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EXERCISE INTENSITY AND THE PROTECTION FROM POSTPRANDIAL VASCULAR DYSFUNCTION IN ADOLESCENTS

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ABSTRACT

Background: Acute exercise transiently improves endothelial function, and protects the vasculature from the deleterious effects of a high fat meal (HFM). We sought to identify whether this response is dependent on exercise intensity in adolescents. **Methods:** Twenty adolescents (10 male, 14.3 ± 0.3 y) completed three 1-day trials: 1) rest (CON); 2) 8x1 min cycling at 90% peak power with 75s recovery (high-intensity interval exercise; HIIE); 3) cycling at 90% of the gas exchange threshold (moderate-intensity exercise; MIE) one hour before consuming a HFM (1.50 $g \cdot kg^{-1}$ fat). Macrovascular and microvascular endothelial function were assessed before and immediately after exercise, and three hours after the HFM by flow mediated dilation (FMD) and laser Doppler imaging (peak reactive hyperaemia; PRH). **Results:** FMD and PRH increased one hour after HIIE (P<0.001, ES=1.20 and P=0.048, ES=0.56) but were unchanged after MIE. FMD and PRH were attenuated three hours after the HFM in CON (P<0.001, ES=1.78 and P=0.02, ES=0.59). FMD remained greater three hours after the HFM in HIIE compared to MIE (P<0.001, ES=1.47) and CON (P<0.001, ES=2.54), and in MIE compared to CON (P<0.001, ES=1.40). Compared to CON, PRH was greater three hours after the HFM in HIIE (P=0.02, ES=0.71) and MIE (P=0.02, ES=0.84), with no differences between HIIE and MIE (P=0.72, ES=0.16). Plasma [triacylglycerol] and [total antioxidant status] were not different between trials. **Conclusions:** Exercise intensity plays an important role in protecting the vasculature from the deleterious effects of a HFM. Performing HIIE may provide superior vascular benefits than MIE in adolescent groups.

INTRODUCTION

It is well established that the atherosclerotic process originates in childhood (58), and that cardiovascular disease (CVD) risk factors in youth are associated with the progression of atherosclerosis during adulthood (40). Endothelial dysfunction is a sentinel event in the progression of atherosclerosis, preceding the development of fatty streaks, and holds prognostic value in predicting CVD end points and patient mortality (59). Conduit artery endothelial function has been shown to be impaired in asymptomatic adolescents with CVD risk factors (16), whilst microvascular function is also impaired in children with clustered CVD risk (36). The ingestion of a high fat meal (HFM) causes a transient period of macro- and micro-vascular dysfunction (5, 54, 70), and given the central role endothelial dysfunction plays in the atherosclerotic process (12), it is likely that repeat exposure of the vasculature to this environment has long-term implications for vascular health.

In adults, acute moderate and high-intensity exercise have transient benefits on macrovascular endothelial function in the fasted and postprandial state (30, 70), with the benefits more pronounced following high-intensity exercise possibly due to favourable changes in total antioxidant status (70). Prior exercise has also been shown to protect the microvasculature from the deleterious effects of a high fat meal in adults (26). In children, cross-sectional evidence suggests that high-intensity exercise may have a positive effect on fasting vascular function (32). Additionally, a single bout of moderate-intensity exercise (54) and sprint interval exercise (53) has been shown to preserve postprandial macrovascular function the following day in adolescent boys. However, the total exercise stimulus in these two studies was not equivalent, and the authors did not include a measure of microvascular function. Therefore, it is currently unknown whether exercise intensity modulates the postprandial macro- and micro-vascular dysfunction observed after a HFM in adolescents, which may have important public health implications as much of the day may be spent in the postprandial state. Furthermore, it has recently been shown that performing even small amounts (~

4 min) of high-intensity exercise is superior than moderate-intensity exercise at modifying cardiometabolic risk factors in youth (15). Considering that few adolescents meet the current recommended minimum of 60 min of moderate-intensity physical activity per day (50), and that habitual physical activity likely declines during adolescence (37, 69), it is pertinent to identify how small volumes of exercise can be optimised for vascular health in this group.

Given the above, this investigation sought to test the hypothesis that a single bout of high-intensity interval exercise (HIIE) provides superior protection of macrovascular function following a HFM compared to a work-matched bout of moderate-intensity exercise (MIE) in adolescents. We also assessed whether postprandial differences in macrovascular function were present at the microvascular level, and if differences in vascular function between trials were related to plasma [triacylglycerol] or total antioxidant status.

METHODS

Twenty 12 to 15-year-old adolescents (10 males) volunteered to take part in this study. Participant assent and parental consent were obtained before participation in the project, which was approved by the institutional ethics committee. Exclusion criteria included the use of any medication or substance known to influence fat metabolism or vascular function.

Body mass, seated height and stature were measured to the nearest 0.1 kg and 0.1 cm respectively. Percentage body fat was estimated using triceps and subscapular skinfold thickness according to Slaughter *et al*. (57) and pubertal status was determined by a self-assessment of secondary sexual characteristics using adapted drawing of the five Tanner stages of pubic hair development (43).

Visit 1: Fitness assessment

The first visit included a validated combined ramp and supramaximal test to exhaustion to establish maximal oxygen uptake ($\dot{V}O_{2\ max}$) (6). Pulmonary $\dot{V}O_2$ was monitored throughout (Cortex Metalyzer III B, Leipzig, Germany) and the gas exchange threshold was identified as the disproportionate increase in carbon dioxide production ($\dot{V}CO_2$) relative to $\dot{V}O_2$. All exercise was performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands).

Visits 2-4: Exercise and postprandial measures

Participants completed three experimental conditions, separated by approximately one week (Figure 1). Following a ~ 12 h overnight fast, participants were transported to the laboratory at 07:45 and rested for 15 min before providing a fasting fingertip capillary blood sample for plasma [triacylglycerol]. Participants then consumed 30 g of commercially available Corn Flakes with 130 mL of skimmed milk, which is unlikely to have influenced endothelial function (71).

At 08:45, participants rested in a darkened, temperature-controlled (24°C) room for 10 min before the simultaneous assessment of macrovascular (flow mediated dilation (FMD)) and microvascular (laser Doppler perfusion imaging (LDI)) function. Immediately afterwards, capillary blood samples were obtained for plasma [triacylglycerol], [3-hydroxybutyrate] and total antioxidant status. These measurements were repeated one hour after exercise (but before the HFM) and three hours after the HFM in order to coincide with peak plasma [triacylglycerol] (67).

At 09:45, one hour after breakfast, participants either: 1) remained seated in the laboratory (CON); 2) performed ~30 min of continuous MIE at 90% of the gas exchange threshold; or 3) completed 23 min of HIIE. These trials were completed on separate days and in a randomised order. The HIIE bout consisted of a 3 min warm up at 20 W, followed by 8 x 1 min intervals at 90% of the peak power determined from the ramp test to exhaustion, interspersed with 75 s of recovery at 20 W,

before a 2 min cool down at 20 W. The duration of the MIE trial was calculated to match the total work performed during the HIIE bout for each participant. Participants provided a rating of perceived exertion (RPE) (73) in the final 10 s of exercise. Participants also completed the 16-point Physical Activity Enjoyment Scale (PACES) (44) immediately after exercise cessation. After their final exercise trial, each participant was asked to identify which exercise bout they preferred. Plasma [triacylglycerol] and total antioxidant status were assessed one hour after the exercise/rest condition. Plasma [3-hydroxybutyrate] was also assessed as a marker of hepatic fatty acid oxidation and very low-density lipoprotein (VLDL) secretion (27). Participants then consumed a milkshake of 3 parts Cornish ice cream and one part double cream between 10:45 and 11:00, which provided ~ 1.50 $g \cdot kg^{-1}$ (80 $kJ \cdot kg^{-1}$) of fat in accordance with other postprandial investigations in this group (54, 67, 68) and our earlier work (11). Plasma [triacylglycerol] was assessed at hourly intervals during the three hour postprandial period. Participants remained seated in the laboratory throughout the postprandial period.

Measures of vascular function

FMD was measured using high resolution ultrasonography (Sequoia 512, Acuson, Siemens Corp, Aspen, USA) with a 13 MHz linear array transducer and in accordance with recent guidelines (19, 61) and our earlier work (25). All FMD analyses were performed by primary investigator who was blinded to the condition. Baseline and post occlusion brachial artery diameter was assessed during end diastole using validated ECG-gating software (Medical Imaging Applications LLC, Coralvile USA) (41, 61). Baseline arterial diameter was measured for 1.5 min. Endothelium-dependent vasodilation was calculated as the percentage increase in arterial diameter after a 5 min ischaemic stimulus (45) induced by rapid forearm pneumatic cuff inflation (Hokanson, Bellevue, USA) (8) to 220 mmHg. The area under the curve for estimated shear rate was calculated from the last 30 s of occlusion until the time of peak dilation (SR_{AUC}) (61). To address concerns about the ratio-scaled

FMD statistic (4), FMD was also allometrically scaled according to published guidelines (3). The between-day coefficient of variation for FMD was 10.5%.

During the FMD protocol, microvascular function was simultaneously assessed using a laser Doppler perfusion imager (Periscan PIM II, Perimed, Järfälla, Sweden) at a reproducible point on the distal third of the forearm (20). High resolution data were collected at 4.33 Hz, and then interpolated to 1 s averages before being smoothed using a 5 s moving average. Resting flux was measured over 2 min before cuff inflation. Peak reactive hyperaemia (PRH) was defined as the highest point after occlusion, and the between-day coefficient of variation was 16.2% for this variable.

Blood analyses

For each blood sample, ~600 μL of capillary blood was collected and centrifuged immediately at 13,000 g for 15 min at 4°C. Plasma was then removed and stored at -80°C for no more than one month. Plasma [triacylglycerol], [3-hydroxybutyrate] and total antioxidant status were quantified in duplicate by enzymatic, colorimetric methods using an assay kit according to the manufacturer's guidelines (Cayman Chemical Company, MI, USA). The within-batch coefficients of variation for plasma [triacylglycerol], [3-hydroxybutyrate] and total antioxidant status were 2.9, 3.8 and 4.2% respectively. The total (TAUC) and incremental (IAUC) area under the curve analyses were performed using the time point immediately before the HFM for plasma [triacylglycerol], and the time point immediately before exercise for plasma [3-hydroxybutyrate] and total antioxidant status.

Control of diet and exercise

With parental supervision, participants were asked to replicate their evening meal prior to each laboratory visit. Participants also completed a food diary during the 48 hour period immediately preceding each visit, which were subsequently assessed for total energy and macronutrient intake

(CompEat Pro, Nutrition Systems, UK). Participants were instructed to avoid strenuous exercise and wear a tri-axial accelerometer on their wrist (GENEActiv, Activinsights Ltd, Cambridge, UK) during the 48 hour prior to each visit. Time spent performing moderate to vigorous activity was determined using established cut points for paediatric groups (48).

Statistical analyses

Descriptive statistics were calculated using SPSS (version 19.0, Chicago, USA) and presented as mean ± SD. Mean differences in descriptive statistics between boys and girls were analysed using independent samples *t* tests. The mean differences in the physiological and perceptual responses of the boys and girls during HIIE and MIE were analysed using paired samples *t* tests. Analysis of plasma [triacylglycerol], [3-hydroxybutyrate] and total antioxidant status, and parameters of macro- and micro-vascular function were performed using a mixed model ANOVA with trial (CON, MIE, HIIE) and sex (male, female) as the main effects. For clarity, the main effects for time and condition are not discussed if the ANOVA output revealed a significant interaction effect. The inclusion of sex into the ANOVA model did not reveal a significant interaction effect for plasma [3-hydroxybutyrate] and total antioxidant status or parameters of macro- and micro-vascular function. Data were subsequently pooled for these outcomes. Pairwise comparisons between means were interpreted using the *P* value and standardised effect sizes (*ES*) to document the magnitude of the effect using the thresholds: small (0.2), moderate (0.5) and large (0.8) (18). Relationships between changes in vascular outcomes and mechanistically important variables were explored using Pearson's correlations.

RESULTS

Baseline participant characteristics are presented in Table 1. The maturation status for boys and girls was as follows; Tanner stage 3, *n*=4 and *n*=1; stage, 4 *n*=4 and *n*=8; stage 5, *n*=2 and *n*=1. No differences in energy intake, individual macronutrient contributions, or time spent performing

moderate to vigorous physical activity were apparent for boys or girls during the 48 hour preceding each laboratory visit (P>0.14, ES<0.20; Table 2).

Table 3 presents the physiological and perceptual data from the exercise trials. The highest $\dot{V}O_2$ achieved during the HIIE condition equated to 93 ± 5% and 96 ± 5% $\dot{V}O_{2\ max}$ for boys and girls respectively. Average length of the MIE trial was 24.9 ± 2.3 min. Nine boys and nine girls indicated that they preferred the HIIE exercise bout.

Blood analyses

Mean differences in plasma [triacylglycerol] during the postprandial period are illustrated in Figure 2A. Mean fasted plasma [triacylglycerol] was lower across all trials in girls (P=0.03, ES=0.96). There was no trial by sex interaction (P=0.44) for TAUC-triacylglycerol, but there was a trend for TAUC-triacylglycerol to be lower in girls across all trials (P=0.05). There was no trial by sex interaction (P=0.58) for IAUC-triacylglycerol.

Mean differences in plasma [3-hydroxybutyrate] are illustrated in Figure 2B. A time by trial interaction (P=0.04) was apparent for plasma [3-hydroxybutyrate], which was elevated three hours after the HFM in HIIE compared to CON (P=0.01, ES=0.59), with no differences between MIE and CON (P=0.16, ES=0.26) or HIIE and MIE (P=0.13, ES=0.29). An increase in TAUC plasma [3-hydroxybutyrate] in HIIE was associated with lower TAUC-triacylglycerol (P=0.01, r =0.61) but not for MIE (P=0.22, r =0.30).

Mean differences in total antioxidant status are provided in Figure 2C. There was no time by trial interaction (P=0.53) or effect of trial (P=0.88), but there was a main effect of time for total antioxidant status (P=0.04). Mean total antioxidant status across conditions was lower after the

HFM compared to baseline (P=0.02, ES=0.39). Changes in total antioxidant status were not related to parameters of vascular function (P>0.05 and r <0.2).

Macrovascular function

Differences in FMD between trials are presented in Figure 3A. There was a time by trial interaction (P<0.001) for FMD. FMD was greater one hour after HIIE (P<0.001, ES=1.20), but unchanged after MIE (P=0.22, ES=0.09) and CON (P=0.99, ES<0.01) compared to before exercise. Consequently, FMD was greater after HIIE compared to MIE (P=0.002, ES=1.14) and CON (P=0.002, ES=1.15), with no difference between MIE and CON (P=0.59, ES=0.15) one hour after exercise.

FMD was greater three hours after the HFM in HIIE compared to MIE (P<0.001, ES=1.47) and CON (P<0.001, ES=2.54), and in MIE compared to CON (P<0.001, ES=1.40). FMD was attenuated after the HFM in CON (P<0.001, ES=1.78) compared to before the meal. FMD remained elevated after the HFM compared to baseline in HIIE (P<0.001, ES=1.56). Differences in SR_{AUC} between trials are provided in Figure 3B. Changes in FMD were not related to SR_{AUC} in any trial. Consequently, FMD was not normalised for SR_{AUC}. There was no time by trial interaction for SR_{AUC} (P=0.25), resting arterial diameter (P=0.11, Figure 3C), or time taken to reach peak dilation (P=0.37).

Microvascular function

Differences in PRH between trials are presented in Figure 3D. There was a time by trial interaction (P=0.002) for PRH. PRH was greater one hour after HIIE (P=0.004, ES=0.82) but unchanged after MIE (P=0.22, ES=0.26) and CON (P=0.27, ES=0.26). Compared to CON, PRH was greater three hours after the HFM in HIIE (P=0.02, ES=0.71) and MIE (P=0.02, ES=0.84), with no difference between HIIE and MIE (P=0.72, ES=0.16). PRH was attenuated three hours after the HFM in CON

(*P*=0.02, *ES*=0.59). There was no effect of trial (*P*=0.15), time (*P*=0.40), or a trial by time interaction (*P*=0.27) for time taken to achieve PRH.

DISCUSSION

The novel findings from this study are: 1) macro- and micro-vascular function were enhanced one hour after HIIE compared to CON and MIE, and remained elevated three hours after a HFM; 2) a single bout of MIE did not alter macro- or micro-vascular function one hour after exercise, but prevented the decline in function observed three hours after a HFM; and 3) the interactions between exercise intensity and vascular function were independent of changes in plasma [triacylglycerol] or total antioxidant status. These data show for the first time that the effect of exercise on postprandial vascular function is dependent on exercise intensity. Specifically, macrovascular function after a HFM is preserved by MIE, and augmented by HIIE. These findings may have a clinically important public health message as a significant proportion of time is spent in the postprandial state, and endothelial function predicts cardiovascular events independently of conventional CVD risk factors (12).

The HFM reduced FMD by 21% in CON, which is consistent with other adolescent (54) and adult (5, 70, 71) data. For the first time in adolescents, we provide evidence that a single bout of MIE performed one hour before a HFM may preserve endothelial function, and that an equivalent bout of HIIE not only prevents this attenuation but improves endothelial function despite no reduction in plasma [triacylglycerol]. Whilst the benefits of prior moderate-intensity (54) and sprint interval (53) exercise on postprandial macrovascular function have been shown to be unrelated to changes in plasma [triacylglycerol] in adolescents, we are the first to identify an independent effect of exercise intensity. Our findings concur with those reported by Tyldum *et al*. (70), however these authors identified that this protective effect of exercise performed the day before a HFM was related to an exercise-induced increase in antioxidant capacity, which we did not observe in this study. It is

known that postprandial lipaemia impairs vascular function via oxidative stress (5), which may reduce nitric oxide bioavailability (72). FMD is considered to be largely nitric oxide dependent (28), but we did not observe an effect of exercise on total antioxidant status, or a relationship between FMD and total antioxidant status. However, Johnson *et al*. (35) also reported no relationship between post exercise FMD and oxidative stress, and this may be related to the limitation of a single measurement of oxidative stress rather than rate of antioxidant depletion (22). Furthermore, the exercise bouts in this study were performed one hour, compared to 16-18 hours (70), before the ingestion of the HFM, and thus the process(es) underlying the response in pro/anti-oxidant state are likely to be mechanistically different. Indeed a recent investigation failed to observe any changes in postprandial antioxidant status after MIE and HIE when exercise was performed one hour after a HFM (14). Additionally, we cannot account for the influence of training status on the changes in pro/antioxidant status following the exercise bouts (10). However, based upon recommended $\dot{V}O_{2\ max}$ cut off values for cardiometabolic health (1), 5 of the boys and 2 of the girls included in this study could be identified as "at risk", and the $\dot{V}O_{2\ max}$ values observed in the present study were typically lower than those reported in trained groups (2).

Previous studies with healthy adults report that FMD either increases (35, 70), decreases (22, 35) or remains unaltered (23, 52) after a single bout of exercise, however these data are difficult to interpret due to inconsistencies in the intensity, duration and modality of exercise, and the timing of the FMD measurement(s) (22). The present study is the first to incorporate a work-matched exercise protocol in order to isolate the influence of exercise intensity on vascular function in adolescents, and our data show that FMD is increased one hour after HIIE but remains unaltered after MIE. In contrast, an exercise intensity dependent decrease in FMD has been shown immediately after cycling in adults (9), and exergaming in children (42). It is likely that this disparity is due to the timing of our FMD measure (one hour vs. immediately after exercise) as the FMD response post exercise is biphasic in nature (23). Indeed, it is thought that the temporary blunting of FMD

observed after high-intensity, but not MIE (9, 35, 42), is the stimulus for subsequent improvements in FMD (47), however no study has yet identified the time course of the FMD response following work-matched exercise in adolescents.

Changes in FMD after exercise have been attributed to differences in baseline arterial diameter and shear rate (22). However, these remained unaltered between trials in the present study and there was no relationship between the magnitude of the FMD response and SR_{AUC}, which is consistent with existing data in children (62) and following exercise in adults (38). However, we did not quantify shear stress during the exercise bouts. Given that the exercise conditions were work-matched, it is likely that the disparate responses in FMD observed post exercise are related to the positive association between brachial artery shear and the intensity of cycling exercise (29, 63). This has been shown to play a leading role in modulating the post exercise FMD response (64, 65), probably due to an upregulation in endothelial nitric oxide synthase and subsequent increase in the bioavailability of nitric oxide (34). We are unable to partition out the influence of the HFM on the postprandial FMD response following MIE and HIIE. For example, it is possible that postprandial FMD could have been higher still following HIIE. However, considering that FMD has been demonstrated to return to baseline 2 hours post high-intensity exercise (35), and the lack of change in total antioxidant status in the present study, it would appear that the inclusion of a HFM 1 hour after exercise did not modulate the post exercise nitric oxide bioavailability. Further study is needed to confirm this.

A novel feature of this investigation was the simultaneous assessment of microvascular function during the FMD protocol. Whilst the endothelium only plays a part of the PRH response (20), impaired microvascular reactive hyperaemia is associated with elevated blood pressure (56), obesity (24), insulin resistance (33), and has been identified in healthy children with clustered CVD risk factors (36). Therefore, it follows that the assessment of PRH as a surrogate of microvascular

function in the current study may provide useful information regarding vascular health in asymptomatic individuals. We observed a significant impairment in postprandial microvascular function in CON, suggesting that a fatty meal presents a global challenge to the vasculature. This dysfunction was prevented in both exercise trials, but not in an intensity-dependent manner. To our knowledge, no other study has identified the effect of exercise intensity on subsequent postprandial microvascular function, however Gill *et al.* observed a similar protective effect of MIE performed the evening before a HFM in adults and this was endothelium-dependent (26).

Prior MIE (67) and HIIE (60) can attenuate postprandial lipaemia in adolescents, however we were unable to replicate these findings in this study, possibly due to our use of a one day protocol (74) and a short (three hour) postprandial observation period. It has been hypothesised that exercise-induced changes in hepatic very low density lipoprotein (VLDL) output may explain some of the reduction in postprandial lipaemia after a HFM (39), particularly when the time between exercise cessation and consumption of the test meal is short due to the delay in the upregulation of lipoprotein lipase (55). Our data would appear to be consistent with this theory, as [3-hydroxybutyrate] was elevated three hours after the HFM in HIIE compared to CON, and significantly correlated with the reduction in TAUC-triacylglycerol, suggesting a shift towards hepatic fatty acid oxidation rather than re-esterification and VLDL synthesis during the HIIE condition (27).

Repeated sprint cycling the day before a HFM has previously been demonstrated to preserve postprandial macrovascular function in adolescents (53). However, these authors reported that one third of the participants failed to complete the exercise protocol. In contrast, all participants in the present study completed the HIIE bout. Furthermore, our data indicate that HIIE was perceived to be more enjoyable than MIE for both boys and girls, despite a greater physiological stress. This is encouraging considering that adolescents rarely sustain exercise for longer than 10 minutes (50),

368 therefore low-volume, high-intensity exercise may be a suitable method of optimising this pattern of
369 activity provided that the exercise is not an "all-out" effort. Further work is needed to identify the
370 long term adherence to a HIIE training intervention in this group, however preliminary evidence is
371 promising (13). Indeed, our data add to a growing body of evidence which indicates that HIIE is a
372 feasible and attractive alternative to MIE in adolescents (11, 21, 49).
373
374 This is the first study to isolate the influence of exercise intensity on postprandial vascular function
375 in adolescents. A further novelty of this study is the simultaneous assessment of microvascular
376 function during the FMD protocol. However, our findings should be interpreted in light of a number
377 of methodological considerations. Firstly, whilst post-occlusive reactive hyperaemia has been used
378 as a marker of microvascular function in adolescents (51), the mechanisms underlying the PRH
379 response to 5 minutes of ischaemia following exercise and a HFM are yet to be fully determined,
380 but likely involve other pathways in addition to changes in endothelial function (20). However,
381 postprandial microvascular function has been shown to be improved following exercise elsewhere
382 and this was endothelium-dependent (26). Therefore, it is likely that some of the improvements
383 observed in macrovascular endothelial function via FMD in the present study are present at the
384 microvascular level. Secondly, we were unable to control for the menstrual cycle, which has been
385 shown to influence FMD in women (31). The median stage of maturity (Tanner 4) suggests that
386 some girls would be pre or post menarche (7), and whilst there was no significant interaction effect
387 of sex on macro- or micro-vascular function in the present study, further work is necessary to
388 explicitly establish whether sex influences this outcome in adolescents and in children. Thirdly, the
389 HFM used in this study has limited ecological validity but provided a metabolic challenge in
390 accordance with other postprandial investigations with adolescents (11, 54, 66, 68). This meal also
391 provided an average of 35 g of sugar, which could plausibly have contributed to the postprandial
392 responses (17), although this is equivocal (46). Future work is needed to identify how prior exercise
393 can alter macro- and micro-vascular function following more habitual fat loads and feeding

regimes. Finally, we were unable to determine endothelial-independent function via a sublingual spray of nitroglycerin (19), and this remains an area of future research.

CONCLUSION

Macro- and micro-vascular dysfunction occur in concert after a HFM in adolescents. We have shown that postprandial vascular function can be preserved after MIE, or improved after HIIE, and these changes were not related to plasma [triacylglycerol] or total antioxidant status. Whilst these findings cannot be extrapolated beyond healthy adolescents, they may have clinical importance as repeat impairment in endothelial function likely plays a key role in the development of CVD, which is known to have its origins in childhood (58). Future work is needed to assess the efficacy of different exercise intensities on postprandial endothelial function in adolescents with risk factors for CVD (e.g. obesity, type I diabetes). Finally, we also report here that HIIE was perceived to be more enjoyable than MIE, despite the greater physiological stress. Taken together, low-volume HIIE may be a feasible and attractive strategy to reduce CVD risk from an early age.

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The authors have no competing interests to disclose

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TABLES

Table 1 Participant characteristics.

	Boys (n = 10)	Girls (n = 10)	P value	ES
Age (y)	14.8 ± 0.2	14.1 ± 0.9	0.06	1.07
Body mass (kg)	61.1 ± 11.9	54.5 ± 9.3	0.19	0.62
Stature (m)	1.69 ± 0.07	1.61 ± 0.09	0.04	0.99
Body fat (%)	10 ± 4	20 ± 4	<0.001	2.50
$\dot{V}O_{2\,max}$ ($L \cdot min^{-1}$)	2.76 ± 0.54	2.03 ± 0.27	0.001	1.71
$\dot{V}O_{2\,max}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	45.5 ± 6.4	37.8 ± 4.5	0.01	1.39
GET ($L \cdot min^{-1}$)	1.40 ± 0.25	1.09 ± 0.20	0.001	1.37
GET (% $\dot{V}O_{2\,max}$)	51 ± 6	54 ± 7	0.39	0.46

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

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

	CON	MIE	HIIE	MIE vs. CON 95% CI	HIIE vs. CON 95% CI	HIIE vs. MIE 95% CI
Moderate-vigorous activity (min day^{-1})	75 ± 30	73 ± 36	75 ± 27	-35 to 19	-37 to 23	-18 to 24
Total energy intake (kcal day^{-1})	1862 ± 427	1980 ± 388	2027 ± 551	-122 to 245	-134 to 455	-171 to 369
Energy from carbohydrates (%)	46 ± 5	47 ± 5	45 ± 5	-1 to 5	-3 to 3	-5 to 2
Energy from fat (%)	37 ± 6	36 ± 4	37 ± 6	-5 to 2	-5 to 2	-4 to 4
Energy from protein (%)	17 ± 4	17 ± 3	18 ± 3	-4 to 2	-1 to 3	0 to 4

CON, control trial; MIE, moderate-intensity exercise trial; HIIE, high-intensity interval exercise trial
95% CI = 95% confidence limits for the true difference
Data have been pooled as ANOVA analysis revealed no main effect for sex

687 **Table 3** Physiological and perceptual responses to exercise conditions.

	MIE	HIIE	*P* value	ES
Boys				
Mean HR ($b \cdot min^{-1}$)	117 ± 7	144 ± 4	<0.001	4.74
Mean HR (% HR_{max})	63 ± 4	77 ± 3	<0.001	3.96
Mean $\dot{V}O_2$ ($L \cdot min^{-1}$)	1.25 ± 0.19	1.59 ± 0.25	<0.001	1.53
Mean $\dot{V}O_2$ (% $\dot{V}O_{2\,max}$)	46 ± 7	58 ± 4	<0.001	2.10
RER	0.89 ± 0.04	1.05 ± 0.04	<0.001	4.00
RPE	4 ± 1	8 ± 1	<0.001	4.00
PACES	53 ± 15	64 ± 7	0.08	0.94
Work performed (kJ)	136 ± 24	136 ± 24	-	-
Energy Expenditure (kJ)	635 ± 100	-	-	-
Girls				
Mean HR ($b \cdot min^{-1}$)	144 ± 13	158 ± 12	0.01	1.12
Mean HR (% HR_{max})	74 ± 6	81 ± 5	0.01	1.27
Mean $\dot{V}O_2$ ($L \cdot min^{-1}$)	1.10 ± 0.09	1.26 ± 0.11	<0.001	1.59
Mean $\dot{V}O_2$ (% $\dot{V}O_{2\,max}$)	55 ± 4	62 ± 5	<0.001	1.55
RER	0.89 ± 0.05	1.04 ± 0.02	<0.001	3.94
RPE	5 ± 2	7 ± 1	0.01	1.26
PACES	54 ± 10	59 ± 7	0.17	0.58
Work performed (kJ)	109 ± 11	109 ± 11	-	-
Energy Expenditure (kJ)	700 ± 82	-	-	-

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689 HR, heart rate; $\dot{V}O_2$, oxygen uptake; MIE, moderate-intensity exercise trial; HIIE, high-intensity
690 exercise trial; *ES* = effect size. Data presented as mean ± SD. $n = 10$ for boys and girls apart from
691 mean HR where $n = 8$ due to loss of telemetric data.
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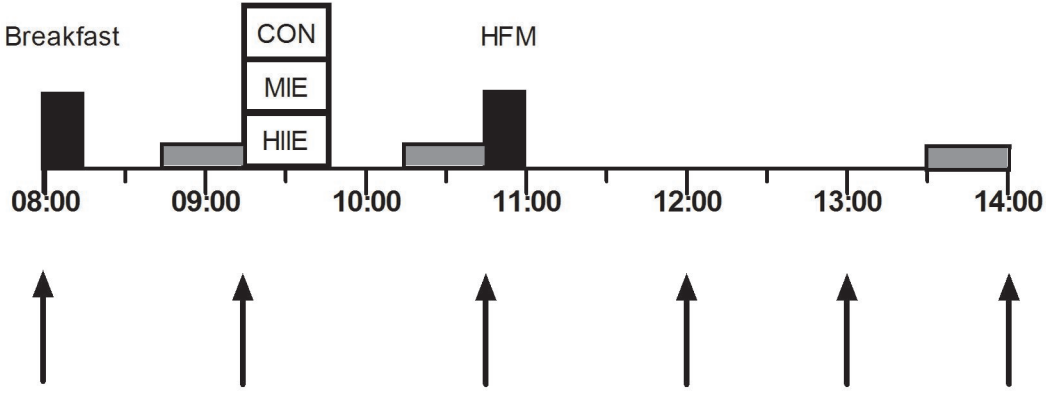
FIGURES

Figure 1. Protocol schematic. CON = rest; MIE = moderate-intensity exercise; HIIE = high-intensity interval exercise. Arrows represent capillary blood samples for plasma [triacylglycerol]; grey boxes represent the assessment of macro- and micro-vascular function and capillary blood samples for plasma [3-hydroxybutyrate] and total antioxidant status; HFM = high fat meal.

Figure 2. Mean plasma [triacylglycerol] (A), [3-hydroxybutyrate] (B) and total antioxidant status (C) for the control (○), moderate-(▲) and high-(■) intensity exercise conditions. Error bars represent the standard deviation. The high fat meal is represented by the black rectangle. **P*<0.05 for HIIE vs CON.

Figure 3. Mean changes in flow mediated dilation (A), area under the curve until peak dilation for shear rate (B), baseline arterial diameter (C) and peak microvascular perfusion (D), for the control (○), moderate-(▲) and high-(■) intensity exercise conditions. Error bars represent the standard deviation. The high fat meal is represented by the black rectangle. Statistical significance between conditions at the same timepoint are described as follows: * HIIE vs CON; $^{\#}$ HIIE vs MIE; † MIE vs CON. Within-condition significant difference from baseline: § HIIE; ‡ CON. Refer to text for specific *P* values.


Breakfast
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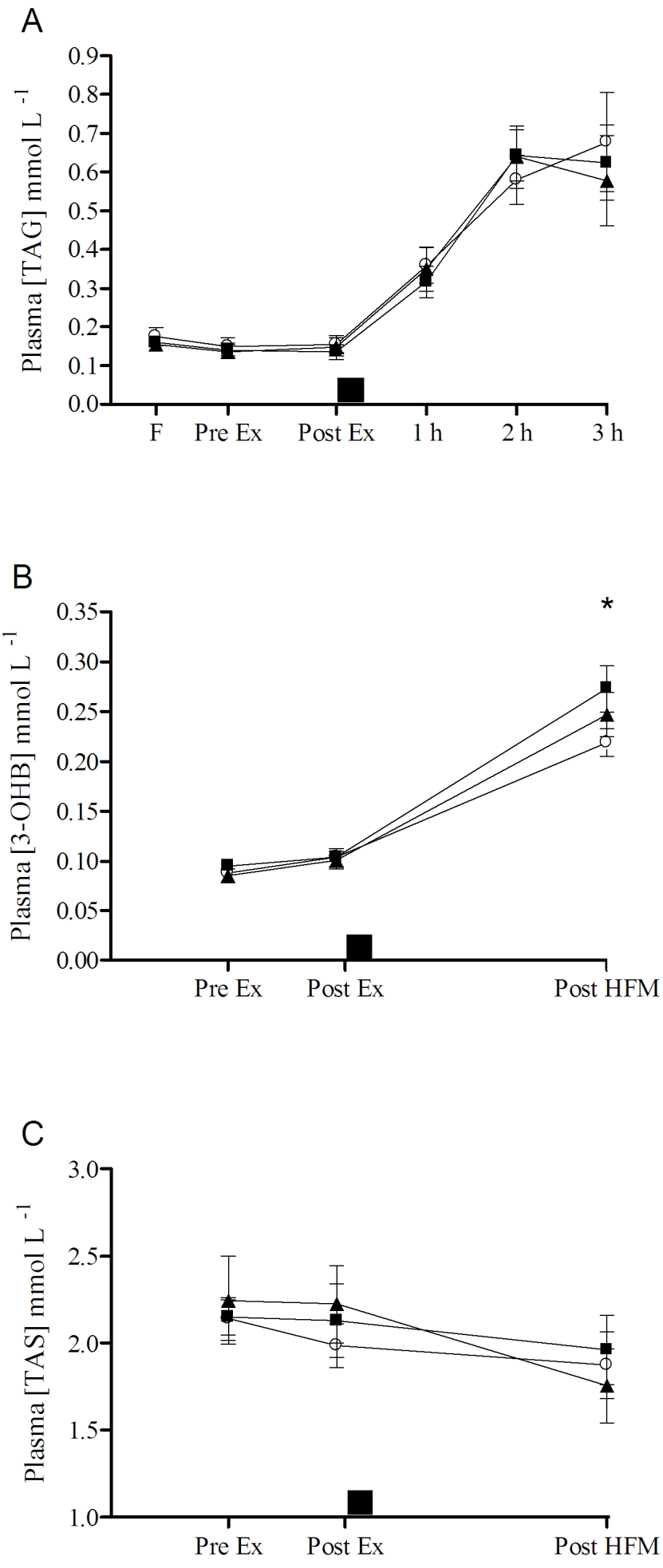
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Plasma [3-OHB] mmol L -1
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Plasma [TAS] mmol L -1
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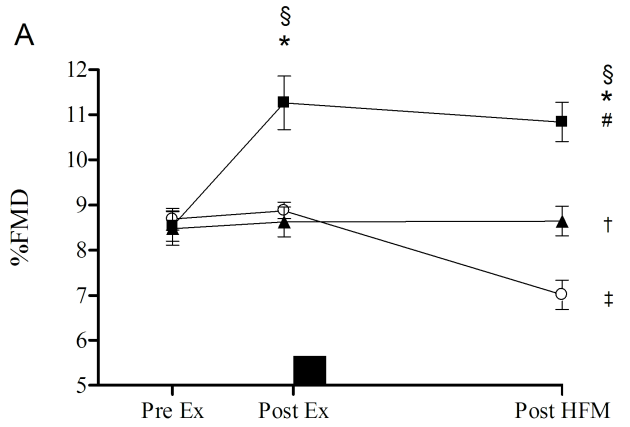
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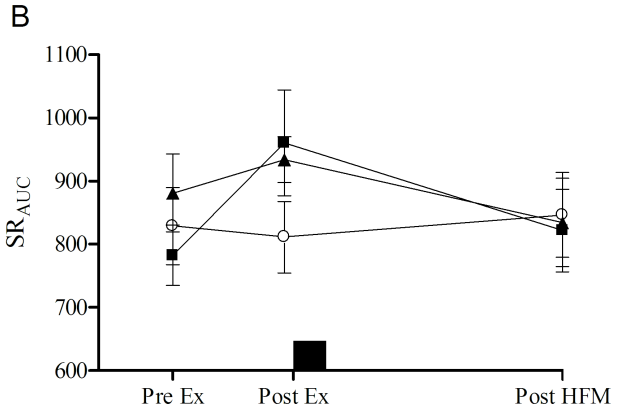
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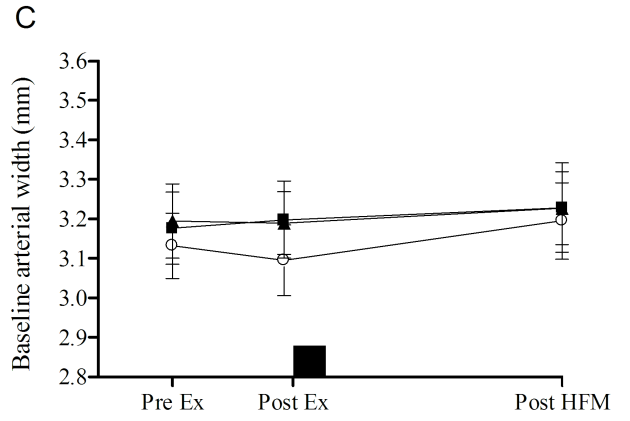
C
Baseline arterial width (mm)
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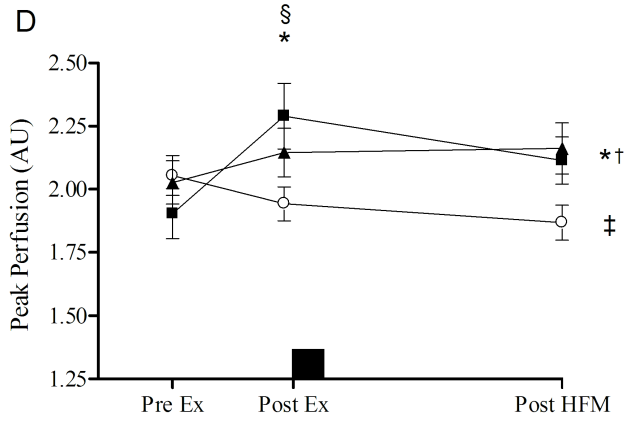
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Peak Perfusion (AU)
2.50
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