity. *Med Sci Sports Exerc*. 2017;49(8):1623–30.
7. Jackson DN, Moore AW, Segal SS. Blunting of rapid onset vasodilatation and blood flow restriction in arterioles of exercising skeletal muscle with ageing in male mice. *J Physiol*. 2010;588(Pt 12):2269–82.
8. Robbins JL, Jones WS, Duscha BD, et al. Relationship between leg muscle capillary density and peak hyperemic blood flow with endurance capacity in peripheral artery disease. *J Appl Physiol (1985)*. 2011;111(1):81–6.
9. Slade JM, Towse TF, Gossain VV, Meyer RA. Peripheral microvascular response to muscle contraction is unaltered by early diabetes but decreases with age. *J Appl Physiol (1985)*. 2011;111(5):1361–71.
10. Womack L, Peters D, Barrett EJ, Kaul S, Price W, Lindner JR. Abnormal skeletal muscle capillary recruitment during exercise in patients with type 2 diabetes mellitus and microvascular complications. *J Am Coll Cardiol*. 2009;53(23):2175–83.
11. Hearon CM Jr., Dinenno FA. Regulation of skeletal muscle blood flow during exercise in ageing humans. *J Physiol*. 2016;594(8):2261–73.
12. Hughes WE, Ueda K, Treichler DP, Casey DP. Rapid onset vasodilation with single muscle contractions in the leg: influence of age. *Physiol Rep*. 2015;3(8):e12516.
13. Carlson RE, Kirby BS, Voyles WF, Dinenno FA. Evidence for impaired skeletal muscle contraction-induced rapid vasodilation in aging humans. *Am J Physiol Heart Circ Physiol*. 2008;294(4):H1963–70.
14. Lawrenson L, Poole JG, Kim J, Brown C, Patel P, Richardson RS. Vascular and metabolic response to isolated small muscle mass exercise: effect of age. *Am J Physiol Heart Circ Physiol*. 2003;285(3):H1023–31.
15. Jacobi B, Bongartz G, Partovi S, et al. Skeletal muscle BOLD MRI: from underlying physiological concepts to its usefulness in clinical conditions. *J Magn Reson Imaging*. 2012;35(6):1253–65.
16. Utz W, Jordan J, Niendorf T, et al. Blood oxygen level–dependent MRI of tissue oxygenation: relation to endothelium-dependent and endothelium-independent blood flow changes. *Arterioscler Thromb Vasc Biol*. 2005;25(7):1408–13.
17. Ledermann HP, Schulte AC, Heidecker HG, et al. Blood oxygenation level–dependent magnetic resonance imaging of the skeletal muscle in patients with peripheral arterial occlusive disease. *Circulation*. 2006;113(25):2929–35.

18. Sanchez OA, Copenhaver EA, Chance MA, et al. Postmaximal contraction blood volume responses are blunted in obese and type 2 diabetic subjects in a muscle-specific manner. *Am J Physiol Heart Circ Physiol*. 2011;301(2):H418–27.
19. Towse TF, Slade JM, Ambrose JA, DeLano MC, Meyer RA. Quantitative analysis of the postcontractile blood-oxygenation-level-dependent (BOLD) effect in skeletal muscle. *J Appl Physiol (1985)*. 2011;111(1):27–39.
20. Seals DR, Walker AE, Pierce GL, Lesniewski LA. Habitual exercise and vascular ageing. *J Physiol*. 2009;587(Pt 23):5541–9.
21. Kingwell BA. Large artery stiffness: implications for exercise capacity and cardiovascular risk. *Clin Exp Pharmacol Physiol*. 2002; 29(3):214–7.
22. Prior SJ, Goldberg AP, Ortmeyer HK, et al. Increased skeletal muscle capillarization independently enhances insulin sensitivity in older adults after exercise training and detraining. *Diabetes*. 2015;64(10):3386–95.
23. Towse TF, Slade JM, Meyer RA. Effect of physical activity on MRI-measured blood oxygen level–dependent transients in skeletal muscle after brief contractions. *J Appl Physiol (1985)*. 2005;99(2): 715–22.
24. Hughes WE, Ueda K, Casey DP. Chronic endurance exercise training offsets the age-related attenuation in contraction-induced rapid vasodilation. *J Appl Physiol (1985)*. 2016;120(11):1335–42.
25. Gibbons RJ, Balady GJ, Beasley JW, et al. ACC/AHA guidelines for exercise testing: executive summary. A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Exercise Testing). *Circulation*. 1997;96(1):345–54.
26. Duteil S, Wary C, Raynaud JS, et al. Influence of vascular filling and perfusion on BOLD contrast during reactive hyperemia in human skeletal muscle. *Magn Reson Med*. 2006;55(2):450–4.
27. Wigmore DM, Damon BM, Pober DM, Kent-Braun JA. MRI measures of perfusion-related changes in human skeletal muscle during progressive contractions. *J Appl Physiol (1985)*. 2004;97(6):2385–94.
28. Lash JM, Bohlen HG. Functional adaptations of rat skeletal muscle arterioles to aerobic exercise training. *J Appl Physiol (1985)*. 1992;72(6):2052–62.
29. Park SY, Ives SJ, Gifford JR, et al. Impact of age on the vasodilatory function of human skeletal muscle feed arteries. *Am J Physiol Heart Circ Physiol*. 2016;310(2):H217–25.
30. Nyberg M, Blackwell JR, Damsgaard R, Jones AM, Hellsten Y, Mortensen SP. Lifelong physical activity prevents an age-related reduction in arterial and skeletal muscle nitric oxide bioavailability in humans. *J Physiol*. 2012;590(21):5361–70.
31. Segal SS. Regulation of blood flow in the microcirculation. *Microcirculation*. 2005;12(1):33–45.
32. Tschakovsky ME, Rogers AM, Pyke KE, et al. Immediate exercise hyperemia in humans is contraction intensity dependent: evidence for rapid vasodilation. *J Appl Physiol (1985)*. 2004;96(2):639–44.
33. Shoemaker JK, Phillips SM, Green HJ, Hughson RL. Faster femoral artery blood velocity kinetics at the onset of exercise following short-term training. *Cardiovasc Res*. 1996;31(2):278–86.
34. Olive JL, Slade JM, Dudley GA, McCully KK. Blood flow and muscle fatigue in SCI individuals during electrical stimulation. *J Appl Physiol (1985)*. 2003;94(2):701–8.
35. Williamson JR, Hoffmann PL, Kohrt WM, Spina RJ, Coggan AR, Holloszy O. Endurance exercise training decreases capillary basement membrane width in older nondiabetic and diabetic adults. *J Appl Physiol (1985)*. 1996;80(3):747–53.
36. Nicklas BJ, Leng I, Delbono O, et al. Relationship of physical function to vastus lateralis capillary density and metabolic enzyme activity in elderly men and women. *Aging Clin Exp Res*. 2008;20(4):302–9.
37. Kruse NT, Hughes WE, Hanada S, et al. Evidence of a greater functional sympatholysis in habitually aerobic trained postmenopausal women. *J Appl Physiol (1985)*. 2018;124(3):583–91.
38. Mortensen SP, Nyberg M, Winding K, Saltin B. Lifelong physical activity preserves functional sympatholysis and purinergic signalling in the ageing human leg. *J Physiol*. 2012;590(23):6227–36.
39. Barrett-O'Keefe Z, Ives SJ, Trinity JD, et al. Endothelin-A-mediated vasoconstriction during exercise with advancing age. *J Gerontol A Biol Sci Med Sci*. 2015;70(5):554–65.
40. Baum O, Gübeli J, Frese S, et al. Angiogenesis-related ultrastructural changes to capillaries in human skeletal muscle in response to endurance exercise. *J Appl Physiol (1985)*. 2015;119(10):1118–26.
41. Gliemann L, Buess R, Nyberg M, et al. Capillary growth, ultrastructure remodelling and exercise training in skeletal muscle of essential hypertensive patients. *Acta Physiol (Oxf)*. 2015;214(2):210–20.
42. Mortensen SP, Nyberg M, Gliemann L, Thaning P, Saltin B, Hellsten Y. Exercise training modulates functional sympatholysis and α-adrenergic vasoconstrictor responsiveness in hypertensive and normotensive individuals. *J Physiol*. 2014;592(14):3063–73.
43. Laurent S, Boutouyrie P. The structural factor of hypertension: large and small artery alterations. *Circ Res*. 2015;116(6):1007–21.