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RESEARCH ARTICLE

High-glucose mixed-nutrient meal ingestion impairs skeletal muscle microvascular blood flow in healthy young men

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Parker L, Morrison DJ, Betik AC, Roberts-Thomson K, Kaur G, Wadley GD, Shaw CS, Keske MA. High-glucose mixed-nutrient meal ingestion impairs skeletal muscle microvascular blood flow in healthy young men. *Am J Physiol Endocrinol Metab* 318: E1014–E1021, 2020. First published April 14, 2020; doi:10.1152/ajpendo.00540.2019.—Oral glucose ingestion leads to impaired muscle microvascular blood flow (MBF), which may contribute to acute hyperglycemia-induced insulin resistance. We investigated whether incorporating lipids and protein into a high-glucose load would prevent postprandial MBF dysfunction. Ten healthy young men (age, 27 yr [24, 30], mean with lower and upper bounds of the 95% confidence interval; height, 180 cm [174, 185]; weight, 77 kg [70, 84]) ingested a high-glucose (1.1 g/kg glucose) mixed-nutrient meal (10 kcal/kg; 45% carbohydrate, 20% protein, and 35% fat) in the morning after an overnight fast. Femoral arterial blood flow was measured via Doppler ultrasound, and thigh MBF was measured via contrast-enhanced ultrasound, before meal ingestion and 1 h and 2 h postprandially. Blood glucose and plasma insulin were measured at baseline and every 15 min throughout the 2-h postprandial period. Compared with baseline, thigh muscle microvascular blood volume, velocity, and flow were significantly impaired at 60 min postprandial (−25%, −27%, and −46%, respectively; all $P < 0.05$) and to a greater extent at 120 min postprandial (−37%, −46%, and −64%; all $P < 0.01$). Heart rate and femoral arterial diameter, blood velocity, and blood flow were significantly increased at 60 min and 120 min postprandial (all $P < 0.05$). Higher blood glucose area under the curve was correlated with greater MBF dysfunction ($R^2 = 0.742$; $P < 0.001$). Ingestion of a high-glucose mixed-nutrient meal impairs MBF in healthy individuals for up to 2 h postprandial.

capillaries; hyperglycemia; microvascular; vascular dysfunction

INTRODUCTION

Defects in insulin action contribute to the pathogenesis of hyperglycemia and the development of cardiometabolic diseases including type 2 diabetes (T2D) and cardiovascular disease (34). Even short-term elevations in glycemia can lead to a transient increase in postprandial oxidative stress, inflammation, impaired bone metabolism, and vascular dysfunction (32, 33, 40)—conditions previously linked to the deterioration of glycemic control and cardiometabolic disease (20, 22, 34). Acute hyperglycemia in rodents (blood glucose levels raised to ~10 mM for 5 h) results in skeletal muscle insulin resistance and impaired insulin-stimulated muscle glucose disposal (11). However, insulin resistance induced through acute hyperglycemia appears to precede defects in canonical insulin signaling suggesting that alternative mechanisms are likely at play (11). Interstitial insulin diffusion and glucose uptake in skeletal muscle are impaired in dogs during moderate hyperglycemia (21). Coupled with the well-established role of the vascular system for glucose homeostasis (20, 39, 45), vascular dysfunction may be one of the driving mechanisms behind acute hyperglycemia-induced skeletal muscle insulin resistance (20).

Cardiac output and macrovascular and microvascular blood flow increase after the consumption of a balanced macronutrient meal, which is thought to synergistically enhance metabolism through the delivery of nutrients and hormones (e.g., insulin and glucose) to peripheral tissues (20, 40, 49). Acute hyperglycemia following high glucose ingestion or intravenous glucose infusion can lead to impaired vascular hemodynamics including impaired endothelial function and increased platelet aggregation and blood viscosity (10, 26, 50, 51). However, high glucose ingestion does not always translate to impaired limb arterial blood flow (1, 37, 40). Furthermore, we have shown that limb arterial blood flow increases after an oral glucose challenge in healthy humans, whereas microvascular blood flow in skeletal muscle is impaired (40). We also reported that physiological hyperinsulinemia in rodents increases muscle microvascular blood flow and glucose uptake before changes in limb arterial blood flow (48). Improvements in glycemic control following 6 wk of resistance exercise training in patients with type 2 diabetes are correlated with improvements in muscle microvascular blood flow, even after adjusting for changes in limb arterial blood flow (39). These findings support the independence of the macrovasculature and microvasculature systems in regulating postprandial metabolism. They also highlight that skeletal muscle microvascular blood flow is an important determinant of insulin-mediated glucose disposal in skeletal muscle.

Muscle microvascular blood flow can account for up to 40–50% of insulin-stimulated glucose disposal in skeletal muscle during euglycemic-hyperinsulinemic conditions (20, 45, 46). However, the contribution of the microvasculature to acute hyperglycemia-induced insulin resistance remains contentious (25). This is likely due to indirect methods for measuring muscle microvascular blood flow and the use of skin microcirculation as a surrogate model for assessing microvascular function in insulin-responsive tissues (20, 25). Furthermore, our team previously showed that the ingestion of a low-glucose mixed-nutrient meal (41 g carbohydrate, 25.1 g as glucose) in healthy humans stimulates muscle microvascular

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perfusion, whereas an oral glucose challenge (50 g glucose) matched for pancreatic insulin secretion leads to impaired muscle microvascular perfusion (40). Whether the inclusion of protein and lipids in a high-glucose load can restore or limit microvascular dysfunction is unknown but may be clinically relevant for minimizing acute hyperglycemia-induced insulin resistance.

We aimed to determine the femoral arterial (macrovascular) and thigh muscle microvascular blood flow responses to a high-glucose mixed-nutrient meal ingested by healthy individuals. It was hypothesized that muscle microvascular blood flow would increase after the ingestion of a high-glucose mixed-nutrient meal containing glucose, eggs, and cheese.

MATERIALS AND METHODS

Participants and screening. This study was approved by the Deakin University Human Research Ethics Committee. Ten healthy young men participated in the study after providing written informed consent. Prior to participation a medical history and health questionnaire was completed, and height, weight, and resting blood pressure were measured. Exclusion criteria for participation included current or previous history of cardiometabolic disease or smoking, medication or vitamin use that may affect vascular function or glycemic control, and musculoskeletal or other conditions that prevent daily activity. Men were recruited to avoid potential confounding effects of sex hormones on glucose metabolism and hemodynamics. Eligible participants were then invited to undergo an experimental session designed to explore the macrovascular and microvascular responses to ingestion of a high-glucose mixed-nutrient meal. Participants refrained from moderate-to-vigorous physical activity (48 h prior), alcohol (24 h prior), and caffeine (12 h) before undergoing the experimental session.

Experimental session. Participants arrived in the laboratory after an overnight fast and rested on a laboratory bed while an intravenous cannula was inserted into the arm. After 30 min of rest, a fasting blood sample was taken, and resting heart rate, femoral arterial blood flow, and muscle microvascular blood flow were measured via a commercial ultrasound machine (iU22; Philips Medical Systems, Andover, MA). Participants were then given a mixed-nutrient meal (10 kcal/kg; 45% carbohydrate, 20% protein, and 35% fat) consisting of eggs, cheese, and 1.1 g/kg glucose powder (84.8 g [77.0, 92.6], mean with lower and upper bounds of the 95% confidence interval) dissolved in 200 mL of water as the only carbohydrate source. The drink was consumed within 1 min, and the meal was consumed within 5 min. Venous blood samples were taken 15, 30, 45, 60, 75, 90, and 120 min after commencement of meal ingestion. Ultrasound measurements of blood flow in the femoral artery, and muscle microvascular blood flow in the thigh, were taken at 60 and 120 min after consumption of the meal.

Femoral arterial blood flow. Diameter and blood velocity and flow of the superficial femoral artery were measured noninvasively using a high-frequency L12-5 linear array transducer interfaced to a commercial ultrasound machine, as described previously (40). In brief, artery diameter was assessed using two-dimensional (2-D) ultrasound linked to a three-lead electrocardiograph system to match diameter measurements to the same phase of the cardiac cycle based on the QRS complex. Velocity was assessed by 2-D Doppler ultrasound. Arterial diameters and velocity were recorded in triplicate. Femoral arterial blood flow (mL/min) was calculated as $\pi r^2 \times$ mean velocity $\times$ 60 min, where radius (r) is in centimeters and mean velocity is in centimeters per second. 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 imaging during Definity (Lantheus Medical Imaging, Australia) contrast agent infusion, as previously performed (40). In brief, this technique involves intravenous infusion of a commercially available contrast agent (Definity) composed of hemodynamically inert perflutren lipid microspheres sufficiently small in size to perfuse muscle capillaries. Ultrasound settings were optimized for humans following a series of in vitro and in vivo experiments involving incremental adjustments of gain, mechanical index, and infusion rates. Settings for mechanical index (0.11 for continuous and 1.30 for flash) and gain (75–76%) were identical for all participants. The ultrasound probe was placed over the vastus lateralis muscle in a position approximately two-thirds distal from the line created between the anterior superior iliac spine and the tip of the patella. The morphology of the leg was then explored to find a suitable position and image of the vastus lateralis muscle in a non-contrast-enhanced mode. Depth and focus were adjusted to accommodate variations in leg morphology (e.g., variations in lean tissue and adipose tissue depth) on an individual basis to achieve an optimal region of interest for muscle microvascular analysis. All settings were kept constant at each time point for each participant.

The contrast agent suspension contained 1 mL of Definity contrast agent added to 19 mL of saline solution (0.9% NaCl). The contrast agent was initially infused by a standard infusion pump at a constant rate of 0.68 mL/min to measure contrast agent concentration in the superficial femoral artery. After 5 min of infusion, to allow for whole body contrast agent equilibrium, the L9-3 linear ultrasound transducer was placed over the femoral artery in cross section (Supplemental Fig. S1; all Supplemental material is available at https://doi.org/10.17605/OSF.IO/7TKDJ), and four 5-s video captures were recorded. The infusion rate was then increased to a constant rate of 2.25 mL/min to measure muscle microvascular perfusion of the right vastus lateralis muscle. The placement of the ultrasound probe over the vastus lateralis muscle was finalized during the first 2 min of contrast agent infusion to ensure that a clear image of the vastus lateralis muscle was achieved and to avoid interference from substantial arterioles and fascia artifacts. The final location of the probe (all 4 corners of the probe holder) was marked on the skin with a permanent marker; this marking persisted throughout the trial and was used for the 60 and 120 min postprandial time points. After 4 min of equilibrium infusion, four 45-s video captures were recorded. All digital recordings were preceded by a high-mechanical index flash to disrupt all current microspheres within the probe line of sight to measure muscle microsphere reappearance kinetics.

Digital images were analyzed off-line using QLAB (Philips Medical Systems, Andover, MA). To measure muscle microvascular blood volume, velocity, and flow, the raw acoustic intensity was background subtracted (0.5-s frame) to eliminate signal from larger vessels and tissue artifacts (40). The acoustic intensity measured from a region of interest was then plotted over time and curve fitted using the equation $y = A\left[1 - e^{-\beta(t-t_b)}\right]$, where y is the acoustic intensity, t is time, t_b 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 $A \times \beta$.

Microvascular perfusion normalization. Arterial microsphere concentration (an indication of total contrast agent infusion and circulation) was measured by calculating a region of interest inside the femoral artery and averaging acoustic intensity over a 3-s time period. Microvascular blood volume (A value; plateau acoustic intensity) may be influenced by potential variations in the contrast agent concentration in the blood pool. Because of the saturation of the microsphere acoustic signal within the artery at the infusion rate selected for assessing muscle microvascular imaging, a submaximal constant infusion was used to determine the microsphere blood pool concentration. The plateau acoustic intensity (A value) was then expressed and normalized to individual percent changes from baseline, within and between participants, in femoral arterial microsphere concentration.

Table 1. *Participant characteristics*

	Value
Age, yr	27 [24, 30]
Height, cm	180 [174, 185]
Weight, kg	77 [70, 84]
Body mass index	24 [23, 25]
Fasting plasma insulin, μU/mL	4.7 [3.4, 6.0]
Fasting blood glucose, mmol/L	4.7 [4.6, 4.8]
Resting systolic blood pressure, mmHg	120 [116, 124]
Resting diastolic blood pressure, mmHg	76 [73, 79]

Values are means with lower and upper bounds of the 95% confidence interval in brackets; $n = 10$ participants.

All muscle microvascular perfusion data are expressed normalized to arterial concentration. Nonnormalized muscle microvascular perfusion data are included as supplemental data and do not change the study outcomes or conclusions.

Blood sampling and analysis. Whole blood was analyzed immediately for blood glucose using an automated analysis system (ABL800 Flex; Radiometer Medical, Copenhagen, Denmark). Venous blood was collected from an antecubital vein with collection tubes containing ethylenediaminetetraacetic acid (EDTA). Blood samples were then separated into plasma by centrifugation (10 min at 1,800 relative centrifugal force, 4°C) and immediately aliquoted and stored at −80°C until analyzed. Plasma insulin was measured via sandwich ELISA (ALPCO Diagnostics, Windham, NH) in duplicate as per the manufacturer's instructions.

Statistical analysis. Data were checked for normality and analyzed using Predictive Analytics Software (PASW v25; SPSS Inc.). Comparison of multiple means was analyzed using a repeated measures analysis of variance (ANOVA) with time (i.e., before and throughout the 2-h postprandial period) as the within-subjects factor. Significant interaction and main effects were explored post hoc using Fisher's least significant difference test. Linear regression analysis was conducted between insulin and glucose area under the curve (AUC) and changes in muscle microvascular blood flow and arterial blood flow (absolute delta between baseline and 120 min postprandial). All data are reported as means ± 95% confidence interval, and statistical analysis was conducted at the 95% level of significance ($P \leq 0.05$).

RESULTS

Participant characteristics. Participant characteristics are reported in Table 1. Participants were apparently healthy with normal fasting blood glucose and insulin levels.

Central, macrovascular, and microvascular blood flow responses. Heart rate significantly increased from baseline to 60 and 120 min postprandial (Fig. 1*A*; $P < 0.05$). Femoral artery diameter, blood velocity, and blood flow significantly increased from baseline to 60 and 120 min postprandial with no differences between 60 and 120 min for any measure (Fig. 1, *B–D*; all $P < 0.05$).

Femoral arterial microsphere concentration did not significantly differ between time points (Fig. 2*A*; $P = 0.617$), indicating that whole body microsphere infusion and concentration were consistent between postprandial measurements. In contrast, muscle microvascular blood volume, velocity, and flow were significantly decreased at 60 and

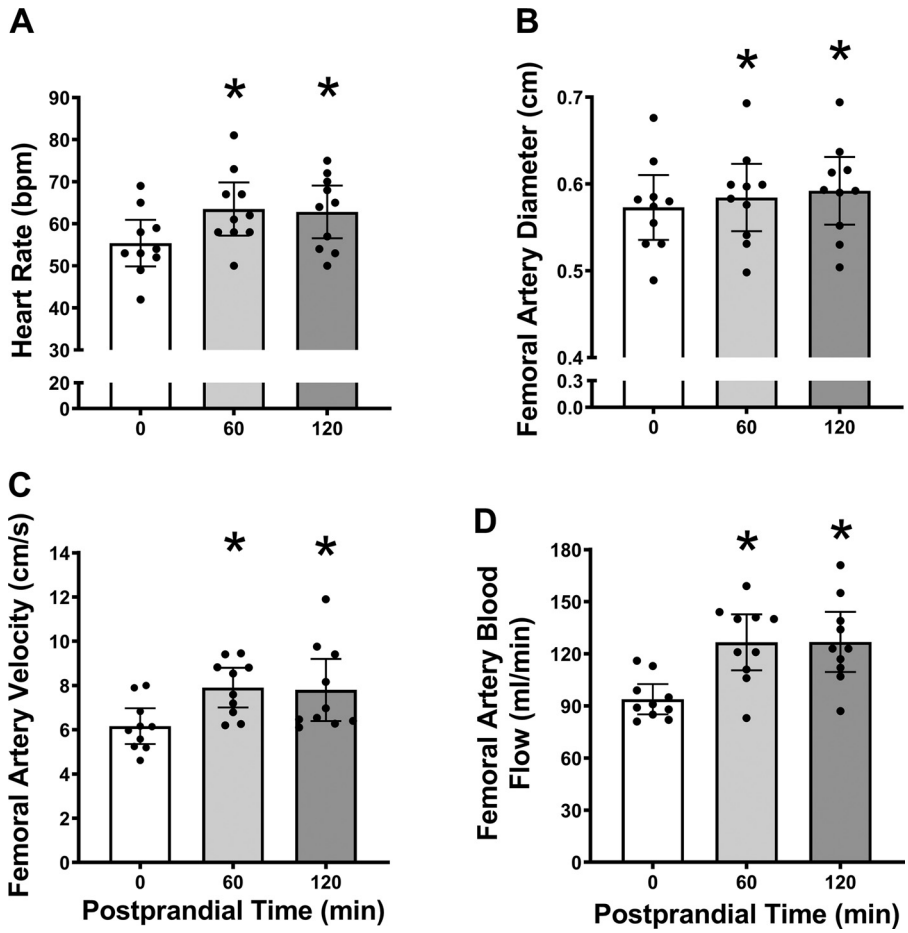

Fig. 1. *A–D*: central hemodynamic responses to ingestion of a high-glucose mixed-nutrient meal. Data are expressed as means and the lower and upper bounds of the 95% confidence interval ($n = 10$ participants) and were statistically analyzed via a one-factor (postprandial time) repeated measures ANOVA. bpm, beats/min. *$P < 0.05$ compared with baseline.

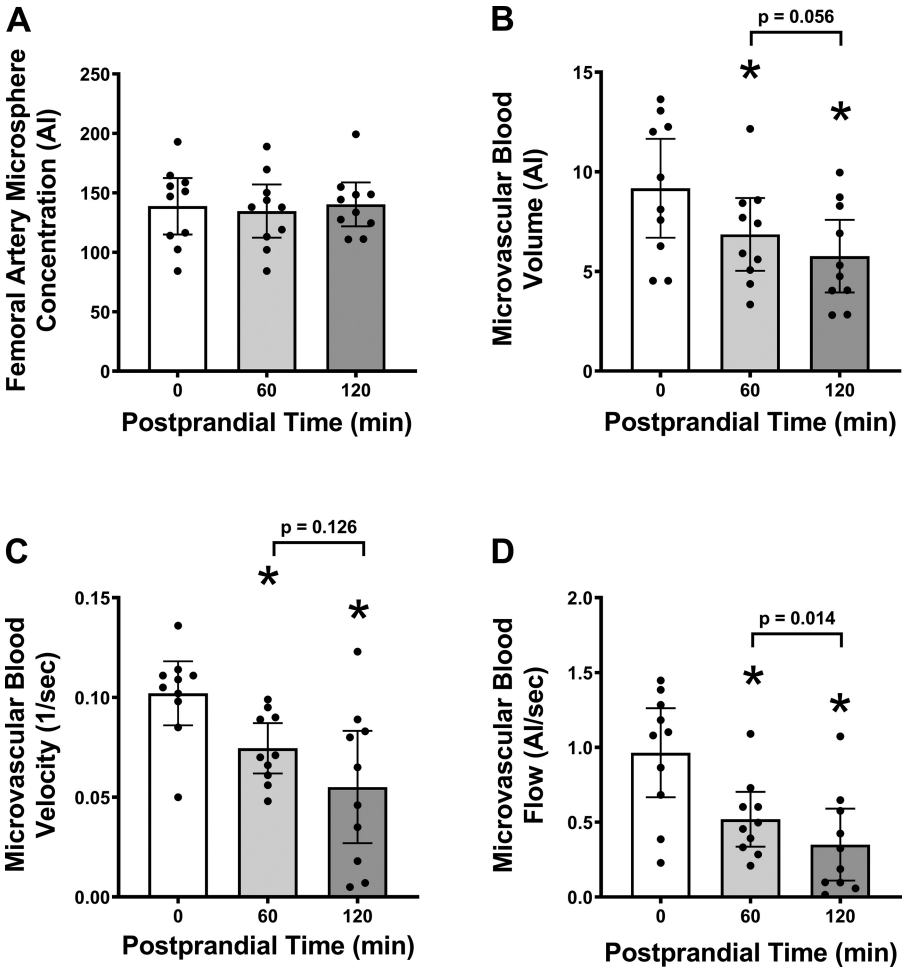

Fig. 2. *A–D*: muscle microvascular responses to ingestion of a high-glucose mixed-nutrient meal. Data are expressed as means and the lower and upper bounds of the 95% confidence interval (n = 10 participants) and were statistically analyzed via a one-factor (postprandial time) repeated measures ANOVA. AI, acoustic intensity. *$P < 0.05$ compared with baseline.

120 min postprandial compared with baseline (Fig. 2, *B–D*; all $P < 0.05$). Muscle microvascular blood flow was decreased to a greater extent at 120 min postprandial compared with 60 min postprandial (Fig. 2*D*; $P < 0.05$). Representative images of muscle microvascular blood flow at baseline and 60 and 120 min postprandial are provided in Supplemental Fig. S1. Nonnormalized microvascular blood flow data followed the same patterns as normalized data and are provided in Supplemental Fig. S2.

Blood glucose and plasma insulin responses. Blood glucose was significantly elevated at 15, 30, and 45 min postprandial compared with baseline (Fig. 3*A*; $P < 0.05$). Plasma insulin was significantly elevated at all postprandial time points compared with baseline (Fig. 3*B*; $P < 0.05$).

Correlations. A significant correlation was detected between higher 2-h blood glucose AUC and greater decrements in muscle microvascular blood flow at both 60 and 120 min after meal ingestion (Fig. 4*A*; $P < 0.01$). A significant but weaker correlation was detected between higher insulin AUC and greater decrements in microvascular blood flow (Fig. 4*B*; $P < 0.05$). In contrast, neither 2-h glucose AUC nor 2-h insulin AUC correlated with changes in femoral arterial blood flow at 60 min or 120 min after the meal (Fig. 4, *C* and *D*; $P > 0.05$). Linear regression analysis between glucose and insulin AUC

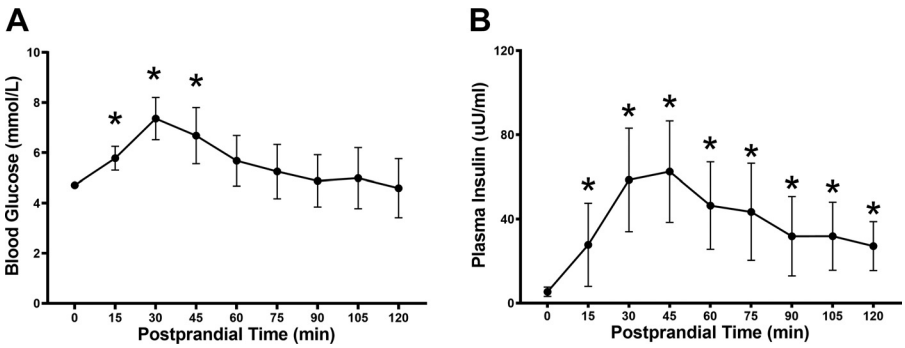

Fig. 3. *A* and *B*: postprandial glucose and insulin responses to ingestion of a high-glucose mixed-nutrient meal. Data are expressed as means and the lower and upper bounds of the 95% confidence interval (n = 10 participants) and were statistically analyzed via a one-factor (postprandial time) repeated measures ANOVA. *$P < 0.05$ compared with baseline.

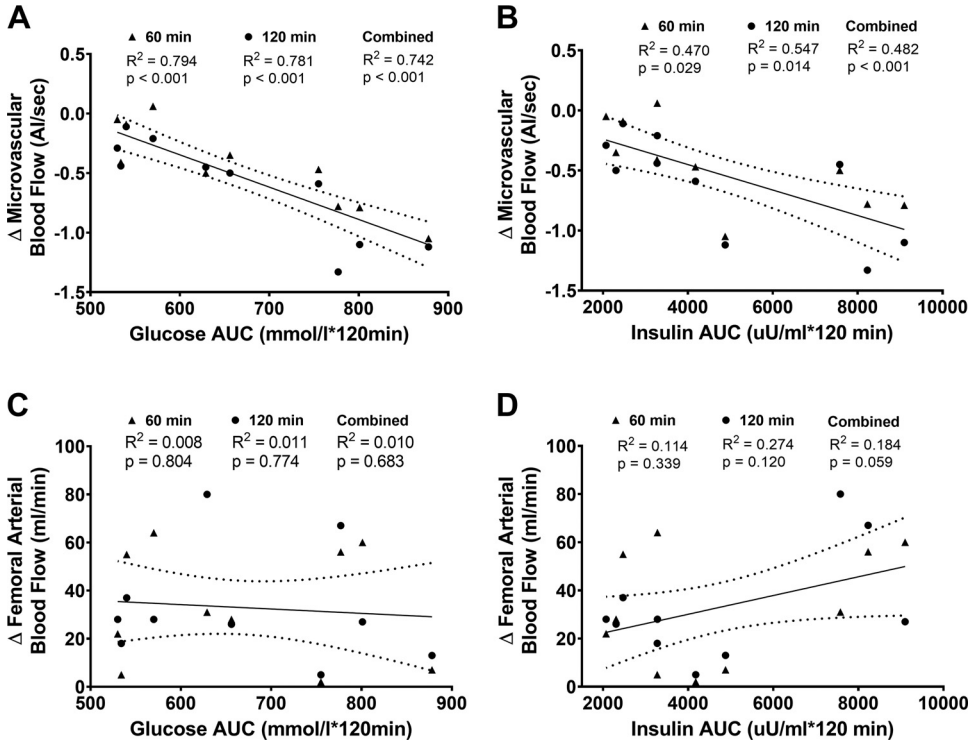

Fig. 4. *A–D*: linear regression analysis between glucose 2-h area under the curve (AUC) and insulin AUC and changes in muscle microvascular and femoral arterial blood flow (delta change from baseline to 60 and 120 min postprandial). The 95% confidence interval is shown for linear regression of the combined data (60 min and 120 min postprandial). $N = 10$ data pairs for 60 and 120 min linear regression analysis. $N = 20$ data pairs for combined linear regression analysis. AI, acoustic intensity.

and nonnormalized microvascular blood flow data are provided in Supplemental Fig. S3.

DISCUSSION

We provide new evidence that high glucose ingestion, combined with lipids and protein (i.e., a high-glucose mixed-nutrient meal), significantly impairs muscle microvascular blood flow for up to 2 h postprandial in healthy individuals. This occurs despite an increase in central hemodynamic measures of heart rate and femoral arterial blood flow. Furthermore, we detected a significant correlation between greater muscle microvascular dysfunction and higher 2-h blood glucose AUC and insulin AUC, supporting the notion that oral glucose ingestion and acute hyperglycemia under real-world (i.e., an actual meal) conditions can lead to microvascular dysfunction in healthy individuals. These findings highlight muscle microvascular dysfunction as a potential contributor to the acute hyperglycemia-induced insulin resistance that has previously been reported (11). Future research is required to explore the effects of hyperglycemia-induced muscle microvascular dysfunction on postprandial glucose metabolism and determine interventions to prevent dysfunction.

Skeletal muscle is the predominant site for glucose disposal during euglycemic-hyperinsulinemic conditions and is a major site for glucose disposal in the postprandial state (29, 43). Among other factors, muscle glucose uptake and transport across the cell membrane require careful coordination between muscle vascular perfusion and muscle insulin signaling (20, 34). For this reason, macrovascular and microvascular blood flow to target tissues such as skeletal muscle is considered to be one of the primary rate-limiting steps for glucose clearance and disposal (5). It was initially conceived that insulin-stimulated increases in macrovascular and microvascular blood flow were coordinated to facilitate skeletal muscle nutrient, hormone, and blood gas exchange between the vasculature and the myocyte. However, because of methodological advances that now allow us to directly measure muscle microvascular function, it is apparent that in many cases, central, macrovascular, and microvascular systems can be differentially and independently regulated to control postprandial metabolism (20).

Following the consumption of a low-glucose mixed-nutrient meal or euglycemic-hyperinsulinemic clamp, both limb arterial blood flow and muscle microvascular blood flow increase (17, 20, 23, 28, 40, 47). We provide new evidence that despite an increase in heart rate and femoral arterial blood flow after ingestion of a high-glucose mixed-nutrient meal, microvascular blood flow in skeletal muscle is significantly impaired for up to 2 h postprandial. Orally ingested glucose is reported to either increase (1, 37, 40) or have no effect on macrovascular blood flow in healthy humans (14, 16). Although intravenous glucose infusion leads to impaired limb arterial blood flow in healthy individuals (10), glucose levels were clamped to a much higher level (15 mmol/L sustained over 2 h) than that observed in the current study (~7.4 mmol/L peak). It is also possible that the intravenous nature of glucose and insulin infusion, bypassing the gut and subsequent release of many gut-derived hormones, is likely to elicit considerably different hemodynamic responses from that of oral macronutrient ingestion (38, 42). For example, a 2-h intravenous hyperglycemic clamp (15 mmol/L) in healthy individuals leads to increased systolic and diastolic blood pressure and decreased leg arterial blood flow (10, 27). This directly contrasts with increased limb arterial and/or central hemodynamic changes (or absence of change) in healthy individuals observed following oral glucose ingestion (50–75 g glucose; 1, 31, 37, 40) and low-moderate glucose mixed-nutrient meal ingestion (9, 17, 40, 47). As such, our findings are in line with previous studies that have explored

the effects of oral macronutrient ingestion on limb arterial blood flow and central hemodynamics.

Our team and others have reported under various conditions of glycemia and hyperinsulinemia that muscle microvascular blood flow can be regulated independently of arterial blood flow (19, 35, 40, 48, 52). We propose that the dissociation between limb arterial blood flow and muscle microvascular blood flow may reflect nonnutritive shunting or redistribution of blood flow within the leg rather than changes in central hemodynamics (4, 20). For example, we previously reported increased muscle microvascular blood flow following a mixed-nutrient meal (41 g carbohydrate, 25.1 g as glucose) and decreased microvascular blood flow following oral glucose ingestion (50 g glucose; 40). This occurred despite similar increases in heart rate and brachial arterial blood flow, similar central systolic blood pressure, and similar decreases in central diastolic and total vascular resistance between the two meals (40). As such, altered central hemodynamics following high-glucose meal ingestion are unlikely to explain the observed decrease in muscle microvascular blood flow in the current study. Furthermore, it has previously been shown that muscle microvascular recruitment can increase in the absence of changes to total limb blood flow following physiological insulin infusion in humans (8) and rodents (48) or very low intensity forearm contraction in humans (47) and rodents (41). Although yet to be confirmed in humans, this macro-micro blood flow dissociation can occur by *1*) redistributing blood between nutritive and nonnutritive capillaries in skeletal muscle, which differ in their diameter and length (with nonnutritive being wider and shorter and therefore acting as a capillary shunt), and/or *2*) redistribution of blood flow from skeletal muscle to other tissues in the leg, e.g., adipose tissue, skin, and bone (2, 3). For example, our team has reported that oral glucose ingestion (50 g glucose) increases microvascular blood flow in abdominal adipose tissue in healthy individuals (12), and increases arm skin blood flow in patients with T2D (39), supporting the potential for redistribution of microvascular blood flow to other regional tissues. The shift from a nutritive blood flow route in the microvasculature to a nonnutritive pathway can lead to lower myocyte glucose transport and metabolism and may in part contribute to acute hyperglycemia-induced insulin resistance (4, 20). However, future research combining compartmental blood flow analysis and glucose disposal is required to confirm this.

Previous studies employing mixed-nutrient meals have largely reported increased muscle microvascular blood flow during the postprandial period (17, 23, 40, 47). In contrast, others have reported either impaired or no change in muscle microvascular blood flow in healthy individuals following ingestion of 75 g and 50 g oral glucose alone (40, 44), respectively. It is possible that the inclusion of protein and fat, as opposed to pure glucose, may contribute to enhanced pancreatic insulin secretion and subsequent stimulation of microvascular perfusion in skeletal muscle (30). However, Russell et al. (40) established in healthy individuals that muscle microvascular blood flow increases after ingestion of a mixed-nutrient meal (25.1 g glucose; total carbohydrate 41 g), whereas it is impaired after an oral glucose challenge (50 g glucose; total carbohydrate 50 g) despite similar postprandial insulin responses. An important difference between our meal and previous mixed-nutrient meals is the much larger glucose content (average of 85 g glucose making up 100% of the carbohydrate content), which was designed to elicit a higher postprandial glucose peak compared with previous studies (~7.4 mmol/L in the current study vs. ~5.9–6.7 mmol/L in other studies). We provide evidence for the first time that the inclusion of lipids and protein, in the form of eggs and cheese, to high glucose oral ingestion does not prevent microvascular dysfunction in healthy individuals. Future research exploring the effect of specific proteins and fats, such as whey protein or saturated and polyunsaturated fatty acids, on acute hyperglycemia-induced muscle microvascular dysfunction is required.

It has been shown that muscle microvascular blood flow and glucose uptake increase during physiological hyperinsulinemia before changes in limb arterial blood flow (48), suggesting that microvascular hemodynamics play an early and primary role in the metabolic actions of insulin. It is possible that microvascular blood flow in the current study may have increased before the impairments measured at 60 min postprandial. However, this is unlikely as others have reported that the increase in microvascular blood flow following insulin stimulation or ingestion of a low-glucose mixed-nutrient meal can remain for up to 120 min (23). On the other hand, the temporal effects of high glucose ingestion on microvascular dysfunction are less clear but occur by 60 min postprandial (40). We extend previous findings by providing new evidence that muscle microvascular blood flow remains impaired for up to 120 min after ingesting a high-glucose mixed-nutrient meal, well after glucose levels have returned to baseline. Future research is required to pinpoint the specific temporal relationship of acute hyperglycemia and muscle microvascular dysfunction.

Acute hyperglycemia can impair postprandial muscle glucose uptake and metabolism (11). The mechanisms remain elusive as acute hyperglycemia-induced insulin resistance appears to occur before defects in muscle insulin signaling (11). Considering the close proximity of the microvasculature to the myocyte and its established role in glucose metabolism (20), it is possible that microvasculature dysfunction, as observed in the current study, may contribute to impaired postprandial glucose disposal. In support, insulin-resistant humans and rodent models exhibit impaired microvascular blood flow responses compared with healthy controls in the postprandial and insulin-stimulated state (6, 7, 17). Likewise, hyperglycemia (increasing blood glucose from 5.5 to 6.7 mmol/L for 5 h) in dogs leads to impaired insulin-mediated glucose uptake in part through impaired interstitial insulin diffusion in skeletal muscle (21). The mechanisms behind impaired hyperglycemia-induced microvascular dysfunction remain unclear but may involve regulatory disturbances between vasodilators and vasoconstrictors of precapillary arterioles that regulate microvascular blood flow in muscle (19, 20). For example, patients with type 2 diabetes exhibit a shift in the nitric oxide and endothelin-1 balance, leading to vasoconstriction and impairment of the microvasculature (15). Intravital microscopy has shown that insulin dilates precapillary arterioles in rat skeletal muscle but, in the presence of hyperglycemia, insulin can lead to the opposite action and vasoconstrict (36). We observed in the current study that postprandial insulin levels (AUC) were negatively associated with microvascular blood flow. This may reflect insulin-induced vasoconstriction of precapillary arterioles during hyperglycemic conditions despite increased femoral artery dilation. We and others have also previously reported

increased systemic oxidative stress in humans following a carbohydrate-rich meal (24, 32), which may lead to reduced nitric oxide bioavailability and subsequent microvascular dysfunction (20, 24, 45). However, systemic markers of oxidative stress and nitric oxide in venous blood are unlikely to adequately reflect or explain mechanisms behind muscle microvascular blood flow, which likely occur at the precapillary arteriole level. Further research is required to confirm the effects of hyperglycemia-induced microvascular dysfunction on postprandial glucose disposal and to explore potential mechanisms of action.

There are several limitations to this study. This study only investigated healthy young men, and thus further research is required to establish and confirm the effects of a high-glucose mixed-nutrient meal on vascular function in women. This study did not include a control (no-meal rest trial) or comparative meal trial (e.g., low-glucose mixed-nutrient meal trial). Nevertheless, our team has previously reported with the same techniques that microvascular blood flow increases following ingestion of a low-glucose mixed-nutrient meal and decreases following an oral glucose challenge (40), and thus the reported changes in vascular function in the current study are likely attributable to the meal itself. Blood pressure was not measured in the current study. However, postprandial blood pressure is reported to be similar between high glucose ingestion and low-glucose mixed-nutrient meal ingestion, despite the two meals eliciting divergent muscle microvascular blood flow responses (40). As such, alterations in blood pressure are unlikely to explain the reported decrease in muscle microvascular blood flow. Another limitation of the current study is that postprandial glucose disposal (whole body or skeletal muscle) following ingestion of the meal was not measured. Others have reported that forearm glucose uptake is enhanced when a 75 g oral glucose load is combined with lipids and protein (13). As such, it is possible that glucose uptake was still enhanced by the inclusion of lipids and protein in the oral glucose ingestion in the current study. Future research is required to investigate glucose kinetics using stable isotope tracers to establish and link microvascular dysfunction to impaired postprandial glucose metabolism under a variety of macronutrient conditions.

In conclusion, we report that a high-glucose mixed-nutrient meal leads to impaired muscle microvascular function for up to 2 h postprandial in healthy individuals. Furthermore, our findings suggest that the inclusion of fat and protein in a high-glucose load does not prevent or restore acute hyperglycemia-induced microvascular dysfunction. Future research should explore whether repeated bouts of acute microvascular dysfunction are linked to long-term aberrant microvascular remodeling and dysfunction observed in vascular-related diseases such as type 2 diabetes and cardiovascular disease.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

L.P., D.J.M., A.C.B., K.R.-T., G.K., G.D.W., C.S.S., and M.A.K. conceived and designed research; L.P., D.J.M., K.R.-T., G.K., and M.A.K. performed experiments; L.P. and D.J.M. analyzed data; L.P., D.J.M., A.C.B., K.R.-T., G.K., G.D.W., C.S.S., and M.A.K. interpreted results of experiments; L.P. prepared figures; L.P. and M.A.K. drafted manuscript; L.P., D.J.M., A.C.B., K.R.-T., G.K., G.D.W., C.S.S., and M.A.K. edited and revised manuscript; L.P., D.J.M., A.C.B., K.R.-T., G.K., G.D.W., C.S.S., and M.A.K. approved final version of manuscript.

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