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ORIGINAL ARTICLE

The relationship between aerobic fitness and low-flow-mediated constriction in older adults

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Abstract

Purpose Aerobic fitness is directly related to favorable vasodilatory (i.e., flow-mediated dilation; FMD) and vasoconstrictor functions (i.e., low-flow-mediated constriction; L-FMC) in young adults. Furthermore, aerobically fit older adults (OA) have larger FMD responses than their less fit peers. However, the relationship between aerobic fitness and vasoconstrictor responsiveness is unknown in OA. We hypothesized that OA who are more aerobically fit will exhibit a greater L-FMC response than their less fit counterparts.

Methods Forty-seven healthy OA (67 ± 5 years) were divided into less (LF; $n = 27$) and more aerobically fit (MF; $n = 20$) groups based on peak oxygen consumption (VO_2peak). VO_2peak was determined from an incremental maximal cycle ergometer test via indirect calorimetry. FMD and L-FMC were assessed in the brachial artery via high-resolution duplex ultrasonography.

Results VO_2peak (18.3 ± 3.2 versus 29.1 ± 5.8 ml/kg/min; $P < 0.001$) and L-FMC were both greatest in the MF versus LF groups (–1.2 ± 0.9 vs. − 0.5 ± 0.6%; $P = 0.01$). Furthermore, the MF group had an enhanced FMD response (5.6 ± 1.5 versus 3.9 ± 1.2%; $P < 0.001$). In the pooled sample, there was a negative correlation ($r = -0.52$; $P < 0.001$) between VO_2peak (22.9 ± 7.0 ml/kg/min) and L-FMC (–0.8 ± 0.8%).

Conclusions In an older population, greater aerobic fitness was associated with a more favorable vasoconstrictor response to low-flow conditions. Interventional or longitudinal aerobic exercise training studies are warranted in this population to determine the impact of training-induced increases in VO_2peak on L-FMC.

Keywords Endothelial function · Vascular aging · Arterial reactivity · Cardiorespiratory fitness

Abbreviations

BMI	Body mass index
CSEP	Canadian Society for Exercise Physiology
CV	Coefficient of variation
DBP	Diastolic blood pressure
ECG	Electrocardiography
FMD	Flow-mediated dilation
MF	More aerobically fit
HR	Heart rate
LF	Less aerobically fit
L-FMC	Low-flow-mediated constriction
MAP	Mean arterial pressure
SBP	Systolic blood pressure
SR	Shear rate
SR_{AUC}	Shear rate area under the curve
VAR	Vasoactive range
VO_2	Volume of oxygen consumption
VO_2peak	Peak volume of oxygen consumption

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Introduction

Aging is associated with chronic low-grade inflammation, increased oxidative stress and an impaired ability to produce endothelial-derived vasodilatory autacoids such as nitric oxide (Ungvari et al. 2010). Collectively, these factors lead to vascular endothelial dysfunction, the development

of atherosclerosis and a greater cardiovascular disease risk (Gimbrone 1995). The flow-mediated dilation (FMD) technique is the gold standard clinical assessment of conduit artery endothelial-dependent vasodilatory function (Grover-Paez and Zavalza-Gomez 2009) and is predictive of future cardiovascular events (Inaba et al. 2010). This non-invasive, high-resolution ultrasound-based procedure measures conduit arterial diameter increases in response to a reactive hyperemia (i.e., increased shear stress) induced by a prior period of distal ischemia (Thijssen et al. 2011). However, the frequently observed endothelial-dependent vasoconstrictor response during the ischemic period, termed low-flow-mediated constriction (L-FMC), is relatively understudied. L-FMC has been shown to be partly mediated through the endothelium (Dawson et al. 2012), via the inhibition of vasodilatory signaling via endothelial-derived hyperpolarizing factor and prostaglandins (Gori et al. 2008), as well as, enhanced vasoconstrictor signaling via endothelin-1 (Spieker et al. 2003). Endothelin-1 levels are typically lower in older adults who are more aerobically fit (Nyberg et al. 2013) and exercise training has been shown to improve vasodilatory signaling of endothelial-derived hyperpolarizing factor (Minami et al. 2002) and prostaglandins (Spier et al. 2007) in aged rodent models. L-FMC may provide additional information regarding the role of reduced shear stress on resting diameter and vascular tone (Gori et al. 2008), is attenuated in individuals with risk factors for coronary artery disease (Harrison et al. 2011), and improves the sensitivity and specificity in detecting patients with cardiovascular disease when complemented with FMD (Gori et al. 2010, 2012). In addition to improving the detection of patients with cardiovascular disease, the combination of L-FMC and FMD, termed vasoactive range (VAR, FMD + L-FMC), has been proposed as a more comprehensive evaluation of vascular health (Black et al. 2003). The VAR encompasses an index of both vasodilation and vasoconstriction and is lower in individuals with cardiovascular disease due to impaired brachial artery FMD and L-FMC responses, which results in a smaller VAR (Harrison et al. 2011).

Aerobic fitness is associated with more favorable brachial artery FMD responses in older populations, who experience age-related declines in peripheral vasculature function (Early et al. 2017). There is a paucity of research investigating the effects of aerobic fitness on L-FMC and VAR despite current FMD guidelines recommending that lumen diameter be measured during the ischemic portion of the FMD test (Thijssen et al. 2011), suggesting that some researchers may be overlooking, or unaware of, this important measure of vasoconstrictor function. Interventional studies have demonstrated that short-term (i.e., 6–8 weeks) continuous aerobic exercise augments brachial artery L-FMC in young healthy adults (Rakobowchuk et al. 2012) and young obese adults (Sawyer et al. 2016). It is unclear whether VAR can be modified via short-term training. However, previous studies have demonstrated either an enhanced (Rakobowchuk et al. 2012) or unchanged (Sawyer et al. 2016) VAR in response to short-term training in young adults. Recently, a cross-sectional study demonstrated that higher estimated peak oxygen consumption (VO_2peak) was associated with greater L-FMC, but not VAR, in the brachial arteries of young men (Bell et al. 2017). It is unknown whether this relationship also exists in older adults.

A limitation in the current state of knowledge regarding the influence of aerobic fitness on vasoconstrictor function and total vessel reactivity is that it has been conducted exclusively in young adult populations (Rakobowchuk et al. 2012; Sawyer et al. 2016; Bell et al. 2017). Older adults experience structural and functional changes to the vascular endothelium that decreases brachial artery vasoconstrictor and vasodilator responsiveness, increasing their risk of cardiovascular events (Black et al. 2009; Norioka et al. 2016). Considering the potential clinical importance of L-FMC and VAR, there is an inherent need to investigate the relationship between aerobic fitness and upper-limb vasoconstrictor function in a population that experiences age-related declines in endothelial function, which may lead to the development of peripheral vascular disease. Herein, the purpose of this study was to test the hypothesis that older adults who are more aerobically fit will exhibit greater brachial artery FMD, L-FMC, and thus VAR, than their lower aerobically fit peers.

Methods

Participants

Forty-seven older adults were recruited from the Active Aging program at Acadia University (Table 1). VO_2peak was assessed via direct calorimetry during a progressive cycle ergometer protocol as described in more detail below. Participants were divided into more aerobically fit (MF; $n = 20$) and less aerobically fit (LF; $n = 27$) groups. LF was defined as a VO_2peak that equated to "poor" according to the Canadian Society for Exercise Physiology (CSEP) age, and sex-specific aerobic fitness classifications (CSEP 2013). Specifically, poor is defined as < 23.5 ml/kg/min for both men and women between the ages of 60 to 69 years. MF was defined as a VO_2peak that equated to "fair" or greater (i.e., $>$ 23.5 ml/kg/min). The breakdown of the specific MF group classifications were as follows: fair ($n = 13$), good ($n = 3$), very good ($n = 3$) and excellent ($n = 1$). Participants were cleared for moderate–vigorous physical activity using the physical activity readiness questionnaire plus (Warburton et al. 2011). Some of the individuals ($n = 21$) from our previously published study investigating the relationship between physical activity and FMD are included in the present study

Table 1 Participant descriptive characteristics

	Less aerobically fit ($n=27$)	More aerobically fit ($n=20$)	Entire sample ($n=47$)
Age (years)	68 ± 5	66 ± 4	67 ± 5
Sex (male, female)	8♂, 19♀	9♂, 11♀	17♂, 30♀
Body mass index (kg/m^2)	27.5 ± 4.6	25.2 ± 3.2	26.5 ± 4.2
Resting heart rate (beats/min)	70 ± 10	66 ± 10	68 ± 10
Systolic blood pressure (mmHg)	129 ± 10	120 ± 9*	125 ± 11
Diastolic blood pressure (mmHg)	74 ± 9	66 ± 8*	71 ± 9
Mean arterial pressure (mmHg)	93 ± 7	84 ± 7*	89 ± 8
Maximum heart rate (beats/min)	148 ± 9	153 ± 10	150 ± 10
Peak RER (VCO_2/VO_2)	1.19 ± 0.11	1.23 ± 0.08	1.21 ± 0.10
Aerobic fitness (mlO_2/kg/min)	18.3 ± 3.2	29.1 ± 5.8*	22.9 ± 7.0

Data are presented as means ± standard deviations

RER respiratory exchange ratio, VCO_2 peak volume of expired carbon dioxide, VO_2 peak volume of oxygen consumed

*$p < 0.05$ versus less aerobically fit

(O'Brien et al. 2018). The protocols and procedures conformed to the Declaration of Helsinki and were approved by the Dalhousie University Health Sciences and Acadia University Research Ethics Boards. Participants were informed of the methods and study design verbally and in writing before providing written informed consent.

Participants had no physical limitations to exercise and a resting blood pressure < 140/90 mmHg. Five participants in the LF group were on Synthroid® for hypothyroidism. Four participants in the LF group were on blood pressure medications. Specifically, participants were prescribed Teveten® (angiotensin-receptor blocker; $n=1$), Adalat (calcium channel blocker; $n=1$); Diuril® (diuretic; $n=1$) and Coversyl® Plus (angiotensin converting enzyme inhibitor + diuretic; $n=1$). One person in the LF group was asthmatic. Participants were requested to continue taking all prescribed medication throughout the duration of the study.

Experimental design

All participants ($n=47$) underwent two separate laboratory visits. Visit 1 involved measurements of height and body mass, which were followed by a graded, maximal cycling exercise test to determine VO_2 peak (see below for details). Visit 2 was dedicated to the assessments of brachial artery vascular function and conducted either before, or between 48 h and 1-week following, the graded exercise test. To minimize confounding influences on endothelial-dependent dilation, vascular assessments were performed 6 h post-prandial, and participants avoided strenuous physical activity, as well as, the consumption of products known to acutely influence FMD responses (e.g., caffeine, chocolate, kiwi, saturated fats, folic acid supplements, antioxidant and multivitamin supplements) for 24 h, consistent with FMD guidelines (Thijssen et al. 2011). All study visits were performed in a thermoneutral environment (21 °C). To control for diurnal variations in blood pressure and vascular function, Visit 2 was performed at the same time of day for the MF and LF groups (Jones et al. 2010).

Experimental protocol

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 (30–50 W), the workload was initially set at 1 W per kilogram of body weight and gradually increased by 15 W every minute 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 primary criterion for the attainment of a VO_2peak was a plateau in VO_2 (change < 2.1 ml/min/kg) despite an increase in workload. In the absence of a plateau, attainment of VO_2peak was based upon a respiratory exchange ratio of ≥ 1.10 or the inability to maintain the required pedal cadence (i.e., 60 revolutions per minute). A VO_{2max} was defined if at least two of the following criteria were observed: (1) a plateau in VO_2 (change < 2.1 ml/min/kg) despite a change in workload, (2) a respiratory exchange ratio ≥ 1.10 and/or

(3) a peak heart rate ≥ 95% age-predicted maximum (i.e., 220—age).

Hemodynamics

Heart rate (HR) was determined via cardiac intervals obtained from lead II of a bipolar electrocardiography configuration. Beat-by-beat systolic (SBP) and diastolic (DBP) blood pressure were measured using finger photoplethysmography (Portapres®; Finapres Medical Systems, Amsterdam, Netherlands). Brachial 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 400 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. (2018), the right brachial artery was imaged 3–5 cm proximal to the antecubital fossa with participants in the supine position. A pressure cuff attached to a rapid cuff inflation system (E20 and AG101, Hokanson®, Bellevue, WA) was positioned around the largest circumference of the forearm (~3 cm distal to the antecubital 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). Simultaneous 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 (Thijssen et al. 2011). Artery lumen diameter and blood flow velocity were measured for a minimum of 2 min before inflation of the pneumatic cuff. The pressure cuff was then rapidly inflated to 250 mmHg for 5 min. Continuous arterial lumen diameter and blood flow velocity recordings were collected throughout the cuff inflation period. Upon release of cuff pressure, lumen diameter and velocity recordings continued for an additional 5 min.

Data analysis

Relative VO_2 data were averaged over 15-s intervals for the duration of the graded exercise protocol. VO_2peak was 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 1/3 SBP + 2/3 DBP.

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 flow 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 (FMD Studio, Cardiovascular Suite, Quipu, Pisa, Italy). This software was used to measure baseline diameter, nadir diameter and peak diameter. All vascular measurements were blindly analyzed by MWO who has demonstrated an intra-tester coefficients of variation (CV) of 2.2%, 2.7%, 3.8% and 4.2% for baseline diameter, nadir diameter, L-FMC% and FMD%, respectively. Blood flow was calculated as mean blood flow velocity × 60 × π × lumen radius^2.

Relative FMD was calculated using the following equation:

$$\text{FMD}\ (\%) = \left[(\text{Post-cuff deflation peak diameter} - \text{baseline diameter}) / \text{baseline diameter} \times 100\%\right].$$

Shear rate (SR, s^{-1}) was defined as [8 × 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, which provides an indication of microvascular function. The correlation between the SR_{AUC} and the FMD response was $r = 0.43$ ($P = 0.003$) in the pooled sample of $n = 47$. As such, FMD data were also normalized to SR_{AUC} to minimize the individual vasodilatory response to reactive hyperemia (Padilla et al. 2008). As described by Atkinson and Batterham (2013), allometric scaling has been recommended to account for differences in arterial diameter. However, the relationship between the natural log of peak FMD diameter and resting diameter across groups yielded an unstandardized β-coefficient that did not deviate from 1 and had an upper 95% confidence interval that was > 1, suggesting allometric scaling of FMD to be unnecessary in the this present study. Specifically, the β-coefficient ± standard error (95% confidence interval) for the FMD measurement was 1.03 ± 0.01 (1.00–1.05). However, allometric criteria were met for L-FMC with a regression value of 0.98 ± 0.01 (0.97–0.999). Allometrically scaled L-FMC data were analyzed using an analysis of covariance model with the natural log of the difference [ln(nadir diameter)—ln(baseline diameter)] as the dependent variable, group (MF, LF) as the fixed factor, and ln(baseline diameter) as the covariate. Significant findings were followed with pairwise comparisons using Bonferroni post-hoc testing. For each group, the allometrically scaled arterial diameter change were back transformed and presented as a percentage change in diameter from baseline.

L-FMC was represented in absolute (mm) and relative (%) terms using the nadir diameter obtained during the final 30 s of the 5-min occlusion period of the FMD test. The following calculations are based on the previous literature (Rakobowchuk et al. 2012; Bell et al. 2017). Relative L-FMC was calculated using the following equation:

$$\text{L} - \text{FMC}\ (\%) = [(\text{Baseline diameter} - \text{nadir diameter})/\text{baseline diameter} \times 100\%].$$

The vasoactive range was calculated as the numerical sum of the absolute values of FMD and L-FMC from baseline and expressed in both absolute (mm) and relative (%) terms. Resting vascular tone was calculated as: tone (%) = [(post-cuff deflation peak diameter – baseline diameter)/(post-cuff deflation peak diameter – nadir diameter) × 100%], as previously documented (Black et al. 2003; Bell et al. 2017).

Statistical analysis

Descriptive variables are presented as means ± standard deviations. Independent samples *t* tests compared baseline characteristics, resting hemodynamics and vascular measures between the LF and MF groups. Effect sizes (ES) were calculated for the vascular measurements between the LF and MF groups (i.e., $MF_{mean} - LF_{mean}$/pooled sample standard deviation). Small, medium and large effect sizes were defined as 0.2, 0.5, 0.8, respectively (Cohen 1992). Pearson product moment correlational analyses were performed between FMD(%), L-FMC (%), FMD + L-FMC (%) and aerobic fitness (VO_{2peak}) in the pooled sample ($n = 47$). Correlational analyses were computed to determine the association between baseline diameter versus FMD(%), L-FMC (%), FMD + L-FMC (%). All data were assessed for normality using a Shapiro–Wilk test and found to be normally distributed. An analysis of covariance model was used to assess the influence of baseline diameter (covariate variable) on relative VAR (dependent variable) between the MF and LF groups (fixed factors). Some participants in the LF group were taking anti-hypertensive medication ($n = 4$), which may have influenced their vascular function. However, the removal of these individuals from the analyses did not change the strength of the correlations between aerobic fitness and our vascular measures, nor the magnitude of the differences between the MF and LF groups. All statistics were completed in SPSS Version 23.0 (IBM, NY) statistical program. Based on previous L-FMC data in young adults (Cohen's $d = 1.06$) (Bell et al. 2017), it was estimated that a minimum of 16 participants in each group were required to achieve sufficient statistical power using a two-tailed alpha = 0.05 and assuming 80% power. Statistical significance was accepted as $P < 0.05$. All data are presented as means ± standard deviations (SD).

Results

Participant characteristics and hemodynamics are summarized in Table 1. Age, BMI, and resting HR were all similar between the MF and LF groups ($P > 0.11$). Compared to the LF group, the MF group had a lower resting SBP, DBP, and MAP (all $P < 0.05$). The MF group had a greater VO_2peak than the LF group ($P < 0.001$). More individuals in the MF group attained the criteria for a VO_2max (60%; $n = 12$) than in the LF group (41%; $n = 11$).

The between group comparisons of vascular measures are presented in Table 2. Baseline, nadir and peak diameters were all larger in the MF group (all $P < 0.05$). There was no difference in resting blood flow velocity, resting shear rate or vascular tone between the groups ($P > 0.17$), but resting brachial artery blood flow was greater in the MF group ($P = 0.04$). The MF older adults had greater FMD (5.7 ± 1.5% vs. 3.9 ± 0.6, $P < 0.001$; ES = 1.29; Fig. 1a) and L-FMC responses (–1.2 ± 0.9% vs. − 0.5 ± 0.6%, $P = 0.01$; ES = − 0.83; Fig. 1b) than the LF group. As such, the VAR (i.e., FMD + L–FMC) was also larger in the MF group ($P < 0.001$; ES = 1.46; Fig. 1c). VAR was still greater in the MF group than the LF group when baseline diameter was included as a covariate (relative difference = 1.9%; $P < 0.001$). The larger L-FMC was not attributed to the larger baseline diameter in the MF groups as the allometrically scaled L-FMC was also greater in the MF versus LF group (–1.1 ± 0.8 vs. − 0.6 ± 0.8%, $P = 0.03$; ES = − 0.63).

The correlations in the pooled sample ($n = 47$) between FMD, VAR and L-FMC versus VO_2peak are presented in Fig. 2. Aerobic fitness was positively correlated to both relative FMD ($r = 0.59$, $P < 0.001$; Fig. 2a) and VAR ($r = 0.69$, $P < 0.001$; Fig. 2c) but negatively associated with relative L-FMC ($r = -0.52$, $P < 0.001$; Fig. 2b). A negative correlation was observed between FMD and L-FMC ($r = -0.34$, $P = 0.02$). Baseline diameter was not correlated to relative FMD ($P = 0.07$) but negatively associated with relative L-FMC ($r = -0.34$, $P = 0.02$) and positively associated with relative VAR ($r = 0.36$, $P = 0.01$).

Discussion

The purpose of this study was to assess the relationship between aerobic fitness and the vasoconstrictor response to low flow in older adults. Consistent with our hypothesis, aerobic fitness was negatively associated with L-FMC

Table 2 Comparison of brachial artery parameters across the pooled sample, more aerobically fit and less aerobically fitness

	Less aerobically fit ($n=27$)	More aerobically fit ($n=20$)	Effect size	Entire sample ($n=47$)
Resting				
Resting diameter (mm)	3.76 ± 0.52	4.21 ± 0.76*	0.70	3.95 ± 0.66
Blood flow velocity (cm/s)	11.8 ± 4.3	12.8 ± 4.6	0.23	12.2 ± 4.4
Blood flow (ml/min)	80 ± 37	104 ± 40*	0.63	90 ± 37
Resting shear rate (s^{-1})	127 ± 50	128 ± 58	0.02	128 ± 53
Tone (%)	89 ± 13	84 ± 12	–0.42	87 ± 13
Flow-mediated dilation				
Peak diameter (mm)	3.91 ± 0.56	4.45 ± 0.80*	0.79	4.14 ± 0.72
Absolute FMD (mm)	0.15 ± 0.06	0.24 ± 0.08*	1.33	0.19 ± 0.08
SR_{AUC}	11,516 ± 3078	13,255 ± 4485	0.45	12,256 ± 3797
Peak FMD:SR_{AUC} ratio (a.u.)	0.14 ± 0.05	0.20 ± 0.10*	0.83	0.17 ± 0.09
Time to peak diameter (s)	64 ± 16	67 ± 23	0.14	65 ± 19
Low-flow-mediated constriction				
Nadir diameter (mm)	3.74 ± 0.51	4.16 ± 0.73*	0.67	3.92 ± 0.64
Absolute L-FMC (mm)	– 0.02 ± 0.03	– 0.05 ± 0.05*	– 0.86	– 0.03 ± 0.04
Vasoactive range				
Absolute FMD + L-FMC (mm)	0.17 ± 0.07	0.29 ± 0.10*	1.37	0.22 ± 0.11

Data are presented as means ± standard deviations or as effect sizes

FMD flow-mediated dilation, SR_{AUC}, shear rate area under the curve to peak dilation, *L-FMC* low-flow-mediated constriction

*$p < 0.05$ versus less aerobically fit

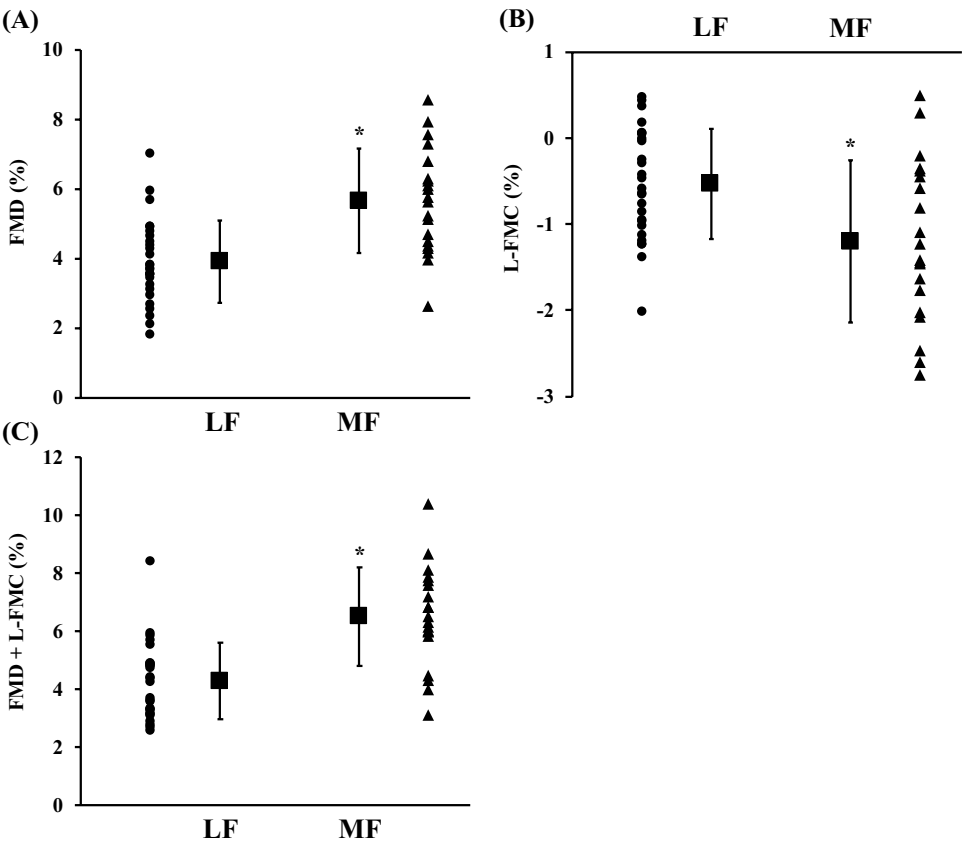

Fig. 1 Comparison of **a** brachial artery relative flow-mediated dilation [FMD, %], **b** low-flow-mediated constriction [L-FMC, %] and **c** vasoactive range [FMD% + L-FMC, %] between less aerobically fit [LF, white bars] and more aerobically fit [MF, black bars] older adults. Data are presented as means ± standard deviations. *$P < 0.05$ versus the lower aerobic fitness group

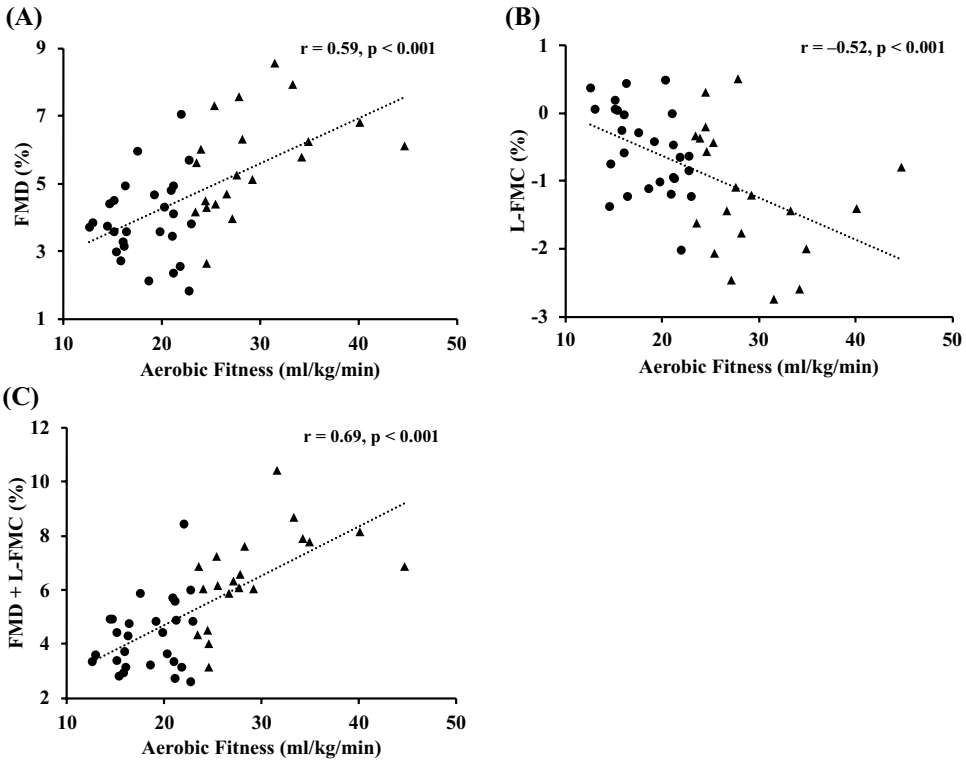

Fig. 2 Pooled sample correlations between aerobic fitness (VO_2peak) with **a** brachial artery relative flow-mediated dilation (FMD, %), **b** relative low-flow-mediated constriction (L-FMC, %), and **c** vasoactive range (FMD + L-FMC, %). Less aerobically fit participants are denoted as circles and more aerobically fit participants as triangles

(Fig. 2b) and proportionally associated with VAR (i.e., FMD + L-FMC; Fig. 2c). The vascular endothelium of more aerobically fit older adults appears to be more sensitive to both reductions and influxes of shear stress than the vasculature of older adults who are less aerobically fit. This represents the first evidence to highlight that greater aerobic fitness has a beneficial effect on both vasodilatory and vasoconstrictor function in an older population.

Our results are consistent with the current literature in that aerobic fitness has been previously associated with augmented vasodilator function (i.e., FMD) in young and older populations (Montero 2015; O'Brien et al. 2018). However, the relationship between aerobic fitness and L-FMC is less clear. Specifically, the only other cross-sectional study demonstrated a moderate negative correlation ($r = -0.50$) between estimated VO_2peak and L-FMC in young men (Bell et al. 2017), which is reflective of the present study that used a maximal aerobic protocol and comprised of older men and women ($r = -0.52$; Fig. 2c). Together, these results lend evidence that the L-FMC frequently observed during the ischemic period of the traditional FMD test is moderately dependent upon aerobic fitness with more aerobically fit older adults exhibiting greater vasoconstriction in response to a low-flow/shear stress stimulus. The L-FMC response remained larger in the MF group in comparison to the LF group when allometrically scaled, suggesting the more favorable vasoconstrictor response is not simply due to the MF having a greater baseline diameter. This observation supports the notion that aerobic fitness is more likely responsible for the greater vasoconstrictor sensitivity in response to low blood flow than artery size in this present study.

Aerobic fitness was proportionally related to VAR in our sample of older adults (Fig. 2b). However, contrary to their hypothesis, the same positive correlation was not observed by Bell et al. (2017) in their population of young healthy men. This difference is likely due to the preserved endothelial function of younger adults that is less sensitive to aerobic fitness status (Montero et al. 2014). The measurement of VAR in older persons, who are at a greater risk of developing cardiovascular disease, may carry prognostic value for the early detection of such disorders (Gori et al. 2010). Our results demonstrate that this vasoactive range is, in part, dependent upon aerobic fitness status. Future research should investigate if this relationship remains valid for older individuals of "excellent" aerobic fitness status, such as in Master's athletes. Of relevance, our sample is representative of a typical older Canadian with an average VO_2peak of ~25 ml/kg/min (Statistics-Canada 2011) increasing the generalizability of our results. Although baseline diameter may have contributed to the larger VAR observed in the MF group compared to the LF group, the relationship was weaker ($r = 0.36$) than the association between aerobic fitness and VAR ($r = 0.69$). VAR was still greater in the MF group than the LF group when baseline diameter was

included as a covariate variable, supporting that aerobic fitness is more likely the mediating factor responsible for the more favorable VAR in this population.

The mechanisms responsible for the enhanced L-FMC in aerobically trained individuals is unclear, but may be due to a greater sensitivity of the vascular endothelium to reductions in shear stress (Rakobowchuk et al. 2012). It is likely that older adults who are more aerobically fit have improved endothelium sensitivity to both reductions and influxes of shear stress in older adults, as evident by the greater L-FMC and FMD (Fig. 1a, b). However, future studies that include markers of endothelial-derived vasodilators (i.e., endothelial-derived hyperpolarizing factor and prostaglandins) and vasoconstrictors (i.e., endothelin-1) are warranted.

As mentioned by Humphreys et al. (2014), L-FMC is an appealing and convenient measure considering FMD guidelines recommend imaging the artery throughout the cuff inflation period (Thijssen et al. 2011). Our study adds to the available evidence by associating aerobic fitness with the 'constrictor-side' of the FMD test and suggesting it, as well as FMD, is greater in older adults who are more aerobically fit. Altogether, accumulating evidence suggests that incorporating a measure of vasoconstrictor capacity may be as important as assessing vasodilator function as an indication of peripheral vascular health in a variety of populations.

Limitations

As with all cross-sectional studies, we are unable to provide direct evidence between exercise training-induced changes in aerobic fitness with changes in L-FMC. However, the present study is the first to investigate the role of aerobic fitness on L-FMC in an older adult population. As such, interventional or longitudinal studies are needed in this population similar to what has been conducted in younger populations (Rakobowchuk et al. 2012; Sawyer et al. 2016). In addition, it is currently unclear if brachial artery L-FMC differs between older men and older women. No differences in relative L-FMC or VAR were observed between men and women in the MF ($P > 0.13$; 9♂, 11♀) or LF groups ($P > 0.60$; 8♂, 19♀). However, the present study was not designed to test if sex influences the magnitude of vasoconstriction in response to low flow. Future studies are needed to determine if sex-differences exist in L-FMC and/or VAR in both younger and older adults.

Conclusion

In an older population, a greater aerobic fitness was associated with a more favorable vasoconstrictor response to low flow, which may represent an independent marker of cardiovascular health. Future studies are needed to determine the clinical relevance of L-FMC as a measure of vascular health and the causal relationships associated with aerobic fitness and both vasoconstrictor capacity and vasoactive range.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to report.

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