CARDIORESPIRATORY FITNESS MODULATES THE ACUTE FLOW-MEDIATED DILATION RESPONSE FOLLOWING HIGH-INTENSITY BUT NOT MODERATE-INTENSITY EXERCISE IN ELDERLY MEN

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SHORT TITLE: Exercise intensity and FMD in elderly males

CONFLICT OF INTEREST: None
DISCLAIMERS: None

FIGURES: 2 **TABLES:** 4

WORD COUNT: 5, 228

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ABSTRACT

Impaired endothelial function is observed with ageing and in those with low cardiorespiratory fitness (VO_{2peak}). Improvements in endothelial function with exercise training are somewhat dependent on the intensity of exercise. While the acute stimulus for this improvement is not completely understood, it may, in part, be due to the flow-mediated dilation (FMD) response to acute exercise. We examined the hypothesis that exercise-intensity alters the brachial (systemic) FMD response in elderly men, and is modulated by VO_{2peak}. Forty-seven elderly men were stratified into lower- (VO_{2peak} = 24.3±2.9 $ml.kg^{-1}.min^{-1}$, n=27) and higher-fit groups (VO_{2peak} = 35.4±5.5 $ml.kg^{-1}.min^{-1}$, n=20) after a test of cycling peak power output (PPO). In randomised order, participants undertook moderate-intensity continuous (MICE; 40% PPO) or high-intensity interval cycling exercise (HIIE; 70% PPO), or no-exercise control. Brachial FMD was assessed at rest, 10 and 60 min after exercise. FMD increased after MICE in both groups [increase of 0.86 % (95% CI, 0.17 to 1.56), P=0.01], and normalised after 60 min. In the lower-fit group, FMD reduced after HIIE [reduction of 0.85 % (95% CI, 0.12 to 1.58), P=0.02), and remained decreased at 60 min. In the higher-fit group, FMD was unchanged immediately after HIIE and increased after 60 min [increase of 1.52 % (95% CI, 0.41 to 2.62), P<0.01, which was correlated with VO_{2peak}, r =0.41; P<0.01]. In the no-exercise control, FMD reduced in both groups after 60 min (P=0.05). Exercise-intensity alters the acute FMD response in elderly men and VO_{2peak} modulates the FMD response following HIIE, but not MICE. The sustained decrease in FMD in the lower-fit group following HIIE may represent a signal for vascular adaptation or endothelial fatigue.

Key Words: exercise, endothelial function, FMD, ageing, cardiorespiratory fitness

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63 **New and noteworthy**

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65 This study is the first to show that moderate-intensity continuous cycling exercise increased
66 FMD transiently before normalisation of FMD after one hour, irrespective of cardiorespiratory
67 fitness level in elderly men. Interestingly, we show increased FMD after high-intensity cycling
68 exercise in higher-fit participants, with a sustained reduction in FMD in lower-fit people. The
69 prolonged reduction in FMD after high-intensity cycling exercise may be associated with future
70 vascular adaptation, but may also reflect a period of increased cardiovascular risk in lower-fit
71 elderly men.

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INTRODUCTION

Ageing is associated with chronic low-grade inflammation, oxidative stress and impaired nitric-oxide (NO) bioavailability that contribute to endothelial dysfunction and large artery stiffness (58, 59). Endothelial dysfunction is considered an important prognostic factor and precursor to the development of atherosclerosis (23, 49), and is strongly associated with the risk of cardiovascular events (23, 61). In addition, endothelial dysfunction is suggested to contribute to other age-associated disorders including cognitive impairment and insulin resistance (64, 66, 76). As such, interventions that prevent or slow the detrimental changes in endothelial function are important in reducing cardiovascular risk and mortality associated with increasing age (60, 61).

Importantly, age-associated endothelial dysfunction, measured using flow-mediated dilation (FMD) of the brachial artery (63), can be attenuated with both regular physical activity (75) and exercise training (16, 24). Results of cross-sectional studies indicate that exercise-trained older adults have preserved endothelial function (17, 42, 48, 53), and reduced cardiovascular disease risk (63), compared with those who are not habitually active. This adaptive response is commonly attributed to the repeated episodes of elevated blood flow, and consequently shear stress, observed during acute exercise that induces vascular adaptation (22).

While the positive impact of chronic aerobic exercise on endothelial function is well described, the significance of the transient changes observed in endothelial function with acute exercise is less clear (15). To elucidate which forms of exercise are most likely to benefit cardiovascular health and function, recent studies have focussed on the acute FMD response and how it is modulated by factors such as exercise intensity. Some evidence suggests that the FMD response

to acute exercise may be bi-phasic, involving an immediate decrease, followed by a transient increase in FMD before returning to baseline levels (15). This may represent the acute initiation of an adaptive response, and be linked to the long-term benefit provided by exercise training on endothelial function at rest (24). This response is suggested to be exaggerated following acute higher-intensity exercise e.g. a larger immediate reduction followed by transient improvement in FMD (3, 11, 15, 33), and may contribute to recent observations of larger improvements in FMD following high-intensity interval exercise (HIIE) compared to moderate-intensity continuous exercise (MICE) training (50, 56). We hypothesize that the bi-phasic FMD response would be further exaggerated in individuals with low cardiorespiratory fitness.

To date, there have been no comparisons of the FMD response to acute exercise between individuals of a higher and lower cardiorespiratory fitness. There is a strong association between a higher cardiorespiratory fitness and maintenance of FMD with aging (42). HIIE training improves cardiorespiratory fitness in healthy elderly adults to a greater extent than MICE training (29), suggesting that it may also modulate the acute FMD response to exercise. Despite this, no study has investigated the influence of a lower and higher cardiorespiratory fitness on the FMD response following acute exercise in the elderly. We therefore aimed to determine whether the effect of acute exercise on FMD differed between MICE and HIIE cycling in elderly males, when controlling for both exercise work and duration. In addition, we assessed the influence of cardiorespiratory fitness on the acute effect of exercise intensity on the FMD response between participants with higher and lower cardiorespiratory fitness. In line with previous findings in the young (3, 11), we hypothesised that acute HIIE would stimulate greater immediate reductions in endothelial function compared to MICE, with subsequent elevation in FMD after 60 min. We

also hypothesised that this overall response would be attenuated in those with a higher cardiorespiratory fitness.

METHODS

Research Design

Participants underwent four laboratory visits, each following an overnight fast, refraining from alcohol and exercise for 24h, and caffeine for 12h, before each visit. Participants consumed a standardised snack (4 oat breakfast biscuits, 20g carbohydrate, 8g fat) 3h prior to attending the laboratory, and the macronutrient content of this snack was unlikely to influence endothelial function (25, 74). Visit 1 consisted of baseline measurements of height, body mass and estimated body composition using bio-impendence scales (BC 545N, Tanita, Australia). After 10 min of supine rest, blood pressure was measured using a manual sphygmomanometer, which was followed by a maximal cycling test to determine cardiorespiratory fitness ($\dot{V}O_{2peak}$) and peak power output (PPO). Experimental visits (2-4) were randomised, counter-balanced and consisted of two separate acute cycling exercise conditions (moderate-intensity continuous vs. high-intensity interval) or a no-exercise control condition. Blood pressure and brachial FMD were assessed at baseline following 20 min of supine rest, and then repeated at 10- and 60-min following exercise/control. Laboratory conditions were standardised for each visit (room temperature: 23 ± 1°C) (67). To control for diurnal variation in blood pressure and vascular function, each visit was performed at the same time of day (34), and separated by 7 days.

Participants

Forty-seven healthy elderly males (mean ± SD, aged 70±5 y; BMI 25.3±3.4 kg.m^2) were recruited from a University Alumni cohort and local advertisement. Participants were screened using a pre-exercise screening questionnaire (1, 52) and included if they were able to exercise and were non-smokers (>12 months no smoking history). Participants were excluded if they were aged >86 years, had a BMI >39, or a chronic cardiovascular or metabolic condition including uncontrolled hypertension, known heart or vascular disease, angina, and atrial fibrillation. During the study, participants were requested to continue to take all prescribed medication. Participants were informed of the methods and study design verbally and in writing before providing written informed consent. The study conformed to the Declaration of Helsinki and was approved by the institutional ethics committees.

Maximal cardiorespiratory cycling test: A maximal incremental cardiorespiratory fitness test was performed in an upright position on an electro-magnetically braked cycle ergometer (Lode Corival, Groningen, Netherlands). Following a 3 min warm up at 0 W, the test began at 20 W and then increased by 10 W each min until volitional cessation. Participants were required to self-select a pedal cadence (between 60 and 90 RPM) and maintain this throughout the test. Expired respiratory gases were collected throughout the test and data were averaged every 15 s (Parvo Medics, UT, USA) for the determination of oxygen consumption (VO_2; mL·kg^{-1}·min^{-1}). Peak VO_2 was determined as the highest 15 s average over the last 60 s of maximal exercise (VO_2peak). Heart rate was measured continuously using 12-lead ECG (Mortara Inc., WI, USA) and recorded, along with perceived exertion (RPE) using the 0-10 Borg scale, during the final 10 s of each stage. All participants reached the criteria for maximum effort based upon attaining >2

of the following: a peak heart rate within 10 bpm of predicted age-related maximum; RPE (>9); a fall in pedal cadence (>10 RPM); a plateau in VO_2 despite an increase in workload; and a respiratory exchange ratio >1.15. Peak power output (W) was then used to establish the exercise intensity in the subsequent test visits.

Acute exercise/control protocols: Following pre-test measurements, participants performed 27 min of upright continuous or interval cycling exercise, or no-exercise control (seated-rest). Both acute exercise protocols commenced with a 3-minute warm-up at 0 W, followed by either 24 min of: *i)* continuous moderate-intensity cycling at 40% PPO, or *ii)* high-intensity interval cycling involving 12 x 60 s bouts at 70% PPO, with each separated by 60 s at 10% PPO. Heart rate and RPE were recorded every 2 min. This design ensured the continuous and interval cycling exercise protocols were duration and work-matched. Control consisted of 27 min of seated-rest with both arms relaxed and rested on a table in front. The total measurement period, and timing between measurements were the same across exercise and control visits. Immediately following exercise/control (<60 s), participants were moved to the supine position and asked to remain supine for post-test FMD measurements (at 10 and 60-min). Right brachial artery blood pressure was measured in triplicate using an automated device (Sphygmocor XCEL, AtCor Medical, NSW, Australia) 10-min before each FMD time-point to negate any effect of cuff inflation on FMD.

Brachial artery flow-mediated dilation: Brachial artery FMD was used as a measure of endothelial function (67). Measurements were performed in the supine position, on the right arm with the cuff placed distal to the olecranon process. High-resolution duplex ultrasound (T3000;

Terason, Burlington, MA) with a 12-MHz multi-frequency linear array probe was used to image the brachial artery at the distal third of the upper arm and simultaneously record the longitudinal B-mode image and Doppler blood velocity trace. The angle of Doppler insonation was 60º. Images were optimised, and settings (depth, focus position and gain) were maintained between FMD assessments within each individual visit, and the location of the transducer was recorded and marked on the skin using an indelible marker. Following a 60 s baseline recording period, the cuff was rapidly inflated to 220 mmHg and maintained for 5 min (D.E. Hokanson, Bellevue, WA). Ultrasound recordings resumed 30 s prior to rapid cuff deflation (<2 s) and continued for 3 min thereafter, in accordance with recommendations (12, 67). All ultrasound scans were performed by the same trained sonographer.

Analysis of brachial artery diameter was performed using custom-designed edge-detection and wall-tracking software, which is largely independent of investigator bias. Recent papers describe the analysis approach in detail (12, 67). Briefly, from recordings of the synchronised artery diameter and blood velocity data, blood flow (the product of lumen cross- sectional area and Doppler velocity) was calculated at 30 Hz. Shear rate (an estimate of shear stress independent of viscosity) was calculated as 4 times mean blood velocity/vessel diameter. This semi-automated software possesses an intra-observer coefficient of variation (CV) of 6.7% and reduces error, with the reproducibility of diameter measurements significantly better than manual methods (68, 77).

Statistical analysis

To differentiate the cohort on the basis of cardiorespiratory fitness, each participant was stratified into lower- ($VO_{2\ peak}$ <27 ml.kg.min^{-1}) and higher ($VO_{2\ peak}$ >31 ml.kg.min^{-1}) fitness (fit) group based on age- and sex-specific normative data (1). These differences in cardiorespiratory fitness were closely aligned with the prior observation that cardiovascular burden and mortality is significantly reduced with a VO_{2peak} >28 ml.kg^{-1}.min^{-1} e.g. 8 METS, in males over the age of 65 (10, 44). A three-way (fitness*protocol*time) linear mixed model (LMM) was employed to analyse changes in FMD parameters [brachial diameter, peak diameter and FMD (mm), FMD (%), time to peak, shear rate area-under-the-curve (SRauc), blood flow,] and blood pressure between the two fitness groups (low and high fitness), across "time" (baseline, 10- and 60-min post) during each protocol (control, moderate- and high-intensity exercise). As variability in the baseline artery diameter and shear rate may influence the magnitude of the FMD response (69), these parameters were included in the analysis as covariates (2, 9). In line with recent recommendations (4-6), we also performed an additional three-way LMM analysis of logarithmically transformed absolute diameter change (difference between peak and baseline diameter as the outcome, in mm), with logarithmically transformed baseline diameter and shear rate again included as covariates, specific to each FMD test. The logged absolute diameter change was then also interpreted in the conventional manner and is presented as "adjusted FMD%" for comparative purposes as suggested (8), in line with recent reports (3, 71). This allometric approach may be more accurate for scaling changes in diameter than percentage change alone, which makes implicit assumptions about the linearity of the relationship between baseline diameter and peak diameter (7). The strength of the relationships between cardiorespiratory fitness and changes in FMD after exercise and/or control were assessed using Pearson correlation coefficient.

Similarly, a three-way LMM analysis was used to detect any differences in heart rate, blood pressure and perceived exertion in response to the acute protocols between the two fitness groups (low- and high-fit), across time (at 2 and 6 minute intervals for HR/RPE and BP, respectively) during each protocol (control, moderate- and high-intensity exercise). Statistically significant interactions were further investigated with multiple comparisons using the least significant difference approach (46, 55). Analyses were conducted using the Statistical Package for Social Sciences (Version 22; IBM SPSS Inc., Chicago, IL). Statistical significance was delimited at $P \leq 0.05$ and exact P values are cited (P values of "0.00" are reported as "<0.01"). Data are presented in the text as mean (95% confidence interval, 95%CI) unless otherwise stated.

Results

Baseline:

Participant characteristics.

Participant characteristics are presented in Table 1. Participant age was higher in the lower-fit compared to the higher-fit group [mean difference of 3 years (95% CI, -1 to 6), P=0.05]. Approximately one quarter of the participants were hypertensive (30% and 26% in the lower and higher fitness groups, respectively) and all hypertensive participants were taking blood-pressure controlling medication. Resting heart rate was lower in the higher-fit compared to lower-fit [mean difference 6 $b.min^{-1}$ (95% CI, 2 to 10), P = 0.01], but there were no differences in resting blood pressure or anthropometric variables between lower- and higher-fit groups.

Cardiorespiratory fitness.

There was a mean difference of 11 $ml.kg^{-1}.min^{-1}$ (95% CI, 8 to 13, $P<0.01$) in $VO_{2\ peak}$ and 50 Watts (95% CI, 30 to 70, $P<0.01$) between higher and lower-fit groups.

Heart rate, mean arterial pressure and perceived exertion during the exercise protocols

Heart rate responses were normalised for peak heart rate obtained during the cardiorespiratory fitness test. Heart rate was significantly higher during high-intensity exercise [mean 65 $\%HR_{peak}$ (95% CI, 62 to 68 %,)] compared to moderate-intensity exercise [mean 58 $\%HR_{peak}$ (95% CI, 55 to 61%, $P<0.01$)], whilst both were elevated compared to control [mean 37 $\%HR_{peak}$ (95% CI, 34 to 40), $P<0.01$]. There was no effect of fitness on the heart rate responses ($P=0.24$). Similarly, mean arterial pressure was higher during high-intensity exercise [mean change of 18 mmHg (95% CI, 14 to 20)] compared to moderate-intensity exercise [mean change of 14 mmHg (95% CI, 11 to 16), $P=0.02$] whilst both were elevated compared to control [mean change 5 mmHg (95% CI, 6 to 10), $P<0.01$]. There was no effect of fitness on the mean arterial pressure responses ($P=0.45$). RPE was higher during the HIIE [mean RPE 4 AU (95% CI, 3 to 5)] compared to moderate-intensity exercise [mean RPE 3 AU (95% CI, 2 to 4, $P<0.01$)]. There was no effect of fitness on the RPE responses ($P=0.58$).

Brachial artery flow-mediated dilation

Baseline flow-mediated dilation.

The coefficient of variation for baseline FMD% across the three visits in this study was 11.8±3.9 %, which is similar to those previously reported (10.1-14.7%) (70, 77). Using test-retest data from our control condition (baseline and 10 min post) we established that the within-day CV%

for FMD% was 8.06±7.50 %. There were no differences in resting (pre-exercise/control) brachial diameter, FMD_{mm}, FMD%, or SR_{AUC} across the three separate testing days (Table 2; $P>0.05$).

Effect of fitness on baseline flow-mediated dilation.

There was no significant difference in resting FMD% between the lower- (Table 3a) and higher-fit groups (Table 3b) [mean difference of 0.2 % (95% CI, -0.8 to 0.9), $P=0.82$]. SR_{AUC} was significantly higher in the lower-fit compared to the higher-fit group [mean difference of 3.2 $10^3 \cdot s^{-1}$ (95% CI, 1.3 to 6.3), $P=0.04$], despite no differences in baseline diameter between fitness groups [mean difference of 0.2 mm (95% CI, -0.6 to 0.8), $P=0.13$]. Furthermore, time to peak diameter was significantly longer in the lower-fit compared to the higher-fit group [mean difference of 10 s (95% CI, 1 to 17), $P=0.02$].

Effect of exercise intensity on the acute flow-mediated dilation response to exercise:

Baseline and recovery (10 and 60 min post) brachial FMD% and associated variables are detailed in Tables 3a and 3b for the lower- and higher-fit groups, respectively. For clarity, post-hoc *P* values are reported only in the text. Delta FMD% data are summarised in Figure 1, which shows the change in FMD% from baseline during recovery (10 and 60 min post). Further, individual responses in delta FMD% are displayed in Figure 2.

In both fitness groups, FMD decreased by 0.74 % (95% CI, -1.34 to -0.03) after 60-min of recovery in control compared to baseline ($P=0.05$). There was no effect of fitness on this response. There was a significant fitness x condition x time interaction for FMD% ($P=0.01$). FMD% was significantly reduced compared to baseline following high-intensity exercise in the lower-fit group at both 10 min [mean reduction of 0.85 % (95% CI, 0.12 to 1.58), $P=0.02$) and 60 min post [mean reduction of 0.72 % (95% CI, 0.02 to 1.46), $P=0.05$] (see Table 3a). In the

higher-fit group, a negligible change in FMD% was observed 10 min after high-intensity exercise [mean difference of 0.13 % (95% CI, -0.73 to 0.98), *P*=0.77], however there was a significant increase in FMD % compared to baseline after 60-min of 0.84 % (95% CI, -0.12 to 1.69; *P*=0.05) (see Figure 1). The improved FMD% response following HIIE elicited a mean difference of 1.52 % (95% CI, 0.41 to 2.62) after 60 min in the higher-fit compared to the lower-fit group (*P*=0.01; Table 3a and 3b). In support of this difference between fitness groups, the delta change in FMD% after high-intensity exercise at 60 min was significantly correlated with VO_{2peak} (r = 0.41; P<0.01). Furthermore, in the higher-fit group, FMD% was elevated after 60-min compared to moderate-intensity and control protocols [mean difference of 0.92% (95% CI, 0.05 to 1.78, *P*=0.01) and 1.54% (95% CI, 0.65 to 2.42, *P*=0.02) (Table 3b). These changes in FMD% were also observed for absolute FMD (mm), with an increase 60-min following high-intensity exercise in the higher-, but not lower-fit group (*P*=0.04; Table 3a and 3b).

FMD% increased significantly from baseline 10 min after moderate-intensity exercise [mean change of 0.86 % (95% CI, 0.17 to 1.56), *P*=0.02; Figure 1], and returned to baseline levels after 60 min [mean difference to baseline of 0.30 % (95% CI, -0.59 to 0.53),] with no effect of fitness on the response [mean between fitness group difference of 0.43 % (95% CI, -0.28 to 1.13), *P*=0.23; r = -0.13, *P*=0.38]. Furthermore, the FMD% response 10-min after moderate-intensity exercise was increased compared to the high-intensity response [mean difference of 1.15 % (95% CI, 0.58 to 1.72), *P*<0.001] and control [mean difference of 1.23 % (95% CI, 0.72 to 1.88), *P*<0.001] in both fitness groups (Figure 1). In the lower-fit group, an increase in FMD% was observed 10 min after moderate-intensity exercise compared to the reduction observed after

high-intensity exercise [mean difference of 1.34 % (95% CI, 0.60 to 2.09), $P<0.001$] and control [mean difference of 0.99% (95% CI, 0.23 to 1.75), $P = 0.01$] (Table 3a).

We also present covariate "adjusted FMD%" values (Table 3a/b). This analysis was consistent with our initial observations in FMD%, with a significant interaction between condition, fitness and time ($P=0.04$). Post-hoc analysis revealed significant differences between the lower- and higher-fit groups 60-min after HIIE ($P<0.01$).

Blood flow and shear rate responses

Resting blood flow was significantly elevated 10 min following both exercise protocols compared to control ($P<0.01$), and was higher following high-intensity exercise compared with moderate-intensity [mean difference of 0.36 $mL.s^{-1}$ (95% CI, -0.03 to 0.66), $P=0.05$]. There was no effect of fitness on the blood flow responses to exercise ($P=0.79$) (Table 3a and 3b). Shear rate demonstrated a similar pattern where it was elevated 10 min after both exercise protocols compared with control ($P=0.01$), and was higher immediately after high-intensity compared to moderate-intensity exercise [mean difference of 17.38 10^3 s^{-1} (95% CI, -3.86 to 38.62), $P=0.01$]. There was no effect of fitness on the shear rate responses after exercise ($P=0.78$) (Table 3a and 3b).

Heart rate and blood pressure responses after exercise

There was a condition x time interaction for HR, SBP and MAP (Table 3a and 3b; $P<0.01$). Heart rate was elevated by 9 $b.min^{-1}$ (95% CI, 8 to 12) and by 13 $b.min^{-1}$ (95% CI, 11 to 15) 10 min after moderate-intensity and high-intensity exercise, respectively, compared to rest. MAP

was 5 mmHg (95% CI, 3 to 8) and 6 mmHg (95% CI, 3 to 9) higher 10-min after moderate- and high-intensity exercise, respectively, compared to rest.

Discussion

Primary findings

To our knowledge, this is the first study to investigate the acute effects of exercise intensity and cardiorespiratory fitness on endothelial function in elderly men. The main findings from this study indicate that the acute effects of leg exercise on brachial FMD are dependent on both the intensity of exercise and cardiorespiratory fitness in elderly men. We observed an immediate increase in FMD following MICE that normalised after 60 min in both fitness groups. In contrast, FMD decreased immediately and 60 min following HIIE in the lower-fit group, whereas FMD increased after 60 min in the higher-fit group. We also observed reductions in FMD in both groups following prolonged rest during the control assessment.

Exercise-intensity and post-exercise FMD in elderly men

The FMD response to acute exercise is suggested to be bi-phasic (15), with an inverse relationship between exercise-intensity and the recovery in brachial artery endothelium-dependent function observed in some (11, 33) but not all studies (3, 62). We attempted to capture the time-course response by measuring FMD immediately (10 min post) and 60 min after exercise in the elderly and found an exercise intensity-dependent decrease in endothelial function immediately after high-intensity exercise, which is consistent with previous findings in young (11, 33), hypertensive (39) and peripheral arterial disease patients (35). Conversely, we found an

immediate increase in endothelial function after short-term moderate-intensity exercise, which has been observed in one (33), but not all (3, 11) studies in younger individuals, and following 30 min of walking exercise in healthy middle-aged adults (13). The immediate improvement in FMD after MICE of 40% PPO in this study contrasts the finding of no-change in FMD following cycling exercise at 50% HR_{max} in albeit, younger healthy individuals (11). This difference in findings may be due to the degree of baseline endothelial dysfunction in elderly compared to younger adults, with greater improvements in acute FMD observed after exercise in coronary artery disease patients with a lower baseline FMD (14). Moreover, the increase in FMD after moderate-intensity exercise normalised after 60 min which is similar in younger adults (33).

In line with the suggested effect of higher-intensity exercise (>70% HR_{max}) on the bi-phasic FMD response, we observed an increase in FMD 60 min after HIIE compared to normalisation of FMD after MICE in the higher-fit elderly adults. This contrasts with a report by Currie *et al.* (2012), who found an increased FMD after both high- and moderate-intensity exercise in coronary artery disease patients. However, unlike the study by Currie and colleagues, our exercise protocols were duration and work matched, which is important as the dose of exercise affects FMD independent of intensity (33). Our study reports intensity-dependent, dose-matched differences in the bi-phasic FMD response in elderly adults. We provide further support that exercise intensity modulates acute endothelial function (3, 11, 19, 33), in healthy elderly adults.

Acute FMD, cardiorespiratory fitness and vascular adaptation

The rationale for assessing the acute response of endothelial function to exercise relates to the potential impact of repeated bouts of exercise on vascular adaptation (24), but whether the

391 immediate increase or decrease in FMD after exercise in this study is important for future
392 vascular adaptation in the elderly is unknown. Padilla *et al.* (2011) suggest recurring periods of
393 exercise-induced transient endothelial impairment may represent a beneficial stimulus that
394 contributes to longer-term improvements in vascular function and structure, a concept known as
395 *hormesis*. That is, the initial challenge, e.g. acute reductions in FMD, leads to activation of
396 beneficial adaptive processes (45). The acute exercise-intensity dependent reductions in FMD we
397 observed in this study may be linked to the recent observation that HIIE training is likely more
398 effective than MICE training in improving conduit artery endothelial function (50). Therefore
399 improving FMD immediately after moderate-intensity exercise (which normalised after 60 min)
400 may not lead to beneficial long-term vascular adaptation with training. Interestingly, we
401 observed that cardiorespiratory fitness modulates the bi-phasic response of FMD to high-, but not
402 moderate-intensity exercise in the elderly. The sustained reductions in FMD in the lower fit
403 individuals after high-intensity exercise may be the signal required for future vascular adaptation
404 observed following training and increases in fitness (45, 65).
405
406 Our study is the first to directly assess the effect of cardiorespiratory fitness levels on acute
407 changes in FMD following exercise in the elderly. The positive relationship between exercise
408 training and endothelial function is well established (41, 42), whilst cardiorespiratory fitness is
409 related to training status (37) and can be modified through changes in routine physical activity
410 (26, 43). In support of this, acute reductions in FMD have been reported in sedentary, but not
411 active adults after both leg-press exercise (47), and maximal running (30). Whether the
412 similarities observed in the reduced FMD response after HIIE in the present study reflect the low

overall physical activity levels or the impact of low activity on reductions in cardiorespiratory fitness is not known.

Physiological significance

The acute changes of ~0.85% in FMD up to 60 minutes in this study are in line with previous studies that reported changes in FMD between 0.6-2.3% in young healthy and individuals with cardiovascular disease (11, 14, 30, 62). Our current understanding of the physiological significance in the magnitude of the acute, transient changes observed in FMD are limited, and we are guided by longitudinal evidence suggesting changes in FMD are associated with changes in cardiovascular risk; with an absolute increase in FMD of 1% associated with a ~9-17% reduction in cardiovascular risk, independent of traditional cardiovascular risk factors (23, 32). It is plausible that larger responses in acute FMD, such as the prolonged reductions in FMD observed in the lower-fit after HIIE in this study, may lead to greater eventual vascular adaptations; however this is yet to be established.

Potential mechanisms

The mechanisms responsible for exercise-induced, intensity-dependent changes in FMD have been proposed to include alterations in oxidative stress, inflammation, reactive oxygen species (19, 31), shear stress and shear pattern, blood pressure, baseline artery diameter, endothelin-1 expression (28), increased sympathetic nervous activity (27), or vasoconstrictors (15). As we did not assess mechanisms of FMD changes, we can only speculate on the possible causes. We covariate-controlled for exercise-induced changes in artery diameter and shear stress, so this is unlikely to be the cause of our observed differences. NO bioavailability (54), and shear stress

patterns during exercise are known to directly contribute to changes in FMD (21, 70, 73). Large increases in brachial antegrade shear stress occur during cycling exercise (21) and are associated with improved FMD (73), whilst increases in oscillatory shear and/or retrograde flow lead to reductions in FMD (57). Increases in oscillatory flow are observed early during cycling exercise (21), but may also be augmented during interval exercise used in this study, due to the stop-start nature of the high-intensity modality. This may explain the immediate improvement in FMD after MICE compared to the reduced FMD immediately following HIIE. Reductions in FMD immediately after exercise of higher-intensity, and not moderate-intensity exercise, may be related to the negative impact of induced hypertension on FMD (18, 40). We observed a larger increase in blood pressure during HIIE compared to MICE in this study, irrespective of fitness level. Interestingly, a training –associated protection against the drop in FMD exists following increases in blood pressure, albeit during resistance exercise (47), which may be linked to our observation of a prolonged reduction in FMD following HIIE in the lower-, but not, higher-fit individuals.

Sedentary time and acute FMD in the elderly

Studies investigating the acute effect of exercise intensity on endothelial function do not commonly assess FMD across the same measurement period using a non-exercise control. This study is unique in that it offers the opportunity to assess changes in FMD during extended periods of sedentary time in the elderly. We observed a reduction in brachial artery FMD after ~120 min of "sedentary time' (baseline rest+protocol+recovery) which is not reported in younger individuals after 6 hours of prolonged sitting (51). As sitting time increases all-cause and cardiovascular mortality risk in older adults (38), the vascular effects of prolonged sitting

warrants investigation. In line with recent evidence (51), we showed that reductions in FMD with sedentary time can be attenuated with short-term moderate-intensity exercise. However, we also found that high-intensity exercise in lower-fit individuals led to a similar decline in FMD to that of prolonged supine rest. This suggests that prescribing moderate-intensity in lower-fit elderly individuals might be considered before progressing to higher-intensity exercise as cardiorespiratory fitness improves.

Cardiorespiratory fitness and baseline FMD in the elderly

A modest association exists between cardiorespiratory fitness and basal endothelial function, independent of age and health status (41). Similarly, aerobically trained middle-aged and older adults have preserved endothelial function compared to those who are sedentary (16, 17, 42, 48, 53), however in this study investigating FMD in the elderly there was no difference in resting brachial artery FMD between lower- and higher-fit groups. This may be due to normalised FMD in the higher-fit following increases in artery diameter and structural remodelling observed with exercise training (36, 72) with a tendency for a larger arterial diameter in the higher-fit compared to the lower-fit group. It is also possible that a "ceiling" effect exists on basal FMD in the elderly, as no improvements in FMD were reported following short-term training in older, higher-fit adults despite increases in $\dot{V}O_{2peak}$ (20).

Clinical relevance

Ischemic events typically occur in the elderly who have known cardiovascular risk factors and/or disease. It is known that regular physical activity and exercise training throughout the lifespan has cardio-protective and vascular effects. Recently, HIIE has become popular for its potential

for additional cardiovascular benefits with shorter bouts of exercise, including improved endothelial function (50). Our findings highlight the *exercise paradox*, where those who are at the greatest risk of adverse responses to acute exercise, have the most to gain from regular exercise (37). Elderly individuals with low fitness and endothelial function who exhibit further reductions in FMD 60 min after higher-intensity exercise may be at increased, acute cardiovascular risk. The acute reduction in FMD was not observed following MICE irrespective of fitness level. Whether the acute reduction is necessary to induce vascular adaptation (see *hormesis,* discussed above) (45, 65) and represents a potential danger period where the vascular system may be less responsive to stress is unknown. However, higher fitness in this study did attenuate the reduction in FMD observed following HIIE, suggesting there may be an adaptive or tolerance response with improvements in cardiorespiratory fitness. However, in the elderly who are of a lower fitness and/or those who already exhibit vascular dysfunction, this type of exercise may need to be treated with caution due to the potential that vascular dysfunction is transiently exacerbated. Importantly, whether the differences in the FMD response to different acute exercise intensities reported here has longer-term consequences on endothelial function and/or CV risk in healthy elderly individuals needs to be determined.

Study limitations

In future studies, it would be interesting to have prolonged FMD measurements e.g. 2h-24h after exercise to establish whether the bi-phasic pattern is delayed or persistent in the lower-fit compared to higher-fit individuals, particularly after high-intensity exercise. A limitation of our study is that we included controlled-hypertensive participants. Despite observing no difference in resting FMD between controlled-hypertensive and normotensive individuals, we cannot rule out

the potential confounding influence of hypertension on the findings. Further, we cannot rule out the potential influence of anti-hypertensive, statin and beta-blocker therapy on the current findings, and further work should focus on the direct impact of medication on acute post-exercise FMD. As we have not reported the physical activity of participants, we cannot exclude the possibility that genetic or behavioural differences contribute to the different levels of fitness and the observed findings. We did not include measures of potential mechanisms involved in the changes in FMD we observed, and further studies are required to fully explain our findings of an interaction between exercise intensity and fitness on the acute FMD response to exercise.

Conclusions

In conclusion, the present study illustrates the effect of exercise intensity on acute FMD responses in elderly men. Furthermore, we highlight the importance of cardiorespiratory fitness on the acute FMD response following high-intensity exercise. Increases in FMD after MICE normalised quickly. Conversely, there were prolonged increases in FMD after HIIE in those with a higher-fitness, whereas lower-fitness individuals exhibited sustained decreases in endothelial function. This decrease in FMD may represent the signal for an adaptive vascular response and/or endothelial fatigue in untrained elderly individuals. Further studies on the acute effects of exercise intensity on endothelial function will be important to establish if the same effect exists in elderly females, and to investigate the link between changes in FMD with acute exercise and the potential for chronic adaptation with exercise training in the elderly.

Grants

Funding from the National Health and Medical Research Council (1022752, 1021416, 1020955, 1003707 and 1000967) and Queensland Government supported this work. Professor Golledge

holds a Practitioner Fellowship from the NHMRC, Australia (1019921), and a Senior Clinical Research Fellowship from the Queensland Government, Australia. Professor Green's research is supported by an NHMRC Principal Research Fellowship (1090914).

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Tables

Table 1. Participant characteristics.

Data are presented as mean±SD. Significance value $P \leq 0.05$. *CRF, cardiorespiratory fitness; BMI, body mass index; SBP, systolic blood pressure; DBP, systolic blood pressure;* VO_{2peak}*, peak oxygen uptake; RER, respiratory exchange ratio*

Table 2. Comparison of baseline FMD indices between testing visits.

Data are presented as mean±SD. Significance value $P \leq 0.05$. *FMD, flow-mediated dilation; SRauc, shear rate area-under-the-curve.*

Table 3. Flow-mediated dilation and hemodynamic indices at rest, 10 min and 60 min following control or acute exercise in lower-fit elderly.

Data are presented as mean±SD for a) lower-fit and b) higher-fit. Significance value $P \leq 0.05$. A fitness x time x condition significant interaction was observed for FMDmm ($P=0.04$), FMD% ($P=0.01$) and 'adjusted FMD%'($P=0.04$). For clarity, post-hoc *P* values are reported in the text only. *significantly different to baseline $^{\#}$significantly different to control $^{\alpha}$significantly different between moderate- and high-intensity. *FMD; flow-mediated dilation; SRauc, shear rate area-under-the-curve; TTP, time-to-peak diameter; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.*

Figure

Figure 1. Delta FMD % from baseline at a) 10-minutes post and b) 60-minutes post in control, moderate-intensity and high-intensity exercise in both lower-fit (*open-bars*) and higher-fit (*dark bars*) elderly individuals.

Error bars represent SD. Significance value $P \leq 0.05$. Post hoc analysis revealed ^{a} control 60-min ΔFMD% was significantly reduced compared to exercise ($P=0.01$), ^{b} ΔFMD% significantly increased 10-min after moderate-intensity compared to high-intensity exercise ($P=0.02$), ^{c} ΔFMD% significantly improved in the higher-fit compared to the lower-fit group 60-min after high-intensity exercise ($P=0.01$). *FMD, Flow-mediated dilation.*

Figure 2. Mean (white squares) and individual (lines) delta FMD% from baseline at 10 and 60 minutes after high-intensity (A-B), moderate-intensity (C-D) and control (E-F) protocols in both higher- and lower-fitness groups.

Significance value $P \leq 0.05$; $^{\#}$ significant change from baseline FMD%. *FMD*, Flow-mediated dilation

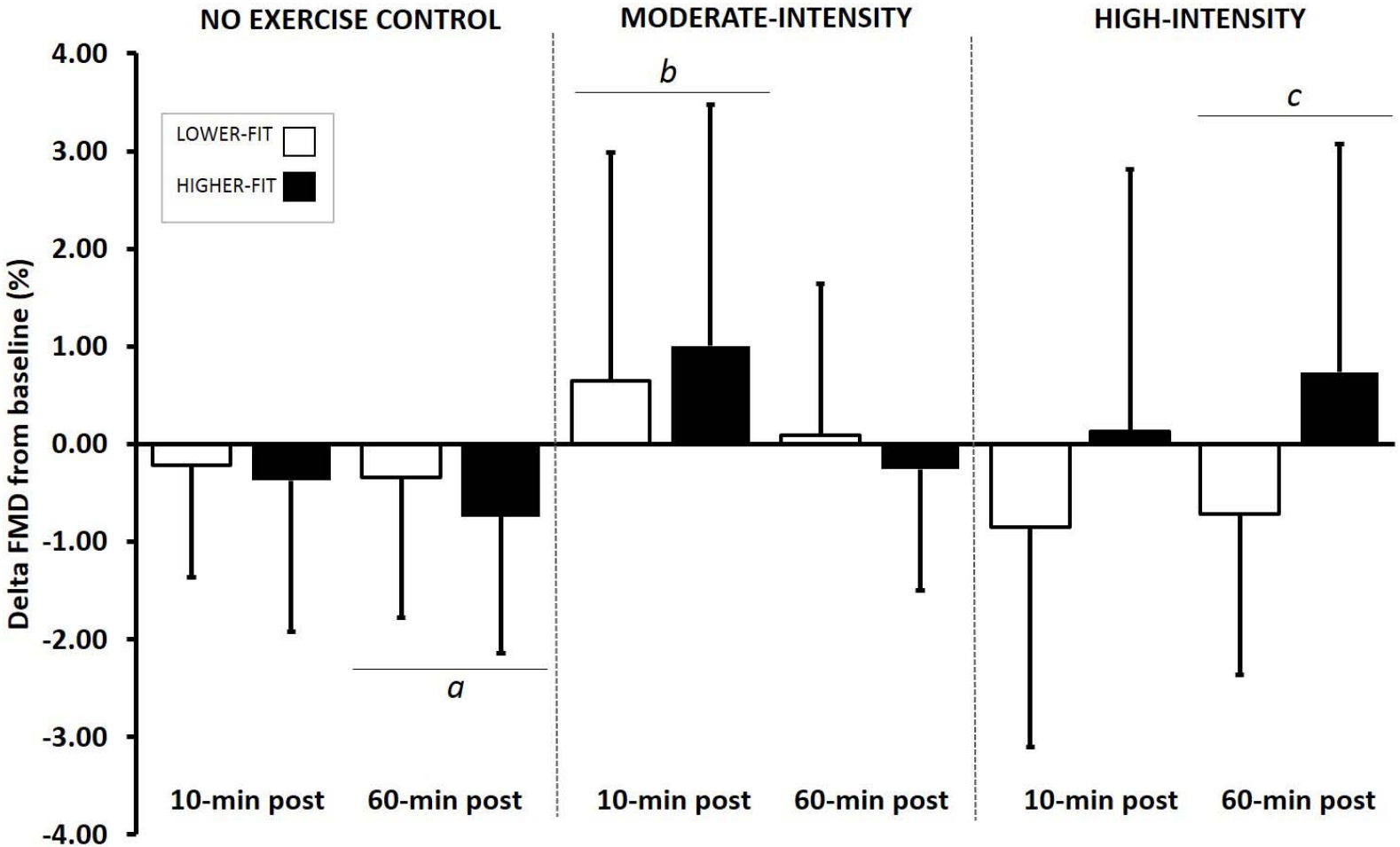
Table 1.	**All (*n*=47)**	**Lower-CRF (*n*=27)**	**Higher CRF (*n*=20)**	***P* value (lower *vs.* higher)**
Demographics				
Age (years)	70±5	72±5	69±5	0.05
Hypertensive (%)	31	29	26	-
Anthropometric measurements				
Height (m)	1.74±0.08	1.72±0.08	1.76±0.09	0.27
Weight (kg)	76.4±11.5	76.3±12.5	76.5±10.3	0.96
BMI ($kg.m^{-2}$)	25.3±3.4	25.5±3.4	24.9±3.3	0.52
Body fat (%)	24.7±5.9	25.8±6.0	23.3±5.8	0.17
Waist:Hip ratio	0.92±0.08	0.92±0.08	0.92±0.07	0.71
Hemodynamic variables				
Resting heart rate (bpm)	55±7	58±7	52±7	0.005
Brachial SBP (mm Hg)	125±15	124±14	126±12	0.66
Brachial DBP (mm Hg)	72±8	72±9	72±7	0.87
Medication classification				
ARB / ACE inhibitors (%)	23	22	19	-
Antiplatelets (%)	6	7	4	-
Beta-blockers (%)	4	7	0	-
Calcium channel blockers (%)	11	7	11	-
Statins (%)	30	40	11	-
Cardiorespiratory fitness				
VO_2 peak :				
Absolute ($L.min^{-1}$)	2.22±0.63	1.85±0.39	2.71±0.56	<0.001
Relative ($mL.kg^{-1}.min^{-1}$)	29.0±6.96	24.3±2.9	35.4±5.5	<0.001
Peak heart rate (bpm)	151±15	146±15	156±10	0.02
Age-predicted (%)	100±10	102±12	97±6	0.08
RER (AU)	1.18±0.11	1.19±0.13	1.16±0.08	0.16
Peak Power (Watts)	160±40	140±30	190±40	<0.001

Table 2.	CONTROL	MODERATE-INTENSITY	HIGH-INTENSITY	*P* value (condition)
Baseline FMD test				
Diameter (mm)	4.82±0.62	4.81±0.66	4.81±0.58	0.79
FMD (mm)	0.02±0.01	0.02±0.01	0.02±0.01	0.32
FMD (%)	4.71±1.57	4.86±1.58	4.89±1.45	0.50
FMD SR_{AUC} (10^3 s^{-1})	13.8±5.7	13.7±7.6	14.6±7.1	0.29

3 a) LOW-FIT	CONTROL (NO EXERCISE)			MODERATE-INTENSITY CONTINUOUS EXERCISE			HIGH-INTENSITY INTERVAL EXERCISE		
	Pre	Post	Post (60 min)	Pre	Post	Post (60 min)	Pre	Post	Post (60 min)
Flow-mediated dilation									
Diameter (mm)	4.6±0.6	4.6±0.6	4.5±0.6*	4.6±0.6	4.7±0.6*#	4.6±0.6	4.6±0.6	4.7±0.6*#	4.6±0.7
FMD (mm)	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.03±0.01*#a	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01
Rest blood flow ($mL.s^{-1}$)	1.2±0.7	1.2±0.6	0.8±0.7*	1.2±0.6	1.8±0.9*	0.8±0.6	1.2±0.7	2.1±1.4*#	0.9±0.6
Peak blood flow ($mL.s^{-1}$)	4.8±2.2	4.5±2.3	4.0±2.6*	4.8±2.0	5.5±2.1*#	4.7±2.6	5.2±2.8	6.0±2.5*#a	4.9±2.8
FMD SR_{AUC} (10^3 s^{-1})	14.1±5.9	13.4±7.4	13.3±6.5*	15.0±8.2	17.6±8.1*#	14.7±8.0	15.5±7.0	18.3±7.6*#a	15.0±7.9
TTP diameter (s)	66±27	67±35	74±36*	72±31	64±27	73±46	69±34	71±32	67±40
FMD (%)	4.7±1.6	4.4±1.7	4.1±1.6*	4.7±1.6	5.4±1.9*#	4.8±1.7	4.8±1.4	4.0±2.2*#a	4.1±1.3*a
Adjusted FMD (%)	4.5±1.6	4.2±1.5	4.0±4.6*	4.5±1.9	5.1±1.7*#	4.5±1.7	4.9±1.4	3.9±2.1*#a	4.2±1.2*a
Heart rate and blood pressure									
Heart rate (bpm)	59±10	56±8	55±7	58±7	68±9*	58±6	58±8	71±13*#a	59±8
SBP (mm Hg)	124±15	130±15	129±15	125±14	133±13*	126±15	124±12	132±14*	124±11
DBP (mm Hg)	72±9	76±9	74±9	73±9	75±9	74±11	73±9	76±10	74±9
MAP (mm Hg)	87±8	91±9	90±9	88±10	93±9*	89±12	88±10	93±11*	88±9

3 b) HIGH-FIT	CONTROL (NO-EXERCISE)			MODERATE-INTENSITY CONTINUOUS EXERCISE			HIGH-INTENSITY INTERVAL EXERCISE		
	Pre	Post	Post (60 min)	Pre	Post	Post (60 min)	Pre	Post	Post (60 min)
Flow-mediated dilation									
Diameter (mm)	5.0±0.7	4.9±0.6	5.0±0.6	5.0±0.7	5.1±0.7*#	5.0±0.6	4.9±0.5	5.1±0.6*#	5.0±0.6
FMD (mm)	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.03±0.01*#a	0.02±0.01	0.02±0.01	0.02±0.01	0.03±0.01*#a
Rest blood flow ($mL.s^{-1}$)	1.1±0.9	0.9±0.6	0.7±0.6*	1.2±0.9	1.9±1.0*#	1.0±0.8	1.2±0.9	2.2±1.1*#a	1.0±0.6
Peak blood flow ($mL.s^{-1}$)	5.0±2.7	4.4±2.7	3.5±1.9*	4.7±2.6	5.1±2.4*#	4.9±2.0	5.0±2.9	6.2±1.9*#a	4.7±2.2
FMD SR_{AUC} (10^3 s^{-1})	10.2±5.6	10.1±5.9	9.3±5.6*	11.6±6.5	13.7±7.3*#	12.0±3.5	13.2±7.1	15.5±7.3*#a	12.7±5.2
TTP diameter (s)	57±24	61±26	69±33*	60±21	54±18	56±23	62±32	58±32	58±27
FMD %	4.8±1.6	4.4±1.0	4.1±1.3	5.1±1.5	6.1±2.5*#a	4.9±1.3	4.9±1.5	5.0±2.6	5.7±2.0*#a
Adjusted FMD (%)	4.6±1.4	4.4±1.1	3.8±1.6	5.0±1.6	5.9±2.0*#a	4.6±1.6	4.9±1.4	4.8±2.3	5.5±1.6*#a
Heart rate and blood pressure									
Heart rate (bpm)	51±7	48±6	49±8	52±7	61±8*	52±6	52±7	64±7*#a	53±6
SBP (mm Hg)	126±12	133±13	132±12	127±12	136±11*	125±13	126±10	135±12*	125±13
DBP (mm Hg)	72±7	75±8	75±8	72±7	76±7	72±8	73±9	76±7	72±8
MAP (mm Hg)	87±7	90±8	89±8	88±8	93±8*	86±10	87±6	94±7*	87±8

Fitness x time $P = 0.37$
Condition x fitness $P = 0.04$
Condition x time $P < 0.01$
Fitness x condition x time $P = 0.01$



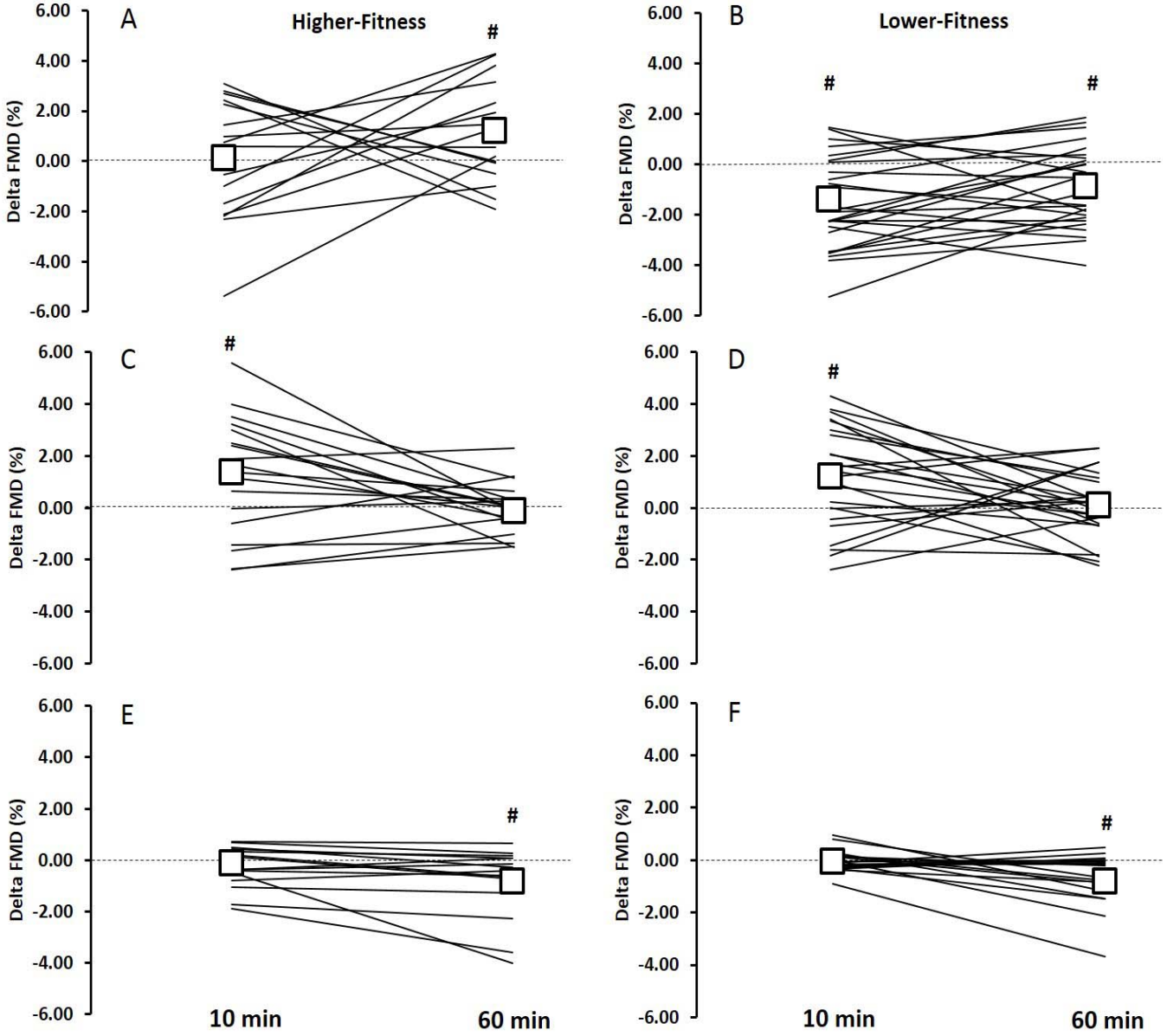


Figure 2. Mean (white square) and individual (lines) delta FMD% from baseline at 10 and 60 minutes after high-intensity (A-B), moderate-intensity (C-D) and control (E-F) protocols in both higher- and lower-fitness groups. Significance value P≤0.05; # significant change from baseline FMD%. *FMD*, Flow-mediated dilation