The acute effect of high- and moderate-intensity interval exercise on vascular function before and after a glucose challenge in adolescents

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New Findings

What is the central question of this study?

What is the effect of high-intensity and moderate-intensity interval running on macro- and microvascular function in a fasted state and following a glucose challenge in adolescents?

What is the main finding and its importance?

Both macro- and microvascular function were improved after interval running independent of intensity. This finding shows that the intermittent exercise pattern and its associated effect on shear are important for vascular benefits. In adolescents, macrovascular function was enhanced after an acute glucose load. However, the effect of chronic glucose consumption on vascular function remains to be elucidated.

ABSTRACT

Interventions targeting vascular function in youth are an important strategy for the primary prevention of cardiovascular diseases. This study examined, in adolescents, the effect of high-intensity interval running (HIIR) and moderate-intensity interval running (MIIR) on vascular function in a fasted state and postprandially after a glucose challenge. Fifteen adolescents (13 male, 13.9 ± 0.6 y) completed the following conditions on separate days in a counterbalanced order: 1) 8 x 1-min HIIR interspersed with 75 s recovery; 2) distance-matched amount of 1-min MIIR interspersed with 75 s recovery; and 3) rest (CON). Macro- (flow-mediated dilation, FMD) and microvascular (peak reactive hyperaemia, PRH) function were assessed immediately before and 90 min post exercise/rest. Participants underwent an oral glucose tolerance test (OGTT) 2 h post exercise/rest before another assessment of vascular function 90 min post OGTT. Following exercise, both HIIR and MIIR increased FMD (P = 0.02 and P = 0.03, respectively) and PRH (P = 0.04, and P = 0.01, respectively) with no change in CON (FMD: P = 0.51; PRH: P = 0.16) and no significant differences between exercise conditions. Following the OGTT, FMD increased in CON (P < 0.01) with no changes in HIIR and MIIR (both P > 0.59). There was no change in PRH after the OGTT (all P > 0.40). In conclusion, vascular function is improved after interval running independent of intensity in adolescents. Acute hyperglycaemia increased FMD, however, prior exercise did not change vascular function post OGTT in youth.

Keywords: Cardiovascular disease, running, FMD, hyperglycaemia, OGTT

INTRODUCTION

Although the symptoms of cardiovascular disease (CVD) start to manifest in adulthood, the process of atherosclerosis begins in childhood (McGill et al., 2000). Endothelial dysfunction represents the initial stage in the atherosclerotic process (Vanhoutte, 2009) and can be non-invasively assessed by flow-mediated dilation (FMD) (Celermajer et al., 1992; Thijssen et al., 2011; Thijssen et al., 2019). Detrimental changes to endothelial function initially occur in the microvasculature before impairments can be observed in the larger conduit arteries (Pinkney, Stehouwer, Coppack, & Yudkin, 1997) and the additional assessment of microvascular function in the form of peak reactive hyperaemia (PRH) has been advocated and shown to be reliable in young people (Bond, Williams, & Barker, 2017). Hence it is important to study the effectiveness of interventions targeting vascular function in youth with the aim of primary prevention of CVD.

Observational data suggest that vigorous physical activity is a more important stimulus than physical activity at lower intensities for improving both traditional risk factors (Barker et al., 2018; Carson et al., 2014) and FMD (Hopkins et al., 2009) in youth. High-intensity interval exercise represents a method of delivering vigorous physical activity and is linked to a myriad of CVD health benefits (Bond, Weston, Williams, & Barker, 2017; Eddolls, McNarry, Stratton, Winn, & Mackintosh, 2017). It has been demonstrated that high-intensity interval exercise in the form of 8 x 1 min cycling at 90% peak power acutely improves both macro- and microvascular function (Bond, Hind, Williams, & Barker, 2015) and protects the vasculature from the deleterious effects of a high-fat meal (Bond, Gates, et al., 2015), more than moderate-intensity continuous cycling in healthy adolescents. Bond, Hind, et al. (2015) reported that FMD was increased 1 and 2 h after an acute bout of high-intensity interval exercise whereas FMD remained unchanged following a work-matched bout of moderate-intensity continuous exercise in adolescents. These data suggest the existence of an exercise intensity effect on vascular function in youth. When comparing the effect of different exercise intensities, studies usually contrast high-intensity interval exercise with moderate-intensity exercise delivered in a continuous form (Ramos, Dalleck, Tjonna, Beetham, & Coombes, 2015). However, the ability of this experimental design to

manipulate the influence of exercise intensity has been challenged (Jimenez-Pavon & Lavie, 2017) as this paradigm fails to control for how the exercise is delivered (i.e. interval exercise) when comparing different intensities. To achieve this, studies need to manipulate exercise intensity whilst controlling for the way the exercise bout is delivered. No study has investigated the effect of interval exercise at different intensities on vascular function in the context of an acute bout of exercise in a paediatric population.

Recently, concerns have been raised about the consumption of sugar sweetened beverages in youth as it is associated with an increased risk of CVD (Vos et al., 2017). In their systematic review and meta-analysis, Loader et al. (2015) reported that vascular function is impaired following acute hyperglycaemia in both healthy and cardiometabolic diseased populations. However, data on the influence of hyperglycaemia on vascular function in paediatric populations are equivocal, with reports of vasodilation in adolescents with type 1 diabetes (Dye, Huang, Bauer, & Hoffman, 2012), an increase in FMD in both normal-weight and overweight children (Dengel, Kelly, Steinberger, & Sinaiko, 2007) and no change in endothelial function in obese children and adolescents (Metzig et al., 2011). These findings also contrast data on a high-fat meal challenge in youth, as during hypertriglyceridaemia both resting macro- and microvascular function are impaired (Bae et al., 2001; Sedgwick et al., 2013). Additionally, an acute bout of high-intensity interval cycling protects endothelial function in adolescents from the harmful effect of a high-fat meal (Bond, Gates, et al., 2015). Currently, no data are available on exercise and vascular function in response to a glucose challenge in a paediatric population. Given the recently shown reduction of glycaemic excursions after an oral glucose tolerance test (OGTT) in adolescents after exercise (Cockcroft et al., 2015), it could be hypothesized that any potential alterations in vascular function following an OGTT in youth could be preserved or even augmented with prior exercise.

The aims of the study were to address the following in a healthy adolescent population: 1) to investigate the effect of an acute bout of high-intensity interval running (HIIR) and distance-matched moderate-intensity interval running (MIIR) on macro- and microvascular function; and 2) to explore the effect of a glucose load on macro- and

microvascular function and whether this response is modified by prior MIIR and HIIR. It was hypothesized that HIIR would lead to greater improvements in vascular function than MIIR. Despite the equivocal reports regarding the effect of hyperglycaemia on vascular function in previous paediatric studies (Dengel et al., 2007; Dye et al., 2012; Metzig et al., 2011), we hypothesized based on the study by Dengel et al. (2007) that the glucose load would have no detrimental effect on vascular function and that prior exercise would elevate vascular function after the glucose challenge in an intensity dependent manner.

METHODS

Ethics approval

The study conformed to the standards established by the Declaration of Helsinki and was approved by the Sport and Health Sciences Ethics Committee, University of Exeter (160217/B/04). The study was not registered in a database. Before commencement of the project, details of the study and associated risks and benefits were explained, and written participant assent and parental consent were obtained.

Experimental design

A convenience sample of fifteen 13- to 15-year olds (two girls) volunteered to participate in this study. The study required four visits to the laboratory and included a within-measures design with counterbalanced delivery of experimental conditions, separated by approximately 1 week. Anthropometric and fitness measurements were taken on visit 1 and the experimental conditions were implemented on visits 2-4. All exercise was performed on a motorised treadmill (Woodway PPS 55 Sport, Woodway GmbH, Weil am Rhein, Germany) set at a gradient of 1% to account for the energy cost of outside running (Jones & Doust, 1996).

Visit 1: Anthropometric and fitness assessment

Stature and body mass of the participants were measured to the nearest 0.1 cm and 0.1 kg, respectively, using standard procedures. Skinfold thickness measurements were taken in triplicate at the subscapular and triceps sites (Harpenden Skinfold Caliper, HaB International Ltd., Southam, UK) and then averaged to estimate body fat percentage using the ethnicity, sex and puberty specific equations by Slaughter et al. (1988). Body mass index (BMI) was calculated and age and sex specific cut-points were used to classify participants as overweight or obese (Cole, Bellizzi, Flegal, & Dietz, 2000). Pubertal status was estimated through self-assessment of secondary sexual characteristics using adapted drawings of the five stages of pubic hair development (Morris & Udry, 1980).

After a familiarisation session, which included jogging and safely dismounting the treadmill, participants were asked to perform a combined incremental and supramaximal running test to exhaustion to determine maximum aerobic speed (MAS), maximum oxygen uptake ($\dot{V}O_{2max}$) and gas exchange threshold (GET). Following a warm-up period of 3 min of walking at 4 $km \cdot h^{-1}$, the test started at an initial speed of 6 $km \cdot h^{-1}$ and increased by 0.5 $km \cdot h^{-1}$ every 30 s until volitional exhaustion (Thackray, Barrett, & Tolfrey, 2013). The running speed at exhaustion was defined as the MAS. After exhaustion, participants continued to walk on the treadmill for 5 min at 4 $km \cdot h^{-1}$ before resting seated for further 5 min. Subsequently, participants performed a supramaximal running test to exhaustion to confirm $\dot{V}O_{2max}$ achieved in the previous test (Barker, Williams, Jones, & Armstrong, 2011; Rowland, 1993). The treadmill speed was adjusted to the MAS achieved in the incremental test with the gradient set at 5 %. Participants were asked to run for as long as possible. During the tests, participants wore a facemask which was connected to an on-line gas analyser (Cortex Metalyzer III B, Cortex Biophysik GmbH, Leipzig, Germany) to monitor gas exchange and ventilation variables. The GET was identified as the first disproportionate increase in CO_2 production relative to $\dot{V}O_2$ and verified using plots showing an increase in expired ventilation ($\dot{V}E$)/$\dot{V}O_2$ with no increase in $\dot{V}E/\dot{V}CO_2$. $\dot{V}O_{2max}$ was identified as the highest 10 s average in $\dot{V}O_2$ during the incremental test or the supramaximal bout. Heart rate (HR) was continuously measured using short-range telemetry (Polar Electro,

Kempele, Finland). Maximum HR (HR_{max}) was defined as highest HR achieved during the incremental test or the supramaximal bout.

Visit 2-4: Experimental visits

An overview of the experimental visits is presented in Figure 1. Following an overnight fast of at least 12 hours, participants were transported by car to the laboratory at 08:00. On the first experimental visit, a fingertip capillary blood sample was taken after participant arrival. At 08:30, participants rested in a supine position in a darkened, temperature-controlled room (24°C) for ~ 10 min before the simultaneous assessment of macrovascular (FMD) and microvascular (laser Doppler Perfusion monitoring) function (Bond, Williams, et al., 2017). At 09:00, participants completed one of the following three experimental conditions in a counterbalanced order:

1) HIIR: Participants completed 8 x 1 min intervals at 90% MAS interspersed with 75 s of walking at 4 $km \cdot h^{-1}$.
2) MIIR: Participants performed an individually calculated number of intervals of 1 min (or less) to match the distance of the HIIR condition. Each interval was completed at 90% of the speed at the GET and separated by 75 s of walking at 4 $km \cdot h^{-1}$.
3) Control (CON): Participants remained seated in the laboratory for 30 minutes and engaged in sedentary activities (e.g. playing board games, watching DVDs etc.).

All exercise conditions started with a 3 min warm-up and finished with a 2 min cool-down at 4 $km \cdot h^{-1}$.

After the experimental condition, participants remained seated in the laboratory and pursued sedentary activities for 90 min before another simultaneous assessment of macro- and microvascular function. Two hours after completion of the experimental condition, participants consumed 75 g glucose dissolved in 300 mL of water with capillary blood samples taken at 0, 10, 20, 30, 60, 90, and 120 min relative to glucose ingestion for the assessment of blood glucose (Dalla Man et al., 2005). A final assessment of macro- and microvascular function was performed 90 min after the glucose load as previous studies with adults reported a nadir for FMD one hour

(Kawano et al., 1999; Zhu, Zhong, Yu, & Li, 2007) or two hours (Title, Cummings, Giddens, & Nassar, 2000) post OGTT.

Macrovascular function

Macrovascular function was assessed in the brachial artery of the left arm. High resolution Doppler and B-mode images of the brachial artery were simultaneously assessed (Sequoia 512, Acuson, Siemens Corp, Aspen, USA) with a 13 MHz linear array transducer in duplex mode, in accordance with recent guidelines (Thijssen et al., 2019) and our earlier work (Bond, Williams, et al., 2017; Kranen, Bond, Williams, & Barker, 2018). Following a ~ 10 min acclimatization period to the temperature-controlled room (24°C) in the supine position, baseline arterial diameter was measured for 1 min. Endothelium-dependent vasodilation of the brachial artery was measured for 3 min after a 5 min ischaemic stimulus induced by rapid forearm pneumatic cuff inflation (Hokanson, Bellevue, WA, USA) to 220 mmHg. Baseline arterial diameter and post-occlusion brachial artery diameter were assessed during end diastole using validated ECG-gating software (Medical Imaging Applications LLC, Coralville, IA, USA) (Mancini, Yeoh, Abbott, & Chan, 2002; Thijssen et al., 2011; Thijssen et al., 2019). FMD was calculated using the following equation:

$$FMD\ (\%) = \frac{Peak\ post-occlusion\ diameter\ -\ Mean\ baseline\ diameter}{Mean\ baseline\ diameter}\ x\ 100\%$$

In order to minimize error in subsequent measurements, the location of the transducer on the first scan was marked on the arm. All analyses were performed by the same investigator who was blinded to the measurement condition. The within-day (control day) and between-day (baseline measurements across days) coefficients of variation for FMD data presented in the current study were 5% and 22%, respectively.

The area under the curve for estimated shear rate (SR_{AUC}) was calculated from the time of cuff deflation until peak dilation (Pyke & Tschakovsky, 2005). In line with other paediatric data reported by our laboratory (Bond, Gates, et al., 2015; Bond, Hind, et al., 2015) and others (Thijssen, Bullens, et al., 2009), preliminary analyses using Pearson's correlation coefficient (*r*) revealed that there were no consistently significant

relationships between SR_{AUC} and FMD. Consequently, FMD was not normalised for shear. However, shear data are presented separately in compliance with the current guidelines (Thijssen et al., 2019). As no consistently significant correlations between FMD and baseline artery diameter were present, allometric scaling was not undertaken (Atkinson & Batterham, 2013).

Microvascular function

Microvascular function was assessed simultaneously during the FMD protocol using a laser Doppler perfusion monitor (moorVMS-LDF, Moor Instruments, Axminster, UK). Two optic probes with 8 collecting fibres in a 2 mm ring with a central delivery fibre were attached with adhesive stickers 3 cm apart to the distal third of the forearm (Cracowski, Minson, Salvat-Melis, & Halliwill, 2006). The placement of the probes on the arm was marked for replication in following measurements. Data were collected at 40 Hz and data of the two probes were averaged for further analysis. The outcome measure was PRH, which was defined as the highest point after cuff deflation in relation to the baseline average. The within-day and between-day coefficients of variation for PRH data presented in the paper were 24% and 19%, respectively.

Blood analyses

Following arrival at the laboratory on the first experimental visit, a fingertip capillary blood sample was taken for the analysis of total cholesterol, high-density lipoprotein (HDL) and triacylglycerol (TAG) (CardioChek PA, BHR Pharmaceuticals Ltd., Nuneaton, UK). Further fingertip capillary blood samples (~ 200 µL) were collected into a heparin/fluoride coated microvette (CB 300 FH tubes, Sarstedt AG & Co., Nümbrecht, Germany) before and after the OGTT and analysed immediately for blood glucose (YSI 2300 Stat Plus Glucose and L-Lactate Analyzer, YSI Inc., Yellow Springs, OH, USA). Samples were analysed in duplicate and the mean was used for subsequent analyses. Changes in blood glucose following the OGTT were analysed using the total and incremental area under the curve (tAUC, iAUC) employing the

trapezium rule (GraphPad Prism, GraphPad Software Inc., San Diego, USA). It has previously been shown that fasting glucose, 2 h glucose and tAUC for glucose can be assessed reliably with a coefficient of variation of less than 7% in adolescents (Cockcroft, Williams, Jackman, Armstrong, & Barker, 2017).

Control for confounding variables

With parental supervision, participants were asked to replicate their evening meal prior to each laboratory visit. Furthermore, they also completed a food diary recording consumed food/drink type and amount during the 48 hours period preceding each visit, which were subsequently assessed for total energy and macronutrient intake (CompEat Pro; Nutrition Systems, Banbury, UK). Participants were also asked wear a triaxial accelerometer on the wrist of their non-dominant hand (GENEActiv; Activinsights Ltd, Cambridge, UK) during the 48 h prior to each visit. Data were collected at 100 Hz and then analysed according to manufacturer's recommendations. Time spent performing moderate-to-vigorous physical activity was determined using validated cut points for paediatric groups (Phillips, Parfitt, & Rowlands, 2013).

Statistical Analyses

All data are presented as mean and standard deviation (SD) unless otherwise stated. Descriptive statistics were used to analyse participant characteristics. Repeated-measures ANOVA with condition and time as the independent variables were employed to examine the macro- and microvascular responses as well as the blood glucose response following the OGTT. Significant main or interaction effects were further analysed using paired samples t-tests and interpreted using the *P* value and standardised effect sizes (ES), with the latter used to determine the magnitude of the observed effect according to the following: trivial (< 0.2), small (0.2), moderate (0.5), and large (0.8) (Cohen, 1988). Individual changes for FMD and PRH pre and post experimental condition (FMD Δ 1 and PRH Δ 1, respectively) and post experimental condition and post OGTT (FMD Δ 2 and PRH Δ 2, respectively) were calculated for each condition

(CON, MIIR and HIIR) and the differences between conditions were tested using one-way repeated measures ANOVA. Statistical significance was accepted when $P < 0.05$. IBM SPSS Statistics software (Version 24; IBM Corporation, Armonk, NY) was used for all statistical analyses.

RESULTS

Characteristics for participants ($n = 15$) are presented in Table 1. One participant was categorized as overweight. Maturity status for boys and girls was as follows: stage 2, $n = 3$ and 0, stage 3, $n = 1$ and 1, stage 4, $n = 8$ and 1, stage 5, $n = 1$ and 0, respectively. All participants completed the exercise bouts with no adverse events. Details of the exercise conditions and cardiorespiratory responses are described in Table 2. There was no difference in distance run between the conditions, however, MIIR was ~31 % longer in duration than HIIR ($P < 0.001$, ES = 5.52).

Diet records and physical activity data are presented in Table 3. No significant mean differences in total energy intake ($P = 0.81$), individual macronutrient contribution (all $P > 0.05$), or time spent performing moderate-to-vigorous physical activity ($P = 0.97$) were apparent during the 48 h preceding each visit. Only seven participants had complete physical activity data.

Macrovascular function

One participant was excluded from the FMD analysis due to poor image quality. Macrovascular function data are illustrated in Figure 2. There was no significant effect of condition ($P = 0.66$) or interaction ($P = 0.067$) but a significant effect of time ($P = 0.003$) for FMD. Following the experimental condition, FMD did not change in CON ($P = 0.51$, ES = 0.04) but increased in both MIIR ($P = 0.026$, ES = 0.66) and HIIR ($P = 0.024$, ES = 0.57) compared to baseline. In CON, FMD increased significantly following the OGTT ($P = 0.009$, ES = 0.83) but remained unchanged in MIIR ($P = 0.74$, ES = 0.07) and HIIR ($P = 0.60$, ES = 0.16). FMD Δ 1 was significantly greater in MIIR ($P = 0.048$, ES = 0.88) and HIIR ($P = 0.036$, ES = 0.87) than CON with no

difference between exercise conditions (P = 0.62, ES = 0.14). In contrast, FMD Δ 2 was significantly lower in MIIR (P = 0.003, ES = 0.94) and HIIR (P = 0.041, ES = 0.92) compared to CON. There was no significant difference in FMD Δ 2 between MIIR and HIIR (P = 0.88, ES = 0.07).

A significant condition by time interaction effect (P = 0.006) for baseline diameter was observed. In CON, brachial artery baseline diameter decreased post experimental condition compared to the first assessment (P = 0.042) with further reduction post OGTT (P = 0.009). In MIIR and HIIR, baseline diameter did not change (MIIR: P = 0.63 and P = 0.37, respectively; HIIR: P = 0.43 and P = 0.065, repectively). With regards to peak artery diameter, the ANOVA revealed no significant effect of condition (P = 0.25), time (P = 0.34) or a condition by time interaction (P = 0.10). Similarly, no significant effect of condition (P = 0.16), time (P = 0.07) or condition by time interaction (P = 0.08) was detected for SR_{AUC}.

Microvascular function

PRH responses are depicted in Figure 3. No significant difference between conditions (P = 0.72) and no interaction effect (P = 0.95) was found for PRH. However, PRH changed significantly over time (P = 0.003). Post exercise, PRH increased significantly in MIIR (P = 0.01, ES = 0.42) and HIIR (P = 0.044, ES = 0.33) compared to baseline, with no change in CON (P = 0.16, ES = 0.44). There was no change in PRH following the OGTT (CON: P = 0.97; MIIR: P = 0.80; HIIR: P = 0.41). The ANOVA showed no significant effect of condition (P = 0.83), time (P = 0.14) or condition by time interaction (P = 0.86) for PRH Δ.

Blood glucose

The changes in blood glucose following the OGTT are presented in Figure 4. There was no effect of condition (P = 0.161) or interaction (P = 0.068), however, the ANOVA revealed a significant main effect of time (P < 0.001). In all conditions, blood glucose increased significantly (all P < 0.05) until 30 min post OGTT, and then decreased. No

significant effect of condition for the tAUC (P = 0.088) was detected, however, there was a moderate reduction in tAUC following HIIR compared to CON (P = 0.053, ES = 0.52) and a decrease compared to MIIR (P = 0.052, ES = 0.43) with no difference between MIIR and CON (P = 0.70, ES = 0.10). The ANOVA revealed no significant condition effect for the iAUC (P = 0.145) following the OGTT, however, the iAUC was significantly lower for HIIR than MIIR (P = 0.017, ES = 0.49).

DISCUSSION

The main finding of the current study was that HIIR and distance-matched MIIR equally improved both macro- and microvascular function 90 min post exercise in adolescents, which does not support the hypothesis of an exercise-intensity dependent effect of acute running exercise on vascular function in youth. A further novel finding was that acute hyperglycaemia augmented FMD during the control condition, but this postprandial increase in FMD was not observed following HIIR and MIIR, due to the existing elevated state post exercise. In contrast, the glucose load did not cause an alteration in microvascular function.

Acute exercise

Previously, Bond, Hind, et al. (2015) reported a significant increase in FMD 1 h and 2 h post high-intensity interval cycling but no change following moderate-intensity continuous cycling in adolescents, suggesting the existence of an exercise intensity effect on vascular function in youth. In contrast, a significant increase in FMD was observed 90 min after both MIIR and HIIR in the current study. This inter-study discrepancy between Bond, Hind, et al. (2015) and our study following moderate-intensity exercise may be explained by two important differences between the studies and their impact on vascular function: the delivery of moderate-intensity exercise (continuous versus interval form) and the exercise modality (cycling versus running).

The repeated shear stimulus produced by exercise training is thought to be important for eventual positive vascular adaptations (Dawson, Green, Cable, & Thijssen, 2013) with

reports of region-specific changes in shear patterns in response to exercise (Green, Hopman, Padilla, Laughlin, & Thijssen, 2017). The shear pattern during the exercise bouts in the current study are likely to have been different to that of Bond, Hind, et al. (2015) due to differences in blood flow in the brachial artery during intermittent vs. continuous exercise, respectively. For example, Lyall, Davies, Ferguson, Porter, and Birch (2019) reported greater brachial artery anterograde shear rate during continuous exercise and no difference in retrograde shear between continuous and intermittent exercise matched for intensity and duration protocols in adults. However, the oscillatory shear index was higher in the interval bouts than during the continuous bout (Lyall et al., 2019) which may have promoted a greater release of endothelial NO (Green et al., 2005). Furthermore, interventions that are designed to alter shear rate fluctuations have observed concomitant changes in FMD in the intervention arm, with no changes in the control arm (Holder et al., 2019), suggesting intermittent changes in shear rate may improve vascular function. Thus, it is possible that the intermittent nature of the exercise bouts performed in the current study may lead to vasodilation and thereby the observed increase in FMD after both MIIR and HIIR. In contrast, Bond, Hind, et al. (2015) reported an augmentation in FMD only following HIIE with no changes after continuous moderate-intensity exercise. Our observations therefore suggest that the fluctuation in shear rate caused by the alternation between work and recovery intervals, regardless of intensity may be responsible for the augmented FMD.

In adults, different exercise modalities have been shown to produce different shear rate patterns (Thijssen, Dawson, et al., 2009). Unlike Bond, Hind, et al. (2015) who used cycling as exercise modality, participants in the current study performed running bouts and presented an enhanced FMD following both MIIR and HIIR. Therefore, it is also plausible to attribute the improved FMD response following HIIR and MIIR to the exercise modality of running. Although systemic vessels in the inactive limb also show improvements in vascular function from exercise (Green, Maiorana, O'Driscoll, & Taylor, 2004), the vessels in the non-active limb are exposed to increased retrograde shear (Green et al., 2005), leading to a reduction in NO bioavailability (Ziegler, Bouzourène, Harrison, Brunner, & Hayoz, 1998). The additional arm movement during running may provide a greater stimulus to the brachial artery than isolated lower limb exercise like cycling and may therefore prevent the aforementioned increase in

retrograde shear observed in non-active limbs. However, we were not able to measure blood flow and shear rate during the running bouts due to its technically challenging nature.

The exercise pattern (continuous vs. intermittent) and/or the modality (cycling vs. running) could also explain the differences in microvascular function in the present study compared to the findings by Bond, Hind, et al. (2015). Whereas PRH increased following MIIR and HIIR in the current investigation, Bond, Hind, et al. (2015) observed greater PRH immediately, 1 h and 2 h post high-intensity interval exercise but only an immediate elevation in PRH after moderate-intensity exercise with a return to baseline values 1 h and 2 h afterwards. The findings by Tsai and Intaglietta (1993) endorse the significance of the intermittent stimulus for changes in vascular function. By using mathematical simulation, the authors demonstrated that an improvement in tissue oxygenation is achieved with variations of capillary blood flow rather than continuous flux in the microvasculature which can be compared to the intermittent exercise stimulus in contrast to continuous exercise. In another study with healthy male adults (Hodges, Stewart, Davison, & Cheung, 2017), participants presented an elevation in cutaneous microvascular reactivity caused by the implementation of an episodic shear stress intervention. However, the lack of a comparable continuous stimulus in the aforementioned study impedes the conclusion that the intermittent nature of the shear stress is of most importance.

Effect of glucose load

A novel finding is that FMD increased significantly 90 min post glucose load in CON in the current study. This is in contrast to the systematic review and meta-analysis by Loader et al. (2015), which concluded that macrovascular function is impaired during hyperglycaemia in both healthy and clinical populations. It is believed that such an impairment in FMD is caused by increases in oxidative stress and inflammation following a glucose load which reduce NO production and bioavailability (Loader et al., 2015). However, the majority of the studies included in this review were based on adults; only three out of the 39 studies represented research within youth and close

scrutiny of the literature suggests vascular dysfunction following an acute glucose load does not apply to paediatric populations. Akin to our observations, Dengel et al. (2007) studied the effect of an OGTT on FMD in 16 overweight and 15 normal-weight children and reported a significant increase in FMD over time in both groups. Unfortunately, the authors did not provide a possible explanation of their finding. Compared to adults, adolescents demonstrate an exaggerated insulin response during an OGTT (Tricò, Natali, Arslanian, Mari, & Ferrannini, 2018) and exhibit temporary insulin resistance which is related to pubertal status (Ball et al., 2006). As insulin is known to be an effective vasodilator and more than 65% of insulin-mediated dilation is NO dependent (Steinberg, Brechtel, Johnson, Fineberg, & Baron, 1994), it could be hypothesized that a greater insulin release following the glucose load caused the observed increase in FMD in youth. Also, the findings of Metzig et al. (2011) indicate that in obese children and adolescents an OGTT does not induce rises in oxidative stress and inflammation. Thus, in the present study an elevated insulin-mediated NO production may have been maintained in the absence of oxidative stress and inflammation, resulting in increased NO bioavailability and thereby improved FMD. However, this explanation is currently speculative as we were not able to measure insulin or markers of oxidative stress and inflammation, and represents an avenue for further research.

Cockcroft et al. (2015) reported a significant reduction in plasma glucose tAUC and iAUC following high-intensity interval and moderate-intensity continuous cycling which was not observed following exercise in the current investigation; however, there was a trend for a reduced tAUC after HIIR compared to MIIR and CON. Besides the differences of exercise mode and modality, the timing of the exercise bout with regards to the OGTT was different in the study by Cockcroft et al. (2015) compared to the current investigation and may explain the diverging findings. In contrast to CON, FMD did not change in MIIR and HIIR following the OGTT. Previously, it was demonstrated that exercise protects from vascular dysfunction after a high-fat meal in adolescents (Bond, Gates, et al., 2015; Sedgwick, Morris, Nevill, & Barrett, 2015) and adults (Tyldum et al., 2009). In the present investigation, no vascular dysfunction was detected following the glucose load, on the contrary, vascular function improved after the OGTT in CON. It appears that the observed reduction in baseline diameter in CON was prevented by the exercise in the other conditions. Similar to CON, peak diameter

remained unchanged in MIIR and HIIR, resulting in an unmodified FMD response. However, FMD had already increased following exercise before the ingestion of the glucose load. Given the previous findings by Cockcroft et al. (2015) of a significantly reduced insulin response following an OGTT performed after exercise, it could be hypothesized that the exercise bouts in the current study decreased insulin release which may have lowered the potential vasodilatory stimulus following the OGTT. Alternatively, it could be hypothesized that FMD was already augmented to such an extent by the prior exercise bout that any potential to increase FMD further after the glucose load was diminished.

The OGTT had no effect on microvascular function in the current study as PRH was not altered following any of the conditions. It was previously shown that a high-fat meal caused a reduction in PRH in a control condition which was precluded by high-intensity interval exercise and moderate-intensity exercise in adolescents (Bond, Gates, et al., 2015). Similar to the current observation in adolescents, microvascular function was preserved during acute hyperglycaemia in adults (Loader et al., 2015). Therefore, unlike the difference in the macrovascular function between adults and adolescents following a glucose load, the response of the microvasculature to an OGTT seems to be consistent across age groups. As PRH is not NO mediated (Wong, Wilkins, Holowatz, & Minson, 2003), it was suggested that the preservation of the microcirculation could be attributed to the importance of other chemical intermediaries responsible for perfusion in the microvasculature (Loader et al., 2015), such as endothelium-derived hyperpolarizing factor and prostaglandin I_2 (Feletou & Vanhoutte, 2006). Further investigations are needed to clarify the mechanisms of the microvascular response to an OGTT.

Considerations and limitations

This is the first study to investigate the effects of both high- and moderate-intensity exercise delivered in an interval form on vascular function in youth before and after an oral glucose challenge. The strengths of the study comprise the distance-matched running bouts, control of previous physical activity and diet before the experimental visits, and measurement of both macro- and microvascular function. Nevertheless, there

are some limitations. There is an obvious sex imbalance in recruited participants (2 female vs. 13 male participants), which was attributed to the unsuccessful efforts of recruiting female participants. However, Bond, Hind, et al. (2015) reported that there were no differences in macro- and microvascular function between boys and girls at baseline and following exercise. Furthermore, the glucose load administered in the current study is not characteristic of habitual sugar sweetened beverage consumption in which the main sugar source is either sucrose or high fructose containing sugars. However, sugar sweetened beverages are not the only source of sugar in diets and glucose is still a common component of the diet and represents a standardised metabolic challenge.

Conclusion

The present study shows that a single bout of HIIR and distance-matched MIIR similarly augment macro- and microvascular function in adolescents. Improvements in vascular function following acute interval running are therefore independent of intensity, suggesting the intermittent exercise pattern is more important than the exercise intensity of running. In addition, acute hyperglycaemia through ingestion of a glucose load increased FMD with no changes in microvascular function. Exercise (HIIR or MIIR) before a glucose load had no effect on macro- and microvascular function. Further studies are required to understand the interaction between glucose, exercise and vascular function in youth and the underpinning mechanisms.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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AUTHOR CONTRIBUTIONS

All the work was done in the Children's Health and Exercise Research Centre, Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter. SHK, CAW, and ARB designed the experiments; SHK, RSO and BB contributed to data collection and analysis; SHK and ARB drafted the manuscript and RSO, BB and CAW critically appraised it. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors approved the final version of the manuscript, all persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Table 1. Participant descriptive characteristics.

	Mean ± SD	**Min**	**Max**
Age (y)	13.9 ± 0.6	13.1	15.0
Stature (m)	1.62 ± 0.10	1.47	1.85
Body mass (kg)	50.5 ± 12.9	34.8	80.3
BMI ($kg \cdot m^{-2}$)	19.1 ± 3.2	14.5	26.7
Body fat (%)	12.3 ± 6.6	4.8	25.9
MAS ($km \cdot h^{-1}$)	15.0 ± 2.1	10.5	17.5
TTE (s)	79 ± 17	54	119
HR_{max} ($b \cdot min^{-1}$)	198 ± 9	183	213
$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	2.44 ± 0.49	1.79	3.63
$\dot{V}O_{2max}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	49.5 ± 6.4	36.0	56.0
GET ($L \cdot min^{-1}$)	1.69 ± 0.32	1.25	2.44
GET ($mL \cdot min^{-1} \cdot kg^{-1}$)	34.5 ± 5.3	25.0	43.0
GET (%$\dot{V}O_{2max}$)	70 ± 4	64	78
Blood glucose ($mmol \cdot L^{-1}$)	4.15 ± 0.43	3.21	5.07
Total cholesterol ($mmol \cdot L^{-1}$)	3.57 ± 0.48	2.82	4.23
HDL ($mmol \cdot L^{-1}$)	1.37 ± 0.31	0.88	1.92
TAG ($mmol \cdot L^{-1}$)	0.71 ± 0.25	0.57	1.42

BMI, body mass index; MAS, maximum aerobic speed; TTE, time to exhaustion in supramax test; HR_{max}, maximum heart rate; $\dot{V}O_{2max}$, maximal oxygen uptake; GET, gas exchange threshold; HDL, high-density lipoprotein; TAG, triacylglycerol.

Table 2. Details of exercise conditions and cardiorespiratory responses.

	MIIR	**HIIR**	***P***	**ES**
Speed ($km \cdot h^{-1}$)	8.6 ± 1.0	13.6 ± 1.9	< 0.001	3.29
Distance (m)	2725 ± 251	2725 ± 251	1.00	0.00
Duration (min)	28:35 ± 1:45	21:45	< 0.001	5.52
Intervals (*n*)	10.7 ± 0.8	8	< 0.001	4.77
Average HR ($b \cdot min^{-1}$)	127 ± 15	155 ± 12	< 0.001	2.06
Average HR (%HR_{max})	64 ± 7	78 ± 4	< 0.001	2.46
Peak HR ($b \cdot min^{-1}$)	151 ± 16	187 ± 13	< 0.001	2.47
Peak HR (%HR_{max})	76 ± 7	94 ± 5	< 0.001	2.96
Average $\dot{V}O_2$ ($L \cdot min^{-1}$)	1.11 ± 0.24	1.51 ± 0.31	< 0.001	1.44
Average $\dot{V}O_2$ (%$\dot{V}O_{2max}$)	46 ± 3	62 ± 4	< 0.001	4.53
Peak $\dot{V}O_2$ ($L \cdot min^{-1}$)	1.60 ± 0.38	2.17 ± 0.49	< 0.001	1.30
Peak $\dot{V}O_2$ (%$\dot{V}O_{2max}$)	65 ± 5	89 ± 7	< 0.001	3.95

HIIR, high-intensity interval running; MIIR, moderate-intensity interval running; ES, effect size; HR, heart rate; HR_{max}, maximum heart rate; $\dot{V}O_2$, oxygen uptake; $\dot{V}O_{2max}$, maximal oxygen uptake.

Table 3. Average physical activity (n = 7) and food consumption (n = 15) in the 48 h preceding the experimental visits.

	CON	**MIIR**	**HIIR**	***P***
MVPA (min·day^{-1})	126 ± 83	125 ± 57	133 ± 75	0.97
Total kcal (kcal·day^{-1})	2004 ± 712	2105 ± 722	1952 ± 696	0.81
Carbohydrate (%)	50.5 ± 10.0	48.8 ± 7.2	49.4 ± 7.7	0.86
Fat (%)	32.4 ± 5.5	32.5 ± 6.0	32.9 ± 6.2	0.98
Protein (%)	15.8 ± 4.6	16.8 ± 3.5	15.8 ± 4.5	0.75

CON, control condition; MIIR, moderate-intensity interval running; HIIR, high-intensity interval running; MVPA, moderate-to-vigorous physical activity.

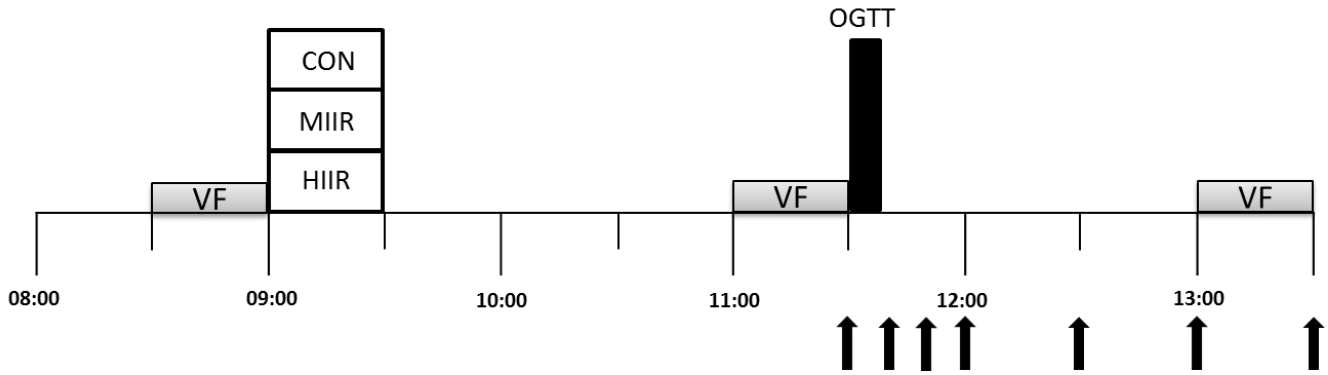


Figure 1. Schematic of study protocol for the experimental conditions (visits 2-4). VF, assessment of macro- (flow-mediated dilation) and microvascular (peak reactive hyperaemia) function; CON, control condition; MIIR, moderate-intensity interval running; HIIR, high-intensity interval running; OGTT, oral glucose tolerance test; Arrows depict capillary blood samples for blood glucose.

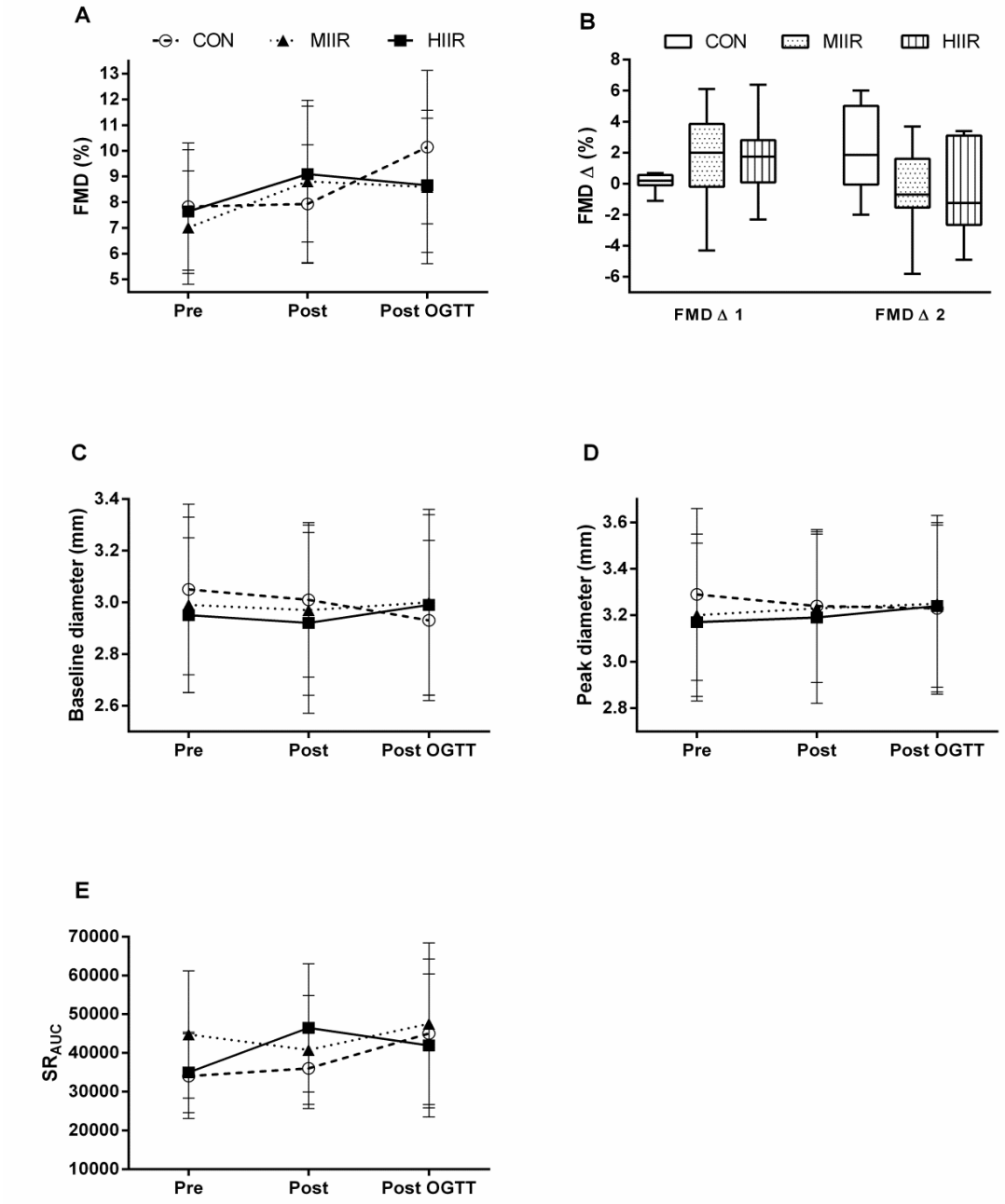


Figure 2. Differences in macrovascular function pre and post experimental condition and post oral glucose tolerance test (OGTT) (n = 14). CON, control condition; MIIR, moderate-intensity interval running; HIIR, high-intensity interval running. Data are shown as mean ± SD. Please see text for sign. differences within and between conditions. **A** FMD % **B** FMD Δ pre and post experimental condition (FMD Δ 1) and post experimental condition and post OGTT (FMD Δ 2) **C** Baseline artery diameter **D** Peak artery diameter **E** Area under the curve for shear rate (SR_{AUC}).

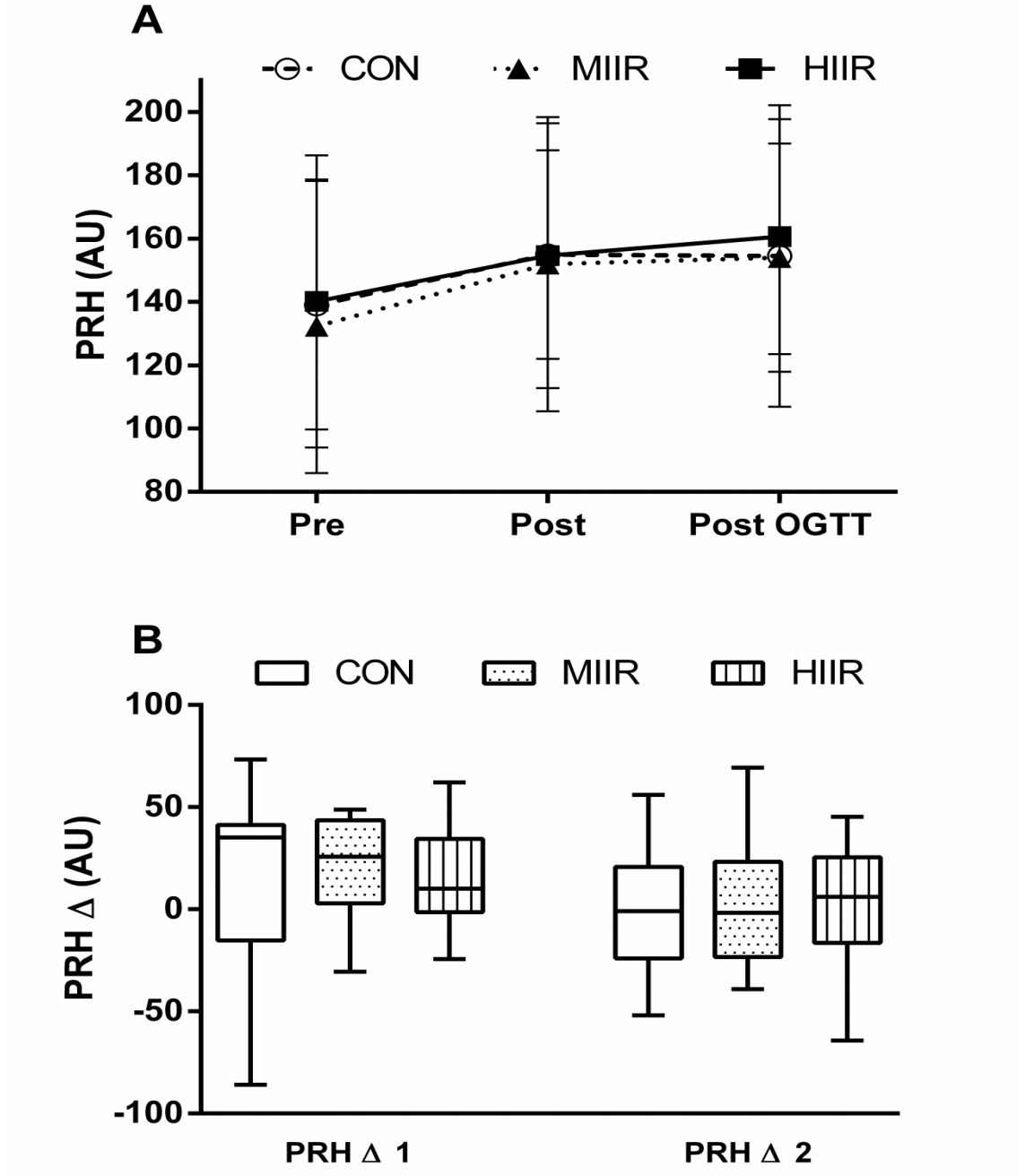
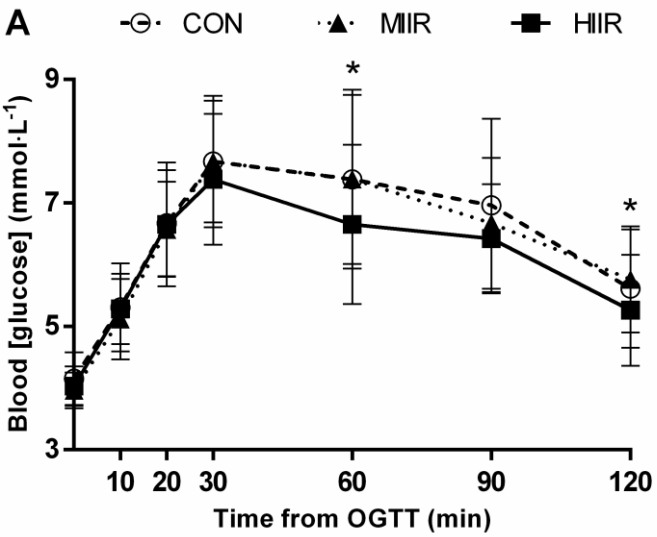
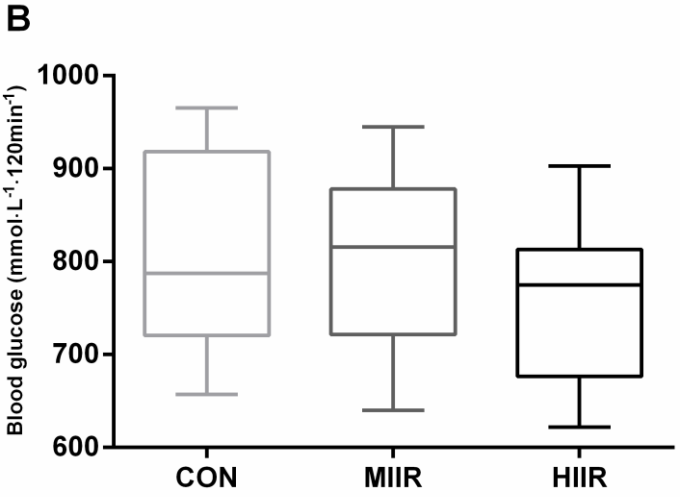


Figure 3. Differences in microvascular function pre and post experimental condition and post oral glucose tolerance test (OGTT). CON, control condition; MIIR, moderate-intensity interval running; HIIR, high-intensity interval running. Data are shown as mean ± SD. Please see text for sign. differences within and between conditions. **A** Peak reactive hyperaemia (PRH) **B** PRH Δ pre and post experimental condition (PRH Δ 1) and post experimental condition and post OGTT (PRH Δ 2).


A
CON
MIIR
HIIR
Blood [glucose] (mmol·L-1)
9
7
5
3
10
20
30
60
90
120
Time from OGTT (min)
*
*


B
Blood glucose (mmol·L-1·120min-1)
1000
900
800
700
600
CON
MIIR
HIIR

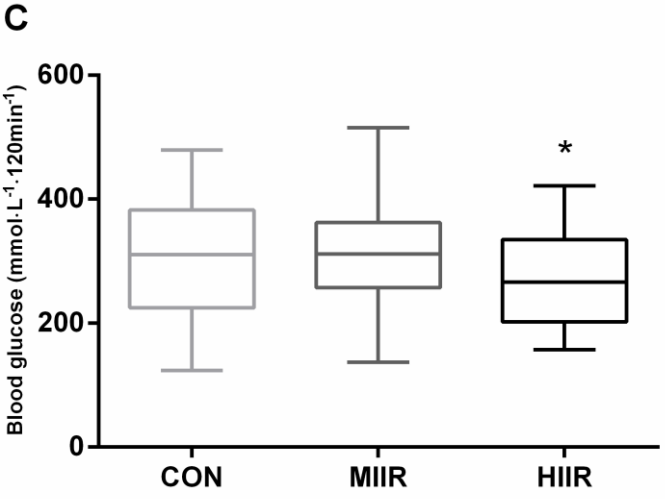

C
Blood glucose (mmol·L-1·120min-1)
600
400
200
0
CON
MIIR
HIIR
*

Figure 4. Blood glucose response following OGTT (oral glucose tolerance test) displayed over time (**A**), using the total area under the curve (AUC) (**B**) and the incremental AUC (**C**). CON, control condition; MIIR, moderate-intensity interval running; HIIR, high-intensity interval running. Data are shown as mean ± SD. * Significant difference between MIIR and HIIR.