

## The acute effect of exercise intensity on vascular function in adolescents

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**Short title:** Exercise intensity and vascular function in adolescents

## ABSTRACT

**Introduction:** Impairments in vascular function are present in asymptomatic youths with risk factors for cardiovascular disease. Exercise can promote vascular health in youth, but the effect of exercise intensity and the time course in response to acute exercise are unknown.

**Methods:** Twenty adolescents (10 male,  $14.1 \pm 0.3$  y) on separate days, and in a counter-balanced order: 1) cycled at 90% of the gas exchange threshold (moderate-intensity exercise; MIE); 2) completed 8x1 min cycling at 90% peak power with 75 s recovery (high-intensity interval exercise; HIIE). The duration of MIE ( $25.8 \pm 2.1$  min) was work-matched to HIIE (23.0 min). Macro- and micro-vascular function were assessed before, immediately post, and 1 and 2 hours after exercise by flow mediated dilation (FMD) and laser Doppler imaging (total reactive hyperaemia). **Results:** FMD was attenuated immediately after HIIE ( $P < 0.001$ ,  $ES = 1.20$ ) but not MIE ( $P = 0.28$ ,  $ES = 0.26$ ). Compared to pre-exercise, FMD was elevated 1 and 2 hours after HIIE ( $P < 0.001$ ,  $ES = 1.33$  and  $P < 0.001$ ,  $ES = 1.36$ ) but unchanged in MIE ( $P = 0.67$ ,  $ES = 0.10$  and  $P = 0.72$ ,  $ES = 0.08$ ). Changes in FMD were unrelated to shear or baseline arterial diameter. Compared to pre-exercise, total reactive hyperaemia was always greater after MIE ( $P < 0.02$ ,  $ES > 0.60$  for all) and HIIE ( $P < 0.001$ ,  $ES > 1.18$  for all). Total reactive hyperaemia was greater in HIIE compared to MIE immediately after ( $P = 0.03$ ,  $ES = 0.67$ ) and 1 hour after ( $P = 0.01$ ,  $ES = 0.62$ ) exercise, with a trend to be greater 2 hours after ( $P = 0.06$ ,  $ES = 0.45$ ). **Conclusion:** Exercise intensity is positively associated with macro- and micro-vascular function 1 and 2 hours after exercise. Performing HIIE may provide superior vascular benefits than MIE in adolescents.

**Key words:** cardiovascular disease, endothelial function, youth, time course

## 1 INTRODUCTION

2 Whilst the clinical manifestations of CVD are not detectable until adulthood, it is well  
3 established that the atherosclerotic process originates in the first decade of life (32). Impaired  
4 vascular function is thought to precede structural adaptations to the vessel wall (44), and both  
5 macro- and micro-vascular function have been shown to be impaired in asymptomatic  
6 adolescents with CVD risk factors (8, 19). Therefore, interventions which improve vascular  
7 function in young people are warranted.

8

9 Data are available demonstrating that time spent performing vigorous-, but not moderate-,  
10 intensity physical activity is related to improved macrovascular function (17) and attenuated  
11 cardiometabolic risk (7) in youth. Additionally, exercise interventions have been shown to  
12 improve macrovascular function in obese adolescents (41). It has been suggested that changes  
13 in vascular function after a single exercise bout provide the foundation for these chronic  
14 adaptations (3, 12). Consequently, there is value in identifying the acute vascular responses to  
15 a single bout of exercise.

16

17 Previous studies with adults report conflicting results on the effects of acute exercise on  
18 macrovascular function, with some reporting increases (16, 18), decreases (3, 18) and no  
19 change (3) in flow mediated dilation (FMD). However, differences between exercise loads,  
20 modalities, the timing of the post exercise FMD measurement(s) (12) and the problems  
21 associated with reporting the ratio-scaled FMD statistic (1), currently limit our understanding  
22 of the FMD response to an acute bout of exercise. To our knowledge, only one study has  
23 assessed FMD immediately post exercise in young people (22). These authors reported that  
24 FMD immediately decreased after high-intensity, but not low-intensity, exergaming, and  
25 concluded that repeating high-intensity exergaming may provide a stimulus for favourable

26 macrovascular adaptations. However, the exercise bouts were not work-matched in this study  
27 and FMD was only assessed immediately post exercise. Given that changes in vascular  
28 function within ~ 2 hours of exercise are thought to be biphasic in nature (12), it is important  
29 to document the time course of the change in vascular function after a single bout of exercise  
30 in youth to establish the influence of exercise intensity on the FMD response.

31

32 An impairment in microvascular reactive hyperaemia has been identified in asymptomatic  
33 children with clustered CVD risk (19) and it is thought that microvascular dysfunction may  
34 play a primary role in the pathogenesis of insulin resistance (25). Microvascular function has  
35 been shown to be elevated in adolescent football players compared to their untrained peers  
36 (29), however we are not aware of any study which has isolated the acute effect of exercise  
37 intensity on microvascular function in young people or adults. Furthermore, post exercise  
38 changes in microvascular reactive hyperaemia have been shown to be unrelated to FMD (31).  
39 Therefore, it is inappropriate to adopt post exercise changes in FMD as a surrogate of  
40 microvascular function.

41

42 The purpose of this investigation was to test the hypothesis that macrovascular function is  
43 immediately impaired, and then subsequently improved, following high-intensity interval  
44 exercise (HIIE), but remains stable following a work-matched bout of moderate-intensity  
45 exercise (MIE) in adolescents. A secondary aim was to identify the effect of exercise  
46 intensity on the time course of the microvascular response following exercise.

47

## 48 **METHODS**

49 Twenty 12 to 15-year-old adolescents (10 males) volunteered to take part in this study.  
50 Written participant assent and parental consent were obtained before participation in the

51 project, which was approved by the institutional ethics committee. Exclusion criteria included  
52 the use of any medication or substance known to influence fat metabolism or vascular  
53 function.

54

## 55 **Experimental overview**

56 This study required three visits to the laboratory and included a within-measures design. All  
57 exercise tests were completed using an electronically braked cycle ergometer (Lode  
58 Excalibur Sport, Groningen, the Netherlands).

59

### 60 **Visit 1: Fitness assessment**

61 Participants were habituated to the cycle ergometer before completing a combined ramp and  
62 supramaximal test to exhaustion to establish maximal oxygen uptake ( $\dot{V}O_{2\text{ max}}$ ) (2).  
63 Pulmonary  $\dot{V}O_2$  was monitored throughout (Cortex Metalyzer III B, Leipzig, Germany) and  
64 the gas exchange threshold was identified as the disproportionate increase in carbon dioxide  
65 production ( $\dot{V}CO_2$ ) relative to  $\dot{V}O_2$  and an increase in expired ventilation ( $\dot{V}E$ )/ $\dot{V}O_2$  with no  
66 increase in  $\dot{V}E/\dot{V}CO_2$ . All exercise was performed on an electronically braked cycle ergometer  
67 (Lode Excalibur Sport, Groningen, the Netherlands).

68

### 69 **Visits 2 and 3: Exercise interventions**

70 Participants completed two experimental conditions, separated by approximately one week.  
71 Following a ~ 12 h overnight fast, participants were transported to the laboratory at 08:00 and  
72 then consumed 30 g of commercially available Corn Flakes with 130 mL of skimmed milk.  
73 The macronutrient contribution of this breakfast is unlikely to have influenced endothelial  
74 function (40).

75 At 08:45, participants rested in a darkened, temperature-controlled (24°C) room for 15 min  
76 before the simultaneous assessment of macrovascular (flow mediated dilation (FMD)) and  
77 microvascular (laser Doppler perfusion imaging (LDI)) function (methods described below).

78

79 At 09:15, one hour after breakfast, participants completed on separate days and in a  
80 randomised order: 1) ~ 30 min of continuous MIE at 90% of the gas exchange threshold; or  
81 2) 23 min of HIIE (4). The HIIE bout consisted of a 3 min warm up at 20 W, followed by 8 x  
82 1 min intervals at 90% of the peak power determined from the ramp test to exhaustion,  
83 interspersed with 75 s of recovery at 20 W, before a 2 min cool down at 20 W. The duration  
84 of the MIE trial was calculated to match the total work performed during the HIIE bout.  
85 Participants provided a rating of perceived exertion (RPE) (43) in the final 10 s of exercise,  
86 before completing the 16-point Physical Activity Enjoyment Scale (PACES) (23)  
87 immediately after the exercise. After their final exercise trial, each participant was asked to  
88 identify which exercise bout they preferred.

89

90 Macro- and micro-vascular function were reassessed immediately after exercise cessation,  
91 with further measures 1 and 2 hours post exercise to facilitate comparison between extant  
92 literature in adults (12). Participants remained seated and were inactive at all times other than  
93 during the exercise bouts.

94

### 95 **Measures of vascular function**

96 FMD was measured using high resolution ultrasonography in duplex mode (Sequoia 512,  
97 Acuson, Siemens Corp, Aspen, USA) using a 12-14 MHz linear array transducer in  
98 accordance with recent guidelines (33) and our earlier work (4). Baseline and post occlusion  
99 brachial artery diameter was assessed during end diastole using validated ECG-gating

100 software (Medical Imaging Applications LLC, Coralvile USA) (10, 21). Baseline arterial  
101 diameter was measured for 1.5 min. Endothelium-dependent vasodilation was calculated as  
102 the percentage increase in arterial diameter after a 5 min ischaemic stimulus induced by rapid  
103 forearm pneumatic cuff inflation (Hokanson, Bellevue, USA) to 220 mmHg (33). The  
104 between-trial coefficient of variation for FMD was 9.7%.

105  
106 During the FMD protocol, microvascular function was simultaneously assessed using a laser  
107 Doppler perfusion imager (Periscan PIM II, Perimed, Järfälla, Sweden) at a reproducible  
108 point on the distal third of the forearm (11). High resolution data were collected at 4.33 Hz,  
109 and then interpolated to 1 s averages before being smoothed using a 5 s moving average.  
110 Peak reactive hyperaemia (PRH) was defined as the highest point after occlusion. The total  
111 hyperaemic response was calculated in by determining the area under the post-occlusive  
112 reactive hyperaemic curve minus the baseline (pre-occlusion) blood flow (expressed as a  
113 percentage of PRH), multiplied by the time taken for reactive hyperaemia to return to  
114 baseline (42). When calculated in this manner, the post-occlusive hyperaemic response is  
115 known to be nitric oxide independent (42), and accounts for differences in baseline skin  
116 perfusion. The between-trial coefficient of variation for PRH and the total hyperaemic  
117 response was 13.3 and 21.7% respectively.

118

### 119 **Standardisation of diet and physical activity**

120 With parental supervision, participants were asked to replicate their evening meal prior to  
121 each laboratory visit. Participants also completed a food diary during the 48 hour period  
122 immediately preceding each visit, which were subsequently assessed for total energy and  
123 macronutrient intake (CompEat Pro, Nutrition Systems, UK). Participants were instructed to  
124 avoid strenuous exercise and wear a tri-axial accelerometer on their wrist (GENEActiv,

125 Activinsights Ltd, Cambridge, UK) during the 48 hour prior to each visit. Time spent  
126 performing moderate to vigorous activity was determined using established cut points for  
127 paediatric groups (13).

128

### 129 **Statistical analyses**

130 The primary outcome for macro-vascular function was the difference between log-  
131 transformed peak and baseline arterial diameter, adjusted allometrically for baseline diameter  
132 (1). Data were analysed using a linear mixed model with a random intercept (accounting for  
133 repeated measures within participants) plus fixed effects for condition (moderate/ high  
134 intensity), time (pre, post, 1-hour, 2-hour), and their interaction. As appropriate for a  
135 crossover trial, we also adjusted for any period effect. Differences on the log-scale were  
136 back-transformed to provide percent (ratio) effects. Point estimates are presented together  
137 with 95% confidence intervals. Additionally, the area under the curve for estimated shear rate  
138 was calculated from the last 30 s of occlusion until the time of peak dilation ( $SR_{AUC}$ ) (15),  
139 however FMD was not related to  $SR_{AUC}$  at rest or at any point post exercise in either trial ( $P$   
140 = 0.21 to 0.80,  $r = -0.1$  to 0.4) which is consistent with other paediatric data (4, 34).  
141 Consequently, FMD was not normalised for  $SR_{AUC}$ .

142

143 Descriptive statistics were calculated using SPSS (version 19.0, Chicago, USA) and  
144 presented as mean  $\pm$  SD. Mean differences in descriptive statistics between boys and girls  
145 were analysed using independent samples  $t$  tests. The mean differences in the physiological  
146 and perceptual responses of the boys and girls during HIIE and MIE were analysed using  
147 paired samples  $t$  tests. Parameters of macro- and microvascular function were analysed using  
148 a mixed model ANOVA with trial (MIE, HIIE) and sex (male, female) as the main effects.  
149 The inclusion of sex into the ANOVA model did not reveal a significant interaction effect for



150 parameters of macro- and micro-vascular function. Data were subsequently pooled for these  
151 outcomes. Pairwise comparisons between means were interpreted using the *P* value, 95%  
152 confidence intervals and standardised effect sizes (*ES*) to document the magnitude of the  
153 effect using the thresholds: small (0.2), moderate (0.5) and large (0.8) (9). Relationships  
154 between changes in vascular outcomes and mechanistically important variables were  
155 explored using Pearson's correlations.

156

## 157 **RESULTS**

158 Baseline participant characteristics are presented in Table 1. The maturation status for boys  
159 and girls was as follows; Tanner stage 2, *n*=1 and *n*=0; stage 3, *n*=3 and *n*=0; stage 4, *n*=5 and  
160 *n*=7; stage 5, *n*=1 and *n*=3. No differences in energy intake, individual macronutrient  
161 contributions, or time spent performing moderate to vigorous physical activity were apparent  
162 for boys or girls during the 48 hour preceding each laboratory visit (*P*>0.50, *ES*<0.20; Table  
163 2).

164

165 The physiological and perceptual data from the exercise trials are presented in Table 3. All  
166 participants completed both exercise trials. The highest  $\dot{V}O_2$  achieved during the HIIE  
167 condition equated to  $96 \pm 5\%$ . Average length of the MIE trial was  $25.8 \pm 2.1$  min. Nine boys  
168 and eight girls indicated that they preferred the HIIE exercise bout.

169

### 170 **Macrovascular function**

171 Baseline arterial diameter,  $SR_{AUC}$  and FMD are illustrated in Figure 1. A time by trial  
172 interaction was present for FMD (*P*<0.001). No differences in mean FMD at baseline were  
173 apparent between trials (*P*=0.62, 95% CI -1.2 to 0.7, *ES*=0.12). Compared to baseline, FMD  
174 was attenuated immediately after HIIE (*P*<0.001, 95% CI -4.4 to -2.3, *ES*=1.20), but was

175 unchanged immediately following MIE ( $P=0.28$ , 95% CI -1.5 to 0.4,  $ES=0.26$ ).  
176 Consequently, FMD was lower in HIIE compared to MIE immediately post exercise  
177 ( $P<0.001$ , 95% CI -3.4 to -1.6,  $ES=1.57$ ). FMD was not different to baseline 1 hour ( $P=0.67$ ,  
178 95% CI -0.8 to 1.2,  $ES=0.10$ ) and 2 hours ( $P=0.72$ , 95% CI -0.8 to 1.1,  $ES=0.08$ ) after MIE,  
179 however FMD was greater than baseline after HIIE at these time points ( $P<0.001$ , 95% CI  
180 1.7 to 3.7,  $ES=1.33$  and  $P<0.001$ , 95% CI 1.8 to 3.7,  $ES=1.36$ , respectively). Consequently,  
181 FMD was greater in HIIE compared to MIE 1 hour ( $P<0.001$ , 95% CI 1.8 to 3.8,  $ES=1.31$ )  
182 and 2 hours ( $P<0.001$ , 95% CI 1.8 to 3.8,  $ES=1.33$ ) post exercise. Changes in FMD post  
183 exercise were not related to age, maturity (Tanner stage) or aerobic fitness in either MIE or  
184 HIIE ( $r<0.43$  and  $P>0.10$  for all).

185

186 There was a main effect of time ( $P<0.001$ ), but not trial ( $P=0.28$ ), or time by trial interaction  
187 ( $P=0.75$ ) for  $SR_{AUC}$ . Pairwise comparisons revealed that  $SR_{AUC}$  was elevated immediately  
188 after exercise compared to baseline in MIE ( $P<0.001$ , 95% CI 206 to 564,  $ES=1.20$ ) and HIIE  
189 ( $P=0.001$ , 95% CI 205 to 704,  $ES=1.31$ ). There was also a trend for  $SR_{AUC}$  to be greater 1  
190 hour after MIE ( $P=0.06$ , 95% CI -10 to 358,  $ES=0.55$ ) and HIIE ( $P=0.08$ , 95% CI -27 to 394,  
191  $ES=0.64$ ) compared to baseline.  $SR_{AUC}$  was not different from baseline 2 hours after exercise  
192 for either trial ( $P>0.14$ ,  $ES<0.36$  for both).

193

194 There was a main effect of time ( $P<0.001$ ), but not trial ( $P=0.68$ ), or time by trial interaction  
195 ( $P=0.09$ ) for baseline arterial diameter. Baseline arterial diameter was greater immediately  
196 after exercise compared to pre exercise values in MIE ( $P=0.03$ , 95% CI 0.01 to 0.22,  
197  $ES=0.32$ ) and HIIE ( $P=0.01$ , 95% CI 0.05 to 0.35,  $ES=0.51$ ). Baseline diameter was not  
198 different from pre exercise values at any other point in either trial ( $P>0.21$ ,  $ES<0.20$  for all).

199

## 200 **Microvascular function**

201 Differences in parameters of microvascular function are presented in Figure 2. There was a  
202 main effect of trial ( $P=0.002$ ) and time ( $P<0.001$ ) for PRH, but no time by trial interaction  
203 ( $P=0.14$ ). There were no differences between trials in mean PRH at baseline ( $P=0.51$ , 95%  
204 CI -0.18 to 0.09,  $ES=0.12$ ). Compared to baseline, PRH increased immediately after MIE  
205 ( $P=0.048$ , 95% CI 0.02 to 0.46,  $ES=0.72$ ) and HIIE ( $P<0.001$ , 95% CI 0.26 to 0.61,  
206  $ES=1.16$ ). PRH was greater in HIIE compared to MIE immediately after ( $P=0.02$ , 95% CI  
207 0.05 to 0.44,  $ES=0.73$ ) and 1 hour after exercise ( $P=0.002$ , 95% CI 0.13 to 0.48,  $ES=0.67$ ).  
208 There was also a trend for PRH to be greater in HIIE 2 hours after exercise ( $P=0.08$ , 95% CI  
209 -0.03 to 0.42,  $ES=0.43$ ).

210

211 There was a main effect of trial ( $P=0.01$ ) and time ( $P<0.001$ ) for the total hyperaemic  
212 response, but no time by trial interaction ( $P=0.17$ ). There were no differences in total  
213 hyperaemic response between trials at baseline ( $P=0.65$ , 95% CI -28 to 18,  $ES=0.12$ ).  
214 Compared to baseline, the total hyperaemic response was greater at all times after MIE  
215 ( $P<0.02$  and  $ES>0.60$  for all) and HIIE ( $P<0.001$  and  $ES>1.18$  for all). The total hyperaemic  
216 response was greater in HIIE compared to MIE immediately after ( $P=0.03$ , 95% CI 3 to 57,  
217  $ES=0.67$ ) and 1 hour after exercise ( $P=0.01$ , 95% CI 12 to 72,  $ES=0.62$ ), with a strong trend  
218 for a statistical difference 2 hours after exercise ( $P=0.06$ , 95% CI -1 to 56,  $ES=0.45$ ).

219

## 220 **DISCUSSION**

221 The purpose of this investigation was to establish the effect of exercise intensity on macro-  
222 and micro-vascular function in adolescents, and to document the time course of the response.  
223 The novel findings from this study are: compared to baseline, 1) FMD is attenuated  
224 immediately following a single bout of HIIE but not MIE; 2) FMD is elevated 1 and 2 hours

225 after HIIE, but unchanged in MIE; 3) PRH and total hyperaemic response are both increased  
226 during the 2 hours immediately following MIE and HIIE, and the magnitude of this increase  
227 is greater after HIIE than MIE. This is the first study to isolate the effect of exercise intensity  
228 and include serial measures of vascular function in adolescents after a single bout of exercise.  
229 The findings indicate that exercise intensity has an independent effect on macro- and micro-  
230 vascular function in young people, which likely have important implications for vascular  
231 health.

232

### 233 **Macrovascular function**

234 Our data demonstrate that an immediate post exercise nadir in FMD is present following  
235 HIIE but not MIE, which is consistent with work-matched data in adults (3, 18) and the only  
236 available data in young people (22). Mills *et al.* (22) hypothesised that this attenuation in  
237 FMD after high-intensity exercise might precede an increase in FMD, and might therefore be  
238 considered to be beneficial. However, these authors did not include serial measures of FMD  
239 in their investigation, and evidence of this response in endothelial function post exercise is  
240 scarce (18). Furthermore, the “high-intensity” exergaming trial included by Mills *et al.*  
241 elicited a mean  $\dot{V}O_{2\text{ peak}}$  of  $3.6 \pm 2.5$  metabolic equivalents, which the authors correctly  
242 classify as moderate-intensity (24). Therefore, the present study extends the work by Mills *et*  
243 *al.* and, to our knowledge, is the first to confirm that the initial impairment in FMD following  
244 high-intensity exercise precedes an increase in macrovascular function, and that this  
245 improvement is present at least two hours later. Thus, exercise which elicits a greater acute  
246 challenge on the vasculature may be associated with larger increases in FMD in adolescents,  
247 and the evidence of a biphasic response in FMD post high-intensity exercise is compelling.

248

249 Our failure to observe any changes in FMD immediately after MIE is consistent with the data  
250 provided by Mills *et al.* following “low-intensity” exergaming (22), however we extend their  
251 findings and report that endothelial function remained unchanged during the 2 hours that  
252 followed. Interestingly, the lack of change in FMD in the hours after MIE is consistent with  
253 some (3, 18), but not all (16, 39) data in healthy adults. However, in addition to differences in  
254 exercise stimulus, timing of the FMD measurement and interpretation of the ratio-scaled  
255 FMD statistic (1, 12), an independent effect of training status (16) has been observed on the  
256 acute FMD response. Furthermore, evidence suggests that age might modulate vascular  
257 reactivity to the FMD protocol (34). Although we were unable to confirm a potential  
258 confounding effect of age, maturity (Tanner stage) or aerobic fitness on the change in FMD  
259 post MIE and HIIE, it appears that a direct comparison between our findings with apparently  
260 healthy adolescents and the available adult literature may be problematic.

261

262 Shear (when expressed as  $SR_{AUC}$ ) is thought to be the main stimulus underlying the FMD  
263 response in healthy adults at rest (26). However, the relationship between  $SR_{AUC}$  and FMD is  
264 not as robust following exercise (20). Indeed, we report here that FMD remained elevated in  
265 the hours following HIIE despite a steady decline in  $SR_{AUC}$ . The relationship between  $SR_{AUC}$   
266 and FMD has been shown to be weak in young people even at rest (34), a finding also  
267 observed in this study. It is therefore not surprising that differences in the FMD response 1  
268 and 2 hours post exercise were independent of changes in  $SR_{AUC}$ . Considering that baseline  
269 arterial diameter remained unchanged 1 and 2 hours following MIE and HIIE, and that we  
270 followed recent statistical guidelines designed to partition out the influence of vessel calibre  
271 (1), our findings are also not explained by this factor. We are therefore unable to identify the  
272 mechanism(s) underlying the disparity in FMD response presented here. It has been  
273 speculated elsewhere that the initial impairment in FMD immediately following exercise

274 relates to an increase in oxidative stress (12, 18), which would reduce the bioavailability of  
275 nitric oxide (6). Whilst we did not measure this outcome, an increase in oxidative stress  
276 following high-intensity exercise is not consistent with the augmented FMD response  
277 observed 1 and 2 hours after HIIE. Conversely, an exercise-intensity dependent increase in  
278 total antioxidant status has been reported during the hours following work-matched HIIE but  
279 not MIE (39), which would prevent the reduction in nitric oxide bioavailability associated  
280 with an increase in exercise-induced oxidative stress. However, this is not a consistent  
281 finding (16, 18), and we have previously reported that changes in FMD 1 hour after identical  
282 HIIE in adolescents were not related to total antioxidant status (4). Alternatively, given that  
283 the exercise bouts were work-matched in the present study, our data may be explained by a  
284 positive association between the intensity of exercise and subsequent activity of endothelial  
285 nitric oxide synthase. Indeed, data in adults demonstrate that brachial artery shear increases  
286 with the intensity of cycling exercise (35), and this has been demonstrated to play a leading  
287 role in the post exercise FMD response (36). We did not quantify brachial artery shear during  
288 the exercise bouts as this is technically challenging during HIIE. However, we have  
289 previously observed a reduction in postprandial systolic blood pressure in the 5 hours after  
290 HIIE, but not MIE, in adolescents (5), which would be consistent with an upregulation in  
291 endothelial nitric oxide synthase activity.

292

293 An interesting finding of the present study is that the magnitude of the increase in FMD  
294 observed 1 hour after HIIE was also present after 2 hours. Further study is needed to identify  
295 the precise decay in this favourable response after high-intensity exercise, although this  
296 benefit has been reported the following day in adults (39). Additionally, we have previously  
297 observed that a similar increase in FMD is present 4 hours after exercise despite the  
298 consumption of a meal which impaired FMD in a non-exercise control trial (4), whilst

299 Sedgwick *et al.* reported an increase in postprandial FMD the day after repeated sprint  
300 cycling in adolescent boys (30). Therefore, a single bout of HIIE appears to provide a potent  
301 stimulus for macrovascular health, and may provide superior health benefits compared to  
302 MIE if repeated on a regular basis. Indeed, high-intensity interval training has been  
303 demonstrated to be more effectual in promoting macro-vascular function than moderate-  
304 intensity training in adults at risk of vascular dysfunction (37), and offer superior  
305 improvements in FMD than a multi-disciplinary approach in overweight adolescents (38).  
306 Furthermore, only time spent performing vigorous-, but not moderate-, intensity exercise is  
307 related to vascular function in children (17).

308

### 309 **Microvascular function**

310 A novel feature of this investigation was the simultaneous assessment of post-occlusive  
311 reactive hyperaemia in the cutaneous circulation (11) during the FMD protocol. We have  
312 demonstrated that microvascular function is improved following both MIE and HIIE, and that  
313 the magnitude of this improvement is greater following HIIE. Furthermore, PRH and the total  
314 hyperaemic response to occlusion remained elevated 2 hours after exercise.

315

316 Our data show that transient improvements in microvascular function are possible following  
317 exercise without concomitant changes in FMD. No association has been demonstrated  
318 between FMD and reactive microvascular hyperaemia in adults post exercise (31),  
319 presumably because the post-occlusive cutaneous response is not mediated by nitric oxide  
320 (42). Our finding that micro-, but not macro-, vascular function was improved in the hours  
321 after MIE is probably testament to the different mechanisms underlying the post-occlusive  
322 hyperaemic response in our investigation, i.e. only the latter is NO-mediated (42).  
323 Furthermore, the microvascular post-occlusive response may include both endothelial-

324 independent and dependent pathways (11). It is therefore likely inappropriate to adopt  
325 measures of macrovascular health as an indication of global vascular function, especially as  
326 the earliest changes in vascular function due to the metabolic syndrome may be specifically  
327 linked to the capillary and arteriole beds, rather than the larger, conduit arteries (25). As a  
328 result, simultaneously assessing microvascular function alongside FMD may offer a novel  
329 insight regarding the effects of exercise intensity on vascular health.

330

331 We are the first to show that a single bout of MIE or HIIE can improve microvascular  
332 function in the hours following exercise, and that HIIE may provide a superior benefit. Whilst  
333 we were unable to identify the time course of the decay in these favourable responses post  
334 exercise, Gill *et al.* reported that endothelium-dependent microvascular function remained  
335 elevated 16-18 hours after 90 minutes of walking at 50%  $\dot{V}O_{2\text{ max}}$  in adults (14). Therefore,  
336 repeating a single bout of exercise may have some utility in promoting microvascular  
337 function the following day, although this needs to be confirmed in adolescents. Conversely,  
338 there is evidence suggesting that the intensity of habitual physical activity may not influence  
339 microvascular endothelial function in adolescents (27). However, this study determined  
340 microvascular function by means that are considered to be NO-dependent, which is  
341 mechanistically disparate from our assessment (42). Currently, no study has identified the  
342 efficacy of HIIE training on microvascular health in asymptomatic adolescents. Further study  
343 is therefore needed to identify whether the acute benefits in microvascular function observed  
344 in the present study translate into meaningful benefits in this group with time.

345

### 346 **Considerations**

347 This is the first study to isolate the effect of exercise intensity on vascular function in  
348 adolescents. The strengths of this investigation include a work-matched design, control of



349 prior physical activity and dietary factors, serial measures of macro- and micro-vascular  
350 function and allometric scaling of the FMD statistic. However, apart from reporting  $SR_{AUC}$   
351 and baseline arterial diameter, we are not able to provide any mechanistic data which could  
352 potentially explain the changes in vascular function following MIE and HIIE. A further  
353 limitation is that we were unable to measure the time course of these changes beyond 2 hours  
354 post exercise. Thus, the rate of decay in microvascular function following MIE and HIIE, and  
355 macrovascular function following HIIE remains unknown. We also cannot rule out that an  
356 increase in skin temperature following exercise influenced our measure of microvascular  
357 function. However, this unavoidable confounding effect is likely limited to the time point  
358 immediately post exercise as participants were acclimatised to the temperature-controlled  
359 ( $24^{\circ}\text{C}$ ) room for all other vascular measures. Furthermore, our analysis of the post-occlusive  
360 reactive hyperaemic response accommodates differences in baseline perfusion (42). Finally,  
361 we are unable to comment on the interaction between exercise intensity and diurnal variation  
362 in FMD. Data in adults suggests that FMD could decline by  $\sim 1\%$  from baseline values over  
363 the course of our measurement period (28). However, the magnitude of this effect is far  
364 lower, and in the opposite direction, than the change observed following HIIE in the present  
365 study.

366

## 367 **CONCLUSION**

368 Our data indicate that the intensity of exercise has an independent effect on macro- and  
369 micro-vascular function in adolescents. Specifically, macrovascular function was improved in  
370 the hours after HIIE but not MIE. Additionally, both exercise bouts promoted microvascular  
371 function, although the magnitude of this increase was greater after HIIE. Therefore, it is  
372 likely that repeating high-intensity exercises may provide superior health benefits and lower

373 cardiovascular disease risk than moderate-intensity activities. Given that HIIIE was deemed to  
374 be more enjoyable than MIE, HIIIE may provide an attractive, alternative to traditional MIE.

375

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378 mediated dilation data. We are also grateful to the staff and participants at Exmouth  
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380

## 381 **DISCLOSURES**

382 The authors have no conflicts of interest to disclose.

383

384 The results of the present study do not constitute endorsement by the ACSM.

385

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504 **TABLES**505 **Table 1:** Participant characteristics

|  | Boys ( <i>n</i> = 10) | Girls ( <i>n</i> = 10) | <i>P</i> value | <i>ES</i> |
|--|-----------------------|------------------------|----------------|-----------|
| Age (y)  | 14.1 ± 0.3            | 14.1 ± 0.3             | 0.72           | 0.00      |
| Body mass (kg)   | 61.6 ± 15.9           | 54.9 ± 4.6             | 0.23           | 0.57      |
| Stature (m)  | 1.66 ± 0.10           | 1.65 ± 0.08            | 0.82           | 0.11      |
| $\dot{V}O_{2\max}$ (L·min <sup>-1</sup> )                    | 2.77 ± 0.80           | 2.04 ± 0.36            | 0.02           | 1.18      |
| $\dot{V}O_{2\max}$ (mL·min <sup>-1</sup> ·kg <sup>-1</sup> ) | 44.8 ± 6.4            | 37.1 ± 5.3             | 0.01           | 1.26      |
| GET (L·min <sup>-1</sup> )                                   | 1.36 ± 0.35           | 1.08 ± 0.17            | 0.04           | 1.02      |
| GET (% $\dot{V}O_{2\max}$ )                                  | 49 ± 4                | 53 ± 6                 | 0.11           | 0.78      |

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507  $\dot{V}O_2$ , oxygen uptake; GET, gas exchange threshold; *ES* = effect size. Data presented as mean  
508 ± SD

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513 **Table 2:** Accelerometer and food diary data during the 48 hours preceding each trial

|   | MIE        | HIIE       | <i>P</i> value | <i>ES</i> |
|---|------------|------------|----------------|-----------|
| Moderate-vigorous activity (min day <sup>-1</sup> ) | 38 ± 12    | 36 ± 15    | 0.50           | 0.15      |
| Total energy intake (kcal day <sup>-1</sup> )       | 1945 ± 301 | 1887 ± 341 | 0.59           | 0.18      |
| Energy from carbohydrates (%)                       | 47 ± 5     | 47 ± 5     | 0.84           | <0.01     |
| Energy from fat (%)                                 | 38 ± 4     | 38 ± 6     | 0.95           | <0.01     |
| Energy from protein (%)                             | 15 ± 4     | 15 ± 3     | 0.73           | <0.01     |

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515 MIE, moderate-intensity exercise trial; HIIE, high-intensity interval exercise trial

516 95% CI = 95% confidence limits for the true difference

517 Data have been pooled as ANOVA analysis revealed no main effect for sex

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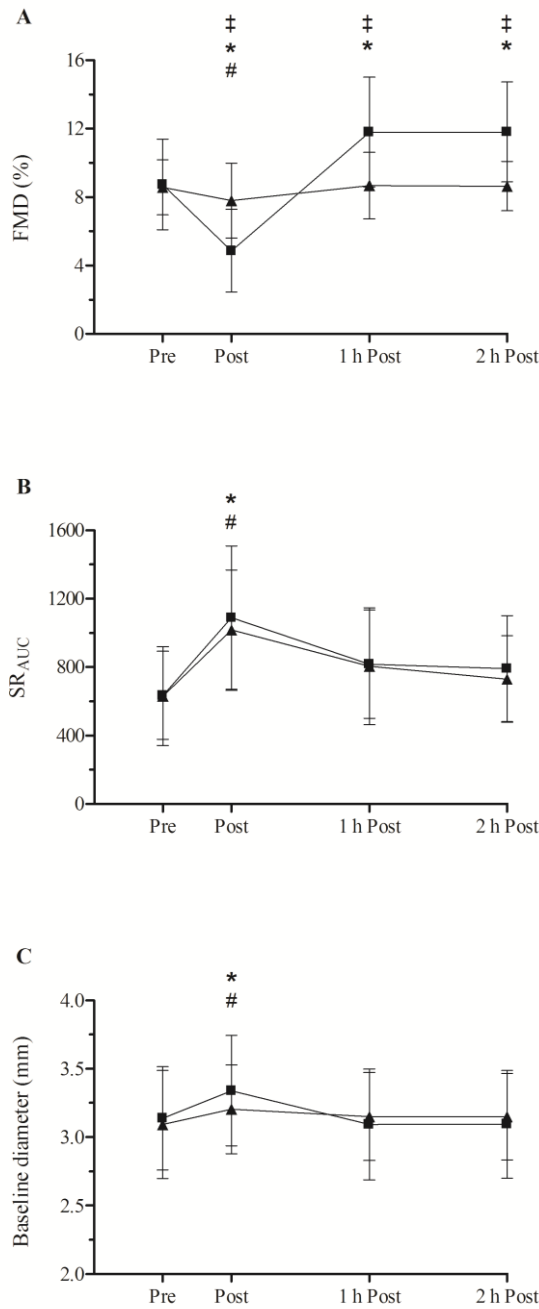
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524 **Table 3:** Physiological and perceptual responses to MIE and HIIE

|   | MIE         | HIIE        | <i>P</i> value | ES   |
|---|-------------|-------------|----------------|------|
| Mean HR (b·min <sup>-1</sup> )*           | 129 ± 14    | 150 ± 14    | <0.001         | 1.50 |
| Mean HR (% HR <sub>max</sub> )*           | 66 ± 6      | 77 ± 6      | <0.001         | 1.83 |
| Mean $\dot{V}O_2$ (L·min <sup>-1</sup> )  | 1.19 ± 0.26 | 1.49 ± 0.37 | <0.001         | 0.94 |
| Mean $\dot{V}O_2$ (% $\dot{V}O_{2\max}$ ) | 51 ± 8      | 63 ± 7      | <0.001         | 1.60 |
| RER                                       | 0.91 ± 0.05 | 1.03 ± 0.06 | <0.001         | 2.17 |
| RPE                                       | 4 ± 2       | 7 ± 1       | <0.001         | 1.90 |
| PACES                                     | 57 ± 9      | 65 ± 7      | <0.001         | 0.99 |
| Work performed (kJ)                       | 117 ± 18    | 117 ± 18    | -              | -    |
| Energy Expenditure (kJ)                   | 770 ± 182   | -           | -              | -    |

525  
 526 HR, heart rate;  $\dot{V}O_2$ , oxygen uptake; MIE, moderate-intensity exercise trial; HIIE, high-  
 527 intensity exercise trial; *ES* = effect size. Data presented as mean ± SD and pooled for sex. *n* =  
 528 20 apart from \* where *n* = 18 due to loss of telemetry

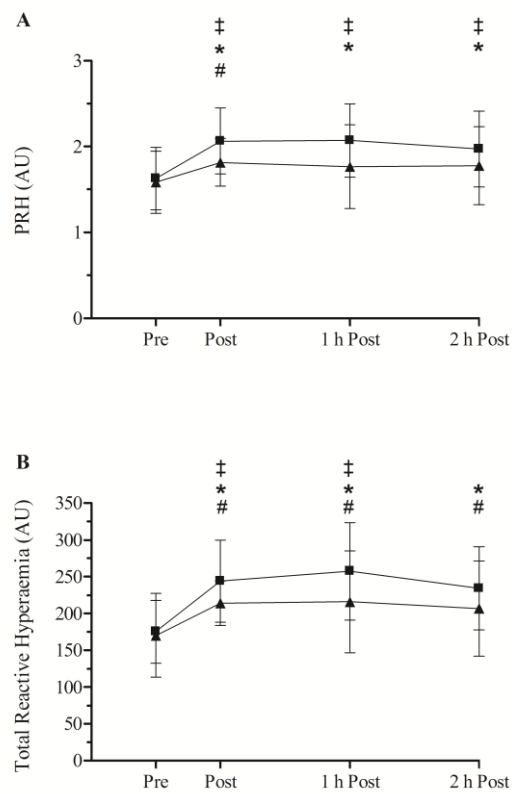
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548 **Figure 1** Mean differences in macro-vascular function pre and post moderate-intensity  
 549 exercise (▲) and high-intensity interval exercise (■). FMD, flow mediated dilation; SR<sub>AUC</sub>,  
 550 area under the curve for shear. Error bars represent the standard deviation. Significant  
 551 difference from pre exercise is denoted by # for moderate-intensity exercise and \* for high-  
 552 intensity interval exercise. ‡ denotes significant difference between exercise trials. Refer to  
 553 text for specific *P* values.

554



555

556 **Figure 2** Mean differences in micro-vascular function pre and post moderate-intensity  
 557 exercise (▲) and high-intensity interval exercise (■). PRH, peak reactive hyperaemia; AU,  
 558 arbitrary units. Error bars represent the standard deviation. Significant difference from pre  
 559 exercise is denoted by # for moderate-intensity exercise and \* for high-intensity interval  
 560 exercise. ‡ denotes significant difference between exercise trials. Refer to text for specific *P*  
 561 values.

562