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Title: Prior exercise enhances skeletal muscle microvascular blood flow and mitigates microvascular flow impairments induced by a high-glucose mixed meal in healthy young men

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Running Title: Prior exercise and postprandial muscle microvascular blood flow.

Key words: Exercise, glycemic control, vascular function.

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Key points summary

- Exercise, insulin-infusion, and low-glucose mixed-nutrient meal ingestion increases muscle microvascular blood flow which in part facilitates glucose delivery and disposal. In contrast, high-glucose ingestion impairs muscle microvascular blood flow which may contribute to impaired postprandial metabolism.
- We investigated the effects of prior cycling exercise on postprandial muscle microvascular blood flow responses to a high-glucose mixed-nutrient meal ingested 3 and 24 h post-exercise.
- Prior exercise enhanced muscle microvascular blood flow and mitigated microvascular impairments induced by a high-glucose mixed meal ingested 3 h post-exercise, and to a lesser extent 24 h post-exercise.
- High-glucose ingestion 3 h post-exercise leads to greater postprandial blood glucose, non-esterified fatty acids, and fat oxidation, and a delay in the insulin response to the meal compared to control.
- Effects of acute exercise on muscle microvascular blood flow persist well after the cessation of exercise which may be beneficial for conditions characterized by microvascular and glycemic dysfunction.

Abstract.

Exercise, insulin-infusion, and low-glucose mixed-nutrient meal ingestion lead to increased muscle microvascular blood flow (MBF), whereas high-glucose ingestion impairs MBF. We investigated whether prior cycling exercise could enhance postprandial muscle MBF and prevent MBF impairments induced by high-glucose mixed-nutrient meal ingestion. In a randomized cross-over design, eight healthy young men ingested a high-glucose mixed-nutrient meal (1.1 g glucose/kg body weight; 45% carbohydrate, 20% protein, and 35% fat) after an overnight fast (no-exercise control) and 3 h and 24 h after moderate-intensity cycling exercise (1 h at 70-75% VO_{2peak}). Skeletal muscle MBF, measured directly by contrast-enhanced ultrasound, was lower at 60 min and 120 min postprandial compared to baseline in all conditions ($p<0.05$), with a greater decrease occurring from 60 min to 120 min in the control (no-exercise) condition only ($p<0.001$). Despite this meal-induced decrease, MBF

was still markedly higher compared to control in the 3 h post-exercise condition at 0 min (pre-meal; 74%, p=0.004), 60 min (112%, p=0.002) and 120 min (223%, p<0.001), and in the 24 h post-exercise condition at 120 min postprandial (132%, p<0.001). We also report that in the 3 h post-exercise condition postprandial blood glucose, non-esterified fatty acids (NEFA), and fat oxidation were substantially elevated, and the insulin response to the meal delayed compared to control. This likely reflects a combination of increased post-exercise exogenous glucose appearance, substrate competition, and NEFA-induced insulin resistance. We conclude that prior cycling exercise elicits long-lasting effects on muscle MBF and partially mitigates MBF impairments induced by high-glucose mixed-nutrient meal ingestion.

Dr Lewan Parker is a NHMRC & National Heart Foundation Early Career Fellow at the Institute for Physical Activity and Nutrition (IPAN), Deakin University. His current research explores how vascular dysfunction and oxidative stress contribute to poor cardiometabolic health and reduced quality of life in patients with type 2 diabetes and cardiovascular disease. Dr Parker is also currently exploring whether interventions, such as antioxidant treatment and home-based exercise training, can be used to improve cardiac, microvascular and metabolic health and quality of life in cardiometabolic disease patients.

Introduction. Cardiac output, large artery and microvascular blood flow in skeletal muscle increase following the ingestion of a balanced mixed-nutrient meal (Waaler & Eriksen, 1992;

Russell *et al.*, 2018). This coordinated increase in blood flow plays a critical role in postprandial metabolism by facilitating the delivery of nutrients and hormones (e.g., insulin and glucose) to peripheral tissues including skeletal muscle (Keske *et al.*, 2016). In contrast, impaired vascular function and peripheral blood flow have been linked to impaired substrate metabolism and is observed in states of insulin resistance including acute hyperglycemia and hyperlipidemia, and chronic cardiometabolic conditions including obesity and type 2 diabetes (Clerk *et al.*, 2006; Liu *et al.*, 2009; Loader *et al.*, 2015; Keske *et al.*, 2016; Russell *et al.*, 2017).

Muscle microvascular blood flow plays a key role in postprandial metabolism and is suggested to account for up to 40 - 50% of insulin-stimulated glucose disposal in skeletal muscle when assessed using the hyperinsulinemic-euglycemic clamp technique (Vincent *et al.*, 2003; Vincent *et al.*, 2004; Bradley *et al.*, 2013; Keske *et al.*, 2016). In rodents, physiological hyperinsulinemia leads to increased muscle microvascular blood flow and glucose disposal prior to eliciting increases in limb arterial blood flow (Vincent *et al.*, 2002). We and others have also reported that muscle microvascular blood flow can be altered independent of changes in large artery blood flow under various conditions of glycemia and hyperinsulinemia (Rattigan *et al.*, 1997; Vincent *et al.*, 2002; Zhang *et al.*, 2004; Eggleston *et al.*, 2007; Keske *et al.*, 2017; Russell *et al.*, 2018). These findings support muscle microvascular blood flow as an important precursor, and potential rate-limiting step, for insulin-mediated glucose disposal.

Skeletal muscle contraction and exercise are potent stimuli for increasing muscle microvascular blood flow (Vincent *et al.*, 2006; Durham *et al.*, 2010; St-Pierre *et al.*, 2012), and are well-known to enhance insulin sensitivity and glycemic control throughout the 24-h post-exercise recovery period (Mikines *et al.*, 1988; Brestoff *et al.*, 2009; Ortega *et al.*, 2015; Parker *et al.*, 2016a; Morrison *et al.*, 2018). Research by Sjoberg *et al.* (2017) established that 1 h of single-legged knee extensor exercise in healthy young adults enhances insulin-mediated leg glucose uptake and microvascular blood flow 4 h later during euglycemic hyperinsulinemic clamp conditions. Furthermore, the enhancement in insulin action post-exercise was largely prevented when microvascular blood flow was blunted via the co-infusion of a nitric oxide (NO) synthase inhibitor (Sjoberg *et al.*, 2017). These findings demonstrate that enhanced insulin-mediated microvascular blood flow contributes to the

insulin sensitizing effects of acute exercise, at least during hyperinsulinemic euglycemic clamp conditions. Whether prior exercise can enhance postprandial (meal-related) microvascular blood flow and metabolism have yet to be determined. This is important, as the exogenous and endogenous glycemic and hormonal responses elicited by orally ingested nutrients and intravenous insulin and glucose infusion are markedly different (Mingrone *et al.*, 2020). Therefore, the microvascular responses likely vary when comparing intravenous infusions and orally ingested nutrients (Roberts-Thomson *et al.*, 2020).

Microvascular dysfunction can be induced via stimuli such as hyperglycemia and hyperlipidemia which is linked to insulin resistance and cardiometabolic diseases including type 2 diabetes (Liu *et al.*, 2009; Keske *et al.*, 2016; Russell *et al.*, 2017). Compared to lean humans, muscle microvascular blood flow is impaired in obese adults following a euglycemic-hyperinsulinemic clamp or ingestion of a mixed-nutrient meal (Clerk *et al.*, 2006; Keske *et al.*, 2009), reflecting similar reports from rodent models of insulin resistance (Wallis *et al.*, 2002; Clerk *et al.*, 2007; St-Pierre *et al.*, 2010). Even in healthy individuals acute hyperglycemia impairs muscle microvascular blood flow (Russell *et al.*, 2018; Parker *et al.*, 2020). For example, the ingestion of a low-glucose mixed-nutrient meal (41 g carbohydrate, 25.1 g as glucose) in healthy adults increases microvascular blood flow, whereas an oral glucose challenge (50 g of glucose) matched for postprandial plasma insulin levels impairs postprandial muscle microvascular blood flow (Russell *et al.*, 2018). Furthermore, our team also reported in healthy males that the ingestion of protein (~39 g) and lipids (~30 g) alongside a high-glucose load (1.1 g/kg bodyweight of glucose) leads to a similar decrease in muscle microvascular blood flow which persists for up to 2 h postprandial (Parker *et al.*, 2020). In both studies greater glycemic excursions were correlated with a greater decrease in muscle microvascular blood flow (Russell *et al.*, 2018; Parker *et al.*, 2020), supporting the notion that acute-hyperglycemia can transiently impair muscle microvascular perfusion in healthy adults.

Previous studies show that acute aerobic exercise has the potential to mitigate and prevent vascular dysfunction, including impaired forearm cutaneous microvascular blood flow, caused by high-carbohydrate and high-fat meal ingestion (Zhu *et al.*, 2007; Tyldum *et al.*, 2009; Varsamis *et al.*, 2018). However, the effect of prior aerobic exercise on microvascular blood flow in skeletal muscle following high-glucose ingestion is unknown. Furthermore,

research has reported a dynamic and complex relationship between prior exercise and postprandial carbohydrate and lipid metabolism, both of which may be influenced by factors such as meal composition, the post-exercise fasting period, and the intensity and duration of exercise (Krzentowski *et al.*, 1982; Bielinski *et al.*, 1985; Folch *et al.*, 2001; Marion-Latard *et al.*, 2003). For example, fat oxidation is elevated whereas carbohydrate oxidation is lowered in response to meal ingestion and a euglycemic hyperinsulinemic clamp in the hours following aerobic exercise (Krzentowski *et al.*, 1982; Bogardus *et al.*, 1983; Bielinski *et al.*, 1985). To our knowledge research has yet to explore the effects of prior exercise on substrate metabolism following high-glucose mixed-nutrient meal ingested 3 h and 24 h post-exercise.

The primary aim of this study was to use contrast enhanced ultrasound to investigate the effects of prior exercise on muscle microvascular blood flow following high-glucose, mixed-nutrient meal ingestion 3 h and 24 h post-exercise. A secondary aim was to measure postprandial substrate metabolism of the meal ingested 3 and 24 h post-exercise.

It was hypothesized that compared to control, prior exercise would increase postprandial muscle microvascular blood flow and prevent high glucose, mixed meal-induced microvascular blood flow impairments 3 h and 24 h post-exercise. It was also hypothesized that prior exercise would lead to greater postprandial fat oxidation and lower carbohydrate oxidation compared to control when the high-glucose mixed nutrient meal was ingested 3 h and 24 h post-exercise.

Methods.

Participants and screening. This study was approved by the Deakin University Human Research Ethics Committee and conformed to the standards set by the *Declaration of Helsinki*, except for registration in a database.. Eight healthy young males completed the current study after providing written informed consent. Screening included a medical history and health questionnaire, the International Physical Activity Questionnaire (IPAQ) short-form, and measurements of height, weight and resting blood pressure. IPAQ scores for participants were calculated in accordance with the current 2005 scoring protocol and are expressed as either low, moderate, or high-levels of weekly physical activity (www.ipaq.ki.se). Participants were excluded if they had a previous history of cardiometabolic disease or smoking, were taking medications or vitamins that may have

affected vascular or glucoregulatory measures, or had musculoskeletal or other conditions that prevented daily activity. Males were recruited to minimize potential confounding effects of sex hormones on glucose metabolism and hemodynamics. Participant characteristics are shown in Table 1. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Eligible participants were invited to undergo a graded cycling exercise test, and three experimental conditions designed to explore the effects of prior exercise on glucoregulatory and macro and microvascular responses to ingestion of a high-glucose mixed-nutrient meal. The meal was ingested at rest after an overnight fast with no prior exercise (control condition), and on separate occasions at 3 h and 24 h after a single bout of aerobic cycling exercise (3 h and 24 h post-exercise conditions). The order of the control and exercise conditions were randomly allocated by block randomization with a minimum 1-week washout period (Figure 1). Participants refrained from moderate to vigorous physical activity (48 h prior), alcohol (24 h prior), and caffeine (12 h) prior to undergoing the experimental testing sessions. Participants completed a 24-h diet diary on the day prior to their first trial, which they then replicated for their subsequent testing conditions. Macro and microvascular blood flow responses to the meal at rest (control trial; n = 10) were previously published (Parker *et al.*, 2020). Data from the eight participants who completed all experimental conditions (i.e., the control trial, and the 3 h and 24 h post-exercise trial) are reported in this study.

Graded Cycling Exercise Test. Prior to commencement of the experimental trials, participants attended the research laboratory and underwent a graded exercise test to volitional exhaustion to determine their maximal aerobic capacity (VO_{2peak}) and power output (W_{max}). The test was performed on a cycle ergometer (Lode Excalibur Sport, Lode, Groningen, The Netherlands) beginning at 50 Watts which was increased by 25 Watts every 3 min for the first three increments, and then increased every 1 min until volitional exhaustion. Heart rate (Polar, USA) and indirect calorimetry (Quark RMR Gas Analyzer, Cosmed, Italy) was measured during the exercise test.

Control condition. Participants arrived in the research laboratory after an overnight fast, a cannula was inserted into an antecubital fossa vein, and a resting venous blood sample was taken (Figure 1). After a 30 min rest period, indirect calorimetry was measured via a silicon

face mask interfaced to a metabolic cart (Quark RMR Gas Analyzer, Cosmed, Italy) for 20 min to determine resting respiratory exchange ratio (RER), energy expenditure (EE) and substrate utilization. After completing indirect calorimetry measures, resting femoral artery and muscle microvascular blood flow were assessed. Participants then underwent a 2-h mixed meal challenge which involved the ingestion of a high-glucose mixed-nutrient meal (10 kcal.kg^{-1}; 45% carbohydrate, 20% protein, and 35% fat) consisting of eggs, cheese and 1.1 g glucose.kg body weight^{-1} (87.6 ± 9.7 g [mean ± SD]) dissolved in 200 ml of water as the only carbohydrate source in the meal. The drink was consumed within 1 min followed by the meal which was consumed within 5 min. Indirect calorimetry and macro and microvascular blood flow were measured at 60 and 120 min postprandial, and venous blood samples taken every 15 min throughout the 120 min postprandial period (Figure 1).

3 h and 24 h post-exercise condition. On a separate day, participants arrived fasted in the research laboratory and a venous blood sample was taken prior to undergoing 60 min of cycling exercise on a cycle ergometer (Lode Excalibur Sport, Lode, Groningen, The Netherlands). Expired gas was measured at 10-min intervals and the workload was adjusted to achieve the target intensity of 70 - 75% VO_{2peak} (73.3 ± 5.0 % VO_{2peak}). A 3-min warm-up and cool-down at 50% VO_{2peak} was performed before and after the 60-min cycling session. After the cool-down, participants rested on a bed for 3 h and then underwent the 2-h mixed meal challenge as described above. Venous blood samples were taken immediately after the exercise session, at 1, 2 and 3 h post-exercise, and throughout the 2-h postprandial period. Indirect calorimetry and macro and microvascular blood flow were measured before meal ingestion (3 h post-exercise), and 60 and 120 min postprandial. Venous blood samples were taken before meal ingestion, and every 15 min throughout the 120 min postprandial period.

On the following day, 24 h after the 60 min cycling exercise session, participants again arrived fasted in the research laboratory and underwent the same 2-h mixed meal challenge. Blood samples, indirect calorimetry, and macro and microvascular blood flow were measured as described above.

Postprandial indirect calorimetry. Energy expenditure and substrate utilization were measured via indirect calorimetry using a face mask interfaced to an 18 mm turbine and metabolic cart designed for measuring resting metabolic rate (Quark RMR system; Cosmed, Albano Laziale, Rome, Italy). The face mask was placed on the participant for a 20-min

period before meal ingestion, and at 40 and 100 min postprandial. The average of the final 10 min sampling period, corresponding to approximately 60 and 120 min postprandial, was used to determine EE and RER. Substrate oxidation rates were calculated using the non-protein respiratory quotient where CHO oxidation rate = $4.585*VCO_2 - 3.226*VO_2$; Fat oxidation rate = $1.695*VO_2 - 1.701*VCO_2$, with VO_2 and VCO_2 expressed in liters per minute (L/min) and oxidation rates in grams per minute (g/min) (Peronnet & Massicotte, 1991).

Femoral artery blood flow. A commercial ultrasound machine and high frequency L12-5 probe (iU22 Philips, Bothell, WA, USA) was used to measure diameter and blood velocity, and to calculate blood flow of the superficial femoral artery. Diameter measurements were conducted using 2D ultrasound and recorded at the peak of the QRS complex using a 3-lead electrocardiograph system. Time-averaged mean arterial blood velocity was assessed by 2D Doppler ultrasound. Arterial diameter and velocity were recorded in triplicate. Femoral artery blood flow (mL/min) was calculated as Πr^2 x mean velocity x 60 min, where radius (r) is cm and mean velocity is cm/s. The probe location and all ultrasound settings were recorded and replicated for each subsequent ultrasound measure.

Muscle microvascular blood flow. Microvascular blood flow was measured via real-time contrast enhanced ultrasound, as previously described (Parker *et al.*, 2020). In brief, 1 ml of DEFINITY® (Lantheus Medical Imaging, Tullamarine, VIC, Australia) contrast agent was added to 19 ml of saline solution (0.9% NaCl) and intravenously infused at a constant rate of 0.68 ml/min. After 5 min of infusion to allow for whole-body contrast agent equilibrium, an L9-3 linear ultrasound transducer was placed in a cross-section over the superficial femoral artery and four 5-s video captures were recorded to measure microsphere concentration within the femoral artery. The ultrasound probe was then relocated to directly over the Vastus Lateralis muscle in a cross-section and the infusion rate was increased to a constant rate of 2.25 ml/min to measure muscle microvascular perfusion. After 4 min of infusion for whole-body equilibrium, four consecutive 45-s video captures were recorded. Each capture was preceded by a high mechanical index flash to disrupt all contrast agent microspheres within the probe line-of-sight to measure microsphere re-appearance kinetics. Settings for mechanical index (0.11 for continuous and 1.30 for flash) and gain (75-76%) were previously optimized in our lab in humans and kept identical for all participants. Ultrasound depth and focus were adjusted on an individual basis in the first trial to ensure that an optimal region

of interest was acquired for muscle microvascular analysis. Probe location and all ultrasound settings were recorded and kept constant within and between conditions for each participant.

Digital images were analyzed using Qlab software (QLAB, Philips Healthcare, Andover, MA, USA). The raw acoustic intensity was background subtracted (0.5 second frame) to eliminate signal from larger vessels and tissue artefacts, as previously done (Russell *et al.*, 2018; Parker *et al.*, 2020). The background subtracted acoustic intensity acquired from the Vastus Lateralis region of interest was then plotted over time and curve fitted using the equation $y = A(1-e^{-\beta(t-tb)})$, where "y" is acoustic intensity, "t" is time, "tb" is the background time, "A" is the plateau of acoustic intensity (a measure of microvascular blood volume), and "β" is the rate constant (a measure of microvascular capillary refilling rate). Microvascular blood flow was calculated by A × β.

Microvascular Blood Flow Normalization. Minor variations in the contrast agent concentration may occur and be influenced by factors such as total body blood volume and body composition. As such, the microsphere concentration within the superficial femoral artery was measured and used to normalize microvascular blood flow data within and between participants, as previously done (Parker *et al.*, 2020). In brief, the acoustic intensity from a region of interest inside the superficial femoral artery was measured and averaged over a 3-second period for all time-points. The plateau acoustic intensity (A value) within and between participants was then expressed and normalized to individual fold changes from the pre-meal (0 min) value in the control condition.

Blood sampling and analysis. Whole blood was analyzed immediately for blood glucose and lactate using an automated analysis system (ABL800 FLEX®; Radiometer Medical, Copenhagen, Denmark). Venous blood was collected from an antecubital vein and collection tubes containing ethylenediaminetetraacetic acid. Blood samples were separated into plasma by centrifugation (10 min at 1800 Relative Centrifugal Force, 4°C) and immediately aliquoted and stored at -80°C until analyzed. Plasma insulin was measured via ELISA assay (ALPCO Diagnostics, Windham, NH, USA) and plasma non-esterified fatty acids (NEFA) were measured via colorimetric assay (NEFA C Wako; Oxoid, Dardilly, France), in duplicate as per the manufacturer's instructions. Area under the time curve (AUC) was calculated using the

trapezoidal rule. AUC is expressed as the total 2 h AUC (0-2 h), and additionally broken down into the 1st (0-1 h) and 2nd (1-2 h) hours of the measured postprandial period.

Statistical analysis. Data was checked for normality and analyzed using GraphPad Prism (v8.4.2). Statistical analysis involved either a one-factor or two-factor repeated measures analysis of variance (ANOVA) with "time" and "condition" as the within-subjects factors. Significant interaction and main effects were explored post-hoc using Fisher's Least Significant Difference test. Statistical analysis was conducted at the 95% level of significance ($p \leq 0.05$). Data are expressed as mean ± SD or median with interquartile range as indicated. Line graphs are presented as mean ± SEM.

Results.

Exercise and post-exercise recovery responses. Main effects of time were detected for heart rate ($p < 0.001$), blood lactate ($p = 0.002$), blood glucose ($p = 0.003$), plasma insulin ($p = 0.008$) and NEFA ($p < 0.001$; Table 2). Heart rate, blood lactate, and plasma NEFA were elevated throughout the 3-h post-exercise recovery period compared to baseline, whereas blood glucose and plasma insulin levels were lowered by exercise.

Microsphere infusion concentration and normalization of muscle blood flow data. No condition ($p = 0.768$), time ($p = 0.705$), or interaction effects ($p = 0.832$) were detected for femoral artery microsphere concentration at the infusion rate of 0.68 ml/min (Figure 2A), indicating that contrast agent infusion and microsphere concentration in the total blood pool were similar within and between participants and experimental conditions. Normalizing muscle microvascular blood flow to the arterial concentration pool resulted in the same outcomes as non-normalized data (data not shown but available from authors on request). As such, normalized data have been presented throughout. The no-exercise control condition postprandial responses (glucose, insulin, femoral artery and microvascular blood flow) represent a subset of the data previously published (Parker *et al.*, 2020).

Muscle microvascular blood flow. Main effects of time ($p < 0.001$) and condition ($p = 0.003$) were detected for muscle microvascular blood volume (Figure 2B). Compared to 0 min (pre-meal), muscle microvascular blood volume decreased by ~21% at 60 min and by ~27% at 120 min postprandial irrespective of the condition ($p = 0.002$ and $p < 0.001$, respectively). Compared to the control condition, microvascular blood volume was elevated by ~54% in

the 3 h post-exercise condition and elevated by ~25% in the 24 h post-exercise condition ($p < 0.001$ and $p = 0.063$, respectively). Microvascular blood volume was elevated by ~23% in the 3 h post-exercise condition compared to the 24 h post-exercise condition ($p = 0.041$). There was no interaction effect ($p = 0.060$).

Main effects of time ($p = 0.001$) and condition ($p = 0.002$) were detected for muscle microvascular blood velocity (Figure 2C). Compared to 0 min (pre-meal), muscle microvascular blood velocity decreased by ~27% at 60 min and by ~33% at 120 min postprandial irrespective of the condition ($p = 0.003$ and $p < 0.001$, respectively). Compared to control, microvascular blood velocity was elevated by ~50% in the 3 h post-exercise condition ($p < 0.001$), but not in the 24 h post-exercise condition ($p = 0.349$). Microvascular blood velocity was elevated by ~35% in the 3 h post-exercise condition compared to the 24 h post-exercise condition ($p = 0.005$). There was no interaction effect ($p = 0.458$).

An interaction effect ($p = 0.005$) was detected for muscle microvascular blood flow (Figure 2D). Compared to 0 min (pre-meal), microvascular blood flow was lower at 60 min and at 120 min postprandial in the control ($p = 0.012$ and $p < 0.001$, respectively), 3 h post-exercise ($p = 0.018$ and $p = 0.002$, respectively), and 24 h post-exercise conditions ($p = 0.005$ and $p = 0.024$, respectively). Microvascular blood flow decreased further from 60 min to 120 min postprandial in the control condition only ($p < 0.001$). Compared to the control condition, microvascular blood flow was elevated in the 3 h post-exercise condition by ~74% at 0 min (pre-meal; $p = 0.004$), by ~112% at 60 min ($p = 0.002$), and by ~223% at 120 min postprandial ($p < 0.001$); and elevated by ~132% at 120 min postprandial in the 24 h post-exercise ($p < 0.001$). Microvascular blood flow was elevated in the 3 h post-exercise condition compared to the 24 h post-exercise condition by ~61% at 0 min and by ~77% at 60 min postprandial ($p = 0.025$ and $p = 0.007$, respectively). Representative images of muscle microvascular perfusion assessed by contrast-enhanced ultrasound are presented in Figure 3.

To determine the extent of the microvascular impairment with the meal and the impact of prior exercise on this impairment, the data was also expressed as percent change from baseline (Table 3). An interaction effect ($p = 0.026$) was detected for muscle microvascular blood flow when expressed as a percent change from baseline (Table 3). Microvascular

blood flow decreased further from 60 min to 120 min postprandial in the control condition only ($p = 0.002$). As such, compared to the control condition impairments in muscle microvascular blood flow at 120 min postprandial occurred to a lesser extent in both the 3 h ($p = 0.006$) and 24 h post-exercise conditions ($p = 0.001$). No effects of time, condition, or interactions were detected for muscle microvascular blood volume ($p = 0.163$, $p = 0.210$, and $p = 0.062$, respectively) and velocity ($p = 0.137$, $p = 0.213$, and $p = 0.085$, respectively).

Heart rate and femoral artery diameter, blood velocity and flow. Main effects of time ($p < 0.001$) and condition ($p < 0.001$) were detected for heart rate (Figure 4A). Compared to baseline, heart rate increased at 60 min and 120 min postprandial irrespective of the condition ($p < 0.001$). Heart rate was elevated in the 3 h post-exercise condition compared to the control and 24 h post-exercise conditions ($p < 0.001$). Heart rate was similar between control and 24 h post-exercise conditions ($p = 0.516$). There was no interaction effect for heart rate ($p = 0.232$)

A main effect of time ($p < 0.001$) was detected for femoral artery diameter (Figure 4B). Compared to baseline, the femoral artery diameter increased at 60 min and 120 min postprandial irrespective of the condition ($p = 0.004$ and $p < 0.001$, respectively). There were no condition ($p = 0.326$) or interaction effects ($p = 0.786$).

Analysis of femoral artery blood velocity and flow revealed main effects of time (both $p < 0.001$) and condition ($p = 0.008$ and $p = 0.006$, respectively) (Figure 4C-D). Compared to baseline, femoral artery blood velocity and flow increased at 60 min and 120 min postprandial irrespective of the condition (all $p < 0.001$). Femoral artery blood velocity and flow were elevated by ~21 - 29% in the 3 h post-exercise condition compared to the control (velocity, $p = 0.003$; flow, $p = 0.002$) and 24 h post-exercise conditions (velocity, $p = 0.012$; flow, $p = 0.017$). There were no interaction effects for femoral blood velocity ($p = 0.368$) and flow ($p = 0.353$).

Glucose, insulin, NEFA and lactate. An interaction effect ($p < 0.001$) was detected for blood glucose (Figure 5A). Blood glucose was elevated above baseline at 15 - 60 min postprandial in all conditions (all $p < 0.05$), remained elevated above baseline throughout the entire 120 min postprandial period in the 3 h post-exercise condition (all $p < 0.05$), and decreased below baseline at 105 and 120 min postprandial in the 24 h post-exercise condition (all $p <$

0.05). Postprandial blood glucose was greater in the 3 h post-exercise condition at 45 - 120 min postprandial compared to the same time-points in the control and 24 h post-exercise conditions (all $p < 0.05$); and lower in the 3 h post-exercise condition at 15 min postprandial compared to the 24 h post-exercise condition ($p = 0.066$). Main condition effects were detected for glucose AUC for the total 2-h ($p = 0.045$) and 2nd postprandial hour ($p = 0.021$; Figure 5B). Glucose AUC for both the total 2-h and 2nd postprandial hour were greater in the 3 h post-exercise condition compared to the control ($p = 0.022$ and $p = 0.013$, respectively) and 24 h post-exercise condition ($p = 0.043$ and $p = 0.015$, respectively).

An interaction effect ($p = 0.008$) was detected for plasma insulin (Figure 5C). Plasma insulin was elevated above baseline at 15 min postprandial and remained elevated throughout the entire 120 min postprandial period in all conditions (all $p < 0.001$). Plasma insulin was lower in the 3 h post-exercise condition prior to meal ingestion (0 min), and at 15 and 30 min postprandial compared to the same time-points in the control and 24 h post-exercise conditions (all $p < 0.05$); and lower in the 24 h post-exercise condition at 120 min postprandial compared to control ($p = 0.085$) and 3 h post-exercise conditions ($p = 0.074$). An effect of condition was detected for insulin AUC for the 1st postprandial hour ($p = 0.018$), whereas insulin AUC for the total 2-hour ($p = 0.637$) and 2nd postprandial hour ($p = 0.794$) were similar between conditions (Figure 5D). Insulin AUC for the 1st postprandial hour was lower in the 3 h post-exercise condition compared to the control ($p = 0.018$) and 24 h post-exercise conditions ($p = 0.007$).

An interaction effect ($p < 0.001$) was detected for plasma NEFA (Figure 6A). Plasma NEFA was greater in the 3 h post-exercise condition compared to the control and 24 h post-exercise conditions at 0 min (pre-meal), and 15 - 75 min postprandial (all $p < 0.05$). Plasma NEFA was also greater in the 24 h post-exercise condition compared to the control condition at 15, 30, 45, and 120 min postprandial (all $p < 0.05$). Plasma NEFA decreased below pre-meal levels (0 min) at 15 min postprandial in the control ($p < 0.001$) and 24 h post-exercise conditions ($p = 0.068$), and at 30 min in the 3 h post-exercise condition ($p < 0.05$), and then remained suppressed throughout the 120 min postprandial period in all conditions (all $p < 0.001$). Main condition effects were detected for NEFA AUC for the total 2-hour ($p = 0.005$) and 1st postprandial hour ($p = 0.006$; Figure 6B). NEFA AUC for both the total 2-hour and 1st postprandial hour was greater in the 3 h post-exercise condition compared to control ($p =$

0.002 and p = 0.010, respectively) and 24 h post-exercise conditions (p = 0.009 and p = 0.007, respectively). NEFA AUC for the 1st postprandial hour was elevated in the 24 h post-exercise condition compared to the control condition (p = 0.090).

An interaction effect (p < 0.001) was detected for blood lactate (Figure 6C). Compared to baseline, blood lactate decreased at 15 min postprandial in the control and 3 h post-exercise conditions (p < 0.001 and p = 0.035, respectively), and then increased and remained elevated throughout the 120 min postprandial period in all conditions (all p < 0.01). Blood lactate was lower at baseline in the 24 h post-exercise condition compared to the control and 3 h post-exercise conditions (p < 0.001); higher at 15 min postprandial in the 3 h post-exercise condition compared to the control and 24 h post-exercise conditions (p < 0.001 and p = 0.002, respectively); higher at 30 min in the 3 h post-exercise condition compared to the 24 h post-exercise condition (p = 0.069); and lower at 60 min postprandial (p = 0.049) and 75 min postprandial (p = 0.058) in the 3 h post-exercise condition compared to control. No condition effects were detected for blood lactate for total 2-hour (p = 0.831), 1st postprandial hour (p = 0.465), and 2nd postprandial hour AUC measures (p = 0.376; Figure 6D).

Indirect calorimetry. Main effects of time (p < 0.001) and condition (p < 0.001) were detected for postprandial RER (Figure 7A). Compared to 0 min (pre-meal), RER was elevated at 60 min and 120 min postprandial irrespective of the condition (p < 0.001). Values for RER were lower in the 3 h post-exercise condition compared to the control and 24 h post-exercise conditions (p < 0.001), and were lower in the 24 h post-exercise condition compared to the control condition (p = 0.032). No interaction effect was detected for RER (p = 0.384).

An interaction effect (p = 0.036) was detected for postprandial EE (Figure 7B). Compared to the control condition, EE was elevated at 0 min (pre-meal) in the 3 h post-exercise and 24 h post-exercise conditions (p = 0.002 and p = 0.058, respectively). Compared to 0 min (pre-meal), EE was elevated at 60 min and 120 min postprandial in the control and 24 h post-exercise conditions (all p < 0.001).

An interaction effect (p = 0.002) was detected for postprandial carbohydrate (CHO) oxidation (Figure 7C). Compared to 0 min (pre-meal), CHO oxidation was elevated at 60 min

and 120 min postprandial in all conditions (all $p < 0.001$). A further increase in CHO oxidation was observed from 60 min to 120 min postprandial in the 3 h post-exercise condition only ($p = 0.057$). CHO oxidation was lower in the 3 h post-exercise condition at all timepoints compared to both control and 24 h post-exercise conditions (all $p < 0.001$). CHO oxidation was also lower in the 24 h post-exercise condition at 0 min ($p = 0.041$) and 120 min postprandial ($p = 0.041$) compared to control.

Main effects of time ($p = 0.008$) and condition ($p < 0.001$) were detected for postprandial fat oxidation (Figure 7D). Compared to 0 min (pre-meal), fat oxidation was lower at 60 min ($p = 0.008$) and 120 min ($p = 0.005$) postprandial irrespective of the condition. Fat oxidation was elevated in the 3 h ($p < 0.001$) and 24 h post-exercise conditions ($p = 0.008$) compared to control, and was greater in the 3 h post-exercise condition compared to the 24 h post-exercise condition ($p < 0.001$). No interaction effect was detected for fat oxidation ($p = 0.092$).

Discussion. We provide novel evidence that prior cycling exercise in healthy young men increases skeletal muscle microvascular blood flow and femoral arterial blood flow for up to at least 3 h post-exercise. Despite a high-glucose meal-induced decrease in all conditions, muscle microvascular blood flow remained almost 2-fold higher above control-meal levels throughout the 2-h postprandial period when the meal was ingested 3 h post-exercise, supporting our hypothesis. Furthermore, the meal-induced impairments in muscle microvascular blood flow observed at 120 min postprandial in the control condition occurred to a lesser extent in the 3 h and 24 h post-exercise conditions. Postprandial blood glucose, non-esterified fatty acids (NEFA), and fat oxidation were substantially elevated, whereas the time-course of the insulin response to the meal was delayed in the 3 h post-exercise condition. We conclude that prior exercise acutely enhances muscle microvascular blood flow, while elevating postprandial glucose, fat oxidation, and NEFA levels following high-glucose mixed meal ingestion.

Previous research has reported elevated muscle and limb arterial blood flow in the hours following treadmill and one-legged knee-extensor exercise (Durham *et al.*, 2010; Sjoberg *et al.*, 2017). In support, we provide new evidence that 1 h of cycling exercise at ~75% VO_{2peak} leads to elevated muscle microvascular blood flow (~74% increase above basal resting

levels) and femoral arterial blood flow for up to at least 3 h post-exercise. Skeletal muscle microvascular blood flow is also reported to increase in response to insulin-infusion and following ingestion of a balanced (low-moderate glucose load) mixed-nutrient meal (Vincent *et al.*, 2006; Keske *et al.*, 2009; Liu *et al.*, 2009; Sjoberg *et al.*, 2017; Russell *et al.*, 2018). This coordinated increase in microvascular hemodynamics facilitates substrate metabolism via the delivery of nutrients and hormones to, and eventual uptake by, target cells such as the myocyte (Keske *et al.*, 2016). Here we show for the first time that compared to a no-exercise control meal, muscle microvascular blood flow is elevated at 0 min (pre-meal), and 60 and 120 min postprandial when a high-glucose mixed nutrient meal is ingested 3 h post-exercise, and elevated at 120 min postprandial when the meal is ingested 24 h post-exercise. These findings are important, as the insulin sensitizing effects of acute exercise are well-known to be observed for up to 24 - 48 h post-exercise (Mikines *et al.*, 1988; Brestoff *et al.*, 2009; Ortega *et al.*, 2015; Parker *et al.*, 2016a; Parker *et al.*, 2017; Morrison *et al.*, 2018). In addition to the well-characterized increase in distal insulin signaling in skeletal muscle after exercise (Parker *et al.*, 2016b; Sjoberg *et al.*, 2017; Parker *et al.*, 2019), research by Sjoberg *et al.* (2017) established the importance of muscle microvascular blood flow for enhancing post-exercise insulin-mediated glucose disposal when measured by euglycemic hyperinsulinemic clamp. Similar to the current findings, muscle microvascular blood flow was elevated 4 h after one-legged knee exercise, and remained elevated during euglycemic hyperinsulinemic conditions compared to the rest-control leg (Sjoberg *et al.*, 2017). Importantly, co-infusion of the nitric oxide synthase (NOS) inhibitor (L-NMMA) substantially blunted limb arterial and muscle microvascular blood flow alongside the suppression of glucose uptake in the exercised leg. In contrast, distal insulin signaling in skeletal muscle was largely unaffected by L-NMMA infusion, suggesting that both muscle microvascular blood flow and insulin signaling are required for the post-exercise increase in glucose disposal (Sjoberg *et al.*, 2017). These findings, combined with rodent research (Vincent *et al.*, 2003; Vincent *et al.*, 2004), support that microvascular hemodynamics in muscle play a critical role in insulin-stimulated glucose disposal and occur at least in part via an NO dependent pathway. Future research will be required to confirm this in the current high-glucose mixed-nutrient meal human model.

In addition to elevated postprandial muscle microvascular blood flow 3 h post-exercise, we report that postprandial blood glucose was elevated and the early time-course postprandial insulin response was delayed. These findings support previous reports from studies that have explored post-exercise glucoregulatory responses to a meal or glucose ingestion (Krzentowski *et al.*, 1982; Rose *et al.*, 2001; Knudsen *et al.*, 2014; Lin & Borer, 2016; Varsamis *et al.*, 2018). For example, postprandial plasma glucose is greater in men with normal glucose tolerance, and similar in men with impaired glucose tolerance or type 2 diabetes when an oral glucose tolerance test is performed immediately after cycling exercise (1 h at 50% W_{peak}) (Knudsen *et al.*, 2014). Likewise, Rose *et al.* (2001) reported greater postprandial glucose excursions in healthy trained men when glucose (75 g) was ingested 30 min after cycling exercise (~55 min at 70% VO_{2peak}). Increased postprandial glucose excursions after exercise are suggested to occur through increased appearance of exogenous glucose in an effort to replenish muscle glycogen stores (Maehlum *et al.*, 1978; Rose *et al.*, 2001; Knudsen *et al.*, 2014). In support, high-glucose ingestion (100 g) 15 minutes after exhaustive exercise leads to a 50 - 300% increase in splanchnic glucose output (Maehlum *et al.*, 1978), whereas the contribution of endogenous glucose in the post-exercise postprandial period is suggested to be minimal (Rose *et al.*, 2001; Knudsen *et al.*, 2014). Importantly, research using stable isotopic tracers have shown that whole-body glucose disposal is still enhanced with prior exercise despite elevated blood glucose levels (Rose *et al.*, 2001; Knudsen *et al.*, 2014). It is therefore possible, and even likely that whole-body glucose disposal in the current study was also increased with prior exercise. However, future studies using tracers to directly link postprandial glucose flux to postprandial muscle microvascular blood flow after exercise will be required to address this.

Acute hyperglycemia and hyperlipidemia can lead to a transient impairment in vascular endothelial function and/or blood flow (Beckman *et al.*, 2001; Tripathy *et al.*, 2003; Liu *et al.*, 2009; Tyldum *et al.*, 2009; Loader *et al.*, 2017). We and others have previously reported that ingestion of a high-glucose load (~50 - 85 g of glucose) in healthy young adults leads to impaired muscle microvascular blood flow despite increased limb arterial blood flow (Russell *et al.*, 2018; Parker *et al.*, 2020). We provide new evidence that this divergent response between limb arterial and muscle microvascular blood flow persists even when high-glucose is ingested 3 h and 24 h post-exercise. The divergent femoral arterial and

muscle microvascular blood flow responses to the meal are unclear, but may reflect redistribution of blood flow from the muscle microvasculature to the skin, adipose tissue, tendons, connective tissue, and/or bone (Clark *et al.*, 2000; Clark, 2008; Russell *et al.*, 2017). In addition to the approximate two-fold elevation in basal and postprandial microvascular blood flow 3 hours after exercise compared to control, our findings indicate that prior exercise partially mitigates the magnitude of impairment in muscle microvascular blood flow induced by high-glucose mixed-nutrient meal ingestion. These findings reflect similar reports that prior cycling exercise (30 min at 75% HR_{peak}) can partially prevent impaired forearm cutaneous microvascular blood flow, as estimated by laser speckle contrast imaging and post-occlusive reactive hyperemia, when a high-carbohydrate drink (1 g/kg bodyweight sucrose) is ingested immediately after exercise (Varsamis *et al.*, 2018). Furthermore, we observed a similar attenuation in the impairment of microvascular blood flow in the 24 h post-exercise condition at the 120 min postprandial timepoint. These findings suggest that the effects of acute exercise on muscle microvascular blood flow persist well after the cessation of exercise. This may prove to be important for ageing populations (Hildebrandt *et al.*, 2017) and diseases characterized by microvascular dysfunction including type 2 diabetes (Sacre *et al.*, 2015), heart failure (Dhakal *et al.*, 2015; Upadhya *et al.*, 2015), and peripheral arterial disease (Kundi *et al.*, 2017). Regular exercise training has been shown to increase skeletal muscle capillary density and eNOS signaling in obese individuals alongside improved glycemic control (Cocks *et al.*, 2016; Scott *et al.*, 2019), and increases postprandial microvascular blood flow responses to an oral glucose challenge (50 g glucose) in T2D patients (Russell *et al.*, 2017), health benefits likely occurring through a combination of both acute and chronic exercise-induced vascular adaptations. Future research investigating whether different exercise modes, intensities and/or volume can provide even greater protection against acute hyperglycemia-induced vascular dysfunction is warranted.

In support of our hypothesis we report that postprandial NEFA levels and fat oxidation were greater, and carbohydrate oxidation lower, in the 3 h post-exercise condition when compared to the control condition. This observation also occurred in the 24 h post-exercise condition, albeit to a lesser extent. These findings support previous reports of elevated post-exercise NEFA, increased postprandial glucose excursions, a delayed postprandial insulin response, and increased fat oxidation when glucose (100 g of ^{13}C-glucose) was ingested

after prolonged exercise in healthy adults (3 h treadmill exercise at 50% VO_{2max}) (Krzentowski *et al.*, 1982). Although elevated post-exercise NEFA levels are a common observation (Krzentowski *et al.*, 1982; Delmas-Beauvieux *et al.*, 1999; Rose *et al.*, 2001; Long *et al.*, 2008; Lin & Borer, 2016), NEFA levels are generally suppressed to control levels within 30 min of meal ingestion and therefore suggested to play only a minor role in elevating post-exercise postprandial glycemia (Krzentowski *et al.*, 1982; Rose *et al.*, 2001; Long *et al.*, 2008). Nevertheless, in the current study there was a progressive increase in NEFA levels throughout the 3 h post-exercise recovery period which remained elevated for at least 75 min throughout the postprandial period. This may be a potential effect of the prolonged 3 h post-exercise fasting period in the current study versus glucose ingested immediately or 30 min post-exercise in other studies (Krzentowski *et al.*, 1982; Rose *et al.*, 2001). We also detected a shift from carbohydrate oxidation towards greater fat oxidation during the post-exercise recovery period which, like others have reported (Bielinski *et al.*, 1985; Folch *et al.*, 2001; Marion-Latard *et al.*, 2003), persisted throughout the post-exercise postprandial period. Elevated post-exercise lipid oxidation is a well-documented phenomena reflecting substrate competition, likely through the Randle "glucose-fatty acid cycle" (Randle, 1998), which can lead to reduced post-exercise exogenous and total glucose oxidation (Krzentowski *et al.*, 1982; Bielinski *et al.*, 1985; Randle, 1998; Folch *et al.*, 2001). Combined with reports of increased glucose appearance and the favoring of splanchnic glucose release for post-exercise muscle glycogen replenishment (Maehlum *et al.*, 1978; Hamilton *et al.*, 1996; Rose *et al.*, 2001; Knudsen *et al.*, 2014), it is likely that a combination of substrate competition and hormonal factors are responsible for the observed increase in post-exercise glycemia.

Postprandial blood glucose and insulin were similar between the 24 h post-exercise and no-exercise control conditions. This contradicts previous reports of improved insulin sensitivity in the days following acute exercise in healthy individuals (Mikines *et al.*, 1988; Brestoff *et al.*, 2009; Ortega *et al.*, 2015; Morrison *et al.*, 2018). However, Bogardus *et al.* (1983) reported a similar finding where the insulin-sensitizing effects the day after exercise are absent when participants are fed 100 g of glucose 3 h post-exercise. In light of previous findings (Bogardus *et al.*, 1983), the high-glucose ingested 3 h post-exercise in the current study may have led to faster and more complete glycogen replenishment compared to other

studies where habitual meal regimes are resumed after exercise (Morrison *et al.*, 2018). Additionally, and in contrast to others (Ortega *et al.*, 2015; Morrison *et al.*, 2018), we also observed an elevation in postprandial NEFA and fat oxidation 24 h post-exercise, a possible result of the preceding 3 h post-exercise trial which may have also precluded improvements in 24 h postprandial glucose and insulin.

Limitations: This study recruited males to avoid the potential confounding effects of sex on hemodynamics and postprandial glycemic control (Huxley & Kemp, 2018; Yardley *et al.*, 2018). As such, future research is required to confirm these findings in females. A strength of the current study is the use of a high-glucose mixed nutrient meal to investigate how prior exercise influences postprandial microvascular blood flow. Our findings provide new evidence that postprandial microvascular blood flow is influenced by prior exercise, and supports the growing consensus that glucoregulatory function is dynamically altered in response to exercise and oral macronutrient ingestion (Rose *et al.*, 2001; Knudsen *et al.*, 2014; Lin & Borer, 2016; Varsamis *et al.*, 2018). However, in the absence of tracers to directly measure postprandial glucose flux we are unable to directly draw conclusions between post-exercise muscle hemodynamics and glucose disposal. Future research using stable isotope tracers will be required to confirm the physiological role that increased post-exercise muscle microvascular blood flow has on postprandial glucose flux. The participants in the current study were self-reported to be untrained but recreationally active, ranging from low to high levels of weekly physical activity. Training status has been reported to influence postprandial microvascular blood flow responses to high-glucose ingestion in T2D patients (Russell *et al.*, 2017). However, the influence of training status in healthy individuals is unknown. Future research will be required to investigate and compare postprandial muscle microvascular responses in well-trained, recreationally active, and sedentary healthy individuals.

Finally, our test meal involved both liquid (glucose solution) and solid phase components (eggs and cheese), and the calorie content was created relative to body weight (10 kcal.kg^{-1}; average of 795 kcal [3326 kj]). In contrast, other studies exploring postprandial muscle microvascular perfusion have used liquid test meals (both glucose and mixed-nutrient meals) with a much lower set calorie content (200 - 480 kcal [837 – 2008 kj] (Vincent *et al.*, 2006; Keske *et al.*, 2009; Liu *et al.*, 2009; Russell *et al.*, 2017; Russell *et al.*, 2018)). It is

possible that differences in meal phase (liquid versus solid) and calorie content (low versus high) could affect postprandial muscle microvascular responses, as these factors are reported to influence postprandial heart rate, cardiac output, total limb blood flow, and metabolic rate (Sidery & Macdonald, 1994; Habas & Macdonald, 1998). Nevertheless, our findings support that muscle microvascular impairments to a high-glucose solid-liquid mixed-nutrient meal lead to similar impairments to that of high-glucose liquid only ingestion (Russell *et al.*, 2018). Further research exploring and comparing the phase (liquid versus solid), the calorie content, and the composition of the meal, and the subsequent magnitude and time-course effects on muscle microvascular perfusion are warranted.

Conclusions. We provide novel evidence that postprandial muscle microvascular blood flow is elevated when moderate-intensity aerobic cycling exercise is performed 3 h prior to ingestion of a high-glucose mixed meal. This occurs despite a meal-induced decrease in muscle microvascular blood flow being observed in all conditions. Furthermore, the high-glucose induced impairment in muscle microvascular blood flow at 120 min postprandial was partially mitigated when the meal was ingested 3 h and 24 h post-exercise. We also provide evidence that postprandial glucose excursions, NEFA and fat oxidation are substantially elevated, and the time-course of the insulin response to the meal delayed, when a high-glucose mixed meal is ingested 3 h post-exercise. The effects of acute exercise on muscle microvascular blood flow persist well after the cessation of exercise which may be beneficial for conditions characterized by microvascular and glycemic dysfunction.

Translational Perspective

Microvascular blood flow along with cardiac output and large artery blood flow play an important role in postprandial metabolism by facilitating the delivery of nutrients and hormones (e.g., insulin and glucose) to peripheral tissues including skeletal muscle. Populations characterized by insulin resistance commonly exhibit vascular dysfunction including impaired skeletal muscle microvascular blood flow responses to meal ingestion. However, this microvascular impairment in skeletal muscle is also observed in healthy adults after the consumption of a high-glucose meal which persists throughout the 2-hour postprandial period. Our findings show that a single session of exercise in healthy adults almost doubles skeletal muscle microvascular blood flow several hours after exercise which persists throughout the postprandial period following high-glucose ingestion. Furthermore,

some of the impairments in skeletal muscle microvascular blood flow elicited by high-glucose ingestion are mitigated when the meal is ingested 3 and 24 hours after exercise. These observations suggest that a single session of exercise has lasting effects on muscle microvascular blood flow which may prove to be beneficial during conditions that elevate blood glucose levels or cause microvascular dysfunction.

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Tables

Table 1. Participant characteristics.

	Mean ± SD *Median (IQR)*
Age (yr)	28 ± 4 28 (25, 30)
Height (cm)	180.1 ± 7.7 180.0 (175.8, 184.0)
Weight (kg)	79.5 ± 9.1 79.3 (70.8, 87.5)
Body Mass Index (kg/m^2)	24.5 ± 1.5 24.3 (23.6, 25.5)
Self-reported Weekly Physical Activity Level (IPAQ)	Low (*n* = 2) Moderate (*n* = 2) High (*n* = 4)
Fasting Plasma Insulin (µU/mL)	4.9 ± 1.9 4.4 (3.8, 6.1)
Fasting Blood Glucose (mmol/L)	4.7 ± 0.2 4.7 (4.5, 4.9)
Resting Systolic Blood Pressure (mmHg)	121 ± 6 121 (117, 126)
Resting Diastolic Blood Pressure (mmHg)	76 ± 4 76 (72, 79)
VO_{2peak} (ml/kg/min)	35.6 ± 8.0 37.9 (25.9, 43.3)
VO_{2peak} (ml/min)	2807 ± 650 2992 (2166, 3363)

W_{max} *(watts)*	272 ± 57
	288 (206, 319)
HR_{peak} *(BPM)*	183 ± 11
	186 (174, 192)

Data are expressed as means ± SD, and the median (interquartile range). *n* = 8 participants. VO_{2peak} and W_{max} are the maximal calculated workload and peak oxygen consumption obtained from the graded exercise test. BPM, beats per minute. IPAQ, international physical activity questionnaire.

Table 2. Post-exercise heart rate and blood responses to aerobic cycling exercise.

	Baseline	*Immediately post-exercise*	*1 h post-exercise*	*2 h post-exercise*	*3 h post-exercise*	*One-way ANOVA*
Heart Rate (BPM)	56 ± 8	**145 ± 22 ***	**72 ± 11 ***	**68 ± 12 ***	**59 ± 10 ***	**p < 0.001**
	56 (50, 64)	143 (130, 167)	73 (60, 82)	68 (56, 81)	56 (52, 69)	
		p < 0.001	p < 0.001	p < 0.001	p = 0.045	
Lactate (mmol/L)	0.69 ± 0.08	**3.56 ± 1.61 ***	**1.00 ± 0.15 ***	**0.88 ± 0.17 ***	**0.86 ± 0.18 ***	**p = 0.002**
	0.70 (0.60, 0.78)	3.70 (2.10, 4.75)	1.05 (0.83, 1.10)	0.85 (0.73, 0.98)	0.85 (0.70, 0.98)	
		p = 0.001	p < 0.001	p = 0.004	p = 0.009	
Glucose (mmol/L)	4.9 ± 0.4	**4.4 ± 0.5 ***	**4.5 ± 0.4 ***	**4.7 ± 0.6 ***	**4.7 ± 0.6 ***	**p = 0.003**
	4.9 (4.7, 5.1)	4.4 (4.1, 4.6)	4.6 (4.4, 4.8)	4.8 (4.4, 4.8)	4.8 (4.6, 4.8)	
		p < 0.001	p = 0.002	p = 0.034	p = 0.034	
Insulin (µU/mL)	4.99 ± 1.94	**2.78 ± 2.36 ***	**3.66 ± 2.03 ***	4.35 ± 2.12	4.11 ± 2.33	**p = 0.008**
	4.78 (3.20, 6.18)	2.49 (1.66, 3.43)	3.13(1.89, 5.15)	3.95 (2.96, 6.44)	3.17 (2.30, 6.05)	
		p < 0.001	p = 0.026	p = 0.271	p = 0.130	
NEFA (mmol/L)	0.15 ± 0.06	0.16 ± 0.05	**0.21 ± 0.09 ***	**0.27 ± 0.10 ***	**0.28 ± 0.13 ***	**p < 0.001**
	0.13 (0.11, 0.21)	0.15 (0.11, 0.22)	0.21 (0.13, 0.25)	0.24 (0.20, 0.29)	0.25 (0.19, 0.35)	
		p = 0.661	p = 0.047	p < 0.001	p < 0.001	

Data are expressed as means ± SD, and the median (interquartile range). n = 8 participants. Data were analyzed using a one-way (time) repeated measures ANOVA. Significant main effects were explored using Fishers LSD test. ***** Bolded values highlight significant differences compared to baseline. The p-values in columns represent comparisons to baseline.

Table 3. Percent change from baseline in muscle microvascular blood flow following high-glucose mixed-nutrient meal ingestion on a rest-control day, and 3 and 24 h post-exercise.

	60 min Postprandial (% change from baseline)	*120 min postprandial (% change from baseline)*	*Two-way ANOVA*		
Microvascular blood volume			Time p = 0.163	Condition p = 0.210	Interaction p = 0.062
Control	-22.5 ± 25.6 -23.5 (-50.0, 2.5)	-36.8 ± 28.2 -37.8 (-60.1, -31.0)	N/A (no post-hoc comparisons conducted)		
3 h post-exercise	-18.5 ± 19.2 -13.3 (-39.7, -0.3)	-33.2 ± 22.6 -34.7 (-47.9, -17.8)			
24 h post-exercise	-14.7 ± 28.5 -17.9 (-33.9, 0.8)	-4.7 ± 25.9 -13.0 (-26.5, 24.3)			
Microvascular blood velocity			Time p = 0.137	Condition p = 0.213	Interaction p = 0.085
Control	-20.8 ± 24.9 -23.0 (-44.6, 5.0)	-53.0 ± 27.7 -54.0 (-81.7, -25.7)	N/A (no post-hoc comparisons conducted)		
3 h post-exercise	-20.7 ± 34.8 -28.3 (-48.0, 18.6)	-18.0 ± 30.6 -17.3 (-48.7, 14.4)			
24 h post-exercise	-29.2 ± 30.8 -30.0 (-51.8, -9.47)	-30.8 ± 23.9 -33.3 (-46.9, -23.7)			
Microvascular blood flow			Time p = 0.042	Condition p = 0.062	Interaction p = 0.026
Control	-38.2 ± 31.1 -46.4 (-60.1, -18.7)	**-70.7 ± 21.6 *** -76.1 (-91.3, -47.8)	<u>60 min vs 120 min:</u> Con, p = 0.002	<u>60 min:</u> Con vs 3 h, p = 0.912	
3 h post-exercise	-37.1 ± 27.4 -42.1 (-53.2, -24.8)	**-44.4 ± 26.6 †** -42.7 (-63.8, -30.7)	3 h, p = 0.544	Con vs 24 h, p =	

24 h post-exercise	-44.1 ± 22.4 -55.8 (-58.9, -22.4)	**-36.2 ± 21.6 †** -46.4 (-53.9, -15.3)	24 h, p = 0.654	0.863 3 h vs 24 h, p = 0.778 <u>120 min:</u> Con vs 3 h, p = 0.006 Con vs 24 h, p = 0.001 3 h vs 24 h, p = 0.441

Data are expressed as means ± SD, and the median (interquartile range). n = 8 participants. Data was analyzed using a two-way ANOVA with "time" (postprandial timepoint) and "condition" (Control, 3 h post-exercise, and 24 h post-exercise) as the within-subjects factors. Significant main effects were explored using Fishers LSD test. *p ≤ 0.05 compared to 60 min postprandial. **†** = p ≤ 0.05 compared to the same timepoint in the control condition. Bolded values highlight significant differences.

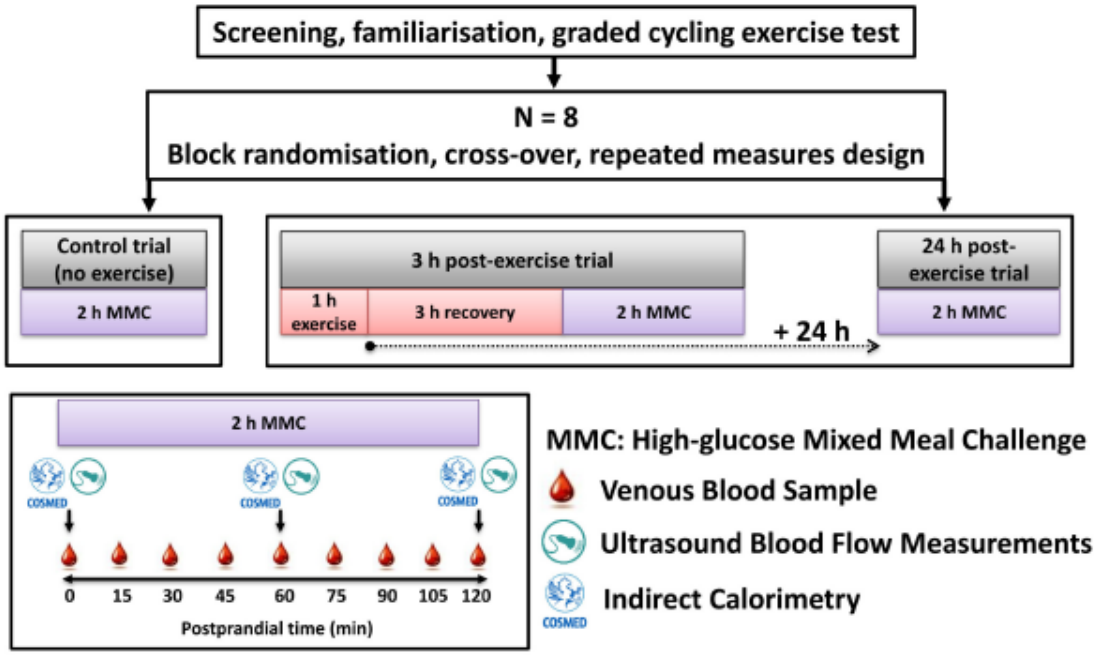

Figure 1. Overview of the randomized crossover study design. Eight young healthy males ingested a high-glucose mixed nutrient meal at rest, and on a separate occasion at 3 h and 24 h after 1 h of moderate-intensity cycling exercise. Metabolic and vascular responses to the meal were measured throughout the postprandial period.

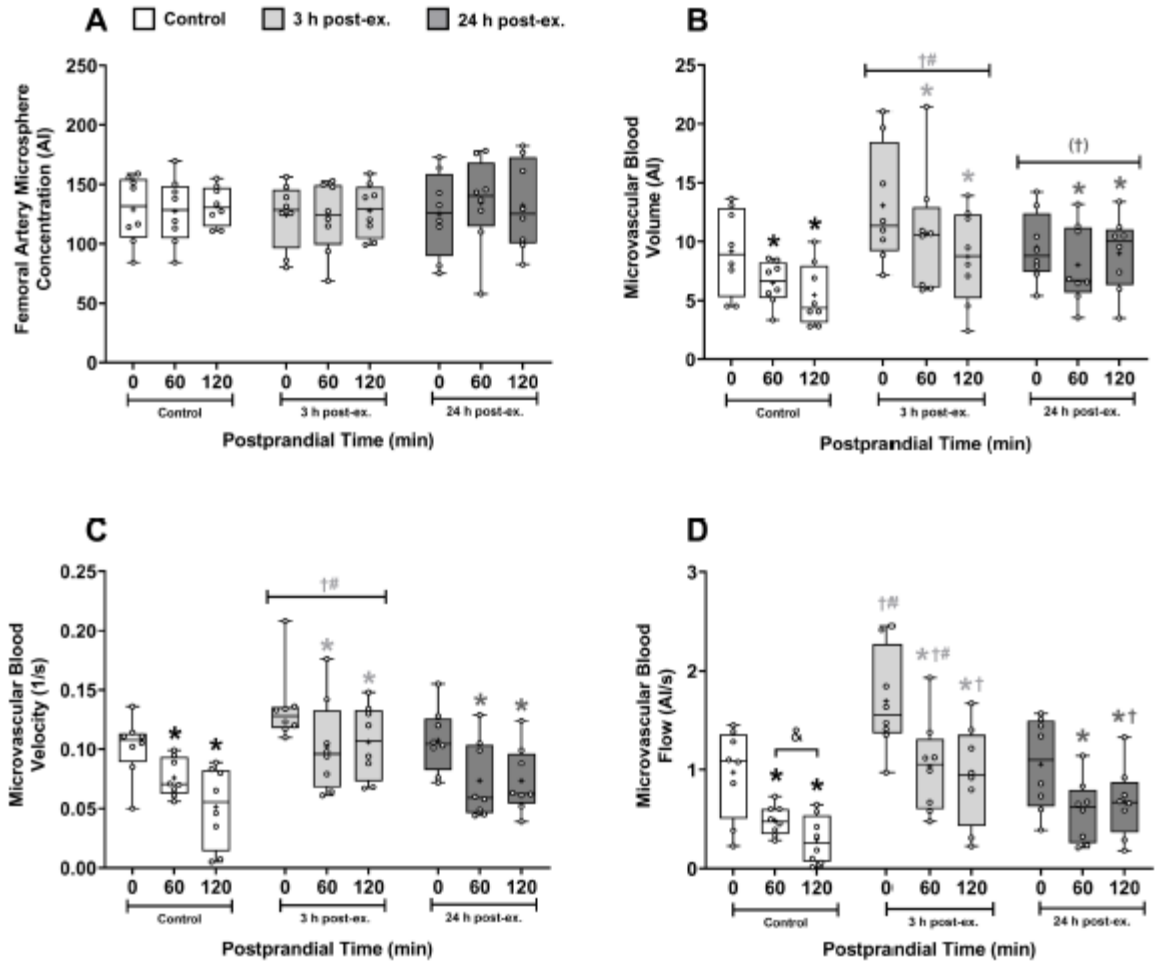

Figure 2. Femoral arterial microsphere concentration (A), and the effects of prior exercise on postprandial muscle microvascular blood volume (B), velocity (C) and flow (D). Data was analyzed using a two-way ANOVA with "time" (postprandial timepoint) and "condition" (Control, 3 h post-exercise, and 24 h post-exercise) as the within-subjects factors. Bar graphs are presented as Box and Whisker plots. The Box represents the interquartile range alongside the median (line) and mean (plus symbol). The Whiskers represent the minimum and maximum range of the data. n = 8 participants. * = $p \leq 0.05$ compared to 0 min (pre-meal) within that condition. † = $p \leq 0.05$ compared to the same timepoint in the control trial. # = $p \leq 0.05$ compared to the same timepoint in the 24 h post-exercise trial. & = $p \leq 0.05$ between the indicated timepoints. Symbols in parenthesis represent $p \leq 0.10$.

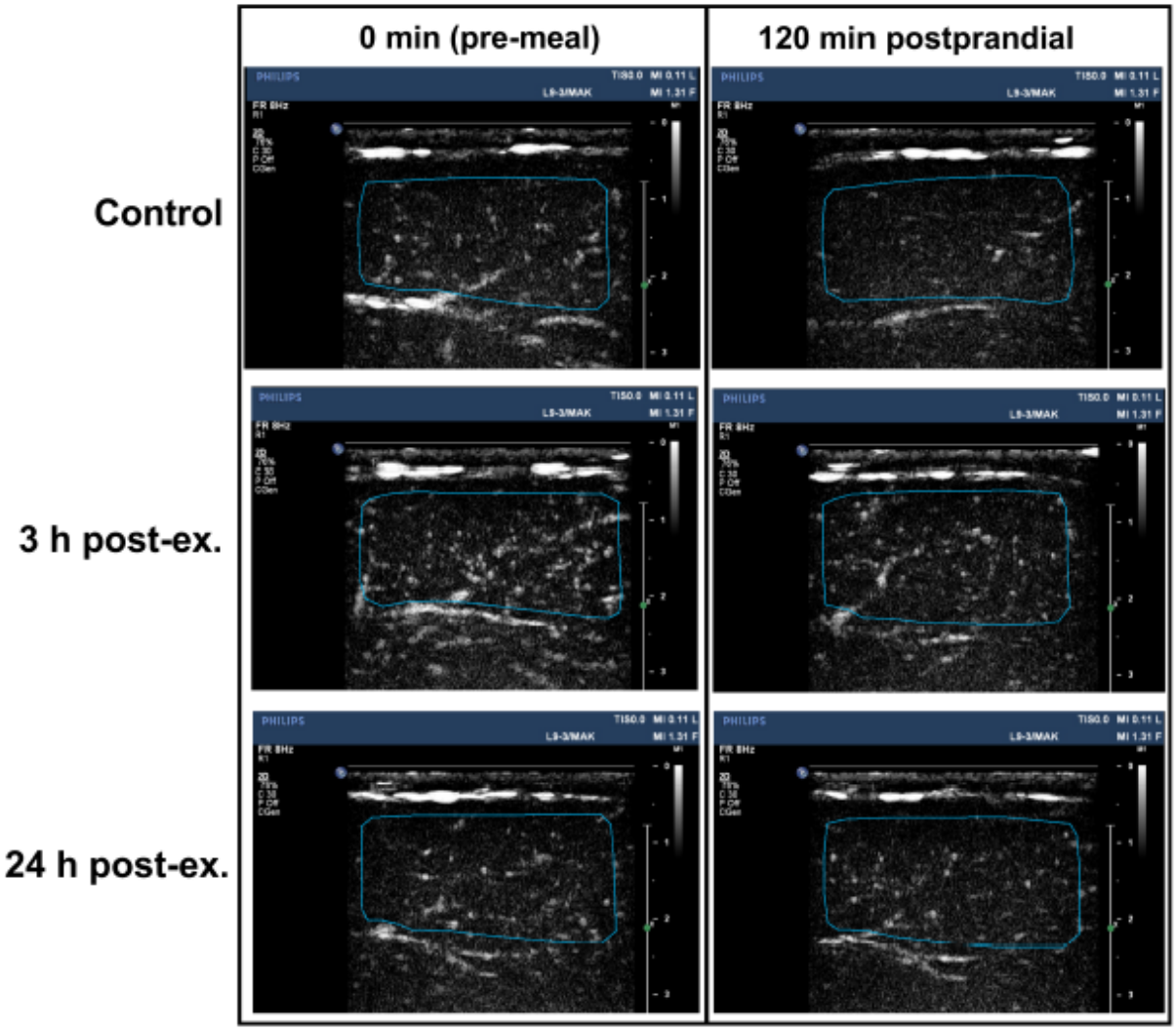

Figure 3. Representative contrast enhanced ultrasound images of muscle microvascular perfusion in a single participant following high-glucose mixed meal ingestion at rest (control), and 3 h and 24 h post-exercise. The rectangle indicates the region of interest that was used to measure the acoustic intensity elicited by circulating microspheres within the muscle capillary network of the Vastus Lateralis muscle in cross-section. The white opacification represents the echogenic microspheres circulating within the muscle microvasculature. Static images provide only a visual representation of microvascular blood volume or capillary recruitment, but not flow dynamics such as velocity or total blood flow. The number of microspheres within the muscle microvascular network can be seen to decrease at 120 min postprandial in the control trial following high-glucose mixed-nutrient meal ingestion. This decrease at 120 min postprandial is attenuated in the 3 h and 24 h post-exercise trials. Furthermore, the number of microspheres in the control and 24 h post-exercise trials are similar at 0 min (pre-meal), but elevated in the 3 h post-exercise trial, indicating the prolonged effects of the prior bout of exercise on muscle capillary recruitment.

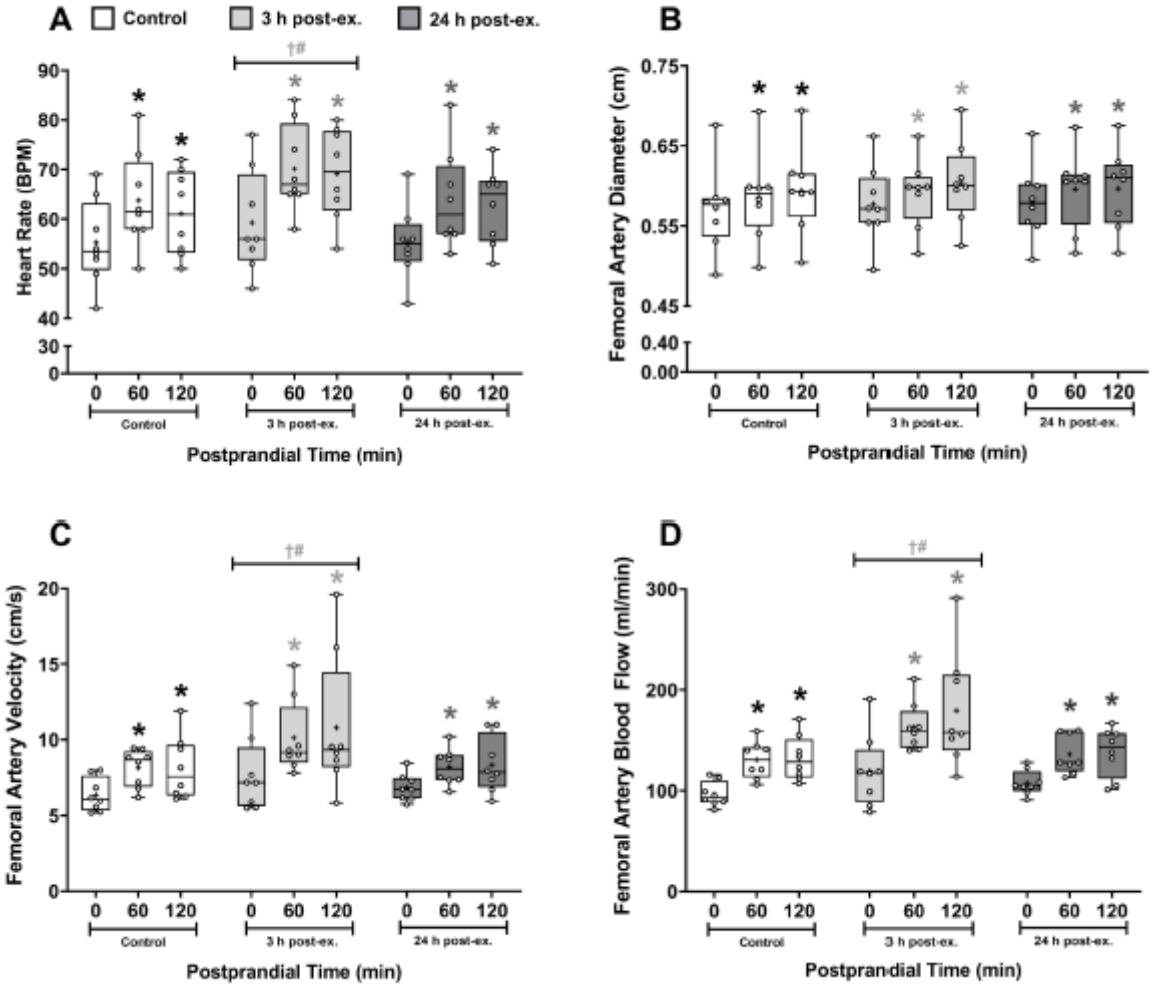

Figure 4. The effects of prior exercise on postprandial heart rate (A), femoral artery diameter (A), blood velocity (C) and blood flow (D). Data was analyzed using a two-way ANOVA with "time" (postprandial timepoint) and "condition" (Control, 3 h post-exercise, and 24 h post-exercise) as the within-subjects factors. Bar graphs are presented as Box and Whisker plots. The Box represents the interquartile range alongside the median (line) and mean (plus symbol). The Whiskers represent the minimum and maximum range of the data. n = 8 participants. ***** = $p \leq 0.05$ compared to 0 min (pre-meal) within that condition. **†** = $p \leq 0.05$ compared to the same timepoint in the control trial. **#** = $p \leq 0.05$ compared to the same timepoint in the 24 h post-exercise trial.

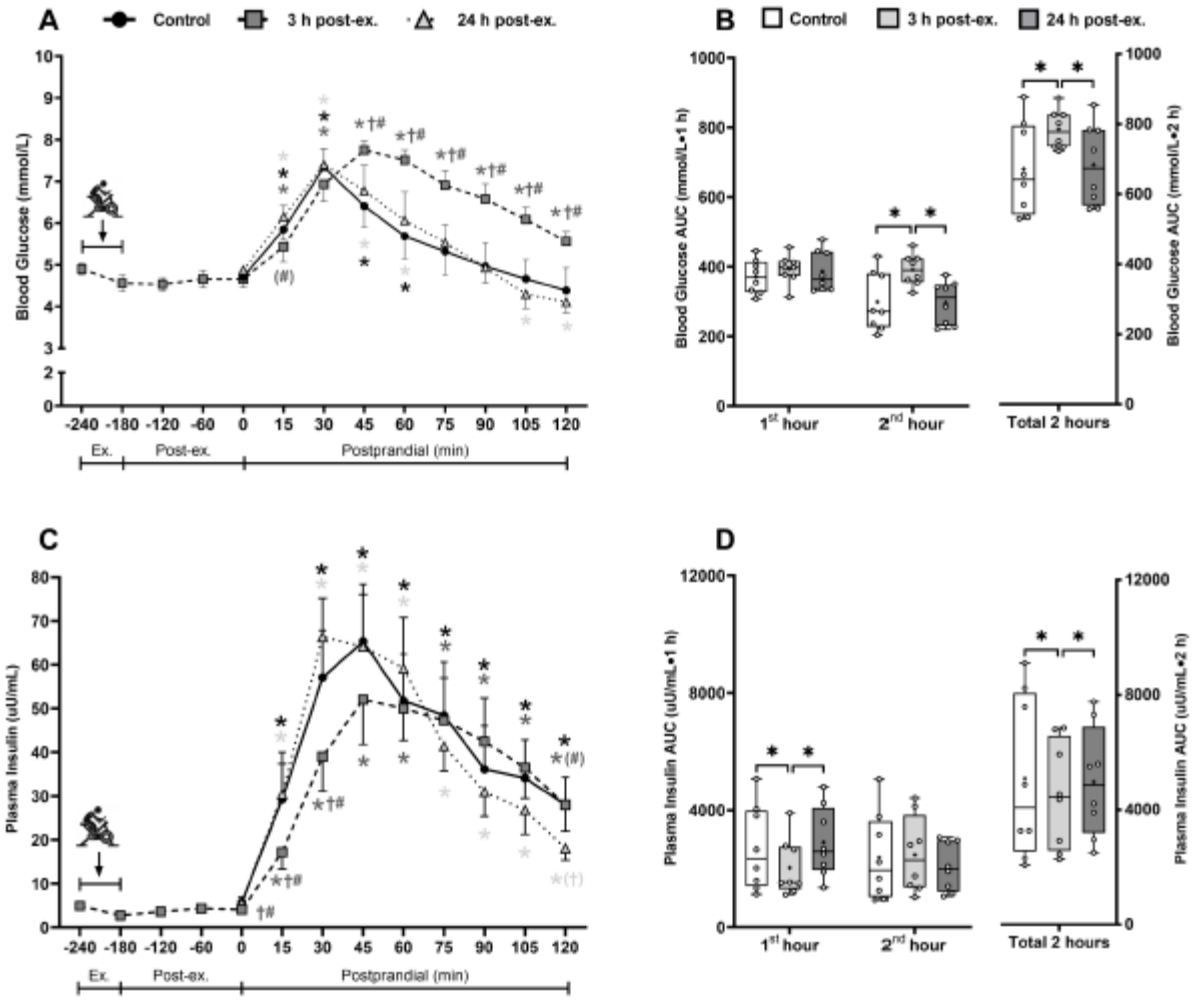

Figure 5. The effects of prior exercise on postprandial blood glucose (A) and glucose AUC (B), and plasma insulin (C) and insulin AUC (D). Data was analyzed using a two-way ANOVA with "time" (postprandial timepoint) and "condition" (Control, 3 h post-exercise, and 24 h post-exercise) as the within-subjects factors. For visual clarity, line graphs are expressed as mean ± SEM. Bar graphs are presented as Box and Whisker plots. The Box represents the interquartile range alongside the median (line) and mean (plus symbol). The Whiskers represent the minimum and maximum range of the data. n = 8 participants. ***** = $p \leq 0.05$ compared to 0 min (pre-meal) within that condition. **†** = $p \leq 0.05$ compared to the same timepoint in the control trial. **#** = $p \leq 0.05$ compared to the same timepoint in the 24 h post-exercise trial.

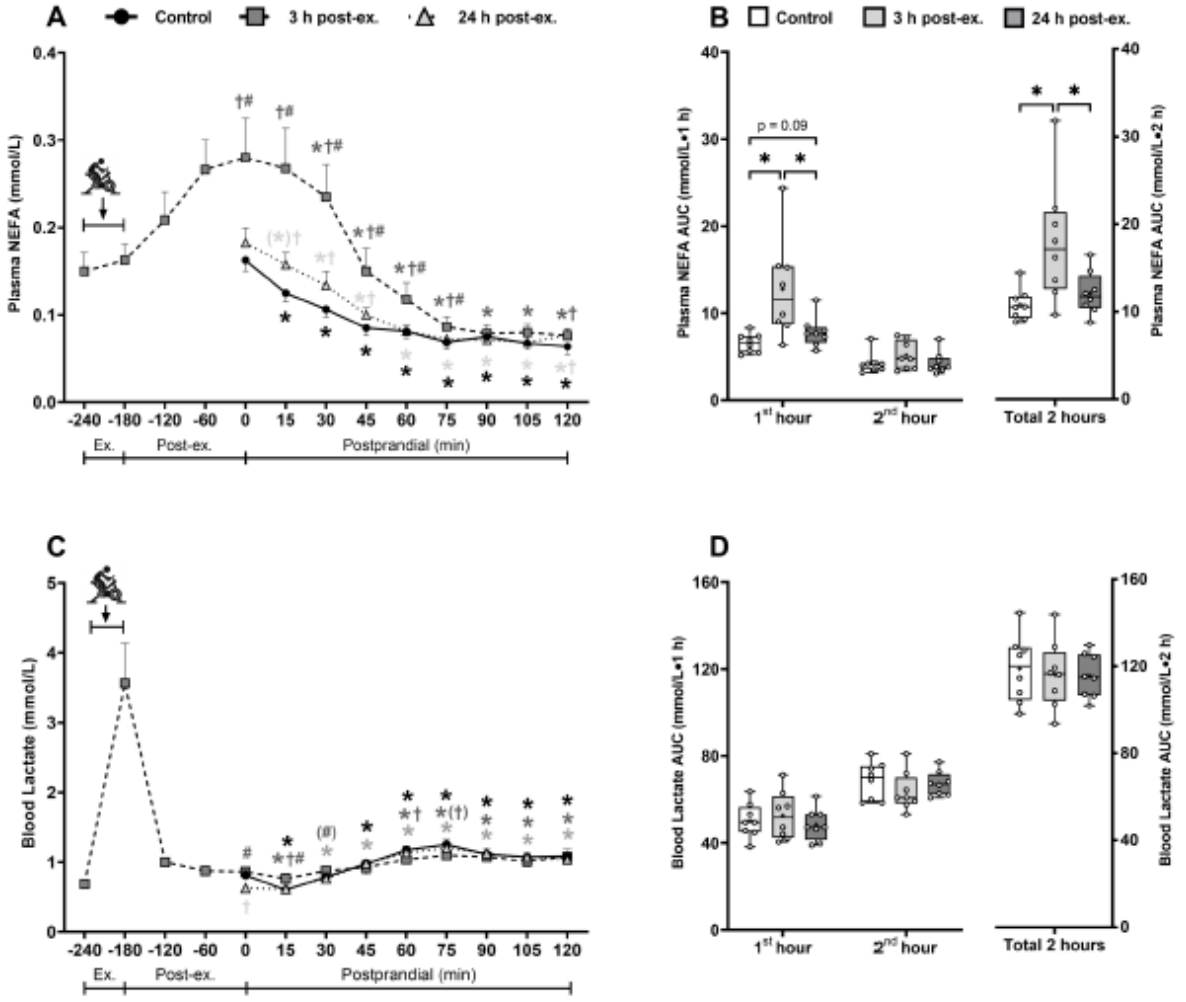

Figure 6. The effects of prior exercise on postprandial plasma NEFA (A) and NEFA AUC (B), and blood lactate (C) and lactate AUC (D). Data was analyzed using a two-way ANOVA with "time" (postprandial timepoint) and "condition" (Control, 3 h post-exercise, and 24 h post-exercise) as the within-subjects factors. For visual clarity, line graphs are expressed as mean ± SEM. Bar graphs are presented as Box and Whisker plots. The Box represents the interquartile range alongside the median (line) and mean (plus symbol). The Whiskers represent the minimum and maximum range of the data. n = 8 participants. ***** = $p \leq 0.05$ compared to 0 min (pre-meal) within that condition. **†** = $p \leq 0.05$ compared to the same timepoint in the control trial. **#** = $p \leq 0.05$ compared to the same timepoint in the 24 h post-exercise trial.

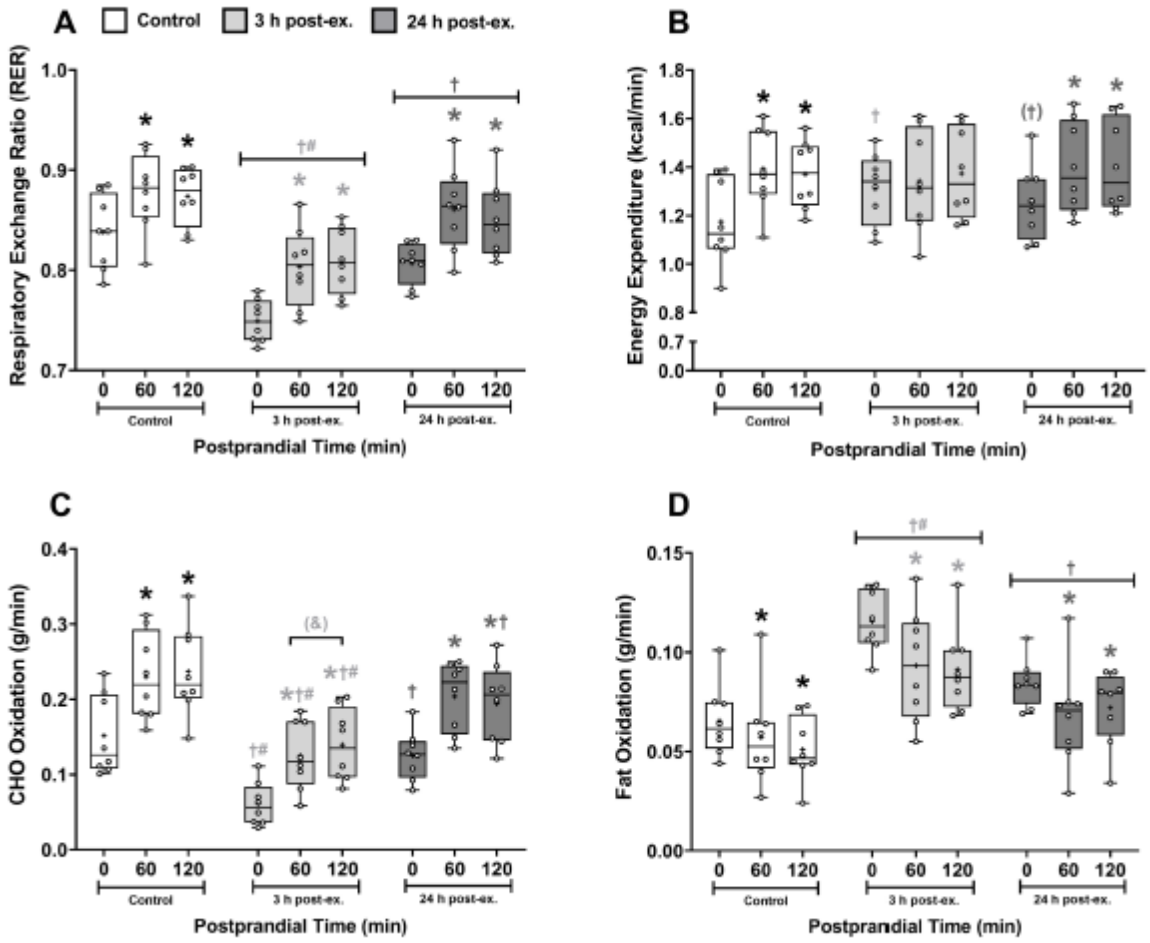

Figure 7. The effects of prior exercise on postprandial respiratory exchange ratio (A), energy expenditure (B), CHO oxidation (C), and fat oxidation (D). Data was analyzed using a two-way ANOVA with "time" (postprandial timepoint) and "condition" (Control, 3 h post-exercise, and 24 h post-exercise) as the within-subjects factors. Bar graphs are presented as Box and Whisker plots. The Box represents the interquartile range alongside the median (line) and mean (plus symbol). The Whiskers represent the minimum and maximum range of the data. n = 8 participants. ***** = $p \leq 0.05$ compared to 0 min (pre-meal) within that condition. **†** = $p \leq 0.05$ compared to the same timepoint in the control trial. **#** = $p \leq 0.05$ compared to the same timepoint in the 24 h post-exercise trial. & = $p \leq 0.05$ between the indicated timepoints. Symbols in parenthesis represent $p \leq 0.10$.

Additional Information

Data availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request. Individual data for blood glucose, plasma insulin, plasma NEFA, and blood lactate are provided in the Statistical Summary Document.

Competing interests: None of the authors have any conflicts of interests to declare.

Author Contributions: The experiments were conducted at Deakin University, Australia. Conception or design of the work: All authors. Data acquisition: LP, DM, KRT, GK, MK. Data analysis and interpretation: All authors. Critically reviewing and revising the manuscript for important intellectual content: All authors. All authors approved the final version of the manuscript, 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, and all persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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