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ABSTRACT

Purpose: It is unclear if high-intensity-interval-training (HIIT) elicits superior improvements in brachial artery (BA) flow-mediated-dilation (FMD) responses (i.e., endothelial-dependent vasodilation) than moderate-intensity-continuous-training (MICT) or resistance training (RT) in otherwise healthy older adults. Whether HIIT enhances lower-limb FMD responses and/or augments low-flow-mediated-constriction (L-FMC; endothelial-dependent vasoconstriction) responses more than MICT or RT is also unknown. We tested the hypothesis that HIIT would improve BA and popliteal artery (POP) FMD and L-FMC responses more than MICT or RT in healthy older adults. **Methods:** Thirty-eight older adults (age: 67±6 yrs) performed 6-weeks of either HIIT [2×20min bouts alternating between 15s intervals at 100% of peak power output (PPO) and passive recovery (0% PPO); n=12], MICT (34min at 60% PPO; n=12), or whole-body RT (8 exercises, 2×10 repetitions; n=14). L-FMC and FMD were measured before and after training using high-resolution ultrasound and quantified as the percent change in baseline diameter during distal cuff occlusion and following cuff release, respectively. **Results:** Resting BA blood flow and vascular conductance (both, $P<0.003$) were greater following HIIT only. HIIT and MICT similarly increased BA-FMD (pre-post: both, $P<0.001$), but only HIIT improved BA L-FMC ($P<0.001$). Both HIIT and MICT similarly enhanced POP FMD and L-FMC responses (both, $P<0.045$). RT did not impact FMD or L-FMC responses in either artery (all, $P>0.20$). **Conclusions:** HIIT and MICT, but not RT, similarly improved lower-limb vasodilator and vasoconstrictor endothelial function in older adults. While HIIT and MICT groups enhanced BA vasodilator function, only HIIT improved resting conductance and endothelial sensitivity to low-flow in the BA. In the short-term, HIIT may be most effective at improving peripheral vascular endothelial function in older adults. **Keywords:** aging; exercise intensity; endothelial-dependent vasodilation; endothelial-dependent vasoconstriction, popliteal artery

INTRODUCTION

Older adults are at an elevated risk of cardiovascular disease due to the development of atherosclerosis, which is initially characterized by an impaired endothelial-dependent dilation in response to physical or chemical stimuli (1). The non-invasive, flow-mediated dilation (FMD) assessment provides clinically relevant information regarding the ability of the endothelium to produce and release nitric oxide (NO) following a reactive hyperemia elicited by a prior period of distal ischemia. Most FMD studies have been performed in the brachial artery (BA), which provides an indication of coronary artery endothelial health (2) and is predictive of future cardiovascular events (3). However, lower limb arteries such as the popliteal artery (POP) are more susceptible to the development of atherosclerosis and peripheral vascular disease than upper-limb arteries (4). In addition to providing a clinically relevant measure of endothelial vasodilator function, the decline in conduit artery diameter observed during the distal ischemic period of the FMD test, termed low-flow mediated constriction (L-FMC), provides an index of endothelial-dependent *vasoconstrictor* function. L-FMC provides information regarding how reduced shear stress influences vascular tone (5) and is lower in older adults and patients with coronary artery disease (6). We have reported that the POP exhibits smaller L-FMC responses than the BA in older adults (7), which highlights the importance of determining whether exercise training impacts vascular endothelial function differently between upper- and lower-limb arteries.

It is well established that aerobic exercise training augments vasodilator function in older adults with chronic disease (e.g., hypertension, heart failure, type 2 diabetes), with high-intensity interval training (HIIT) eliciting superior improvements in BA-FMD than moderate-intensity continuous training (MICT) (8, 9). The superior effects of HIIT have been attributed to larger and more sustained blood flow responses and/or greater reductions in oxidative stress, which

together may enhance endothelial cell sensitivity to shear stress and increase NO bioavailability [as reviewed in (9)]. Most previous reports that have compared the effects of HIIT and MICT on vasodilator function have been conducted solely in the BA of older adults with chronic disease and of a longer duration (i.e., >10 weeks) [as reviewed in (9)]. To date, the only study to directly evaluate the impact of aerobic exercise intensity in older adults free of chronic disease observed no improvement in BA-FMD after 2 weeks of HIIT [10×1-min at 100% peak power output (PPO)] or MICT (40-min at 65% PPO) in post-menopausal women (10). As such, 2-weeks (6 total exercise sessions) may not provide a sufficient stimulus to elicit favorable BA-FMD changes in this population. It is unclear if 6-weeks of aerobic exercise improves BA-FMD in healthy older adults, improves endothelial function in arteries responsible for supplying blood flow to the active limbs during traditional modes of aerobic exercise (e.g., POP), or if HIIT produces greater improvements in FMD than MICT in these arteries. Cross-sectionally, we have demonstrated that cardiorespiratory fitness is moderately related to BA L-FMC (11) and strongly related to POP L-FMC (7) in older adults. Whether or not short-term aerobic exercise training interventions enhances L-FMC in either artery in older adults is unclear, and if HIIT augments the endothelial-dependent vasoconstrictor response more than MICT is also unknown in this population.

It is recommended that older adults engage in resistance training (RT) a minimum of 2 days per week (12). Resistance exercise elicits larger post-contraction blood flow responses than aerobic exercise, but this augmented blood flow response is transient and under high-pressure in comparison to the sustained low-pressure flow observed during aerobic exercise (13–15). Despite these differences in exercise-induced shear stress profiles, RT appears to be an effective stimulus for increasing BA-FMD, albeit less than aerobic training in older adults with chronic disease [as reviewed in (8)]. To date, the influence of short-term RT-induced adaptations on

lower-limb endothelial-dependent vasodilatory or vasoconstrictor responses remains uncertain in healthy older adults.

We investigated the effects of short-term (6-weeks) HIIT, MICT and RT on upper- and lower-limb endothelial function in older adults. We hypothesized that HIIT would elicit superior improvements in BA-FMD and POP-FMD than MICT, and that these aerobic protocols will elicit greater endothelial-dependent vasodilator responses than RT. Based on our previous observations that greater aerobic fitness was associated with larger L-FMC responses in both the BA and POP (7, 11), we anticipated that 6-weeks of HIIT or MICT, but not RT, will augment endothelial-dependent vasoconstrictor function in this population.

MATERIALS AND METHODS

Participants. Thirty-eight older adults (23 females; age 56-83 years) were recruited from the Active Aging program at Acadia University (Table 1). Participants had no physical limitations to exercise and a resting blood pressure <140/90 mmHg. Three participants were on Synthroid for hypothyroidism ($n = 1$ in each group). Four participants were prescribed medications to treat high blood pressure. Specifically, participants were taking Teveten® (angiotensin-receptor blocker; $n = 1$; HIIT), Adalat® (calcium channel blocker; $n = 1$; RT); Diuril® (diuretic; $n = 1$; HIIT) and Coversyl Plus® (angiotensin converting enzyme inhibitor + diuretic; $n = 1$; RT). One person was asthmatic (RT). During the study, participants were requested to continue taking all prescribed medications. Participants were informed of the methods and study design verbally and in writing before providing written informed consent. Participants were randomized to HIIT ($n = 12$), MICT ($n = 12$), or RT ($n = 14$) after the pre-training determination of aerobic fitness and initial vascular testing day. The pre-training vascular endothelial data have previously been presented in a cross-sectional study investigating

L-FMC responses between the BA and POP (7). All protocols and procedures conformed to the Declaration of Helsinki and were approved by the Dalhousie University Health Sciences and Acadia University Research Ethics Boards.

Experimental Design. Participants underwent four separate laboratory visits in total. Days 1 and 2 were conducted pre-training while days 3 and 4 were completed following training. Days 1 and 3 involved measurements of height and body mass, which were followed by a graded, maximal cycling exercise test to determine aerobic fitness (VO_{2peak}). PPO was also recorded and used to establish exercise intensities for the two aerobic training protocols. Days 2 and 4 were dedicated to the assessments of BA and POP vascular function and conducted either prior to, or a minimum of 48-h (or maximum one-week) following the graded exercise tests (see below for details). To minimize known confounding influences on endothelial-dependent dilation, vascular assessments were performed 6-h post-prandial while participants avoided strenuous physical activity, as well as the consumption of products known to acutely influence endothelial responses (e.g., caffeine, chocolate, kiwi, saturated fats, folic acid supplements, antioxidant and multivitamin supplements) for 24-h, consistent with FMD guidelines (16). Participants replicated their diet prior to both the pre-training and post-training vascular measurements. Upon arriving to the laboratory, participants rested in the supine position for a minimum of 20-min prior to the vascular assessments. Vascular function was assessed first in the BA, followed by at least 10-min of rest before the POP measures. All study visits were performed in a thermoneutral environment (21°C). Days 2 and 4 were performed at the same time of day within each participant to control for diurnal variations in blood pressure and vascular function.

Anthropometrics and peak aerobic fitness. Height and weight were measured using a calibrated stadiometer (Health-O-Meter, McCook Il, USA) to the nearest 0.5 cm and 0.1 kg, respectively. An incremental and maximal exercise test on a cycle ergometer (Lode Excalibur

Sport, Groningen, The Netherlands) was administered to determine VO_{2peak} via a mixing chamber-based commercial metabolic system (TrueOne 2400®, Parvomedics Inc., Sandy, UT). Following a 5-min warm-up period of light-intensity cycling (30-50W), the workload was set at 1 watt/kg body mass and gradually increased by 15 watts/min until voluntary exhaustion. Strong verbal encouragement was provided throughout the test. Upon completion of the test, the workload was immediately reduced to the warm-up level for a 5-min cool-down period. The workload of the last completed stage was considered as the PPO, measured in watts.

Training protocols. For the three training protocols, all sessions were supervised and conducted 3 days per week (Mondays, Wednesdays and Fridays) for 6-weeks. As outlined in Figure 1, all participants completed 18 supervised sessions with no participants dropping out of the study. Warm-up and cool-down periods consisted of 5-min at 25% PPO for both the HIIT and MICT protocols. The HIIT protocol was based on a previous study that compared the time to exhaustion, safety, participant preference, and time spent near VO_{2max} in older adults (17). Specifically, the protocol implemented in the present study resulted in the longest time to exhaustion and a similar amount of time spent above $80\% VO_{2max}$ and $90\% VO_{2max}$ versus 3 other HIIT protocols varying in work:rest ratios and recovery intensity (active vs. passive) (17). Furthermore, previous research has demonstrated that this HIIT protocol elicits greater mean VO_2 responses than an isocaloric bout of moderate-intensity continuous exercise performed at 70%PPO (18). For the first 2-weeks, the HIIT protocol consisted of forty, 15-s intervals at 100% PPO interspersed with 15-s of passive recovery. Following a 5-min passive recovery period, a second set of 40 intervals was completed (i.e., 40-min total time). To adjust for anticipated improvements in aerobic fitness and exercise tolerance, the duration of the HIIT protocol was increased to 45-min (2×22.5-min; 2 sets of 45 intervals) for the remaining 4-weeks and workload increased by 15 watts for the final 2-weeks. Training volume was decreased by 10-min

(from 2 sets of 45 intervals to 2 sets of 35 intervals) for the final 2 training sessions to ensure appropriate recovery prior to the post-training assessment of aerobic fitness.

The MICT protocol was based on the American College of Sports Medicine physical activity guidelines that recommends at least 30-min of daily moderate aerobic physical activity (19). Continuous cycling at 60% PPO for ~34-min was initially prescribed. This duration was adjusted to ensure that the MICT protocol was isoenergetic to the HIIT protocol based on the assumption that mechanical efficiency, aerobic fitness and PPO were similar between groups; in that 20-min at 100% PPO expends the same energy as 34-min at 60% PPO. To support this assumption, the HIIT and MICT groups demonstrated similar pre-training VO_{2peak} and PPO (both, P=1.00, Table 1). To accommodate the matched increase in energy expenditure, total exercise time was prolonged to 39-min for the remaining 4-weeks and power output increased by 15W for the final 2-weeks. For the final 2 MICT sessions, participants decreased their cycling time from 39 to 30-min in preparation for the post-training determination of maximal aerobic fitness.

Each RT session began and ended with 3-min of light cycling at 25% PPO. Thereafter, participants completed a total of 8 strength exercises, alternating between muscle groups. The exercises were primarily isokinetic machine-based and included leg press, bench press, hamstrings curl, shoulder press and leg extensions. Cable-exercises included seated row and latissimus pull-down, and bird-dogs (i.e., a core exercise that involves kneeling on the floor and simultaneously extending the hip while flexing the contralateral shoulder). Each participant performed 2×10 repetitions at 70% of perceived one repetition maximum (1RM) for the first 2-weeks. Both sets of each exercise were performed before starting the next exercise with 30-60 seconds of rest between sets. Upper- and lower-body exercises were alternated. Participants were instructed to increase the number of repetitions to 12 *ad libitum*. Once participants were able to

perform 2×12 repetitions, the resistance was proportionally increased to a weight that equated to 10 repetitions of their new estimated 70% of 1RM with the assistance of the supervising Canadian Society for Exercise Physiology Certified Exercise Physiologist.

Hemodynamics. Heart rate (HR) was determined via cardiac intervals obtained from lead II of a standard bipolar limb lead electrocardiogram (ECG). Beat-by-beat systolic (SBP) and diastolic (DBP) blood pressures were measured using finger photoplethysmography (Portapres®; Finapres Medical Systems, Amsterdam, Netherlands). The finger used for recording blood pressure was maintained at heart level throughout the protocol and minor deviations in height between the heart and finger were corrected using the Portapres® height correction unit. Left brachial artery measurements of SBP and DBP were also recorded by an automated patient vital signs monitor (Carescape v100®, General Electric Healthcare) and used to perform a 'physiological calibration' of the Portapres® waveform. All data were sampled continuously at 200 Hz using a PowerLab (PL3508 PowerLab 8/53, ADInstruments, Sydney, Australia) data acquisition system with the exception of the ECG waveform, which was sampled at 1000 Hz. Recordings were displayed in real-time and analyzed offline using LabChart software (ADInstruments, Sydney, Australia).

Vascular Measures. As described in O'Brien et al. (20), the right BA and left POP were imaged with the participants in the supine and prone positions, respectively. The BA was imaged 3-5 cm proximal to the antecubital fossa and the POP was imaged proximal to the bifurcation at or slightly above the popliteal fossa. A pressure cuff attached to a rapid inflation system (E20 and AG101, Hokanson®, Bellevue, WA) was positioned around the largest circumference of the forearm (BA; ~3 cm distal to the antecubital fossa) or lower leg (POP; ~10 cm distal to the popliteal fossa). All images were obtained using a 12-MHz multi-frequency linear array probe attached to a high-resolution ultrasound system (Vivid i, General Electric Healthcare). Blood

velocity signals were recorded in duplex mode at a pulsed frequency of 5-MHz and corrected with an insonation angle of 60° that remained constant throughout the study. The sample volume was adjusted for each participant such that the anterior to the posterior intima were included, as recommended in published guidelines (16). Artery lumen diameter and blood velocity were measured for a minimum of 2-min prior to inflation of the pneumatic cuff. The pressure cuff was then rapidly inflated to 250 mmHg for 5-min. Continuous arterial lumen diameter and blood velocity recordings were collected throughout the cuff inflation period. Upon release of cuff pressure, lumen diameter and blood velocity recordings continued for an additional 5-min.

Following a minimum 10-min of rest after the FMD test, the POP was imaged for 1-min before and 10-min following a sublingual administration of nitroglycerin spray (0.4 mg). This nitroglycerin test provides a measure of endothelial-independent vasodilation (21).

Data Analysis: Relative VO_2 data were averaged over 15-s intervals for the duration of the graded exercise protocol. Maximum or peak VO_2 were considered as the greatest 30-s averaged VO_2. SBP and DBP were determined from the Portapres® waveform as the maximum and minimum waveform values, respectively. These pressures were then used to calculate mean arterial pressure (MAP) using the equation ⅓ SBP + ⅔ DBP. In addition, stroke volume (SV) was derived from the raw finger blood pressure waveforms using the ModelFlow® method incorporated into the non-invasive cardiac output add-on for LabChart® (ADInstruments, Sydney, Australia). Cardiac output (Q) was calculated as the product of HR and SV and total vascular conductance (TVC) as Q÷MAP. Portapres® data (SV, Q and TVC) were not recorded from two participants in the MICT group (1♂, 1♀) due to equipment malfunctions. Hemodynamic data were averaged over at least 5-min of beat-by-beat data recoded immediately prior to the POP-FMD protocol.

Video signals from the ultrasound were exported to a laptop via a video graphics array converter (Epiphan Systems Inc., VGA 2 USB, Ottawa) for offline analysis. Analysis of artery diameter, blood velocity and shear rate (i.e., frictional force of blood flow on the endothelium) were performed using automated commercial edge-detection and wall-tracking software combined with simultaneous Doppler waveform envelope analysis (FMD Studio, Cardiovascular Suite, Quipu, Pisa, Italy). This software was used to measure baseline diameter, nadir diameter (i.e., L-FMC) and peak diameter (i.e., FMD). All vascular measurements were blindly analyzed by a single investigator from the lab, who has demonstrated coefficients of variation of 2.2%, 2.7%, 3.8%, and 4.2% for baseline diameter, nadir diameter, L-FMC% and FMD%, respectively. reliability (7, 11, 20, 22).

Absolute FMD was calculated as the difference (in mm) between the post-cuff deflation peak and baseline diameters. Relative FMD was calculated using the equation: FMD (%) = [(post-cuff deflation peak diameter – baseline diameter) ÷ baseline diameter × 100%]. Blood flow (ml/min) was calculated as mean blood velocity (cm/s) × π × lumen radius (cm)^2 × 60 (s/min). Popliteal and brachial vascular conductance were calculated by dividing POP and BA blood flow by MAP, respectively. All blood flow and MAP data from the corresponding FMD-baseline periods were used for these calculations. Shear rate (SR, s^{-1}) was defined as [4 × mean blood velocity (cm/s)] / diameter (cm). Subsequently, the SR area under the curve (SR$_{AUC}$) was calculated between the start of cuff deflation to the time that peak dilation occurred. Whether or not, and how, FMD responses should be normalized to SR$_{AUC}$ is unclear (16, 23). Our data did not meet the statistical assumptions required to normalize the FMD responses to SR$_{AUC}$ (23, 24) in either the BA or POP [see Figures, Supplemental Digital Content 1 and 2, which contains scatterplots demonstrating the relationship between brachial (http://links.lww.com/MSS/B848) and popliteal (http://links.lww.com/MSS/B849) shear rate area under the curve versus FMD]).

However, SR_{AUC} responses and the relationships between FMD and SR_{AUC} are presented for both arteries, as per recommendations (16, 23).

L-FMC was represented in absolute (mm) and relative (%) terms using the nadir diameter obtained during the final 30-s of the 5-min distal cuff occlusion period. Relative L-FMC was calculated using the equation: L-FMC (%) = [(baseline diameter − nadir diameter) ÷ baseline diameter ×100%]. The vasoactive range (VAR) was calculated as [(post-cuff deflation peak diameter − nadir diameter) ÷ baseline diameter ×100%] and expressed in both absolute (mm) and relative (%) terms.

POP nitroglycerin-mediated vasodilation was calculated as a percentage change from baseline to the peak lumen diameter obtained during the 10-min period following sublingual administration of nitroglycerin. POP nitroglycerin-mediated vasodilation was not conducted in two participants (total participants: HIIT: $n=11$ and MICT: $n=11$)

As described by Atkinson and Batterham (25), allometric scaling of FMD has been recommended to account for differences in baseline arterial diameter. Although based on FMD analysis, we previously demonstrate that baseline diameter is correlated to the L-FMC responses in older adults (7). Allometric scaling is recommended if the relationship between the natural log of peak FMD diameter (or nadir diameter for L-FMC) and resting diameter yield an unstandardized β-coefficient that deviates from 1 and/or have an upper 95% confidence interval <1. These assumptions were met for L-FMC measurements in the BA and POP with β ± standard error (95% confidence intervals) of 0.979 ± 0.01 (0.97-0.99) and 0.989 ± 0.01 (0.98-1.00), respectively. However, the allometric assumptions were not met for BA-FMD [1.036 ± 0.01 (1.01-1.06)] and POP-FMD [1.008 ± 0.01 (0.99-1.03)], suggesting allometric scaling of FMD to be unnecessary in this study. These assumptions remained when checked for each group individually. Allometrically scaled L-FMC were examined using an analysis of covariance

(ANCOVA) model with the natural log of the difference [ln(nadir diameter) – ln(baseline diameter)] as the dependent variable and ln(baseline diameter) as the covariate. Group (HIIT, MICT, RT) and time (pre-training, post-training) were used as a fixed-factors. Statistically significant ANCOVAs were followed up with pairwise comparisons using Fisher's least squares difference *post hoc* testing (25). For each instance, overall group (not individual) allometrically scaled arterial diameter changes were back transformed and presented as a percent change from the baseline diameter, as described in more detail previously (25). Separate ANCOVA models were conducted for each artery.

Statistical Analysis. Participant descriptive characteristics were compared using one-way analysis of variance (ANOVA). The effects of exercise training on resting hemodynamic and vascular measurements were compared using a between-subjects (Group × Time) repeated measures ANOVA. The variance of differences was assessed using Mauchly's test of sphericity and when violated, the Greenhouse-Geisser correction to the degrees of freedom was applied. Bonferroni post-hoc testing was conducted on statistically significant ANOVAs. All data were assessed for normality using a Shapiro-Wilk test, and non-normalized data were appropriately transformed (e.g., log transformation) prior to statistical analysis. Based on the previously observed relationship between aerobic fitness and L-FMC (7, 11), correlational analysis were conducted to determine if the change in aerobic fitness (ΔVO_{2peak}) was related to the change (ΔL-FMC) in either artery. Although not the purpose of the present study, correlational analysis comparing the exercise training induced changes in L-FMC (ΔL-FMC) and FMD (ΔFMD) between the BA and POP are presented in Supplemental Digital Content 3 [see Figure, Supplemental Digital Content 3, Scatterplot showing the relationship between the change in relative flow-mediated dilation (FMD; A) responses and changes in relative low-flow-mediated constriction (L-FMC; B) between the brachial and popliteal arteries,

http://links.lww.com/MSS/B850]. Some participants were taking anti-hypertensive medication (n = 4), which may have influenced their vascular function. Removing these individuals from analyses did not alter the significant findings determined from the full sample (see below). All statistics were completed in SPSS Version 25.0 (IBM, NY). Statistical significance was accepted as $P < 0.05$. All data are presented as means ± standard deviations (SD).

RESULTS

Participant characteristics, systemic hemodynamics and aerobic fitness were similar between the groups at baseline (all, $P>0.29$) and are summarized in Table 1. No pre-post differences were observed for body mass index (RT: $P=0.09$), SV (HIIT: $P=0.08$), CO, TVC (HIIT: $P=0.06$), peak respiratory exchange ratio (HIIT: $P=0.08$), or peak HR (all, $P>0.11$ unless specified) in any of the training groups. Resting HR decreased ($P<0.04$) in the MICT group only. The HIIT group had lower post-training resting SBP, DBP, and MAP (all, $P<0.03$), but MICT only decreased DBP ($P=0.02$). RT did not change any systemic resting hemodynamic measurements (MAP: $P=0.06$; rest of variables, $P>0.11$). Compared to pre-training, all groups had a greater VO_{2peak} and PPO at post-training (both, $P<0.04$).

Brachial Artery Hemodynamics: BA lumen diameter was larger following RT ($P=0.02$), but not HIIT or MICT (both, $P>0.79$; see Table 2). The increase in resting BA diameter lead to a lower resting BA-SR in the RT group ($P=0.048$). Resting BA blood flow velocity and blood flow were increased following HIIT only (both, $P<0.003$), which, resulted in a higher BA-SR ($P=0.01$). The increases in BA blood flow and corresponding decreases in MAP following HIIT resulted in a larger resting BA vascular conductance in this group ($P<0.001$). MICT did not change any resting BA variables (all, $P>0.38$).

Brachial Artery Vasoreactivity: As shown in Table 2, BA peak diameter was greater following training in all three groups (all, $P<0.03$). BA-SR$_{AUC}$ was larger following HIIT and RT (both, $P<0.01$), but not MICT ($P=0.12$). Training did not change the time-to-peak diameter in any group (all, $P>0.37$). Relative BA-FMD was enhanced following HIIT (4.8±1.8% to 6.7±1.3%; $P<0.001$) and MICT (4.7±1.9% to 6.8±1.7%; $P<0.001$), but not RT (4.7±1.4% to 5.0 ± 1.4%; $P=0.52$; Figure 2A).

BA L-FMC was greater following HIIT (-0.9±1.7% to -1.7±1.2%; $P<0.001$), but not MICT (-0.9±1.0% to -1.2±0.9%; $P=0.14$) or RT (-0.8±0.6% to -0.9±0.7%; $P=0.68$; Figure 2B). This observation was unchanged after allometrically scaling values for baseline diameter (HIIT: $P=0.03$; MICT & RT: $P>0.42$; Figure 2C). The change in BA L-FMC was moderately correlated with the change in aerobic fitness ($r = $ -0.51, $P=0.001$; ΔVO$_{2peak}$ vs. ΔBA L-FMC; see Supplemental Digital Content 4, http://links.lww.com/MSS/B851). The greater FMD (HIIT & MICT) and L-FMC (HIIT only), resulted in a greater VAR following both HIIT and MICT (both, $P<0.001$).

Popliteal Artery Hemodynamics: As shown in Table 3, POP lumen diameter was larger after RT ($P=0.02$) but not HIIT or MICT (both, $P>0.62$). Mean POP blood flow velocity (MICT: $P=0.08$), blood flow, resting SR (MICT: $P=0.08$), and POP vascular conductance were unchanged following any of the three-training protocol (all, $P>0.10$ unless specified).

Popliteal Artery Vasoreactivity: A greater POP peak diameter in response to reactive hyperemia was observed after MICT and RT (both, $P<0.02$), but not HIIT ($P=0.052$) (Table 3). All groups had a post-training increase in the SR$_{AUC}$ (all, $P<0.001$) with no changes in the time-to-peak diameter ($P>0.52$). POP-FMD was enhanced following HIIT (3.6±1.9% to 4.9±1.5%; $P<0.001$) and MICT (2.6±1.7% to 4.0± 1.9%; $P<0.001$), but not RT (3.1±1.9% to 3.4±1.8%; $P=0.20$; see Figure 3A).

Similarly, POP L-FMC was increased after HIIT (-0.9±1.1% to -1.8±0.9%; P=0.01) and MICT (-0.8±1.6 to -1.5±0.7%; P=0.045), but was unchanged following RT (-1.0±1.7% to -1.0±1.1%; P=1.00; Figure 2B). However, allometrically scaled POP L-FMC was not statistically greater in the MICT group (-0.4±0.5% to -0.7±0.6%; P=0.16). In contrast, the greater L-FMC response following HIIT remained significant after scaling for baseline diameter (-0.4±0.6% to -0.8±0.6; P=0.047; Figure 3C). The change in POP L-FMC was not correlated to the change in aerobic fitness (r = -0.27, P=0.10; ΔVO_{2peak} vs. ΔPOP L-FMC; see Supplemental Digital Content 4, http://links.lww.com/MSS/B851).

The larger POP-FMD and POP L-FMC responses following HIIT and MICT resulted in a greater VAR in these groups (both, P<0.001), that was not observed following RT (P=0.44). POP nitroglycerin-mediated vasodilation was unchanged following any of the training protocols (all, P>0.33).

DISCUSSION

The purpose of this study was to compare endothelial-dependent vasodilator and vasoconstrictor responses in the brachial and popliteal arteries following short-term HIIT, MICT and RT in healthy older adults. Contrasting with our hypothesis, 6-weeks of HIIT and MICT similarly improved FMD responses in the brachial and popliteal arteries. Adding to the current literature, HIIT enhanced endothelial vasoconstrictor responses in both arteries, but MICT only increased L-FMC in the POP (i.e., to active limbs). Whole-body RT did not improve either measure of vascular endothelial function in the BA nor POP. Favorable increases in resting blood flow and vascular conductance were observed in the BA following HIIT, but not MICT or RT. Similar responses were not identified in the POP. This is the first study to compare the effects of different exercise modalities on both endothelial vasodilator and vasoconstrictor

function in healthy older adults. Furthermore, we report the first data to document vascular endothelial responses to training in the POP of older persons, which experiences different shear stress profiles during cycling exercise training than the BA.

Our results support the notion that the vasculature may be favourably modified by short-term aerobic exercise training in older adults known to experience the deteriorating effects of advanced aging (26), such as chronic low-grade inflammation and increased oxidative stress (27). Adding to the current body of literature, our short-term HIIT and MICT models (but not RT) elicited a sufficient stimulus for modifying both BA-FMD (ΔHIIT: 1.9%; ΔMICT: 2.1%; Figure 2) and POP-FMD (ΔHIIT: 1.3%; ΔMICT: 1.4%; Figure 3). Similar to observations in younger adults (28), short-term (i.e., 6-8 weeks) aerobic training augmented vasodilator function in two arteries that experience very different shear rate stimuli during cycling exercise in our population of older adults. Future research should aim to uncover the time course of these changes and if longer training periods continue to increase vasodilator function. Of particular importance, a 1% increase in relative BA-FMD equates to a 10% reduction in the relative risk of a future cardiovascular event and all-cause mortality (3). Furthermore, BA-FMD has been shown to be predictive of coronary artery function; dysfunction of the coronary arteries precedes or is responsible for most cardiovascular disease (2). In addition, endothelial dysfunction is an early marker of atherosclerosis (1), and the POP is a common site for plaque development (4). Herein, both HIIT and MICT resulted in a clinically significant reduction in cardiovascular disease risk in a relatively short period of exercise training among healthy, older adults.

Of relevance, we did not observe any changes in POP nitroglycerin-mediated vasodilation following any training regimen, suggesting that the improved POP-FMD responses in the HIIT and MICT groups are not attributed to enhanced vascular smooth muscle sensitivity. This finding is consistent with previous literature conducted in the BA, in that a meta-analysis of

26 exercise training studies concluded that BA nitroglycerin-mediated vasodilation was only marginally greater or was unchanged following exercise training (8). Perhaps longer training periods may be needed to combat the diminished POP nitroglycerin-mediated vasodilation associated with advancing age (29). Certainly, the present study was not designed to test the mechanisms responsible for our observed findings, but possible mechanisms behind the improved relative FMD responses is a reduction in oxidative stress (i.e., reactive oxygen species) or resting levels of the potent vasoconstrictor endothelin-1. Aging is associated with exacerbated oxidative stress that promotes the uncoupling of endothelial nitric oxide synthase (30) and endothelin-1 expression (31), which has been shown to be favourably modulated via exercise training (32, 33). Future studies are needed to confirm or refute this hypothesis.

We have previously demonstrated that higher cardiorespiratory fitness is associated with a greater L-FMC response in healthy older adults (7, 11). The present study is the first to determine if an aerobic or resistance exercise training intervention can augment L-FMC in this population. Interestingly, HIIT augmented endothelial-dependent vasoconstrictor function in the BA and POP, but MICT only increased L-FMC in the POP (Figure 2 & Figure 3). Considering that POP L-FMC similarly increased following HIIT and MICT, but not after RT, the increased endothelial sensitivity to low-flow in the popliteal artery may be attributed to the increased shear stress in the active limb arteries during aerobic exercise, which translates to more effective signalling of vasodilatory/vasoconstrictor pathways. With that, our results suggest that the higher shear stress during HIIT versus MICT may be needed to elicit favorable adaptations in the vasoconstrictor side of endothelial function in the BA. L-FMC has been shown to be mediated through the endothelium (34), via the inhibition of vasodilatory signaling via endothelial-derived hyperpolarizing factor and prostaglandins (5), as well as enhanced vasoconstrictor signaling via endothelin-1 (35). Our results suggest that HIIT may be more effective at promoting the

inhibition of these vasodilatory pathways and/or stimulation of the endothelin-1 pathway in response to reductions in shear stress to a greater extent than MICT. The importance of enhancing these pathways and the L-FMC response to overall vascular health and risk of adverse cardiovascular events is currently unknown. Contrary to our previous cross-sectional observations equating cardiorespiratory fitness and vasoconstrictor function (7, 11), aerobic fitness but not L-FMC was improved in all three groups. It appears that other factors independent of increased aerobic fitness are responsible for subsequent enhancements to endothelial-dependent vasoconstrictor function. Of importance, these observations differ between arteries, with the relationship between the change in aerobic fitness and change in L-FMC being moderate-strength in the BA but non-existent in the POP [see Figure, Supplemental Digital Content 4, Scatterplot showing the relationship between the change in aerobic fitness (peak oxygen consumption) with the change in brachial artery relative low-flow-mediated constriction (L-FMC; A) and the change in popliteal artery L-FMC (B) in the pooled sample, http://links.lww.com/MSS/B851]. Furthermore, no relationship was observed for the training-induced changes in L-FMC between the brachial and popliteal arteries [see Figure, Supplemental Digital Content 3, Scatterplot showing the relationship between the change in relative flow-mediated dilation (FMD; A) responses and changes in relative low-flow-mediated constriction (L-FMC; B) between the brachial and popliteal arteries, http://links.lww.com/MSS/B850]. As such, it is plausible that different mechanisms may be responsible for the improvements in L-FMC between the BA and POP.

With advancing age, there is a reduction in BA blood flow (36). Interestingly, HIIT, but not MICT or RT, resulted in a greater resting BA blood flow and shear stress through an increase in blood flow velocity with no corresponding change in resting BA diameter. Higher anterograde and lower retrograde BA blood flow velocity is associated with greater physical function in older

persons (36) and a higher resting shear stress may promote anti-atherogenic gene expression (37). This suggests our HIIT protocol may be a more effective training program than MICT or RT alone at combatting the decline in physical function and progression of atherosclerosis in an aged population.

Few studies have investigated if RT influences vascular function in older adults (8). Our results show that short-term RT did not alter endothelial-dependent vasodilator or vasoconstrictor function in either artery in this population. It may be possible that a longer duration of RT, or more intense RT, may be needed to elicit these functional adaptations. Interestingly, the whole-body RT did increase resting lumen diameter. While the mechanisms behind this are unclear, our observations are consistent in both the BA and POP. This warrants future investigations regarding the influence of transient, high-pressure shear stress patterns on local resting diameter in older adults, who are often encouraged to engage in regular RT (12). Of relevance, resting diameter is influenced by numerous competitive vasodilator and constrictor influences, limiting its use as an index of vascular structure (38). With that, the maximal dilation in response to nitroglycerin, which represents near maximal diameter, has been used as a preferred index of arterial structure (38) and was not influenced by RT in the POP. This suggests that RT likely did not elicit structural changes in this short-term intervention.

Although improvements in BA-FMD were observed in the HIIT and MICT groups, endothelial-independent dilation was not assessed in the BA. However, it is unlikely that 6-weeks is long enough to enhance vascular smooth muscle function in the BA (8). We appreciate that our study is unable to provide information regarding the mechanisms of our observed findings. Future research incorporating blockades of endothelial-dependent vasodilatory and vasoconstrictor pathways pre- and post-training are warranted. With that, we did not observe training induced changes in POP nitroglycerin-mediated dilation (i.e., endothelial-independent

dilation), excluding alterations in vascular smooth cell sensitivity to endothelial-derived substances as a potential mechanism for the enhanced FMD and L-FMC responses. Sex-differences in the vascular responses to exercise training may exist (39). However, the number of men and women in the exercise groups were similar. Future studies should examine the role that sex has on exercise mode- and aerobic exercise intensity-induced vascular responses in older populations. We acknowledge our study did not include a control group, but our repeated measures design allowed participants to serve as their own controls. As well, randomized controlled studies investigating exercise modalities and endothelial function consistently demonstrate no improvement in the control group, as reviewed in (8), which in conjunction with the high-reproducibility of our ultrasound measures suggest the lack of a control group to be a minor limitation that does not take away from our primary findings.

The findings of this study demonstrate that 6-weeks of HIIT and MICT are superior to RT at eliciting improvements in endothelial-dependent dilation in the brachial and popliteal arteries of older adults. While HIIT and MICT enhanced endothelial-dependent vasoconstrictor function in the POP, only the HIIT group exhibited a greater endothelial sensitivity to low-flow and baseline blood flow in the BA. HIIT produces clinically meaningful increases in vascular endothelial function in the short-term, which translates to a reduced risk of cardiovascular disease of a greater magnitude than MICT or RT in older adults. Therefore, exercise training programs aimed at improving peripheral vascular function in healthy older adults should consider implementing HIIT.

Acknowledgements

Conflicts of Interest

The authors report no conflicts of interest. The results of the present study do not constitute endorsement by ACSM. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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FIGURE CAPTIONS

Figure 1. Schematic of the experimental design and each of the high-intensity interval training (HIIT), moderate-intensity continuous training (MICT) and whole-body resistance training (RT) protocols. PPO, peak power output (measured in Watts). *The RT protocol consisted of 8 exercises targeting all major muscle groups for 2 sets of 10 repetitions.

Figure 2. Comparison of brachial artery relative flow-mediated dilation (FMD, %; *A*), relative low-flow mediated constriction (L-FMC, %; *B*), and allometrically-scaled L-FMC (%; *C*) from pre- to post-training following high-intensity interval training (HIIT), moderate-intensity continuous training (MICT) and whole-body resistance training (RT). Data are presented as means ± SD. Individual data are presented in panels *A* and *B*. *, $p < 0.05$ versus pre-training within same training group. Data were analyzed via a between-subjects repeated measures (training group × time) ANOVA with Bonferroni post-hoc testing. HIIT: $n=12$; MICT: $n=12$; RT: $n=14$.

Figure 3. Comparison of popliteal artery relative flow-mediated dilation (FMD, %; *A*), relative low-flow mediated constriction (L-FMC, %; *B*), and allometrically-scaled L-FMC (%; *C*) from pre- to post-training following high-intensity interval training (HIIT), moderate-intensity continuous training (MICT) and whole-body resistance training (RT). Data are presented as means ± SD. Individual data are presented in panel *A* and *B*. *, $p < 0.05$ to pre-training within same training group. Data were analyzed via a between-subjects repeated measures (training group × time) ANOVA with Bonferroni post-hoc testing. HIIT: $n=12$; MICT: $n=12$; RT: $n=14$.

Supplemental Digital Content

Supplemental Digital Content 1.pdf—Scatterplot showing the relationship between the reactive hyperemia (shear rate area under the curve; SR AUC) and brachial flow-mediated dilation (FMD) with data from both pre-training and post-training for each of the high-intensity interval training (HIIT; circles; black regression line), moderate-intensity continuous training (MICT: triangles; black and white regression line) and resistance training groups (RT: squares; grey regression line).

Supplemental Digital Content 2.pdf— Scatterplot showing the relationship between the reactive hyperemia (shear rate area under the curve; SR AUC) and popliteal flow-mediated dilation (FMD) with data from both pre-training and post-training for each of the high-intensity interval training (HIIT; circles; black regression line), moderate-intensity continuous training (MICT: triangles; black and white regression line) and resistance training groups (RT: squares; grey regression line).

Supplemental Digital Content 3.pdf─Scatterplot showing the relationship between the change in relative flow-mediated dilation (FMD; A) responses and changes in relative low-flow-mediated constriction (L-FMC; B) between the brachial and popliteal arteries.

Supplemental Digital Content 4.pdf— Scatterplot showing the relationship between the change in aerobic fitness (peak oxygen consumption) with the change in brachial artery relative low-flow-mediated constriction (L-FMC; A) and the change in popliteal artery L-FMC (B) in the pooled sample. Data is presented for high-intensity interval training (circles), moderate-intensity continuous training (triangles) and resistance training groups (squares).
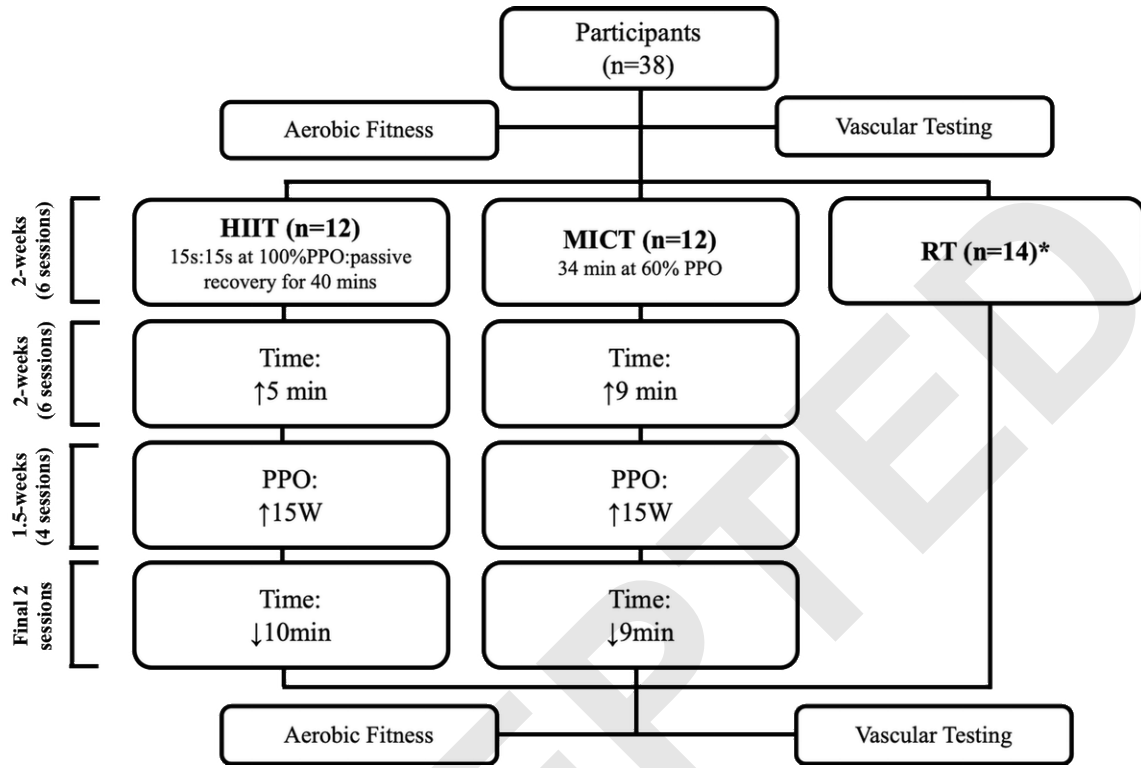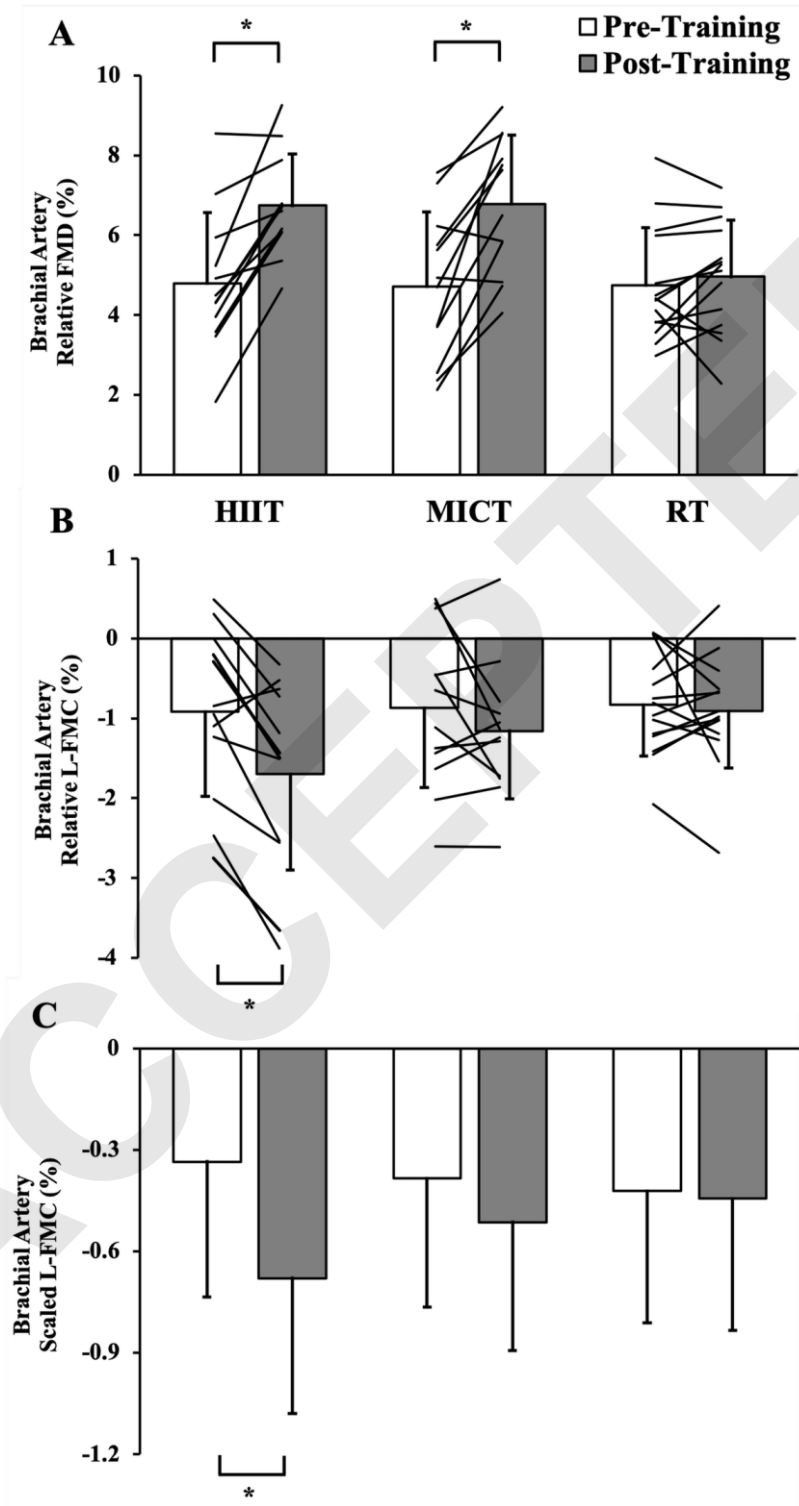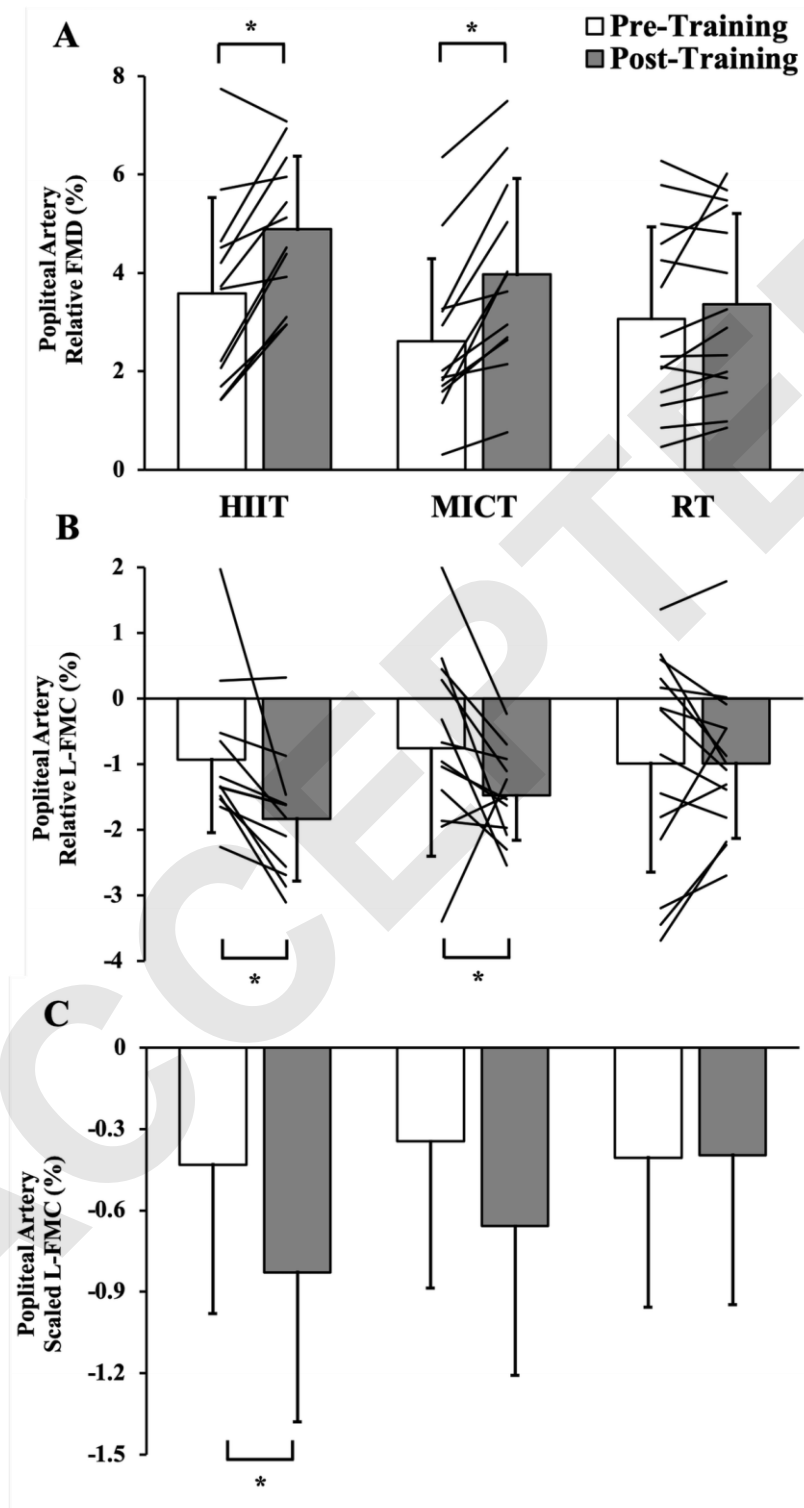
Figure 1

Figure 2

Figure 3

Table 1. Participant descriptive characteristics, resting hemodynamics and aerobic fitness.

	HIIT		MICT		RT	
	Pre-Training	Post-Training	Pre-Training	Post-Training	Pre-Training	Post-Training
Age (years)	68 ± 5		68 ± 6		66 ± 7	
Sex (Male, Female)	5♂, 7♀		4♂, 8♀		6♂, 8♀	
Body Mass Index (kg/m^2)	25.9 ± 3.1	25.8 ± 3.0	25.2 ± 3.6	26.0 ± 3.1	27.2 ± 5.1	27.0 ± 5.1
Heart Rate (beats/min)	70 ± 11	68 ± 10	68 ± 11	65 ± 10*	68 ± 8	66 ± 10
Stroke Volume (ml/beat)[a]	67 ± 27	73 ± 21	71 ± 25	73 ± 23	69 ± 25	74 ± 22
Cardiac Output (L/min)[a]	4.7 ± 2.1	4.9 ± 1.4	5.0 ± 2.0	5.0 ± 1.9	4.7 ± 1.8	4.8 ± 1.4
Systolic Blood Pressure (mmHg)	126 ± 12	120 ± 10*	121 ± 12	121 ± 10	128 ± 9	125 ± 11
Diastolic Blood Pressure (mmHg)	74 ± 9	68 ± 9*	71 ± 9	66 ± 7*	70 ± 11	67 ± 9
Mean Arterial Pressure (mmHg)	91 ± 8	84 ± 8*	87 ± 9	83 ± 8	89 ± 9	85 ± 9
Total Vascular Conductance (ml/min/mmHg)[a]	53 ± 24	59 ± 17	60 ± 28	62 ± 29	54 ± 23	57 ± 18
Aerobic Fitness (mlO$_2$/kg/min)	23 ± 7	28 ± 7*	23 ± 4	29 ± 8*	23 ± 7	26 ± 10*
Peak Power Output (W)	145 ± 39	170 ± 50*	150 ± 36	179 ± 43*	135 ± 60	144 ± 60*
Peak Heart Rate (beats/min)	149 ± 13	151 ± 19	156 ± 7	153 ± 16	150 ± 9	148 ± 20
Peak RER (VCO$_2$/VO$_2$)	1.23 ± 0.06	1.25 ± 0.08	1.22 ± 0.12	1.18 ± 0.12	1.16 ± 0.12	1.17 ± 0.12

Data are presented as means ± standard deviations. HIIT, high-intensity interval training; MICT, moderate-intensity continuous training; RT, resistance training. *, $p < 0.05$ versus pre-training within same training group. [a]n = 10 for MICT group. Between-subject repeated measures (training group × time) ANOVA with Bonferroni post-hoc testing compared pre- versus post-training data within each group.

Table 2. Comparison of brachial artery parameters across the HIIT, MICT and RT protocols

	HIIT		MICT		RT	
	Pre-Training	Post-Training	Pre-Training	Post-Training	Pre-Training	Post-Training
Resting						
Resting diameter, mm	4.28 ± 0.74	4.28 ± 0.72	3.91 ± 0.51	3.91 ± 0.51	3.69 ± 0.63	3.74 ± 0.64*
Mean blood velocity, cm/s	11.8 ± 3.7	14.2 ± 4.2*	12.4 ± 5.0	12.6 ± 4.2	13.3 ± 5.1	12.0 ± 3.8
Blood flow, ml/min	102 ± 43	120 ± 42*	90 ± 38	93 ± 41	83 ± 36	78 ± 28
Resting shear rate, s^{-1}	113 ± 39	137 ± 47*	130 ± 61	131 ± 48	150 ± 62	134 ± 50*
Brachial vascular conductance, ml/min/mmHg[a]	1.1 ± 0.5	1.5 ± 0.5*	1.1 ± 0.5	1.1 ± 0.6	1.0 ± 0.4	0.9 ± 0.3
Flow-Mediated Dilation						
Peak diameter, mm	4.49 ± 0.80	4.58 ± 0.79*	4.10 ± 0.56	4.18 ± 0.57*	3.87 ± 0.69	3.93 ± 0.70*
Absolute FMD, mm	0.21 ± 0.09	0.30 ± 0.09*	0.19 ± 0.08	0.28 ± 0.09*	0.18 ± 0.08	0.20 ± 0.08
SR_{AUC}, a.u.	11589 ± 3998	15334 ± 6124*	12880 ± 4988	14641 ± 4201	12056 ± 2683	14941 ± 4961*
Time-to-peak dilation, s	54 ± 13	55 ± 12	66 ± 21	68 ± 18	63 ± 14	65 ± 11
Low-Flow Mediated Constriction						
Nadir diameter, mm	4.24 ± 0.70	4.20 ± 0.66	3.87 ± 0.49	3.86 ± 0.49	3.66 ± 0.63	3.70 ± 0.64*
Absolute L-FMC, mm	–0.04 ± 0.06	–0.08 ± 0.07*	–0.04 ± 0.04	–0.05 ± 0.04	–0.03 ± 0.02	–0.03 ± 0.03
Vasoactive Range						
Relative VAR, %	5.8 ± 2.4	8.6 ± 2.0*	5.6 ± 2.1	8.3 ± 2.4*	5.7 ± 1.9	6.0 ± 1.8
Absolute VAR, mm	0.25 ± 0.14	0.38 ± 0.14*	0.22 ± 0.11	0.33 ± 0.12*	0.21 ± 0.09	0.23 ± 0.09

Data are presented as means ± standard deviations. HIIT, high-intensity interval training; MICT, moderate-intensity continuous training; RT, resistance training; FMD, flow-mediated dilation; SR_{AUC}, shear rate area under the curve to peak dilation; VAR, vasoactive range. *, $p < 0.05$ to pre-training within training group. [a]n = 10 for MICT group. Between-subject repeated measures (training group × time) ANOVA with Bonferroni post-hoc testing.

Table 3. Comparison of popliteal artery parameters across the HIIT, MICT and RT protocols

|	HIIT		MICT		RT	
	Pre-Training	Post-Training	Pre-Training	Post-Training	Pre-Training	Post-Training
Resting						
Resting diameter, mm	6.35 ± 1.12	6.35 ± 1.13	6.65 ± 2.04	6.63 ± 2.02	6.93 ± 0.86	7.00 ± 0.83*
Mean blood velocity, cm/s	5.3 ± 1.6	5.5 ± 1.8	3.7 ± 2.0	4.2 ± 1.8	4.0 ± 2.3	4.1 ± 2.1
Blood flow, ml/min	111 ± 54	110 ± 56	75 ± 38	83 ± 37	96 ± 55	95 ± 47
Resting shear rate, s^{-1}	35 ± 14	36 ± 15	25 ± 17	28 ± 16	24 ± 15	24 ± 14
Popliteal vascular conductance, ml/min/mmHg[a]	1.2 ± 0.5	1.3 ± 0.7	0.9 ± 0.5	1.0 ± 0.5	1.1 ± 0.7	1.1 ± 0.6
Flow-Mediated Dilation						
Peak diameter, mm	6.59 ± 1.23	6.67 ± 1.23	6.81 ± 2.04	6.88 ± 2.01*	7.16 ± 0.98	7.25 ± 0.95*
Absolute FMD, mm	0.24 ± 0.16	0.32 ± 0.13*	0.17 ± 0.11	0.25 ± 0.12*	0.22 ± 0.15	0.24 ± 0.15
SR_{AUC}, a.u.	6685 ± 2317	8208 ± 2812*	5308 ± 2404	7066 ± 2805*	5485 ± 2030	7429 ± 2485*
Time-to-peak dilation, s	110 ± 32	111 ± 30	111 ± 20	109 ± 21	89 ± 32	88 ± 29
Low-Flow Mediated Constriction						
Nadir diameter, mm	6.29 ± 1.10	6.24 ± 1.13	6.60 ± 2.03	6.53 ± 2.00	6.86 ± 0.76	6.93 ± 0.77*
Absolute L-FMC, mm	–0.06 ± 0.06	–0.11 ± 0.06*	–0.05 ± 0.10	–0.10 ± 0.05*	–0.08 ± 0.13	–0.07 ± 0.09
Vasoactive Range						
Relative VAR, %	4.51 ± 2.36	6.72 ± 1.53*	3.38 ± 2.43	5.46 ± 2.33*	4.06 ± 3.27	4.35 ± 2.61
Absolute VAR, mm	0.30 ± 0.18	0.43 ± 0.13*	0.22 ± 0.15	0.35 ± 0.14*	0.30 ± 0.27	0.32 ± 0.22
Nitroglycerin-Mediated Dilation						
Relative NMD, %[b]	5.89 ± 2.22	6.04 ± 1.88	5.66 ± 2.12	6.00 ± 1.95	5.43 ± 2.30	5.42 ± 2.16
Absolute NMD, mm[b]	0.38 ± 0.19	0.39 ± 0.17	0.35 ± 0.11	0.36 ± 0.10	0.39 ± 0.19	0.39 ± 0.19

Data are presented as means ± standard deviations. HIIT, high-intensity interval training; MICT, moderate-intensity continuous training; RT, resistance training; FMD, flow-mediated dilation; SR_{AUC}, shear rate area under the curve to peak dilation; VAR, vasoactive range; NMD, nitroglycerin mediated dilation. *, $p < 0.05$ to pre-training within training group. [a]n = 11 for MICT group; [b]n=11 for MICT and HIIT groups. Between-subject repeated measures (training group × time) ANOVA with Bonferroni post-hoc testing.
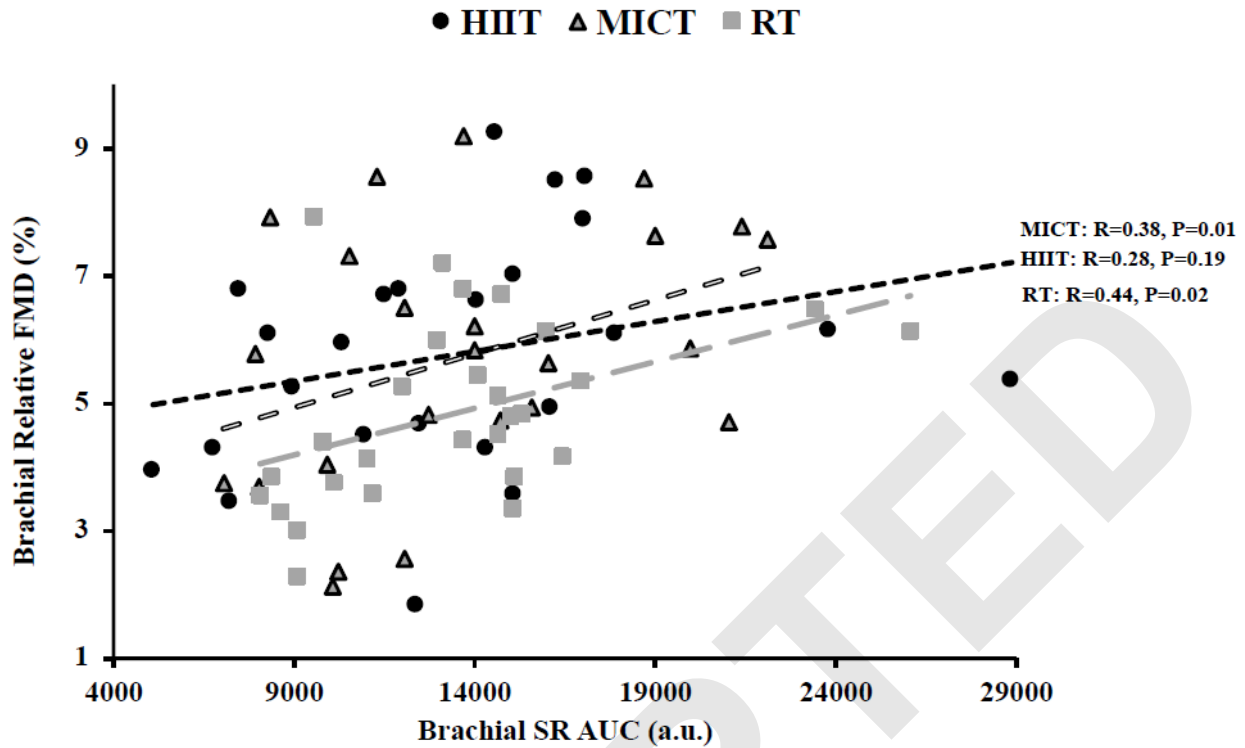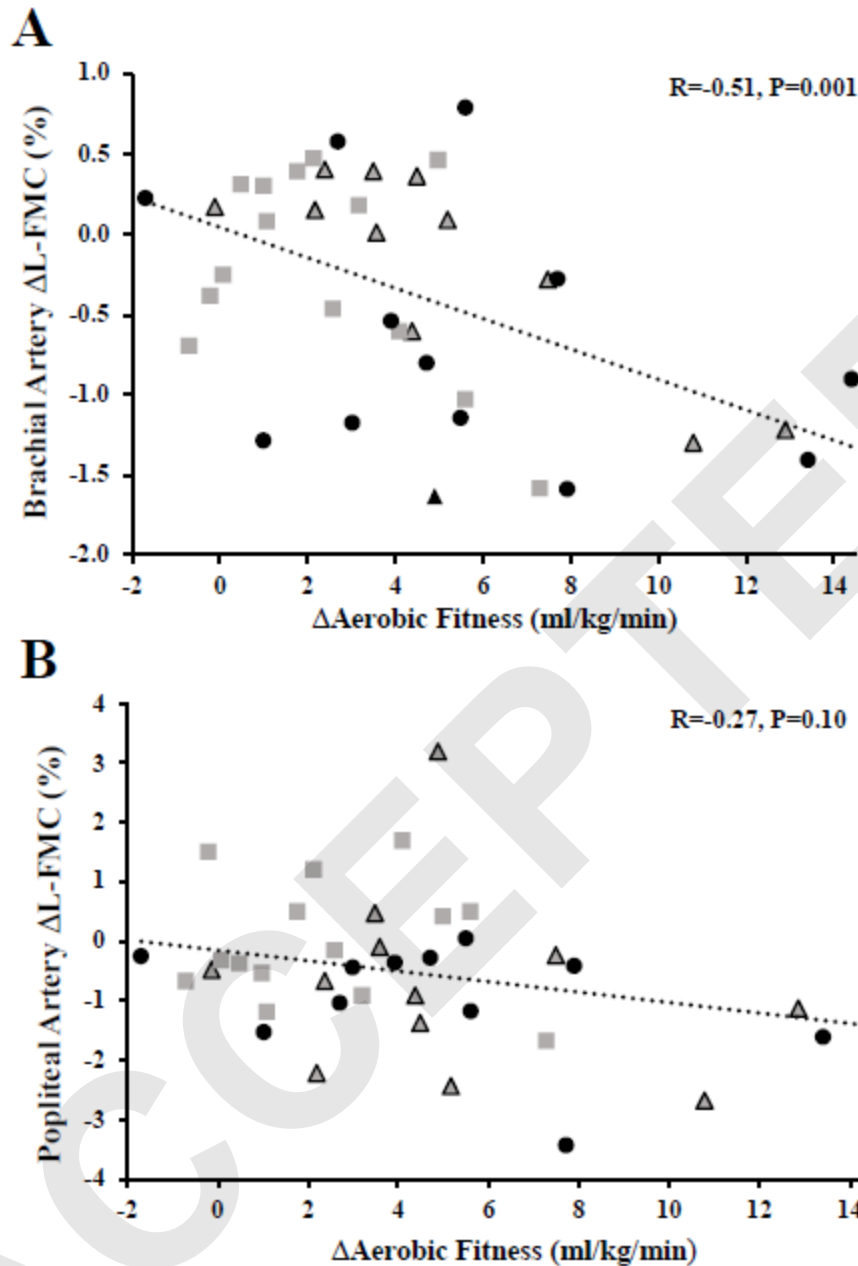
Supplemental Digital Content 1. Scatterplot showing the relationship between the reactive hyperemia (shear rate area under the curve; SR AUC) and brachial flow-mediated dilation (FMD) with data from both pre-training and post-training for each of the high-intensity interval training (HIIT; circles; black regression line), moderate-intensity continuous training (MICT: triangles; black and white regression line) and resistance training groups (RT: squares; grey regression line). The slope (10^4) was 0.93, 1.70 and 1.46 for the HIIT, MICT and RT groups, respectively. The correlation of the pooled sample was r = 0.35 (P=0.002) and the y-intercept was greater than zero with β=3.6±0.6, P<0.001 (95% CI: 2.4-4.8).
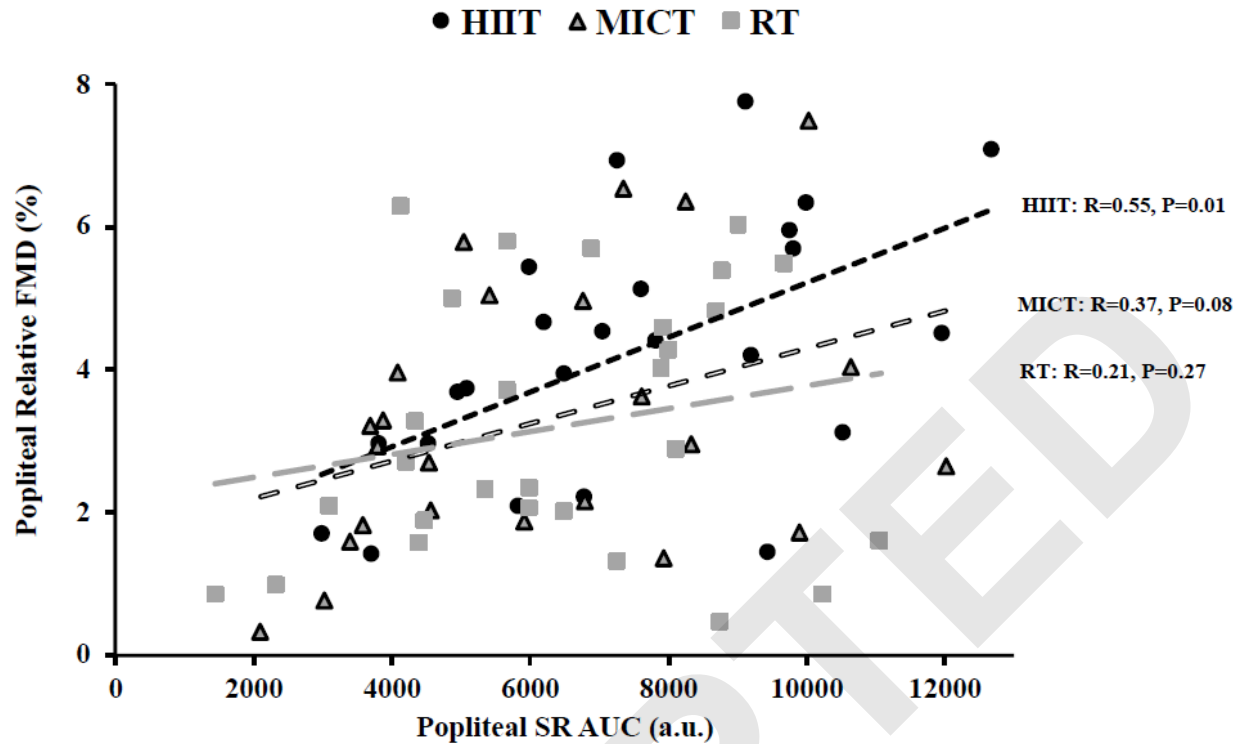
Supplemental Digital Content 2. Scatterplot showing the relationship between the reactive hyperemia (shear rate area under the curve; SR AUC) and popliteal flow-mediated dilation (FMD) with data from both pre-training and post-training for each of the high-intensity interval training (HIIT; circles; black regression line), moderate-intensity continuous training (MICT: triangles; black and white regression line) and resistance training groups (RT: squares; grey regression line). The slope (10^4) was 3.81, 2.62 and 1.60 for the HIIT, MICT and RT groups, respectively. The correlation of the pooled sample was r = 0.40 ($P<0.001$) and the y-intercept was greater than zero with $\beta=1.6\pm0.6$, $P=0.004$ (95% CI: 0.5-2.7).
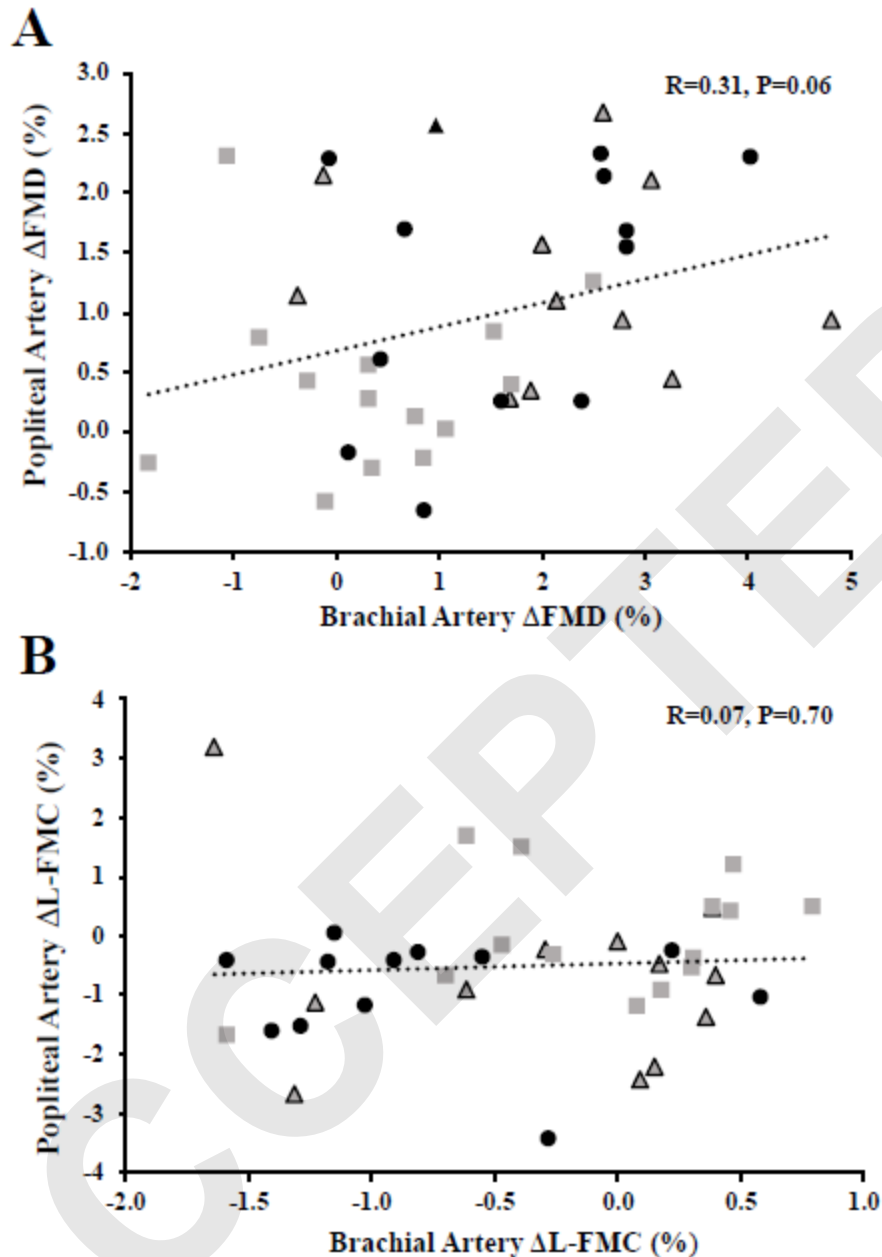
A

B

Supplemental Digital Content 3. Scatterplot showing the relationship between the change in relative flow-mediated dilation (FMD; *A*) responses and changes in relative low-flow-mediated constriction (L-FMC; *B*) between the brachial and popliteal arteries. Data is presented for high-intensity interval training (circles), moderate-intensity continuous training (triangles) and resistance training groups (squares). The unstandardized β ± standard error (95% confidence intervals) were 0.20±0.10, (-0.01 to 0.41; *A*) and 0.11±0.29, (-0.47 to 0.69; *B*).

Supplemental Digital Content 4. Scatterplot showing the relationship between the change in aerobic fitness (peak oxygen consumption) with the change in brachial artery relative low-flow-mediated constriction (L-FMC; *A*) and the change in popliteal artery L-FMC (*B*) in the pooled sample. Data is presented for high-intensity interval training (circles), moderate-intensity continuous training (triangles) and resistance training groups (squares). The unstandardized β ± standard error (95% confidence intervals) were -0.10±0.03, (-0.15 to -0.04; *A*) and -0.09±0.05, (-0.19 to 0.02; *B*).