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Oral Glucose Challenge Impairs Skeletal Muscle Microvascular Blood Flow in Healthy People

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Author contribution statement: M.A.K. and R.D.R were responsible for the conception and design of the research. R.D.R, D.H., T.G. and M.A.K. performed the experiments. R.D.R. and M.A.K analysed the data and drafted the manuscript. All authors (including J.E.S, S.R and S.M.R) contributed to interpretation and writing of the manuscript. R.D.R., and M.A.K. are the guarantors of this work and, as such, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Running head: Post-prandial hyperglycemia and muscle blood flow

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School of Exercise and Nutrition Sciences
Key words: Insulin action, carbohydrate metabolism, metabolic physiology, skeletal muscle, microvascular blood flow.
ABSTRACT

Skeletal muscle microvascular (capillary) blood flow increases in the post-prandial state or during insulin infusion due to dilation of pre-capillary arterioles to augment glucose disposal. This effect occurs independent of changes in large artery function. However, acute hyperglycemia impairs vascular function, causes insulin to vasoconstrict pre-capillary arterioles, and causes muscle insulin resistance in vivo. We hypothesize that acute hyperglycemia impairs post-prandial muscle microvascular perfusion, without disrupting normal large artery hemodynamics, in healthy humans. Fifteen healthy people (5F/10M) underwent an oral glucose challenge (50g glucose) and a mixed meal challenge (MMC) on two separate occasions (randomised, cross-over design). At 1 hr, both challenges produced a comparable increase (6-fold) in plasma insulin levels. However, the OGC produced a 1.5-fold higher increase in blood glucose when compared to the MMC 1-hr post ingestion. Forearm muscle microvascular blood volume and flow (contrast-enhanced ultrasound) were increased during the MMC (1.3- and 1.9-fold from baseline, respectively, p<0.05 for both) but decreased during the OGC (0.7- and 0.6-fold from baseline, respectively, p<0.05 for both) despite a similar hyperinsulinemia. Both challenges stimulated brachial artery flow (ultrasound), and heart rate to a similar extent, as well as yielding comparable decreases in diastolic blood pressure and total vascular resistance. Systolic blood pressure and aortic stiffness remained unaltered by either challenge. Independent of large artery hemodynamics, hyperglycemia impairs muscle microvascular blood flow, potentially limiting glucose disposal into skeletal muscle. The OGC reduced microvascular blood flow in muscle peripherally, and therefore may underestimate the importance of skeletal muscle in postprandial glucose disposal.
INTRODUCTION

Studies using the euglycemic hyperinsulinemic clamp demonstrate that 80% of infused glucose is removed by skeletal muscle (9, 38) indicating that skeletal muscle is an important tissue for glucose disposal following a meal. Contrary to this view, others have shown that glucose uptake by splanchnic tissues matches or exceeds that by skeletal muscle following an oral glucose challenge (OGC) and indicate that skeletal muscle is only responsible for 30-40% of glucose uptake (15, 26). It is now becoming more generally accepted that smaller glucose uptake by skeletal muscle during the OGC or oral glucose tolerance test (OGTT) is more representative of the post-prandial state. This assumption has led some to suggest that muscle is not the predominant site for glucose disposal. However, the OGC or OGTT do not contain all the macronutrients (protein, lipid and carbohydrate) typically found in food (meals) that may impact on glucose distribution.

Notably, Jackson and colleagues demonstrated that glucose disposal by forearm skeletal muscle is greater after a mixed meal challenge (MMC) compared to an OGTT, when adjusted for arterial glucose levels (14). These tests were matched for carbohydrate content however the MMC had a significantly lower blood glucose excursion than the OGTT. These authors suggested that the differences in skeletal muscle contribution to glucose uptake between MMC and OGTT were not due to alteration of the secretion of incretins (gut derived hormones such as glucose-dependent insulintropic polypeptide which are insulin sensitizing) and that other mechanisms are involved (14). A number of animal studies have shown that acute hyperglycemia (blood glucose levels increased by ~2-fold by intravenous glucose infusion – which does not cause secretion of incretins) markedly impairs skeletal muscle glucose disposal in vivo within hours (12, 19). Intriguingly, the onset of muscle insulin resistance by acute glucose infusion is not always accompanied by defects in the insulin signaling cascade in skeletal muscle responsible for glucose uptake (12), suggesting that other mechanisms are
implicated. One possible explanation is hyperglycemic-induced vasoconstriction which reduces the perfusion of the muscle thereby limiting delivery or glucose and insulin to the myocyte. However, this has not been previously investigated.

Insulin is classically known to increase skeletal muscle glucose uptake by binding to insulin receptors on myocytes causing glucose transporter 4 (GLUT4) translocation to the cell surface membrane. However, an additional action of insulin to augment muscle glucose uptake is its action to increase microvascular perfusion (which are predominantly capillaries) thereby improving insulin and glucose delivery to myocytes. This microvascular action of insulin accounts for 40-50% of insulin-stimulated glucose disposal in skeletal muscle (2, 18, 39, 40) and is therefore a significant contributor to glucose homeostasis. We have shown that physiological doses of insulin, whether infused intravenously via a euglycemic hyperinsulinemic clamp or released from the pancreas following a MMC stimulates total limb and microvascular blood flow in skeletal muscle of healthy humans (5, 6, 41) and animals (4, 7, 40, 49). These vascular actions of insulin are fundamentally dependent on the production of nitric oxide (NO) in the vascular endothelium (44). When this microvascular action is acutely blocked with vasoconstrictors (29), pro-inflammatory cytokines (48), elevated free fatty acids (3), or with a nitric oxide synthase (NOS) inhibitor (2, 39, 40), glucose disposal in vivo in skeletal muscle is also markedly impaired. Animal models of insulin resistance (27, 28, 35) and type 2 diabetes (4, 46) have reduced muscle microvascular and metabolic responses to insulin. Obese insulin resistant people also display reductions in skeletal muscle microvascular responses to infused insulin (5) or a MMC (16).

It has been reported that during concomitant hyperglycemia, insulin switches from dilating precapillary arterioles, observed directly using intravital microscopy, to vasoconstricting the microvessels (31). Furthermore, hyperglycemia (25 mM x 24 hrs) impairs insulin-mediated
endothelial NOS activation in cell culture studies (8). Given that pre-capillary arterioles are responsible for the regulation of capillary (microvascular) networks in skeletal muscle, and that insulin recruits the microvasculature in skeletal muscle via NOS-dependent process, we hypothesised that acute hyperglycemia (at levels higher than what we have observed in response to the MMC (16, 41)) would similarly impair microvascular blood flow responses in muscle. Therefore, the purpose of this study was to determine whether ingestion of glucose at a dose that raises plasma insulin levels to those seen with the MMC, but with markedly higher blood glucose levels, impairs skeletal muscle microvascular blood flow but not large vessel hemodynamics in healthy people.
METHODS

This study was approved by the University of Tasmania Human Research Ethics Committee. All participants provided written informed consent. The study was carried out in accordance with the Declaration of Helsinki as revised in 2008. Procedures followed were in accordance with institutional guidelines. A prior power calculation determined that sixteen people would be needed to detect a 30% difference in microvascular blood volume (MBV) between the MMC and the OGC (power = 0.8, $\alpha = 0.05$) (16, 41). To account for a 10% drop-out rate, eighteen healthy people (6 female and 12 male) were recruited through community advertisement between August 2014 and October 2016. Participants were included in the study if they were between 18-60 years, were normal to obese (BMI = 19 – 35 kg/m$^2$) and were weight-stable for the previous 3 months. We recruited people with a wide age and BMI range to reflect the general community. Participants were excluded if they had been diagnosed with diabetes, cardiovascular disease, a BMI $>$35kg/m$^2$, or had a personal history of smoking, cardiovascular disease, stroke, myocardial infarction, uncontrolled blood pressure (seated brachial blood pressure $>$160/100 mmHg), peripheral arterial disease, pulmonary disease, arthritis/muscular skeletal disease, malignancy within past 5 years or severe liver disease. Participants taking statins or anti-hypertensive medications were allowed to participate in the study and were instructed to not change their medication during the course of the study. Fifteen people (5 female and 10 male, 46 ± 12 years) completed the study between October 2014 and October 2016, and data from those participants who did not complete the study were excluded from the final analysis. Two people were dropped from the study due to difficulties in vein cannulation and blood sampling.

Screening Visit
Participants completed a medical questionnaire, and had their blood pressure, height and weight evaluated to confirm eligibility. After determining eligibility, participants were scheduled for their first and second clinic test visits which were conducted between 1 and 8 weeks apart, with most participants completing testing within 4 weeks. Participants were given either the OGC (50g glucose) or MMC at their first visit, and then given the other challenge in the follow-up clinic test visit. Block randomisation was performed where the first half of participants that enrolled into the study were allocated to OGC (50g glucose) first, and the second half of participants that enrolled into the study were allocated to MMC first.

Clinic Testing Visits
Participants fasted for 12 hours and refrained from alcohol and exercise for 48 hrs prior to the clinic visit. A catheter was placed in the antecubital vein of the non-dominant arm for blood draws and microbubble infusion. Baseline vascular and metabolic data was collected prior to either OGC or MMC being administered.

Mixed Meal Challenge (MMC)
A MMC (Table 2) was given to elicit a vascular and metabolic response as described previously (16, 41). Blood samples were taken at fasting and at 15, 30, 60, 90 and 120 minutes post-MMC ingestion.

Oral Glucose Challenge (OGC)
An OGC (50g glucose) was given to elicit a similar insulin response to the MMC. Blood samples were taken at fasting and at 15, 30, 60, 90 and 120 minutes post-OGC ingestion.
Contrast-enhanced ultrasound (CEU) was used to determine muscle microvascular perfusion in forearm skeletal muscle. CEU used a linear array transducer (L9-3) interfaced with an ultrasound system (iU22; Philips Medical Systems, Australia) as conducted previously (33). Microbubbles were diluted in 30ml of saline, and continuously infused intravenously for contrast imaging. Microbubble infusion rate was determined according to body weight (2.0-2.6 ml/min, or 0.03 ml/min/kg body weight) for muscle imaging. After 5 min steady-state infusion, a high energy pulse of ultrasound was transmitted to destroy microbubbles within the region of interest (deep flexor muscle group), and data was acquired in real time, and analysed as previously reported (33, 36). Forearm muscle microvascular responses were measured at baseline and then repeated 1hr following the OGC and MMC. As larger arteries, arterioles, veins and venules have significantly higher blood velocity than the downstream microvascular vessels being imaged, all images were background subtracted (0.5 sec image) to eliminate signal from these larger blood vessels and tissue per se. The resulting signal is located within the microcirculation where >90% are capillaries (5-10 μm diameter) and the remainder from small arterioles and venules (< 40μm diameter). Background-subtracted acoustic-intensity versus time was fitted to the function: \( y = A (1-e^{-\beta(t-t_b)}) \) where: \( y \) is acoustic-intensity at time \( t \), \( t_b \) the background time, \( A \) is plateau acoustic intensity (MBV), and \( \beta \) is the rate constant (a measure of microvascular re-filling rate) as previously published (33, 36). Microvascular blood flow (MBF) was determined by \( A \times \beta \). Analysis of images was not blinded to the primary technician. A large region of interest was selected from the baseline images (which are not expected to be different between post-prandial tests) and the identical region of interest was used for analysis of the post-MMC or post-OGC images. As such, we do not believe that bias played a significant role in the analysis or the interpretation of the data. A second blinded
technician reviewed ~20% of images for quality control to confirm that increases or decreases in microvascular responses were similarly observed. The values from the blinded technician were not used for the final analysis.

**Brachial and large artery hemodynamics**

Brachial artery measurements were made ~10 cm proximal to the antecubital fold using an L12-5 linear array transducer interfaced to an iU22 ultrasound (Philips Medical Systems, Andover, MA). Brachial artery diameter was measured on-line in high definition zoom in triplicate using 2-D imaging of the longitudinal artery (diameter assessed as the distance between each inside edge of the arterial intima). Brachial flow velocity was determined using pulse-wave Doppler quantified by automated tracing software on-line and averaged over 10-12 heart beats. Blood flow was determined using pulse-wave Doppler and brachial flow calculated from the diameter and velocity measurements. Brachial artery responses were measured at baseline and 1hr following the OGC/MMC.

Each participant was fitted with a Mobil-O-Graph monitor validated to measure aortic stiffness, augmentation index, vascular resistance and brachial and central blood pressure (I.E.M. Stolberg, Germany). Recordings were taken in triplicate at baseline and every 10 minutes during the OGC or MMC. Data at baseline and 1hr post-OGC or –MMC were analysed.

**Blood analysis**

Glycosylated hemoglobin (HbA1c), total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) cholesterol, and triglycerides were measured at a nationally accredited pathology laboratory (Royal Hobart Hospital, Hobart, Australia). Blood glucose was measured using a YSI analyzer (Yellow Springs Instruments,
Yellow Springs, OH), and plasma insulin was measured using an enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden). Fasting plasma free fatty acid (FFA) levels were determined using an enzymatic assay kit (Wako Pure Chemical Industries, Osaka, Japan).

**Statistical Analyses**

All data are expressed as means ± SD. Student’s paired t-test was used to compare changes in response to OGC versus MMC. When data were not normally distributed Signed Rank Test was performed. For all continuous variables, a two-way repeated measures ANOVA (interactions: time: 0 and 60 min group: MMC and OGC) followed by a Student–Newman–Keuls post-hoc was performed. Pearson’s bivariate correlation were used to evaluate relationships between variables. Significance was set at p<0.05. Tests were performed using SigmaStat™ statistical program (Systat Software, San Jose, CA, USA).

**RESULTS**

**Participant Characteristics**

Participant characteristics and anthropometrics are given in Table 1. Participants’ ages ranged from 25 – 58 years (46 ± 12 years, mean ± SD). Participants had normal fasting blood glucose (<6.5mM), insulin (<174 pM), and HbA1c [<6.0% (<43mmol/mol)] levels and all had seated brachial blood pressure <150/100 mmHg.

**Glucose, Insulin and FFA Responses**

Blood glucose significantly increased in response to the OGC and the MMC. However, blood glucose concentrations at 15, 30, 60, 90, and 120 min, and the area under the glucose time
curve were markedly higher with the OGC than the MMC (p<0.01) (Figure 1A/B). However, all participants displayed normal blood glucose responses at 1 and 2 hours post OGC consumption, further verifying participants did not have type 2 diabetes. Plasma insulin concentrations were the same between OGC and MMC test at all time points between 0 and 90 min (Figure 1C). During the OGC, insulin concentrations were higher at 120 min versus the MMC (Figure 1C). However, area under the insulin time curve was not different between groups (Figure 1D). FFA levels significantly decreased at 60 mins during the MMC and OGC to a comparable level (Figures 1E and 1F).

**Skeletal Muscle Microvascular Responses**

Baseline MBV, β, and MBF were the same prior to OGC and MMC (Figure 2A, B and C, respectively). The MMC elicited a significant increase in MBV (by 1.3-fold) and MBF (by 1.9-fold) at 1hr post-consumption (p<0.05 for both). However, the OGC caused the opposite effect with both MBV and MBF being significantly impaired (by 0.7- and 0.6-fold, respectively, p<0.05 for both) by 1hr despite a similar level of hyperinsulinemia as the MMC (Figure 1). There were no significant relationships between age and MBF response to the OGC (r=-0.157, p=0.576) or the MMC (r=0.398, p=0.141)

**Brachial and Large Artery Hemodynamics**

Brachial artery diameter significantly increased in response to the MMC, but was absent in response to the OGC (Figure 2D). Brachial artery flow velocity and blood flow increased to a similar extent in both OGC and MMC (Figure 2E and F, respectively).

The OGC and MMC produced similar actions on other cardiovascular hemodynamics. Heart rate increased significantly in response to the MMC or the OGC to a similar extent (Figure
3A). Central diastolic BP and total vascular resistance significantly decreased to a similar extent 1hr post OGC and MMC (Figure 3B and 3D). Central systolic BP, augmentation index and aortic stiffness were unaffected following consumption of the OGC or MMC (Figure 3E, F and C, respectively).

Correlates with peripheral vascular responses

When combining both post-prandial challenges (MMC and OGC), there was a significant negative correlation between ∆MBV and ∆blood glucose levels at 1hr (Figure 4A; r=-0.49, p=0.005). There was also a significant negative correlation between ∆MBF and ∆blood glucose levels at 1hr (Figure 4B; r=-0.44, p=0.014). Linear regression indicate that when post-prandial plasma insulin levels increased to ~240pM this results in a stimulatory effect on MBV and MBF providing that blood glucose levels do not increase by more than 2.4mM above fasting levels, otherwise the microvascular effects are inhibitory. There was no relationship between brachial artery blood flow and degree of glycemia at 1hr (Figure 4C).
DISCUSSION

The current study confirms our previous work demonstrating a stimulatory effect of the MMC on brachial artery blood flow, MBV and MBF in skeletal muscle of healthy people (41). However, we have made the important observation that orally ingested glucose – which raises plasma insulin levels to a similar extent as the MMC – has the opposite effect, impairing microvascular responses (both MBV and MBF) in skeletal muscle while maintaining a stimulatory effect on brachial artery blood flow. Interestingly, both MMC and OGC produced similar effects centrally on heart rate, total vascular resistance and blood pressure. There was a negative correlation between the degree of hyperglycemia and both post-prandial MBV (r=-0.49, p=0.005) and MBF (r=-0.44, p=0.014). Hyperinsulinemia with a concomitant moderate hyperglycemia (increase <2.4mM from fasting level) increased MBV and MBF, whereas the same level of hyperinsulinemia with a concomitant exaggerated hyperglycemia (increase >2.4mM from fasting level) inhibited post-prandial microvascular responses. There was no correlation between degree of hyperglycemia and brachial artery responses post-prandially regardless of the extent of the blood glucose excursion. Therefore, the OGC preferentially restricts microvascular blood flow in skeletal muscle while eliciting the same large artery hemodynamic responses as the MMC.

Skeletal muscle is an important site for glucose disposal in the post-prandial state (38). Our research group has demonstrated that microvascular responses in skeletal muscle play an integral role in insulin-mediated muscle glucose disposal (17, 18). Physiological doses of insulin (euglycemic hyperinsulinemic clamp) stimulate MBV, and this increase is intimately linked with enhanced glucose uptake by muscle in both humans (5, 10) and animal models (7, 40, 43). We have shown that blocking this microvascular action of insulin (e.g. with vasoconstrictors, inflammatory cytokines, or elevated free fatty acids (FFAs)) directly impairs
insulin-mediated skeletal muscle glucose disposal by 40-50% (2, 3, 29, 39, 40, 48). This microvascular impairment in skeletal muscle is also observed during chronic states of insulin resistance and type 2 diabetes in animal models (27, 28, 35) and humans (5, 16, 33). We also see similar stimulating effects of a MMC on the skeletal muscle microvascular responses in healthy humans (16, 41), and impairments during insulin resistance (16) showing that this microvascular action is physiologically important. Here we demonstrate that the consumption of 50g of glucose, which raises plasma insulin levels to a similar extent as the MMC, impairs rather than stimulates both MBV and MBF in skeletal muscle. Jackson and colleagues have demonstrated a greater peripheral (i.e. muscle) glucose uptake after a mixed meal when compared to an OGTT (in their study both challenges had an equivalent 75g carbohydrate load but the OGTT produced a higher blood glucose excursion) (14). Thus, we propose the novel finding that hyperglycemia that accompanies glucose loading impairs microvascular responses in skeletal muscle, and may explain reduced glucose uptake rates following an OGTT compared to a mixed meal in the study by Jackson and colleagues. A limitation of the current study was that rates of muscle glucose uptake were not measured during each of the post-prandial tests.

Elevated blood glucose levels over a prolonged period of time are strongly associated with microvascular complications of type 2 diabetes including neuropathy, retinopathy and nephropathy. This is in part due to the vulnerability of the vascular endothelium to prolonged hyperglycemia. However, a growing body of literature suggests that acute hyperglycemia can also impair vascular function in healthy people (1, 11, 20). Ingestion of glucose (50g glucose, peak blood glucose Δ 4.5mM) impairs brachial artery flow mediated dilation to a similar extent as a high glycemic-index meal (50g carbohydrate, peak blood glucose Δ 4.0mM) when compared to a low glycemic-index meal (50g carbohydrate, peak blood glucose Δ 1.3mM)
Our data indicate that when blood glucose levels rise >2.4 mM post-prandially in healthy people, skeletal muscle MBF and MBV become impaired; whereas small increments in glucose (<2.4mM) post-prandially are accompanied by augmented muscle microvascular responses. The findings from the current study support the above-mentioned studies of the effects of hyperglycemia on vascular function. Importantly, however, we have demonstrated here that the vascular impairment occurs at the microvascular level (which are predominantly controlled by pre-capillary arterioles) rather than at the level of the large vessels controlling total limb flow, which has significant implications for glucose disposal by muscle.

A number of animal studies have shown that intravenous infusion of glucose for 3-5 hrs markedly impairs skeletal muscle glucose disposal in vivo (12, 19) and this impairment occurred without a concomitant decrease in the insulin signaling cascade in skeletal muscle responsible for glucose uptake (12). This suggests that other peripheral mechanisms, such as impaired microcirculation in skeletal muscle may be involved, as insulin dilates pre-capillary arterioles (31). Notably, it has been reported that the microvascular response to insulin is switched from dilation to constriction by the presence of hyperglycemia (31). NOS is essential for insulin’s vascular actions (2, 25, 39, 40, 44), and short-term hyperglycemia (25 mM x 24 hrs) markedly impairs insulin-mediated eNOS activation (8). Acute hyperglycemia has been observed to diminish endothelial vascular responsiveness in healthy humans via activation of protein kinase C β (1) or reduced NO bioavailability (11). Animal and cell culture studies have also demonstrated a direct effect of high glucose exposure to augment production of vasoconstrictors such as endothelin-1 (22) and prostanoids (37). Given that pre-capillary arterioles are responsible for the regulation of capillary networks in skeletal muscle, and this effect is NOS dependent, it is perhaps not surprising that OGC impairs, while the MMC stimulates, microvascular recruitment in skeletal muscle under similar levels of
hyperinsulinemia. Understanding the mechanism by which the OGC limits skeletal muscle microvascular blood flow warrants further investigation.

Both OGC and MMC produced comparable effects on brachial artery blood flow and large artery/central effects on heart rate, total vascular resistance, augmentation index and central blood pressure. It is well established that consumption of food or glucose (OGTT) causes increases in brachial artery blood flow, heart rate and cardiac output (32, 41, 45). Others have shown that an OGT will reduce central blood pressure and augmentation index (47). We have demonstrated under various circumstances that MBV in skeletal muscle increases independent of changes in total limb blood flow (13, 30, 42, 43, 49). Such is the case during physiological doses of insulin (43, 49) and low intensity contraction (13, 42). Conversely, total limb blood flow can also increase without changes in microvascular blood flow (e.g. adrenaline) (30). Therefore, the opposing effects of the MMC and OGC on skeletal muscle MBV and MBF with a comparable increase in total limb blood flow is not surprising. Improvements in muscle MBV and MBF following the MMC may be reflective of the microvasculature system re-routing flow from less nutritive sites to more nutritive for skeletal muscle cells. Therefore, we speculate that the OGC promotes a non-nutritive flow pattern in skeletal muscle.

The OGC and MMC differ in their macronutrient profile (Table 2). Unlike the OGC, the MMC also contains lipid and protein which may also influence muscle microvascular responses. Insulin infusion (euglycemic hyperinsulinemic clamp) (5, 10, 23), ingestion of a MMC (16, 41), or ingestion of an amino acid meal that does not contain any carbohydrate (24), increases microvascular blood flow in skeletal muscle of healthy humans. The common link between these meals is hyperinsulinemia due to amino acid- and glucose-stimulated pancreatic insulin secretion. It is for that reason we carefully formulated our MMC to contain a sufficient amount
of protein and carbohydrate to produce hyperinsulinemia and to match to the hyperinsulinemia seen in response to the 50g glucose load (Figure 1). Elevating plasma FFAs is detrimental for insulin-mediated microvascular blood flow (21), however in the current study FFAs decreased in both OGC and MMC to a similar level (Figure 1). Therefore FFAs play a negligible role in blocking muscle microvascular flow during these post-prandial tests. The OGC decreased microvascular blood flow in skeletal muscle despite a similar level of hyperinsulinemia to the MMC, which has led us to postulate that hyperglycemia is a key player in the microvascular impairment. However, hyperglycemia may not be the only contributing factor to the divergent microvascular responses between the MMC and OGC. The contribution of other macronutrients in the MMC and involvement of other hormone derived mechanism such as gut-derived hormones which have also been shown to stimulate microvascular blood flow in skeletal (34) may also play a role. Although Jackson et al demonstrate that gut-derived hormones did not explain the differences in muscle glucose disposal following a mixed composite meal and an OGC (14), given that some of these hormones are vasoactive it would be important to follow-up to confirm that the changes we are seeing in the current project are related to hyperglycemia and not incretin release. Also, whether lowering the amount of glucose in the oral glucose load to match blood glucose levels seen in the MMC elicits a similar (enhanced) muscle microvascular response is not known and is also important to follow-up.

There are several important implications from this study. Firstly, consumption of high glycemic meals impairs skeletal muscle microvascular blood flow which may limit glucose disposal into skeletal muscle. Indeed, others have demonstrated that peripheral glucose uptake is greater after a mixed meal compared to an OGTT when adjusted for arterial glucose levels (14). Secondly, the contribution of skeletal muscle glucose uptake in the post-prandial state may be
underestimated when using the OGTT as a post-prandial test, and there has been significant
debate as to how much glucose is disposed into skeletal muscle in the post-prandial state. The
euglycemic hyperinsulinemic clamp technique using a physiological dose of insulin (to reflect
post-prandial insulin levels) indicates that ~80% of glucose is disposed in skeletal muscle (9,
38) whereas the OGTT indicates that skeletal muscle is only responsible for 30-40% of glucose
disposal (15, 26). Here we demonstrate that the OGTT may have underestimated the potential
contribution of the skeletal muscle because of restricted glucose delivery to myocytes. Thirdly,
glucose challenges (e.g. OGC or OGTT) do not evoke a normal physiological response
peripherally and therefore using these tests for determining glucose intolerance may require
additional interpretation and perhaps identifying tests that provoke a normal microvascular
blood flow response (e.g. a MMC) need to be strongly considered.
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One person who substantially contributed to the manuscript, but did not fulfil authorship criteria, is named here. The authors would like to specially thank Dr Kazuaki Negishi from the Menzies Institute for Medical Research for his help with intravenous cannulations for the study.

GRANTS

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DISCLOSURES

None.
REFERENCES


FIGURE CAPTIONS

Figure 1. Glucose and insulin response to MMC and OGC. (A) Blood glucose time course, (B) Area under the blood glucose time curve, (C) Plasma insulin time course and (D) Area under the plasma insulin time curve., (E) Plasma free fatty acid (FFA) levels and (F) Change in FFA levels at 60 mins. Data are means ± SD for each group (n=15). Repeated-measures two-way ANOVA was used to determine if there were differences between treatment groups over the time course of the experiment, or Student’s t-test (or Signed Rank Test if data not normally distributed) was used for single point measurements. When a significant difference was found, pair wise comparisons by the Student–Newman–Keuls test was used to determine treatment differences. # P<0.05 versus MMC, *P<0.01 versus 0 min.

Figure 2. Skeletal muscle microvascular and brachial artery responses to MMC and OGC. (A) Skeletal muscle microvascular blood volume (MBV), (B) Skeletal muscle microvascular flow velocity (β), (C) Skeletal muscle microvascular blood flow (MBF), (D) Brachial artery diameter, (E) Brachial artery flow velocity and (F) Brachial artery blood flow. Data are means ± SD for each group (n=15). Repeated-measures two-way ANOVA was used to determine if there were differences between treatment groups over the time course of the experiment. When a significant difference was found, pair wise comparisons by the Student–Newman–Keuls test was used to determine treatment differences. *P<0.05 versus baseline (0 min), # P<0.05 versus MMC. MBV is expressed as acoustic intensity (AI), microvascular filling rate or β is expressed as 1/second, MBF is expressed as acoustic intensity/sec (AI/sec). Figure 3. Large artery hemodynamic responses to MMC and OGC. (A) Heart rate, (B) Total vascular resistance, (C) Aortic stiffness, (D) Central diastolic blood pressure (DBP), (E) Central systolic blood pressure (SBP) and (F) Augmentation index adjusted to heart rate of 75 beats per min. Data are means ± SD for each group (n=15). Repeated-measures two-way ANOVA was used to determine if there were differences between treatment groups over
the time course of the experiment. When a significant difference was found, pair wise comparisons by the Student–Newman–Keuls test was used to determine treatment differences. *P<0.05 versus baseline (0 min).

Figure 4. Relationship between (A) skeletal muscle microvascular blood volume (MBV), (B) microvascular blood flow (MBF), and (C) brachial blood flow (BF) and blood glucose levels in response to a (○) MMC and (●) OGC at 1hr. Pearson’s bivariate correlation were used to evaluate relationships between variables. Equations of the line of best fit are provided. MBV is expressed as acoustic intensity (AI), MBF is expressed as acoustic intensity/sec (AI/sec).
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<tr>
<td>Sex (M/F)</td>
<td>10/5</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.4 ± 2.7</td>
<td>21.7 - 31.8</td>
</tr>
<tr>
<td>Fasting glucose (mM)</td>
<td>4.7 ± 0.4</td>
<td>4.0 - 6.0</td>
</tr>
<tr>
<td>HbA1c % (mmol/mol)</td>
<td>5.4 ± 0.4 (35.1 ± 2.7)</td>
<td>5.0 - 5.7 (31.0 – 39.0)</td>
</tr>
<tr>
<td>Fasting insulin (pM)</td>
<td>41 ± 8</td>
<td>32 - 53</td>
</tr>
<tr>
<td><strong>Plasma lipids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mM)</td>
<td>5.0 ± 1.2</td>
<td>3.7 - 6.8</td>
</tr>
<tr>
<td>LDL (mM)</td>
<td>3.1 ± 0.8</td>
<td>1.6 - 4.5</td>
</tr>
<tr>
<td>HDL (mM)</td>
<td>1.5 ± 0.4</td>
<td>1.1 – 1.9</td>
</tr>
<tr>
<td>Triglyceride (mM)</td>
<td>0.8 ± 0.4</td>
<td>0.5 – 1.7</td>
</tr>
<tr>
<td>FFA (mM)</td>
<td>0.4 ± 0.2</td>
<td>0.2 – 0.8</td>
</tr>
<tr>
<td><strong>Brachial Blood Pressure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>123 ± 12</td>
<td>106 - 148</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>79 ± 8</td>
<td>61 - 95</td>
</tr>
</tbody>
</table>

**Table 1. Participant characteristics.** Data expressed as means ± SD (n=15).

BMI = body mass index; DBP = diastolic blood pressure; FFA = free fatty acid; HDL = high density lipoprotein; LDL = low density lipoprotein; SBP = systolic blood pressure.
Table 2. Macronutrient composition of the MMC and OGC per serving.

<table>
<thead>
<tr>
<th></th>
<th>MMC</th>
<th>OGC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>1272</td>
<td>837</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>21.7</td>
<td>-</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>4.8</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>41.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Sugars (g)</td>
<td>25.1</td>
<td>50.0</td>
</tr>
</tbody>
</table>
Figure 1
Figure 2
Figure 3
Figure 4.