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Vascular function after acute aerobic exercise in adults with and without type 2 diabetes mellitus

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It is unknown whether type 2 diabetes mellitus (T2DM) influences the vascular function response to aerobic exercise. We examined brachial artery flow-mediated dilation (FMD) and flow-mediated slowing (FMS) of pulse wave velocity (PWV), 10-and 60-min after a high-intensity interval exercise (HIIE) and moderate-intensity continuous exercise (MICE) in adults with and without T2DM. Twelve older male adults with T2DM (57–84 years), and twenty-four healthy young and older adults (12 per group, aged 20–40 years and 57–76 years, respectively), completed an acute bout of HIIE, MICE, and a non-exercise condition. FMD was evaluated by the same researcher following standardized guidelines. FMS was calculated from the manufacturer's PWV beta formulas. Central arterial stiffness was estimated via carotid-femoral PWV (cfPWV). %FMD was reduced (d= − 5.94%, 95% CI: − 10.50 to − 1.38%, $p = 0.002$), whereas %FMS increased (d = 4.55%, 95% CI: 0.62 to 8.48%, $p = 0.01$), 10-min after HIIE only in adults with T2DM, normalizing 60-min into recovery. Conversely, %FMD was increased (d = 5.33%, 95% CI: 0.76 to 9.89%, $p = 0.009$) 10-min after MICE only in adults with T2DM. cfPWV remained unchanged following HIIE and MICE in all groups. We report disease-associated vascular function responses to aerobic exercise suggesting both HIIE and MICE uncover transient vascular alterations in older adults with T2DM.

Type 2 diabetes mellitus (T2DM) increases the risk of cardiovascular disease by exacerbating age-associated reductions in nitric oxide (NO) bioavailability, oxidative stress, and chronic low-grade inflammation[1]. These mechanisms collectively contribute to endothelial dysfunction, a pivotal step in the pathogenesis of atherosclerosis[2]. People with T2DM also exhibit stiffening of the central arteries, which is partly a consequence of endothelial dysfunction[3]. In fact, the infusion of L-NMMA, a blocker of endothelial nitric oxide synthase (eNOS), leads to an increase in carotid-femoral (cf.) and brachial pulse wave velocities (PWV)[4,5]. Furthermore, the deceleration response of brachial artery PWV to reactive hyperemia, known as flow-mediated slowing (FMS), shows an inverse correlation with changes in vessel diameter[6]. This underscores the critical role of endothelial function in determining arterial stiffness[6]. Together with flow-mediated dilation (FMD), which reflects NO bioavailability and endothelium-dependent vasodilation, FMS offer complementary insights into vascular health with prognostic value for endothelial dysfunction being increasingly established[7–9]. While FMD assesses endothelial responsiveness, FMS captures dynamic arterial stiffness modulation during reactive hyperemia where the resulting NO-mediated vasodilation is expected to reduce stiffness by the Moens-Kortweg Eq.[8,9]. Notably, evaluating both parameters allows a comprehensive characterization of vascular function to physiological stimuli, especially in populations at increased cardiovascular risk, such as individuals with T2DM.

Acute exercise presents a challenge to the vasculature, a response that may offer insight into the benefits of exercise training, including increases in endothelium-dependent vasodilation[10]. Despite large variability, in young, healthy adults, post-exercise brachial artery FMD typically follows an intensity-dependent biphasic pattern, with high—but not moderate—intensity exercise (>15 min) initially decreasing FMD (10–30-min post) before (supra)normalizing within 1–24 hours[10]. Preliminary evidence suggests that HIIE does not cause a

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biphasic post-exercise FMD response in people with T2DM at the 30-min mark[11]. The unaltered post-exercise FMD is surprising given that cardiorespiratory fitness (CRF) appears to modulate the post-exercise vasomotor responses[12]. Low CRF, common in T2DM[13]is associated with sustained post-exercise reductions in FMD of older adults, while in those who are fit and active with either preserved or augmented responses[12]. The low systemic effort entailed by the cycle ergometer when compared to the treadmill may explain the unaltered post-exercise FMD reported by Francois and Little[11]but FMD responses after treadmill exercise remain to be determined. It is also unknown whether T2DM influences the vascular function response to exercise of different intensities, or whether FMD and arterial stiffness recovery follows an intensity-dependent biphasic pattern akin to that observed in healthy adults. Importantly, research has not yet explored the effects of acute aerobic exercise on brachial artery FMS, limiting insights for optimizing exercise prescriptions, monitoring treatment outcomes, and advancing personalized exercise.

Therefore, we compared the temporal response patterns of brachial artery FMD, FMS and regional estimates of arterial stiffness, following an acute bout of high-intensity interval exercise (HIIE) and moderate-intensity continuous exercise (MICE) (10- and 60-min), in older adults with and without T2DM, as well as young adults. We hypothesised that (1) an intensity-dependent biphasic response pattern in FMD is observed in older adults with and without T2DM; and that (2) HIIE induces a larger decrease in FMD 10-min after exercise than MICE.

Results

Characteristics of the participants

The clinical characteristics are depicted in Table 1. Participants with T2DM showed a higher %fat mass compared to young (difference (d) = 15%; 95% CI: 9 to 20%; $p = 0.002$) and older adults (d = 7%; 95% CI: 2 to 13%, $p = 0.008$). Absolute peak oxygen uptake ($\dot{V}O_{2peak}$) was lower in participants with T2DM compared to young (d = − 2.15, 95% CI: − 2.66 to − 1.64 L.min^{-1}, $p < 0.001$) and older adults (d = − 0.64, 95% CI: − 1.15 to − 0.13 L. min^{-1}, $p = 0.011$). Systolic (SBP) and diastolic blood pressure (DBP) were not different between groups (Table 1). Eight out of 12 participants with T2DM were on exogenous insulin therapy; other self-reported medication in these participants are depicted in Supplemental Table 1.

Aerobic exercise characteristics

Isocaloric HIIE and MICE protocols differed among all groups in number of bouts [$p < 0.001$, $w^2 = 0.73$] and duration [$p < 0.001$, $w^2 = 0.71$], respectively. Participants with T2DM completed the highest number of HIIE bouts [12 (3)] and exercised for longer in MICE [34 (6) min] followed by older [8 (2) bouts; 26 (5) min] and lastly young adults [4 (1) bouts; 16 (2) min]. The peak heart rate (HR) of the last bout of HIIE [$p < 0.001$, $\omega^2 = 0.67$] and the mean HR during MICE [$p < 0.001$, $\omega^2 = 0.60$] was higher in young [HIIE: 176 (10); MICE: 138 (7) b min^{-1}] than older adults [HIIE: 147 (12); MICE: 138 (7) b min^{-1}] and lowest in participants with T2DM [HIIE: 132 (16); MICE: 113 (11) b min^{-1}]. Peak HR at the last HIIE bout and mean HR during MICE was lower in older adults with TD2M compared to those without ($p = 0.044$) The estimated EE difference between the exercise protocols was 4.28 (2.74) kcal and not different between the groups ($p = 0.15$).

Vascular function at rest

Brachial %FMD [F (2, 33) = 0.65, $p = 0.53$] and %FMS [F (2, 33) = 2.41, $p = 0.11$] were similar among groups along with underlying ratio parameters baseline (D_{bas}, $bPWV_{bas}$), peak diameter (D_{peak}) and minimum PWV ($bPWV_{min}$) (Table 2, and 3). Scaling %FMD and %FMS did not change results (Table 3). The shear rate until D_{peak} was not different between the groups, whereas time-to-peak was highest in participants with T2DM compared to young (d = 24 s, 95% CI: 9 to 39 s, $p = 0.001$), but not to older adults (Table 2). Similarly, time-to-min bPWV was highest in participants with T2DM in comparison to young (d = 32 s, 95% CI: 13 to 51 s, $p < 0.001$) and older adults (d = 27 s, 95% CI: 8 to 47 s, $p = 0.004$). %FMD and %FMS were inversely correlated in older participants

Characteristic	Young adults ($n = 12$)	Older adults ($n = 12$)	Older adults with T2DM ($n = 12$)	p-value[1]
Age (years)	27 (4)	64 (5)#	67 (8)#	< 0.001
Height (m)	1.75 (0.05)	1.74 (0.07)	1.68 (0.05)#*	0.01
Weight (kg)	75.8 (7.9)	81.2 (10.2)	84.2 (15.0)	0.20
Body mass index (kg.m^{-2})	24.8 (2.4)	26.7 (2.1)	29.8 (4.8)#	0.003
Waist circumference (cm)	80 (10)	100 (100)#	100 (100)#	< 0.001
Body fat mass (%)	17.5 (5.9)	25.0 (4.8)#	32.3 (5.5) #*	< 0.001
bSBP (mmHg)	125 (10)	121 (16)	132 (12)	0.14
bDBP (mmHg)	77 (10)	74 (12)	77 (10)	0.60
MAP (mmHg)	90 (12)	95 (10)	95 (8)	0.34
Resting HR (b min^{-1})	60 (8)	60 (10)	65 (8)	0.086
HR_{max} (b min^{-1})	188 (9)	157 (11)#	142 (116) #*	< 0.001
$\dot{V}O_{2peak}$ (L min^{-1})	4.2 (0.4)	2.7 (0.6) #	2.0 (0.5) #*	< 0.001
$\dot{V}O_{2peak}$ (mL kg^{-1} min^{-1})	55.5 (7.2)	32.8 (6.5) #	24.0 (4.2) #*	< 0.001

Table 1. Characteristics of the participants. #Indicates differences from young adults. *Indicates difference from older adults.

	Young adults			Older adults			Older adults with T2DM			T	Ex	Gr	Ex*T*Gr
	CON	HIIE	MICE	CON	HIIE	MICE	CON	HIIE	MICE	p (ω^2)	p (ω^2)	p (ω^2)	p (ω^2)
FMD scaled, %													
Pre	5.98 (2.58)	5.54 (2.94)	5.82 (2.52)	6.43 (3.19)	6.28 (3.75)	6.02 (2.86)	5.92 (2.66)	6.04 (3.18)	5.98 (3.84)	0.07 (0.01)	< **0.0001** (0.11)	0.18 (0.04)	**0.002** (0.06)
Post 10	5.12 (2.32)	1.50 (3.97)	4.57 (1.89)	6.79 (3.24)	6.98 (3.89)	7.83 (4.74)	5.84 (2.70)	0.30 (3.79)*# $	11.11 (5.38)*#				
Post 60	5.59 (2.83)	2.06 (2.48)	6.22 (4.58)	6.06 (3.30)	3.12 (4.25)	5.86 (4.87)	6.21 (3.50)	3.88 (4.06)	5.69 (5.58)				
FMD, mm													
Pre	0.26 (0.08)	0.23 (0.11)	0.24 (0.09)	0.26 (0.15)	0.24 (0.16)	0.24 (0.20)	0.23 (0.10)	0.20 (0.14)	0.22 (0.16)	0.52 (0.00)	**0.004** (0.03)	0.25 (0.03)	**0.007 (0.05)**
Post 10	0.21 (0.08)	0.07 (0.16)	0.18 (0.08)	0.27 (0.13)	0.29 (0.17)	0.32 (0.20)	0.23 (0.11)	0.00 (0.16)*#$	0.42 (0.20)*#				
Post 60	0.23 (0.11)	0.24 (0.10)	0.24 (0.18)	0.24 (0.13)	0.25 (0.19)	0.25 (0.23)	0.24 (0.14)	0.26 (0.16)	0.22 (0.22)				
SR_{Dpeak}, s^{-1}													
Pre	216 (100)	180 (87)	170 (76)	173 (132)	176 (54)	142 (102)	175 (66)	201 (70)	164 (32)	0.09 (0.00)	0.28 (0.00)	0.16 (0.00)	**0.03 (0.03)**
Post 10	233 (128)	214 (130)	157 (112)	181 (124)	140 (90)	138 (80)	180 (116)	77 (39)*#$	160 (59)				
Post 60	199 (128)	292 (157)	189 (121)	165 (109)	145 (84)	218 (76)	162 (68)	113 (101)	217 (130)				
SR AUC $s^{-1} \times 10^3$													
Pre	14.2 (4.9)	11.4 (6.7)	11.8 (4.7)	9.3 (7.8)	11.9 (5.3)	10.6 (7.1)	11.5 (5.9)	13.5 (3.6)	13.6 (6.5)	0.048 (0.02)	0.38 (0.00)	0.78 (0.00)	**0.04 (0.03)**
Post 10	14.8 (6.4)	12.7 (3.5)	9.0 (5.6)	10.9 (4.9)	11.6 (7.9)	12.4 (9.6)	13.9 (5.0)	4.9 (2.4)*#$	14.0 (6.8)				
Post 60	14.7 (7.6)	14.9 (3.6)	12.4 (8.0)	13.1 (5.7)	13.4 (8.4)	15.6 (5.0)	12.0 (1.6)	13.1 (6.8)	14.4 (5.0)				
FMD/SR AUC$\times 10^3$													
Pre	0.69 (0.35)	1.05 (0.68)	0.76 (0.34)	1.05 (0.58)	0.70 (0.51)	0.83 (0.66)	0.99 (0.61)	0.52 (0.20)	0.74 (0.92)	0.40 (0.00)	0.052 (0.02)	0.24 (0.04)	0.29 (0.00)
Post 10	0.44 (0.27)	0.20 (0.56)	1.41 (0.77)	0.76 (0.49)	1.07 (1.14)	1.25 (1.04)	0.54 (0.31)	0.06 (0.31)	0.84 (0.62)				
Post 60	1.01 (0.57)	0.49 (0.22)	1.35 (0.95)	0.7 (0.3)	0.67 (0.43)	0.53 (0.35)	0.49 (0.34)	0.80 (0.67)	1.01 (0.75)				
D_{peak} mm													
Pre	4.08 (0.42)	4.14 (0.42)	4.13 (0.36)	4.39 (0.68)	4.62 (0.60)	4.51 (0.71)	4.45 (0.50)	4.32 (0.50)	4.45 (0.48)	0.63 (0.00)	**0.03** (0.02)	0.07 (0.09)	0.91 (0.00)
Post 10	4.01 (0.43)	4.11 (0.45)	3.85 (0.26)	4.44 (0.63)	4.57 (0.44)	4.34 (0.61)	4.47 (0.55)	4.39 (0.47)	4.47 (0.45)				
Post 60	4.09 (0.40)	4.14 (0.44)	4.09 (0.35)	4.36 (0.67)	4.57 (0.55)	4.48 (0.77)	4.54 (0.52)	4.46 (0.49)	4.60 (0.52)				
D_{bas} mm													
Pre	3.81 (0.43)	3.91 (0.41)	3.88 (0.34)	4.13 (0.68)	4.38 (0.59)	4.28 (0.70)	4.22 (0.51)	4.10 (0.52)	4.22 (0.50)	0.64 (0.00)	< **0.001** (0.05)	0.10 (0.07)	**0.02** (0.04)
Post 10	3.81 (0.44)	4.04 (0.35)	3.85 (0.26)	4.17 (0.62)	4.28 (0.45)	4.33 (0.61)	4.24 (0.54)	4.39 (0.49)*# $	4.05 (0.41)*#				
Post 60	3.86 (0.40)	3.91 (0.43)	3.86 (0.29)	4.12 (0.67)	4.32 (0.57)	4.23 (0.62)	4.30 (0.52)	4.17 (0.50)	4.36 (0.54)				
Time to peak, s													
Pre	57 (34)	57 (37)	55 (28)	59 (37)	83 (47)	71 (37)	86 (51)	52 (24)	89 (56)	0.76 (0.00)	0.73 (0.00)	**0.002** (0.28)	**0.02** (0.04)
Post 10	45 (22)	59 (43)	57 (27)	63 (27)	65 (46)	67 (30)	67 (46)	95 (56)*	81 (43)				
Post 60	42 (10)	55 (29)	55 (26)	68 (32)	56 (22)	70 (34)	66 (32)	105 (88)*	57 (32)				

Table 2. Brachial artery endothelial function before and after acute aerobic exercise. *Different from pre ($p < 0.01$). #Different from post 60 measures ($p < 0.01$). $Different from MICE ($p < 0.01$).

with [r (34) = − 0.37, $p = 0.029$] and without T2DM [r (34) = − 0.39, $p = 0.02$] but not in young adults [r (34) = − 0.022, $p = 0.90$].

Carotid-femoral PWV (cfPWV) was higher in participants with T2DM compared to young (d = 3.47 m s^{-1}, 95% CI: 2.04 to 4.89 m s^{-1}, $p < 0.001$) and older adults (d = 1.94 m s^{-1}, 95% CI: 0.44 to 3.44 m s^{-1}, $p = 0.009$). Carotid-dorsalis pedis PWV (cdPWV) was higher in participants with T2DM when compared to young (d = 1.80 m s^{-1}, 95% CI: 0.94 to 2.64 m s^{-1}, $p < 0.001$) but not to older adults (d = 0.29 m s^{-1}, 95% CI: − 0.56 to 1.14 m s^{-1}, $p = 0.68$). Adjustments to $\dot{V}O_{2peak}$, %fat mass and mean arterial pressure (MAP) did not change these results.

Responses of vascular function to acute exercise

Exercise-by-time-by-group interactions were observed in brachial %FMD (Fig. 1) and %FMS (Fig. 2). %FMD was reduced (d = − 5.94%, 95% CI: − 10.50 to − 1.38%, $p = 0.002$) while %FMS was increased (d = 4.55%, 95% CI: 0.62 to 8.48%, $p = 0.01$) immediately after HIIE only in participants with T2DM, returning to baseline values 60-min into recovery. %FMD at 60-min post-HIIE of older adults with T2DM was not different from those of young (d = 1.82%, 95% CI: − 4.21 to 6.00%, $p = 0.995$) and older adults (d = 0.76%, 95% CI: − 5.20 to 4.41%, $p = 0.895$) without T2DM. Participants with T2DM also showed an increase in %FMD (d = 5.33%, 95% CI: 0.76 to 9.89%, $p = 0.009$) after MICE but %FMS remained unchanged (Fig. 2). Scaled %FMD (Table 2) did not change these results, even if D_{bas} showed an exercise-by-time-by-group interaction, with increases in D_{bas} (d = 0.29 mm, 95% 0.01 to 0.58 mm, $p = 0.04$) observed after HIIE but not following MICE. Scaling FMS $bPWV_{bas}$ did not change results, despite the main effects of time and exercise observed in bPWVbas (Table 3). An exercise-by-time-by-group interaction was observed for shear rate until peak diameter (SR_{Dpeak}) [$F(8, 257) = 2.23$, $p = 0.03$, $\omega^2 = 0.04$]. Specifically, only participants with T2DM exhibited SR_{Dpeak} reductions (d = − 123 s^{-1}, 95% CI: − 240 to − 6 s^{-1}, $p = 0.03$) after HIIE but not MICE. Still, controlling for SR AUC Dpeak did not abolish the significant exercise-by-time-by-group interaction for % brachial FMD (Fig. 3, Supplement 2). Time-to-peak diameter showed an exercise-by-time-by-group interaction wherein participants with T2DM required a longer time (d = 53 s, 95% CI: 1 to 104 s, $p = 0.04$) to attain %FMD of resting levels following 60 min into HIIE recovery. cfPWV, cdPWV, and carotid-radial PWV (crPWV) remained unchanged following exercise in all groups (Table 3). Adjustments to $\dot{V}O_{2peak}$, total minutes in moderate-to-vigorous physical activity, %fat mass, and MAP did not change these results. Insulin-stratified analyses, limited to T2DM participants, suggested that FMD (Exercise-by-time-insulin $p = 0.748$) and FMS (Exercise-by-time-insulin $p = 0.190$) responses did not differ between those on insulin and those without (Supplemental Table 3). Neither HIIE nor MICE affected post-exercise BP. However, HR remained elevated 10-min into recovery to a greater extent with HIIE, with similar responses observed across groups (Table 4).

Discussion

We found that HIIE reduced brachial artery FMD and increased FMS in older adults with T2DM, but these effects were only observed within the first 10 min post-exercise. Following a 60-min recovery period, vascular function indices returned to baseline. Conversely, MICE increased FMD but not FMS in older adults with T2DM. These findings refute our hypothesis, indicating that post-acute exercise effects on vascular function are intensity-dependent, but only in older adults with T2DM. Still, the long-term impact of high versus moderate stimuli on vascular function in T2DM remains unclear.

Vascular function at rest

Endothelial function measured through FMD was similar between our adults with and without T2DM, which aligns with some studies[11,14,15] but contrasts with others[16,17]. While older adults with T2DM took their last insulin dose the night before, FMD may still be influenced by insulin therapy, which enhances NO bioavailability via the PI3 kinase/Akt pathway[18,19] and increases peripheral blood flow[18,20]. Therefore, it is not surprising that the key stimulus for FMD, SR_{Dpeak}, was similar between adults with and without T2DM. Despite the similarity in FMD and FMS between adults with and without T2DM, the time-to-D_{peak} and time-to-min bPWV were prolonged in people with T2DM. This is concerning, given the evidence suggesting longer times to achieve vessel dilation are associated with an increased risk of cardiovascular events in patients with T2DM[21]. Notably, FMD was also similar between young and older adults without T2DM. Although aging is generally associated with reductions in FMD[22,23]adults who remain physically active throughout their lifespan demonstrate preserved endothelium-dependent vasodilation (i.e., FMD)[24]. The similar resting FMD in our healthy young and older adults may result from upregulated eNOS and antioxidant enzymes, as well as from preserved shear rate, and the absence of age-related vessel remodelling (i.e., no increase in D_{bas}) as noted in this study[24–26].

In the present study, FMS demonstrated an inverse association with FMD in older adults. These results align with previous work from our group[6]suggesting that FMS could serve as a complementary measure to FMD. Building on the mechanistic link between endothelial function and arterial stiffness modulation during reactive hyperemia, the observed inverse association between FMD and FMS highlights the potential utility of FMS in evaluating vascular health.

Age-related vascular changes were more pronounced in our older adults with T2DM, as evidenced by the higher cfPWV (i.e., ~ 2 m s^{-1}) compared to older adults without T2DM. Chronic hyperglycemia promotes the deposition of advanced glycation end products on arterial walls, leading to collagen cross-linking and impairing proteolysis, which collectively increase arterial stiffness[27]. The similar lower limb arterial stiffness observed in older adults with and without T2DM supports the reported preferential stiffening of central arteries in T2DM[27]. This impedance mismatch may elevate pulsatile energy transmission to vascular beds, contributing to microvascular complications (e.g., neuropathies) and end-organ damage (e.g., kidney, brain).

	Young adults			Older adults			Older adults with T2DM			T	Ex	Gr	Ex*T*Gr
	CON	HIIE	MICE	CON	HIIE	MICE	CON	HIIE	MICE	p (ω^2)	p (ω^2)	p (ω^2)	p (ω^2)
FMS scaled, %													
Pre	− 7.52 (5.01)	− 7.43 (4.03)	− 6.39 (2.95)	− 6.66 (3.16)	− 5.29 (2.28)	− 4.15 (1.08)	− 4.70 (3.03)	− 4.92 (3.62)	− 6.26 (2.17)				
Post 10	− 6.79 (4.23)	− 6.05 (2.96)	− 8.22 (4.14)	− 6.44 (2.47)	− 6.94 (3.52)	− 6.25 (3.57)	− 4.78 (3.43)	− 0.90 (3.16)*#$	− 8.65 (3.21)	0.26 (0.00)	**<0.001** (0.04)	0.06 (0.10)	**0.02** (0.04)
Post 60	− 8.03 (4.94)	− 6.79 (4.48)	− 8.03 (4.48)	− 6.37 (2.70)	− 6.34 (2.84)	− 7.58 (2.84)	− 3.77 (2.91)	− 5.23 (2.84)	− 6.47 (2.84)				
FMS, $m\,s^{-1}$													
Pre	− 0.53 (0.58)	− 0.62 (0.37)	− 0.50 (0.37)	0.56 (0.36)	− 0.38 (0.25)	− 0.33 (0.13)	− 0.51 (0.50)	− 0.51 (0.31)	0.55 (0.18)				
Post 10	− 0.59 (0.42)	− 0.54 (0.46)	− 0.75 (0.39)	− 0.53 (0.28)	0.63 (0.34)	− 0.52 (0.37)	− 0.49 (0.42)	− 0.04 (0.28)*#$	− 0.74 (0.34)	**0.04** (0.02)	**0.05** (0.02)	0.34 (0.00)	**0.02** (0.02)
Post 60	− 0.71 (0.43)	− 0.68 (0.42)	− 0.72 (0.48)	− 0.55 (0.36)	− 0.58 (0.32)	− 0.65 (0.38)	− 0.45 (0.46)	− 0.45 (0.29)	− 0.65(0.53)				
$bPWV_{min}$ m s^{-1}													
Pre	8.20 (0.85)	8.07 (0.80)	8.07 (0.79)	7.97 (1.10)	7.82 (0.80)	7.77 (0.60)	8.54 (0.79)	8.67 (1.02)	8.05 (0.46)				
Post 10	8.41 (0.57)	8.42 (0.61)	8.01 (0.71)	8.15 (0.84)	8.19 (0.90)	7.89 (1.00)	8.66 (0.98)	8.50 (0.69)	7.72 (0.67)	**0.06** (0.04)	**<0.0001** (0.05)	0.39 (0.00)	0.65 (0.00)
Post 60	8.31 (0.62)	8.31 (0.88)	8.35 (0.68)	8.32 (0.86)	8.26 (1.14)	8.09 (1.05)	9.02 (0.79)	8.27 (0.74)	8.02 (0.89)				
$bPWV_{bas}$ m s^{-1}													
Pre	8.74 (0.55)	8.68 (0.72)	8.58 (0.75)	8.54 (1.29)	8.21 (0.88)	8.10 (0.68)	9.05 (1.08)	9.18 (1.10)	8.60 (0.40)				
Post 10	9.00 (0.45)	8.96 (0.71)	8.76 (0.72)	8.67 (0.94)	8.81 (1.01)	8.42 (1.16)	9.14 (1.22)	8.54 (0.70)	8.45 (0.86)	**0.003** (0.04)	**0.03** (0.02)	0.52 (0.00)	0.49 (0.00)
Post 60	9.02 (0.36)	8.99 (0.79)	9.07 (0.96)	8.87 (1.07)	8.84 (1.34)	8.74 (1.13)	9.48 (1.21)	8.73 (0.86)	8.67 (1.12)				
Time-to-min, s													
Pre	88 (56)	69 (58)	70 (56)	77 (35)	96 (50)	75 (43)	113 (58)	97 (46)	111 (53)				
Post 10	84 (53)	70 (56)	93 (51)	100 (68)	105 (74)	75 (45)	124 (53)	107 (79)	104 (52)	0.49 (0.00)	0.39 (0)	**<0.0001** (0.06)	0.79 (0.00)
Post 60	88 (54)	95 (37)	77 (51)	53 (55)	108 (56)	85 (55)	95 (65)	151 (85)	120 (50)				
crPWV, m s^{-1}													
Pre	9.58 (1.03)	8.68 (1.30)	9.08 (1.50)	9.31 (1.22)	9.25 (0.80)	9.58 (1.01)	8.79 (3.04)	9.36 (1.94)	9.44 (1.71)				
Post 10	9.61 (2.50)	9.20 (1.61)	9.36 (1.76)	9.79 (1.47)	9.68 (1.17)	9.43 (1.17)	9.61 (2.16)	9.49 (1.56)	10.02 (3.03)	0.15 (0.00)	0.59 (0.00)	0.98 (0.00)	0.33 (0.00)
Post 60	9.51 (0.68)	9.68 (1.79)	9.34 (1.51)	9.61 (1.44)	9.23 (1.48)	8.83 (0.94)	9.73 (1.87)	9.56 (1.21)	8.76 (2.00)				
cdPWV, m s^{-1}													
Pre	8.51 (0.78)	8.14 (0.86)	7.98 (0.92)	9.33 (1.69)	9.99 (1.32)	9.04 (0.91)	9.96 (1.29)	10.02 (1.65)	10.20 (0.93)				
Post 10	8.75 (0.99)	7.75 (1.29)	8.00 (0.64)	10.29 (1.83)	10.26 (1.46)	9.41 (1.14)	9.87 (0.96)	9.93 (1.35)	10.05 (1.33)	0.22 (0.00)	**0.006** (0.03)	**<0.001** (0.44)	0.58 (0.00)
Post 60	8.74 (1.07)	7.86 (1.20)	7.71 (1.01)	9.46 (1.50)	9.75 (0.93)	9.32 (1.24)	9.94 (1.05)	9.90 (1.17)	9.55 (1.43)				
cfPWV, m s^{-1}													
Pre	7.03 (1.11)	7.09 (1.26)	7.10 (1.21)	8.94 (1.44)	8.71 (0.136)	8.93 (1.68)	10.43 (2.2)	10.88 (2.1)	10.41 (1.88)				
Post 10	7.20 (1.86)	7.42 (1.42)	6.94 (1.06)	9.47 (1.41)	9.54 (1.91)	9.48 (1.61)	10.39 (2.16)	11.15 (2.06)	10.74 (2.12)	0.06 (0.02)	0.72 (0.00)	**<0.001**(0.49)	0.69 (0.00)
Post 60	6.91 (1.02)	7.20 (1.12)	6.93 (1.06)	9.39 (1.55)	9.37 (1.68)	9.42 (1.85)	10.52 (2.28)	9.96 (1.78)	9.93 (1.60)				

Table 3. Brachial artery and regional stiffness responses to reactive hyperemia before and after acute aerobic exercise. *Different from pre ($p < 0.01$). #Different from post 60 measures ($p < 0.01$) $Different from MICE ($p < 0.01$).

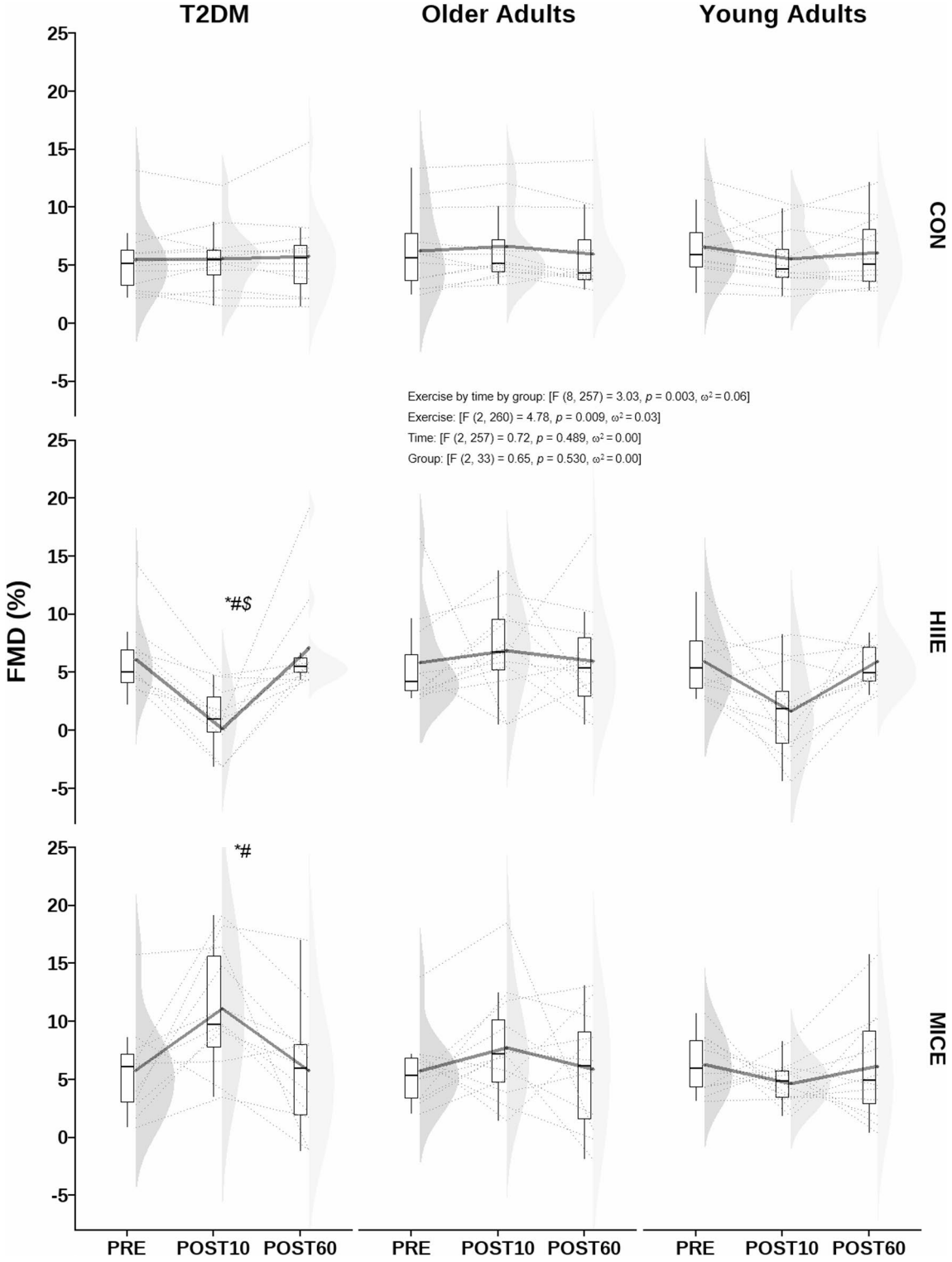

Fig. 1. Post aerobic exercise acute response of brachial artery flow-mediated dilation (FMD) in adults with and without type 2 diabetes mellitus (T2DM). Dashed lines depict individual responses to control (CON), and acute bouts of high-intensity interval (HIEE) and moderate continuous exercise (MICE). *HIIE and MICE post 10 different from exercise pre-measures ($p < 0.01$) #HIIE and MICE post 10 different from CON ($p < 0.01$); $ different from MICE post 10.

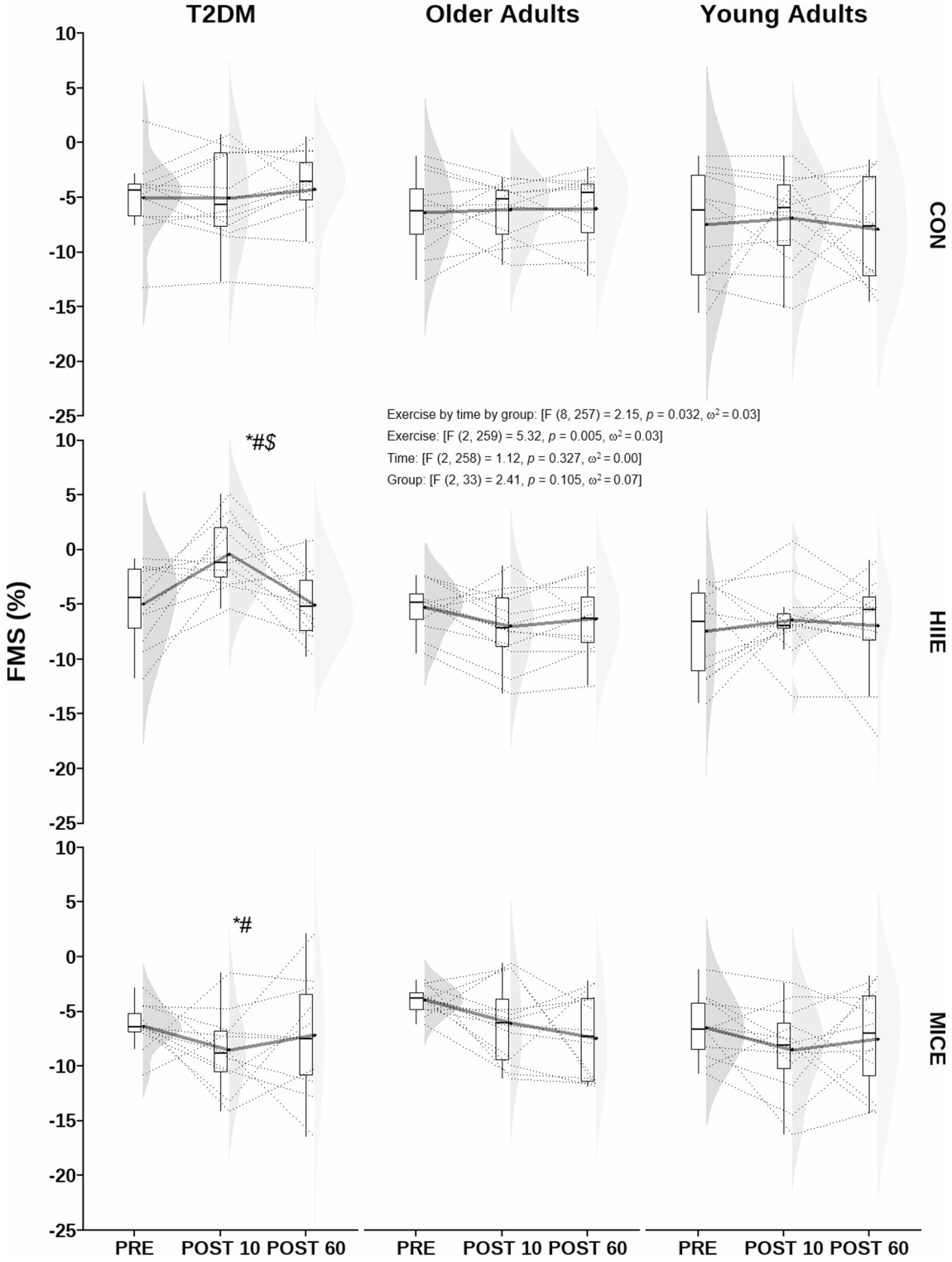

Fig. 2. Post aerobic exercise acute response of brachial artery flow-mediated slowing (FMS) in adults with and without type 2 diabetes mellitus (T2DM). Dashed lines depict individual responses in control (CON), and acute bouts of high-intensity interval (HIEE) and moderate continuous exercise (MICE). *HIIE and MICE post 10 different from exercise pre-measures ($p < 0.01$) #HIIE and MICE post 10 different from CON ($p < 0.01$); $ different from MICE post 10.

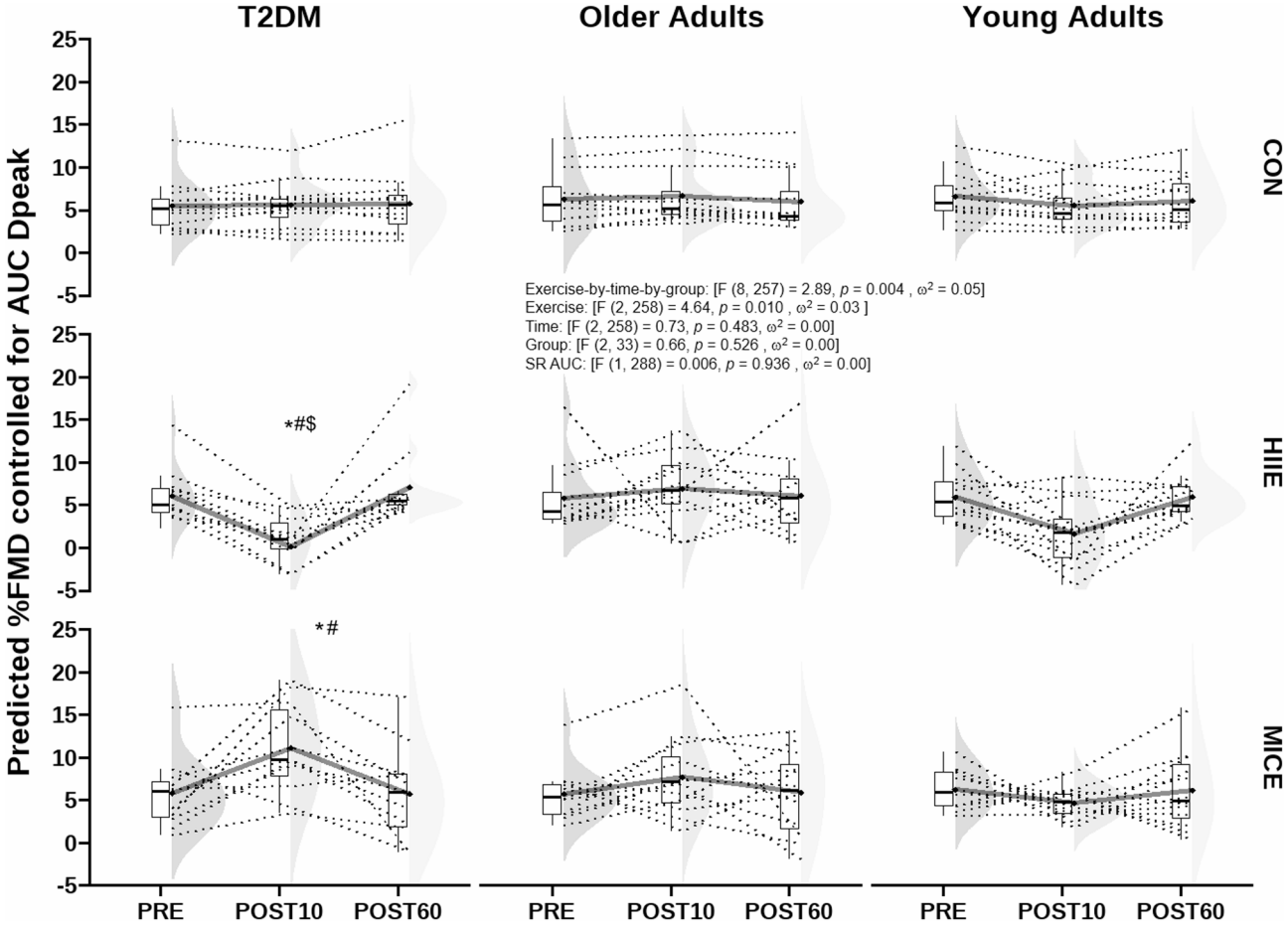

Fig. 3. Post aerobic exercise acute response of brachial artery flow-mediated dilation (FMD) in adults with and without type 2 diabetes mellitus (T2DM) controlled for shear rate area under the curve to peak diameter (SR AUC). Model-predicted values of FMD controlled for SR AUC are depicted. Dashed lines depict individual responses to control (CON), and acute bouts of high-intensity interval (HIEE) and moderate continuous exercise (MICE). *HIIE and MICE post 10 different from exercise pre-measures ($p < 0.01$) #HIIE and MICE post 10 different from CON ($p < 0.01$); $ different from MICE post 10.

Responses of vascular function to acute exercise

Acute aerobic exercise is suggested to elicit an intensity-dependent biphasic response in brachial artery endothelial function[10]. However, recent evidence highlights variable responses to acute exercise, with reports of unchanged[28–31]increased[12,32–34]or decreased[12,28,35–38] FMD after moderate[28–30] or high-intensity exercise[29–31] in young healthy and clinical populations, such as those with metabolic syndrome[39]cardiovascular disease[40–42] and chronic kidney disease[43]. The present study extends this observed heterogeneity in post-exercise FMD response to older adults with T2DM, which may relate to factors, such as disease progression, insulin therapy, timing of exercise measurements, and differences in CRF.

In comparison to our experiment, the study by Francois and Little[11] included participants with T2DM who had a shorter duration of diabetes and were not on exogenous insulin. Exogenous insulin acts as both a sympathoexcitatory and a vasodilatory hormone[44–46], which challenges the interpretation of post-exercise FMD response. CRF is a key modulator of post-exercise FMD responses, with higher CRF associated with preserved FMD or accelerated recovery[11,46] after HIIE[12,47]. Despite the lower CRF of our older adults with T2DM and those of Francois and Little's study, FMD normalized 60-min into recovery or was preserved. It is possible that Francois and Little's [9] failure to measure FMD within the first 30-min after exercise prevented the detection of the FMD biphasic response. Nevertheless, the higher CRF of both young and older adults may, in part, explain the unchanged post-exercise FMD, particularly 10-min post-HIIE. Notably, young adults, although not statistically significant, exhibited a ~ 4% reduction in FMD 10 min post-HIIE, aligning with the intensity-dependent biphasic response[10].

This study showed reductions SR_{Dpeak} 10-min after HIIE, supporting the biphasic response of FMD and the stiffening of the brachial artery (i.e., FMS) to reactive hyperemia, specifically in older adults with T2DM[26]. Mechanistically, HIIE may increase superoxide production, which reacts with NO-yielding peroxynitrite, a potent oxidant, that inactivates NO-mediated vasodilation[37]. Conversely, MICE appears to induce less oxidative stress, than HIIE[37]which may explain increases in FMD observed in older adults with T2DM after MICE, even

	Young adults			Older adults			Older adults with T2DM			T	Ex	Gr	Ex*T*Gr
	CON	HIIE	MICE	CON	HIIE	MICE	CON	HIIE	MICE	p (ω^2)	p (ω^2)	p (ω)	p (ω^2)
SBP, mmHg													
Pre	125 (10)	120 (9)	121 (10)	121 (16)	124 (14)	127 (16)	132 (12)	135 (10)	132 (11)	0.83 (0.00)	0.10 (0.06)	**0.006 (0.27)**	0.947 (0.00)
Post 10	121 (9)	118 (8)	117 (6)	126 (10)	124 (13)	123 (13)	135 (15)	131 (9)	127 (12)				
Post 60	121 (10)	121 (9)	124 (6)	124 (14)	129 (16)	122 (13)	131 (9)	127 (12)	135 (12)				
DBP, mmHg													
Pre	77 (10)	72 (7)	72 (9)	74 (12)	78 (10)	74 (12)	77 (9)	76 (7)	77 (10)	**0.01 (0.02)**	0.46 (0.00)	0.07 (0.09)	0.52 (0.00)
Post 10	71 (10)	70 (6)	68 (9)	73 (13)	77 (8)	77 (11)	77 (6)	77 (10)	78 (9)				
Post 60	71 (12)	71 (8)	68 (11)	73 (11)	73 (10)	70 (12)	77 (7)	74 (10)	77 (10)				
MAP, mmHg													
Pre	93 (10)	88 (7)	88 (7)	90 (12)	93 (9)	92 (12)	95 (8)	96 (4)	95 (9)	0.11 (0.00)	**0.03 (0.02)**	0.009 (0.20)	0.473 (0.00)
Post 10	88 (8)	86 (6)	84 (7)	91 (13)	93 (9)	92 (10)	96 (7)	95 (7)	94 (8)				
Post 60	88 (9)	88 (8)	87 (9)	90 (11)	92 (10)	87 (10)	95 (6)	92 (9)	96 (10)				
HR, b min^{-1}													
Pre	60 (10)	57 (8)	57 (9)	60 (10)	55 (7)	57 (8)	65 (12)	66 (9)	64 (11)	**<0.001 (0.31)**	**<0.001 (0.37)**	**0.03 (0.15)**	0.902 (0.00)
Post 10	60 (8)	77 (11) *#$	68 (9)	51 (6)	72 (12)*#$	65 (10)*#	66 (9)	79 (11)*#$	73 (10)*#				
Post 60	57 (10)	65 (8)	60 (7)	54 (6)	62 (12)	63 (8)	64 (9)	71 (13)	69 (11)				

Table 4. Brachial blood pressure and heart rate before and after acute aerobic exercise. *Different from pre ($p < 0.01$). #Different from post 60 measures ($p < 0.01$). $Different from MICE ($p < 0.01$).

though SR_{Dpeak} was not increased. Although FMD responses can be confounded by changes in D_{bas}[26] and by inter-individual variability in SR, scaling FMD for D_{bas} and controlling for SR AUC Dpeak did not change post-exercise FMD response in older adults with T2DM. When examining FMD responses normalized to SR AUC Dpeak (%FMD/SR AUC Dpeak), the exercise-by-time interaction was abolished, suggesting that FMD was neither increased nor depressed 10-min after acute exercise in older adults with T2DM but rather reflected variations in stimulus. Although no consensus exists on the optimal method to normalize FMD responses, the statistical limitations of FMD-to-SR ratio precludes its use[48]. Our interpretations relied on allometrically scaling FMD to D_{bas} and on SR AUC covariate controlling, though the latter may be unnecessary with appropriate scaling since SR calculation depends on Dbas[49]. Interestingly, FMS increased 10-min after HIIE but remained unchanged after MICE in older adults with T2DM, suggesting that the inverse association between FMD and FMS becomes non-linear after acute exercise. Further research is warranted to clarify the mechanisms underlying this relationship.

Acute exercise-induced BP fluctuations are a plausible mechanism behind post-exercise vascular responses (e.g., FMD)[10,50]. Transient elevations in brachial BP during and immediately after exercise may reduce FMD[50]whereas the onset of post-exercise hypotension (PEH) within 10–20 min may facilitate recovery or potentiate FMD. However, such a mechanism lacks empirical support, particularly for aerobic exercise, further confirmed by our study, as post-exercise BP across groups was unchanged. Post-exercise BP responses are highly variable across populations, with PEH either observed[51–53] or blunted[54–58] in clinical groups, such as people with T2DM and coronary artery disease. In healthy people, it is less common and typically smaller in young adults, becoming more prevalent with age due to increasing hypertension prevalence, but remains inconsistent[59]. Our recent work reflects this variability: one study demonstrated PEH in middle-aged adults[60]while another with a similar cohort did not[57]. Although response heterogeneity stems from the interplay of biological, methodological, and exercise factors (e.g., intensity), in our study, the absence of PEH is likely due to the law of initial values[61]. Based on this law, those with higher resting BP experience greater post-exercise reductions, since resting BP was normal and comparable across groups, the potential for PEH was lower.

Clinical and exercise training implications

Physiological responses to acute exercise are important as they can reveal underlying vascular abnormalities[62]. Resting FMD was similar between adults with and without T2DM; however, a large reduction in FMD (-6%) was observed 10-min after HIIE only in T2DM, which is consistent with the reactivity hypothesis. The clinical relevance of acute exercise-induced changes in FMD is not fully understood. However, a 1% decrease in resting FMD is associated with an 8% increase in the risk of future cardiovascular events[63]. Interestingly, the transient decrease in FMD noted in older adults with T2DM may trigger key responses for subsequent vascular adaptations induced by repetitive exposure to acute exercise in line with the hormesis hypothesis[12]. It remains to be established whether acute reductions in FMD are necessary for upregulation of vascular function in the long term, or if they represent a period of increased cardiovascular risk. Given that FMD was increased 10-min after MICE in people with T2DM, such exercise intensity may hypothetically be safer for acutely upregulating vascular function. Regardless, acute exercise revealed vascular changes only in participants with T2DM during early recovery, but whether vascular function is upregulated to a greater extent with high or moderate exercise is unknown.

Limitations

Several limitations should be acknowledged. First, the absence of measurements for NO and circulating markers of endothelial damage, hinders the ability to provide a detailed mechanistic understanding of vascular function changes after exercise. Additionally, monitoring vascular responses within the first hour may overlook delayed changes (e.g., 1–72 h). Relative rather than absolute exercise intensity was standardized, leading to different exercise prescriptions across participants. While no consensus exists on standardizing exercise doses, many T2DM training studies employ the isocaloric approach [64,65]. Despite conducting a priori power analysis, the substantial inter-individual variability observed in FMD responses, particularly in young adults, may have rendered our inferences underpowered. The use of oral glycemic agents and insulin therapy by most T2DM participants can confound results. Discontinuing medications raises ethical and medical concerns, especially for longstanding T2DM. Analyses of post-exercise vascular outcomes in T2DM participants by exogenous insulin use revealed no apparent differences, still we were underpowered as only four participants were not on insulin. Another important limitation is the lack of a younger group with T2DM, which prevents determining the concomitant effects of aging and T2DM on vascular function response to acute aerobic exercise. Finally, the results apply only to older male adults with and without T2DM.

Conclusions

We found evidence of disease-associated changes in vascular function following acute aerobic exercise among older adults with T2DM. Notably, we observed a reduction in FMD and an increase in FMS immediately after HIIE, with values returning to baseline 60-min into recovery. Conversely, MICE led to an increased FMD in older adults with T2DM, while FMS remained unchanged.

Methods

Participants

Twelve male older adults (aged 57 to 84) with long-standing T2DM [(17 (6) years], 67% insulin-treated, and diagnosed by American Diabetes Association criteria [fasting HbA1 = 7.25% (0.73)] completed the trial as depicted in Fig. 4. Twenty-four healthy males were also recruited and allocated into young ($n = 12$; aged 20 to 40) and older adult groups ($n = 12$; aged 57 to 76). Exclusion criteria for patients with T2DM included smoking, cardiac diseases, and renal/musculoskeletal diseases. All participants completed the PAR-Q + and International Physical Activity (IPAQ) questionnaires. Participants with T2DM were physically inactive based on the IPAQ questionnaire (M = 65, SD = 30 min/week), while healthy participants were physically active, with at least 150 min/week of moderate to vigorous activity [M = 200 (25) min/week]. Exclusion criteria for healthy participants included smoking, physical inactivity, cardiac diseases or cardioactive medication, and renal diseases. Considering the extensive evidence supporting the association of physical inactivity with T2DM, being physically active was considered a key inclusion criterion for young and older adults without T2DM, who served as the control group[66–68]. All participants provided written informed consent. The inclusion of young adults, in this study, is essential to distinguish the effects of aging and T2DM on vascular function. Younger participants act as a baseline for normative vascular responses, facilitating the identification of deviations related to aging and metabolic dysfunction. Given that vascular aging is marked by progressive endothelial dysfunction and arterial stiffening, which are compounded by T2DM, comparisons across these groups will help contextualize the observed responses.

Experimental design

The study was approved by the Ethical Review Board of the Faculdade de Motricidade Humana – Universidade de Lisboa (10/2020) and retrospectively registered in ClinicalTrials.gov (NCT06684912). The study was designed as a randomized, cross-over, repeated-measures experiment and all testing procedures were aligned with the Declaration of Helsinki for Human Research.

In the first visit, all participants performed a cardiopulmonary exercise test (CPET). On following visits, participants complete three block randomized experimental sessions (1:1 allocation ratio) an acute bout of HIIE, and MICE, or a no-exercise (CON) condition, with their assignments blinded until arrival. All experimental sessions occurred in the morning with a minimum of one week between sessions. Participants reported to the laboratory in a fasting state (≥ 4 h) and refrained from vigorous exercise, vitamin supplements, foods/drinks containing caffeine, and alcohol ≥ 24 h before each session. Participants with T2DM ($n = 8$) who were on insulin therapy, none were using short-acting insulin, took their last dose the night before the study visits.

Exercise sessions began and ended with a warm-up and a cool-down, respectively, with a 3-min duration each at 60% of $\dot{V}O_{2reserve}$. Exercise protocols were matched for energy expenditure (EE) of 8 kcal kg^{-1} $week^{-1}$ [64] comprising 3 hypothetical exercise sessions per week. Both protocols were individually tailored using weight and peak oxygen uptake ($\dot{V}O_{2peak}$). An exercise physiologist supervised target intensities with a heart rate monitor (Garmin, US). Post-exercise measurements at 10 and 60 min aimed to characterize the post-exercise biphasic response of the brachial endothelial function[10]. To ensure successful FMD and PWV measurements at 10 min post-exercise, strategies included simultaneous acquisition and waterproof colour markers at arterial pulses and ultrasound probe sites placed during baseline assessments. Additionally, exercise sessions were held in a room adjacent to the testing area, with participants returning immediately post-exercise under researcher supervision.

Interventions

The HIIE was comprised of 1-min exercise bouts at 90% of $\dot{V}O_{2reserve}$ interspersed by 1-min active recovery bouts at 60% $\dot{V}O_{2reserve}$ (1:1). $\dot{V}O_{2reserve}$ as calculated using the following equation: $\dot{V}O_{2reserve} = (\dot{V}O_{2peak} - \dot{V}O_{2rest}) \times I + \dot{V}O_{2rest}$, where I is the exercise intensity in percentage and $\dot{V}O_{2peak}$ is peak oxygen uptake measured

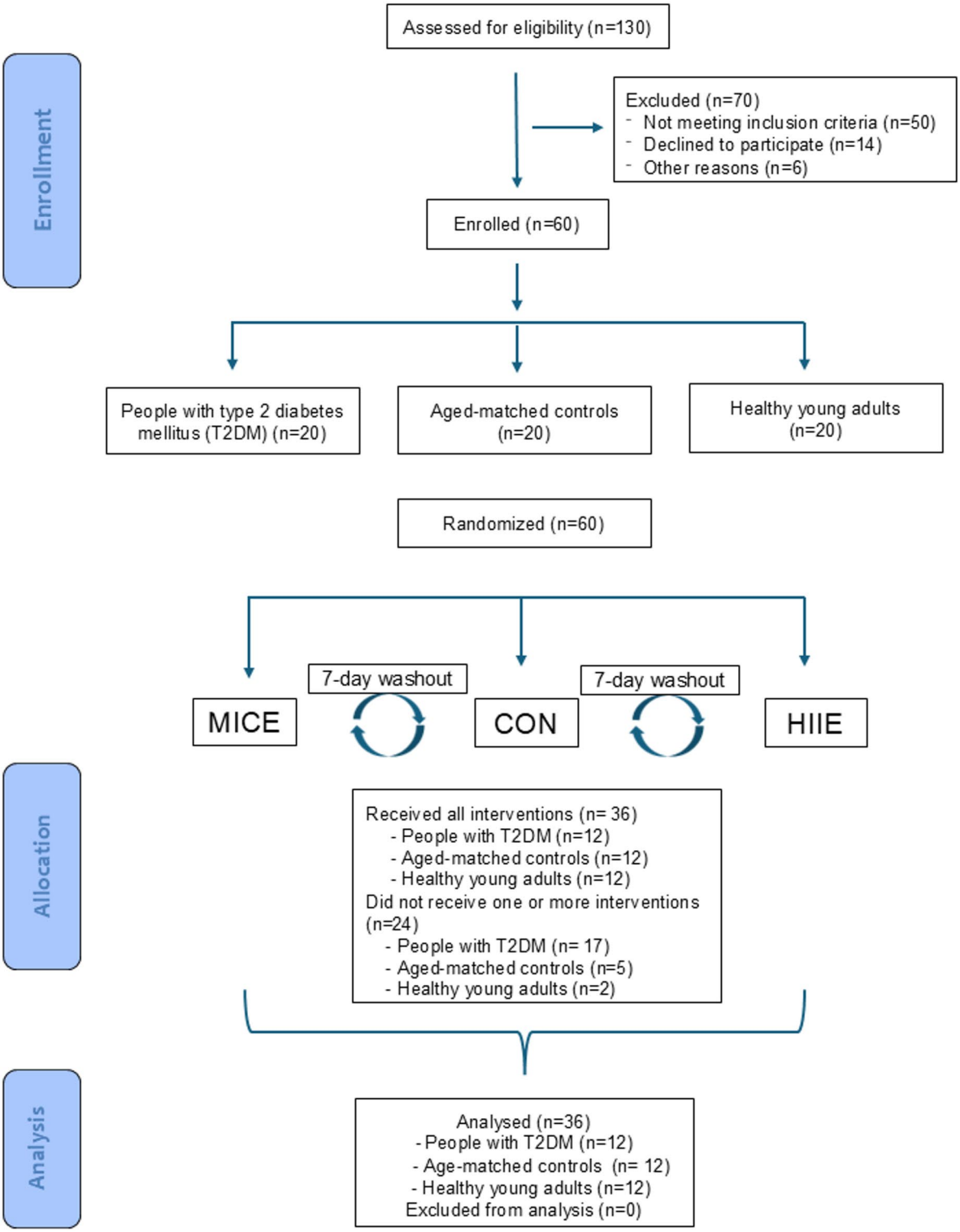

Fig. 4. CONSORT flow chart. Recruitment took place from January 2020 to October 2021. Abbreviations. T2DM, type 2 diabetes mellitus; MICE, moderate continuous exercise; HIIE, high-intensity interval exercise.

during the CPET. Resting oxygen uptake (VO_{2rest}) was assumed equal to one metabolic equivalent (3.5 mL kg min^{-1}) for all participants. The number of bouts of exercise-recovery for each participant was tailored to achieve the desired EE. For example, an 80-kg participant with a $\dot{V}O_{2peak}$ of 30 mL kg^{-1} min^{-1}, would need 6 bouts to match the target EE, assuming 1 L of O_2 uptake equals 5 kcal,

The MICE protocol was set at 60% $\dot{V}O_{2reserve}$ with duration adjusted so that each participant achieved the target EE. The same hypothetical participant as described above, would exercise for 21-min to match the

required EE. The protocols were performed on a motorized treadmill, and the participants walked or ran at the pace required to achieve the target intensity.

Evaluation of vascular function

Brachial artery flow-mediated dilation

Before the baseline vascular measurements participants rested supine for 15-min in a quiet, dimmed and climate-controlled room (22°C). FMD was assessed in the right brachial artery with an ultrasound (Arietta V60, Hitachi Aloka Medical Ltd, Mitakashi, Tokyo, Japan) equipped with a 7.5-MHz linear array probe incorporating a 5-MHz Doppler transducer, placed ~ 4 cm above the antecubital fossa, and held by a mechanical clamp following standard guidelines[48]. Reactive hyperemia was induced by rapid cuff-deflation following a forearm occlusion maintained for 5-min at 250 mmHg. Intraluminal brachial artery diameter was measured with automated edge-detection software[48]. Off-line analyses of FMD were conducted on the in-built software provided by the manufacturer. Baseline diameter (D_{bas}) was averaged in end-diastole during the 60-s of the baseline, whereas peak hyperemic diameter (D_{peak}) was the highest 10-s average interval throughout the 3-minute period of cuff deflation. FMD was calculated both as an absolute change ($\mathrm{FMD\ [mm]} = \mathrm{peak\ diameter}_{\mathrm{post-ischemia}} - \mathrm{D_{bas}}$) and as a relative change ($\%\mathrm{FMD} = \frac{\mathrm{D_{peak}-D_{bas}}}{\mathrm{D_{bas}}} \times 100\%$). %FMD was also allometrically scaled for D_{bas} (scaled FMD) as previously described given the non-linear ratio between D_{peak} and D_{bas}[69]. Additionally, Doppler measurements of blood velocity with an insonation angle of ≤ 60°[48]allowed the estimation of the shear rate as $Shear\ Rate\ \left(s^{-1}\right) = 8 \times \frac{mean\ blood\ velocity}{brachial\ internal\ diameter}$. Blood velocity signals were analysed offline in R to estimate trimmed 95% means shear rate, and trapezoidal area under the curve (AUC) for shear rate until D_{peak}. All measurements were performed by the same researcher with > 100 h of experience with an inter-day measurement coefficient of variation (CV) for %FMD of 14%.

Brachial artery flow-mediated slowing off-line analysis

Brachial pulse wave velocity and FMS were estimated from raw distensibility and blood pressure signals from the FMD procedure using Eq. 1 and Eq. 2. Systolic and diastolic blood pressures required to estimate beta stiffness and PWV were recorded using beat-to-beat finger plethysmography (Finapres, Nova, Amsterdam, The), and were then averaged (95% trimmed) during the FMD procedure. Baseline PWV ($bPWV_{bas}$) was averaged during end-diastole over the last 60-s, whereas post-ischemia pulse wave velocity ($bPWV_{min}$) was averaged over the first 3 min (i.e., 5-s time bins) after cuff-deflation. FMS was calculated as the absolute change ($FMS\ \left[m\ s^{-1}\right] = bPWV_{min} - bPWV_{bas}$) and as the percentage change ($\%FMS = \frac{absolute\ change}{PWV_{baseline}} \times 100\%$)[8].%FMS was allometrically scaled to $bPWV_{bas}$ (scaled FMS) as previously described[69]. All analyses were conducted offline using an R script. All analyses were conducted by the same researcher with an inter-day analysis CV of 21%[70]

$$\beta = \ln\left[\frac{SBP/DBP}{(D_{syst} - D_{diast})/D_{diast}}\right] \quad (1)$$

where, b is beta stiffness; SBP, brachial systolic blood pressure, DBP, diastolic blood pressure; D_{syst}, brachial artery diameter during systole; D_{diast}, brachial artery diameter during diastole.

$$bPWV = \sqrt{((\beta \times DBP)/(2\rho)} \quad (2)$$

.

where bPWV is brachial artery pulse wave velocity; b, beta stiffness, DBP, brachial diastolic blood pressure; and r, is blood density assumed constant (1050 kg/m^3).

Arterial stiffness

cfPWV a marker of central arterial stiffness was measured using a non-invasive automatic device (Complior, Alam Medical, France) with participants lying supine on a cushioned table. Pressure waveforms of the common carotid artery and the femoral artery were recorded using 2 piezoelectric pressure mechanotransducers placed on both arteries. The PWV from the carotid-radial (crPWV) and femoral dorsalis (cdPWV) pedis artery was also measured as indices of upper and lower peripheral stiffness, respectively. The travel distance (d) of pressure waveforms was defined as the tape-measured distance over the body surface between the 2 recording sites of interest with carotid-femoral distance corrected by a 0.8 factor[70]. The pulse transit time (PTT) was automatically calculated using the intersect tangent algorithm of the foot-to-foot method, allowing the PWV estimation as $PWV = \frac{d}{PTT}$. All measurements were performed by the same operator on the left side of the body after 10 pulse waveforms of quality > 90%. Inter-day CVs for cfPWV, crPWV, and cdPWV were 7%, 12%, and 9%, respectively. Brachial mean arterial pressure was calculated as $bMAP = \frac{2}{3}\ DBP + \frac{1}{3}\ SBP$.

Cardiopulmonary exercise testing

An incremental CPET with mixing-chamber gas exchange measurements (K5, Cosmed, Rome, Italy) was conducted on a motorized treadmill. Participants were tested 4 h post-prandial and under regular medication. BP was measured using a sphygmomanometer at rest, at the end of stages, and every minute post-peak effort, while electrical heart activity was continuously monitored via a 12-lead ECG. A certified physician supervised the protocol that started with an initial walking period (3-min) at a self-selected pace (that could be sustained for

20–25 min). Subsequently, the grade was set to 5% and increased by 1% every 2 min until volitional exhaustion[71]. The recovery phase consisted of a 3-min walk period. The increases in velocity were simultaneous with those of grade and were matched for 25 W increments using the following equation: $WR = m \times g \times v \times \sin(\alpha)$, where WR (watts) is work rate; m is body mass (kg); g is the gravitational acceleration (9.81 ms^{-1}), v is the velocity (m s^{-1}), and α is the angle of inclination. Participants were encouraged to exercise until exhaustion, as defined by ≥2 of the following criteria: attaining a $\dot{V}O_2$ plateau (variance of $\dot{V}O_2$ < 2.1 mL kg^{-1} min^{-1} in the last 60-s of the test); HRmax > 90% of the age-predicted maximum heart rate; RER ≥ 1.10; Rating perceived exertion (RPE) ≥ 18 (Borg 6–20); subjective judgment that the participant can no longer safely continue, even after encouragement, unless clinical criteria for earlier test termination was observed. $\dot{V}O_{2peak}$ was defined as the highest $\dot{V}O_2$ value on the final 20-s of exercise.

Body composition

Fat and fat-free masses were estimated using a bioimpedance device (seca mBCA 515, seca gmbh & co. kg, Hamburg, Germany) featuring four pairs of electrodes positioned at each hand and foot that allow impedance to be measured with a current of 100 μA at frequencies between 1 and 1 000 kHz.

Statistics analysis

Based on an estimated medium effect size of 0.25 from the %FMD mean decrease ~ 2.5% of Yoo et al.[38]the a priori power analysis (G-Power Version 3.1.9.3) suggested a total of 36 participants was necessary to detect significant differences between groups and interventions ($\alpha = 0.05$, $1-\beta = 0.80$). All analyses were conducted using R software, version 4.1.2, with a significance level (α) of 0.05.

The data are presented as mean (SD) unless otherwise stated. The normality and homoscedasticity assumptions were verified with the Shapiro-Wilk and Levene tests, respectively, and by visual plot inspection. One-way ANOVAs were used to compare the characteristics of participants and aerobic exercise with Tukey correction for multiple testing. Pearson correlation coefficients calculated over CON measurements were used to test associations between brachial endothelial function and arterial stiffness outcomes.

Exercise-induced changes in main vascular outcomes (e.g., FMD, FMS) were examined with linear mixed models using the restricted maximum likelihood and applying Satterthwaite's method for approximating degrees of freedom for the F test. Fixed effects were defined as time, condition, and group, and the random intercept was defined as each participant. Partial omega squares (ω^2) were calculated for main effects (Exercise (Ex), Time (T), and Group (Gr) and interactions (e.g., Ex*T* G) and interpreted applying Cohen's benchmarks [small ($\omega^2 < 0.05$), medium ($\omega^2 < 0.25$), and large ($\omega^2 > 0.25$) effects sizes]. $\dot{V}O_{2peak}$, %fat mass, and MAP were added to the mixed model as covariates. The %FMD linear mixed model was controlled for SR AUC Dpeak (Supplemental Table 1). Post-hoc comparisons using Tukey's HSD test were performed in the presence of significant main effects and interactions.

Data availability

The data supporting current findings are available from the corresponding author, XM, upon reasonable request.

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Author contributions

JLM drafted the manuscript; JLM, MP, HSC, BF, and XM conceived and designed research; JLM, IA, TS, MP, SL, JM and XM collect all data; JLM analyzed data; JLM, HSC, BF, and XM interpreted results of experiments; JLM prepared figures; SL, HSC, BF, and XM edited and revised manuscript; JLM, IA, TS, MP, SL, JM, HSC, BF and XM approved final version of manuscript.

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Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

The study was approved by the Ethical Review Board of the Faculdade de Motricidade Humana—Universidade de Lisboa. ID: 10/2020; July 3rd, 2020.

Consent for publication

Consent for publication was obtained by the authors. All participants provided written informed consent.

Additional information

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