HHS Public Access

Author manuscript

J Physiol. Author manuscript; available in PMC 2022 February 16.

Published in final edited form as:

J Physiol. 2022 February ; 600(4): 949–962. doi:10.1113/JP281286.

Acute hyperglycaemia enhances both vascular endothelial function and cardiac and skeletal muscle microvascular function in healthy humans

William B. Horton[1], **Linda A. Jahn**[1], **Lee M. Hartline**[1], **Kevin W. Aylor**[1], **James T. Patrie**[2], **Eugene J. Barrett**[1,3]

[1]Division of Endocrinology and Metabolism, Department of Medicine, University of Virginia School of Medicine, Charlottesville, VA, USA

[2]Division of Biostatistics, Department of Public Health Sciences, University of Virginia School of Medicine, Charlottesville, VA, USA

[3]Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, VA, USA

Abstract

High glucose concentrations acutely provoke endothelial cell oxidative stress and are suggested to trigger diabetes-related macro- and microvascular injury in humans. Multiple clinical studies report that acute hyperglycaemia (induced by mixed meal or oral glucose) decreases arterial vascular function in healthy humans. Feeding, however, impacts autonomic output, blood pressure, and insulin and incretin secretion, which may each independently alter vascular function and obscure the effect of acute hyperglycaemia *per se*. Surprisingly, no studies have examined the acute effects of intravenous glucose-induced hyperglycaemia on both macro- and microvascular function while controlling plasma insulin concentrations. In this randomized study of healthy young adults, we compared macrovascular (i.e. brachial artery flow-mediated dilatation, carotid-femoral pulse wave velocity and post-ischaemic brachial artery flow velocity) and microvascular (heart and skeletal muscle perfusion by contrast-enhanced ultrasound) functional responses to euglycaemia and hyperglycaemia. Octreotide was infused throughout both protocols to prevent endogenous insulin release. Acute intravenous glucose-induced hyperglycaemia enhanced brachial artery flow-mediated dilatation ($P = 0.004$), increased skeletal muscle microvascular blood volume and flow ($P = 0.001$), and expanded cardiac muscle microvascular blood volume (P

Corresponding author W. B. Horton: 450 Ray C. Hunt Drive, Box 800136, Charlottesville, VA 22908, USA. wbh2n@virginia.edu.

Author contributions

W.B.H. and E.J.B. contributed to the conception and design of this experiment. W.B.H., L.A.J., L.M.H. and K.W.A. contributed to acquisition of data. W.B.H., K.W.A., J.T.P. and E.J.B. contributed to analysis and interpretation of the data. W.B.H., L.A.J., L.M.H., K.W.A., J.T.P. and E.J.B. contributed to drafting the article or revising it critically for important intellectual content. All authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Additional information

Competing interests

All authors have no competing interests to declare or disclose.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Statistical Summary Document

= 0.014). No measure of vascular function changed during octreotide-maintained euglycaemia. Our findings suggest that unlike meal-provoked acute hyperglycaemia, 4 h of intravenous glucose-induced hyperglycaemia enhances brachial artery flow-mediated dilatation, provokes cardiac and skeletal muscle microvascular function, and does not impair aortic stiffness. Previous findings of acute large artery vascular dysfunction during oral glucose or mixed meal ingestion may be due to differences in study populations and meal-induced humoral or neural factors beyond hyperglycaemia *per se*. (ClinicalTrials.gov number NCT03520569.)

Keywords

cardiac muscle; hyperglycaemia; microvascular perfusion; muscle metabolism; octreotide; skeletal muscle

Introduction

Chronic hyperglycaemia is a major driver of diabetes mellitus (DM) microvascular complications through non-enzymatic glycation and formation of advanced glycation end-products, enhanced reactive oxygen species production, endoplasmic reticulum stress, polyol pathway activation, and other derangements (Shah & Brownlee, 2016; Barrett *et al.* 2017).

Acute (hours-to-days) exposure to hyperglycaemia provokes oxidative stress in cultured human endothelial cells (Brownlee, 2001; Shah & Brownlee, 2016), which may be an initial trigger for DM-related micro- and macrovascular injury. Moreover, absent DM, acute hyperglycaemia (AH) associates with worsened vascular function in a number of clinical settings (DECODE study group, 1999; Ishihara *et al.* 2003; Deedwania *et al.* 2008; Van den Berghe *et al.* 2009). However, it is uncertain whether AH *per se* or the underlying conditions that provoked AH are primarily responsible for this vascular dysfunction.

Loader *et al.* performed a systematic review and meta-analysis of >30 clinical observational studies and controlled trials that examined AH's effect on macrovascular endothelial function (Loader *et al.* 2015). In healthy young adults, AH (either from a mixed meal or oral glucose) decreased flow-mediated dilatation (FMD) in 9 of 11 studies while two reported no significant change. The use of oral glucose or high-carbohydrate meals to induce AH in these studies is complicated by their effects on autonomic nervous system output (Lipsitz *et al.* 1993), blood pressure (Sauder *et al.* 2012), gut hormone release (Holst, 2007) and insulin secretion, which can each alter vascular function and which were not controlled in the 11 studies cited. Surprisingly, the effect of intravenous (I.V.) glucose-induced AH on arterial FMD has been little studied. One study in healthy young adults reported no effect (Bagg *et al.* 2000), while FMD declined in response to I.V. glucose-induced AH in older (Ceriello *et al.* 2008*a*,b) and overweight or obese subjects (Ceriello *et al.* 2008*a*,b; Perkins *et al.* 2015; Joy *et al.* 2016).

Many fewer studies have examined the microvascular responses to AH in healthy humans, and the majority of these studies used resistance arteriolar flow (e.g. responses to acetylcholine, post-ischaemic hyperaemia or cutaneous warming) to quantify oral glucose's effects. Results were near-evenly split between improved (Grasser *et al.* 2014), unchanged

(Charkoudian *et al*. 2002) or diminished (Akbari *et al*. 1998; Russell *et al*. 2018) responses. Once again, contributions by insulin, incretins and autonomic output were neither controlled nor examined in these studies. Two careful clinical studies of healthy young adults used brachial artery glucose infusion to produce local forearm hyperglycaemia without insulin or incretin stimulation and measured forearm blood flow basally and in response to cholinergic stimulation. One reported a flow decrease after 6 h of AH (Williams *et al*. 1998), and the second reported a slight increase at 6, 12 and 24 h (Houben *et al*. 1996). Plasma insulin was maintained at basal levels in each forearm study. Another study of healthy young adults utilized I.V. glucose-induced hyperglycaemia plus somatostatin and basal insulin infusion for 6 h and saw a significant increase in forearm blood flow (van Gurp *et al*. 2005). These studies did not examine macrovascular endothelial function.

Given the multiple, complex physiological responses to meal ingestion, we rationalized that specifically testing AH's direct macro- and microvascular effects would be best approached using I.V. glucose and controlling endogenous insulin release. To our knowledge, such studies have not been previously performed. We studied healthy young adults in an attempt to identify 'normal' vascular responses. To accomplish this, we infused glucose intravenously for 4 h to generate steady-state AH (during co-administration of octreotide (OCT)) and assessed vascular function at three distinct levels of the arterial tree. We measured microvascular perfusion using contrast-enhanced ultrasound in both cardiac and skeletal muscle, resistance arteriolar function using brachial artery post-ischaemic flow velocity (PIFV), and macrovascular function with carotid-femoral pulse wave velocity (cfPWV) and brachial artery FMD. As a control, we tested the effect of euglycaemic OCT infusion on these same vascular endpoints.

Methods

Ethical approval

Each study participant gave written informed consent at their initial visit prior to being carefully screened to verify inclusion/exclusion criteria. All study protocols were approved by the University of Virginia (UVA) Institutional Review Board (Study No. 19948) and met all conditions outlined in the seventh revision of the *Declaration of Helsinki* for use of human volunteers. This trial was registered with ClinicalTrials.gov on 9 May 2018 (ClinicalTrials.gov number NCT03520569).

Recruitment and study population

We recruited study participants by community flyers and digital advertisements. Healthy young adults met inclusion criteria if they were ≥18 and ≤35 years old, had a body mass index of 18–25 kg m^{-2}, and had fasting plasma glucose <100 mg dl^{-1} and blood pressure <140/90 mmHg at time of screening. Subjects were excluded if they were current smokers or quit smoking <5 years ago, had a first-degree relative with type 2 DM, were taking vasoactive medications (e.g. anti-hypertensives, diuretics, statins, etc.), were pregnant (i.e. positive pregnancy test) or nursing, had a history of allergy or prior adverse reaction to OCT or Definity®, or had history of significant premorbid disease that could, in the investigator's opinion, affect outcome measures or subject safety.

Clinical assessment and initial screening

All screening visits and infusion studies were conducted at the UVA Clinical Research Unit (CRU). Screening included a detailed medical history and physical examination along with fasting measures of complete blood count, comprehensive metabolic panel, lipid panel, fasting plasma glucose and serum pregnancy test.

Experimental protocols

Subjects meeting inclusion criteria were randomized to one of two study protocols (Fig. 1) using a 1:1 allocation with a computer-generated sequence program (Urbaniak & Plous, 2013). After randomization, study personnel were blinded to subject and protocol when evaluating outcome measures. In this randomized crossover trial, the infusion protocols were designed to test the effects of OCT alone (protocol A) or OCT with AH (protocol B) on systemic macrovascular and cardiac and skeletal muscle microvascular function. Protocols A and B were performed ≥2 but ≤4 weeks apart for individual subjects. For each protocol, we measured cfPWV, brachial artery FMD and PIFV, and cardiac and skeletal muscle microvascular perfusion immediately before and at the end of the infusion period (Fig. 1). Study participants were instructed to avoid alcohol, exercise and caffeine for 24 h and fast overnight prior to admission to the CRU. Prior to study initiation, we placed I.V. catheters in the right wrist for blood sampling and in the right antecubital fossa for administration of insulin, glucose, OCT and saline. Studies began with a saline (protocol A) or an OCT infusion (protocol B), with simultaneously infused regular insulin (to maintain plasma insulin near basal levels) and variable-rate glucose (to maintain euglycaemia in protocol A and hyperglycaemia in protocol B). We did not replace glucagon or growth hormone, as there is currently no evidence that acutely suppressing basal levels of either hormone affects vascular function.

Protocol A (euglycaemia).

A 90-min saline infusion was initiated, with baseline vascular function measurements obtained during the final 30 min (Fig. 1A). Then, OCT (30 ng kg^{-1} min^{-1}) with basal insulin replacement (0.15 mU min^{-1} kg^{-1}) was infused for 240 min. Plasma glucose (PG) was sampled every 10 min and euglycaemia (EU) was maintained by a variable-rate glucose infusion using the negative feedback principle (DeFronzo *et al.* 1979). We then repeated vascular measurements over the final 30 min of study. Plasma insulin was sampled every 30 min throughout the protocol, and intercellular adhesion molecule-1 (ICAM-1), interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α) and protein carbonyls were sampled at baseline and end-of-study.

Protocol B (acute hyperglycaemia).

OCT with basal insulin replacement was continuously infused for 90 min, with baseline vascular function measurements obtained over the final 30 min (Fig. 1B). Then, a primed, continuous variable-rate 20% dextrose infusion was begun to acutely raise and maintain PG at ~200 mg dl^{-1} using the hyperglycaemic clamp method (DeFronzo *et al.* 1979). PG was sampled every 5 min and plasma insulin every 30 min, with repeat vascular measurements

obtained over the final 30 min of AH. ICAM-1, IL-6, TNF-α and protein carbonyls were again sampled at baseline and end-of-study.

Carotid-femoral pulse wave velocity.

To assess central aortic stiffness, cfPWV was measured non-invasively using a Sphygmacor tonometer (ATCOR USA; Napierville, IL, USA). To minimize the effects of sympathetic activity on cfPWV measurements, participants lay in the supine position for at least 15 min prior to measurement. We measured the distance from the suprasternal notch to the carotid pulse and from the suprasternal notch to the femoral pulse on the same side. For each cfPWV measurement, 10 s of carotid and 10 s of femoral arterial waveforms were recorded. cfPWV measurements were made in duplicate and the mean value was reported. We followed expert consensus (Thijssen *et al.* 2019; Townsend *et al.* 2015) and had the same trained observer obtain all vascular measurements in this study. We assessed cfPWV intraobserver reliability by having the observer record three serial cfPWV measurements on the same subject over a 4-h period. The coefficient of variation was 3.63%, indicating good intraobserver reliability (Shechtman, 2013; Thijssen *et al.* 2019).

Flow-mediated dilatation and post-ischaemic flow velocity.

We measured left brachial artery FMD and left forearm PIVF with the Epiq 7 cardiovascular ultrasound (Philips Medical Systems; Andover, MA, USA) instrument with a linear array probe (L12–3) steadied by a probe-holder as described previously (Jahn *et al.* 2016). FMD images were analysed using Brachial Analyzer (Medical Imaging Applications, LLC; Coralville, IA, USA) edge detection software by study personnel blinded to subject and protocol. We assessed FMD intraobserver reliability by having the same trained observer record eight serial FMD measurements on the same subject over a 4-h period. Coefficient of variation was 7.41%, indicating good intraobserver reliability (Shechtman, 2013; Thijssen *et al.* 2019).

Microvascular perfusion by contrast-enhanced ultrasound.

Cardiac (interventricular septum) and forearm skeletal muscle microvascular perfusion were assessed with an Epiq 7 ultrasound system during steady-state infusion of Definity® microbubbles (Lantheus Medical Imaging; North Billerica, MA, USA) using low mechanical index (MI; 0.10) continuous imaging for 20 s (myocardium) or 30 s (forearm) at a framerate of 15 s^{-1} with a flash at 0.88 MI to initiate a replenishment curve. Four 30-s replenishment curves for forearm and four 20-s replenishment curves for myocardium were acquired at baseline and at the end of the infusion study. Replenishment curves were analysed using Q-Lab (Philips Research; Cambridge, MA, USA) by study personnel blinded to subject and protocol.

Biochemical analyses

Complete blood count, comprehensive metabolic panel, lipid panel, fasting PG, and serum pregnancy tests were assayed at the UVA Clinical Chemistry Laboratory. PG was measured with a YSI 2700 Biochemistry Analyzer (Yellow Springs Instrument Company; Yellow Springs, OH, USA), plasma insulin with an ALPCO insulin ELISA assay (ALPCO; Salem,

NH, USA), and ICAM-1, IL-6, TNF-α and protein carbonyls by ELISA assay (R&D Systems; Minneapolis, MN, USA). All assays were read on a Synergy 2 microplate reader (BioTek; Winooski, VT, USA).

Data storage

Study data were stored in a Research Electronic Data Capture (REDCap) (Harris *et al.* 2019) project file repository hosted at UVA. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Statistical analyses

Sample size.—We used the Hedges g effect size from a prior study that examined the effects of acute hyperglycaemia on brachial artery flow-mediated dilatation in persons without diabetes (Joy *et al.* 2016) to estimate sample size for the current study. This calculation indicated that a total sample size of ≥10 subjects would have >90% power to detect meaningful differences in vascular function.

Outcomes.—The primary macrovascular and microvascular outcomes for each protocol were change in FMD and microvascular blood volume (MBV), respectively. Main secondary outcomes for each protocol included changes in cfPWV, PIFV, microvascular flow velocity (MFV), microvascular blood flow (MBF), plasma insulin and ICAM-1.

Descriptive summarization.—Patient demographics were summarized using common descriptive statistics. The arithmetic mean and standard deviation, median and interquartile range were used to summarize continuous scaled outcome measures.

Data transformation and summarization.—The pre- and post-intervention outcome measurements for each protocol were rescaled to the natural logarithmic scale (i.e. $\log_e$). The analytical outcome data were then derived by subtracting the $\log_e$-transformed pre-intervention outcome measurements from the $\log_e$-transformed post-intervention outcome variable measurements. For all outcome measurements, the point estimate for the mean pre-intervention outcome measurements, the point estimate for the mean pre- to post-intervention outcome measurement change, and the point estimate for the difference between the mean of the pre-intervention outcome measurements and the mean pre- to post-intervention outcome measurement change for each protocol were converted via natural logarithmic antilog transformation (i.e. e^x) to a geometric mean ratio scale.

Outcome measure analyses.—The pre-intervention EU and AH outcome measurements were compared by linear mixed model (LMM) analysis of variance, testing whether pre-intervention measurements were equal. To meet assumptions of the LMM, all outcome measurements were rescaled to the natural logarithmic scale (i.e. $\log_e$). The pre- to post-intervention outcome variable change for each protocol was compared by LMM analysis of covariance. Significance was set at $\alpha = 0.05$ (two-tailed test). Bonferroni adjustment was used to correct for multiple comparisons. All statistical analyses were performed with SAS Studio 3.8 (SAS; Cary, NC).

LMM specification.: The analytical outcome data of each protocol served as the dependent variable measurements of the LMM. An indicator variable to identify the protocol (i.e. A or B) served as one of the LMM independent variables, and the $\log_e$-transformed pre-intervention outcome measurements of protocols A and B served as a second LMM independent variable. Note that the $\log_e$-transformed pre-intervention measurements were included as part of the LMM so that the between-admission comparison of the mean pre- to post-intervention change in the outcome measure could be standardized to a common reference pre-intervention measurement value.

Hypothesis testing.: Primary hypotheses under the null tested whether the mean within-protocol change in each outcome measure was equal to zero. Secondary hypotheses examined whether the mean pre- to post-intervention outcome measurement change was equivalent between protocols after standardizing the comparison to a common reference pre-intervention measurement value. Specific hypotheses were that AH negatively alters FMD, cfPWV, PIFV, MBV, MFV and MBF.

Results

Baseline subject characteristics and demographics

Table 1 details baseline demographics of the 13 study participants. All had normal BMI, blood pressure and fasting PG. Notably, 10 subjects completed both protocols while three subjects completed only the EU protocol. Reasons for withdrawal were that one subject moved out of the geographic area, one experienced abdominal cramping secondary to OCT and one withdrew due to scheduling difficulties.

Plasma insulin, glucose, ICAM-1 and inflammatory biomarker concentrations

Figure 2 shows the time course for mean PG (Fig. 2A) and mean glucose infusion rate (Fig. 2B) throughout each infusion study. Plasma insulin concentrations during EU and AH did not change from baseline within either protocol (Table 2), indicating that the selected OCT and basal insulin doses maintained the basal insulin and glucose milieu during EU and prevented an endogenous insulin secretory response during AH. Furthermore, there were no between-protocol differences in insulin concentrations during either the pre- or post-intervention periods (Table 2). There were also no significant within- or between-protocol changes in ICAM-1, indicating that neither protocol induced endothelial inflammation/dysfunction (Table 2). Plasma IL-6, TNF-α and protein carbonyl levels in these healthy subjects were below the lower limits of detection for the assays used at both the baseline and end-of-study measurements.

Macrovascular function

Figure 3 shows the pre- and post-intervention measures of cfPWV, FMD and PIFV in each protocol. FMD did not change with EU but significantly increased with AH (ratio of geometric mean (RGM): 1.34; 95% CI: 1.11, 1.62; $P = 0.004$) (Table 3). There were no significant pre- to post-intervention changes in cfPWV or PIFV with either protocol.

Microvascular function

Figure 4 shows the pre- and post-intervention measures for skeletal muscle MBV (upper panel), MFV (middle panel) and MBF (lower panel). MBV is given in arbitrary units of video intensity, MFV in 1/s, and MBF is the product of MBV and MFV (video intensity/s). There were no significant changes from baseline in MBV (RGM: 1.10; 95% CI: 0.92, 1.32; $P = 0.273$), MFV (RGM: 1.19; 95% CI: 0.95, 1.48; $P = 0.124$), or MBF (RGM: 1.31; 95% CI: 0.89, 1.93; $P = 0.162$) during 4 h of EU. By contrast, AH increased skeletal muscle MBV (RGM: 1.68; 95% CI: 1.37, 2.06; $P < 0.001$), MFV (RGM: 1.39; 95% CI: 1.08, 1.79; $P = 0.012$) and MBF (RGM: 2.34; 95% CI: 1.51, 3.63; $P = 0.001$) above baseline (Table 3). MBV, MFV and MBF declined with AH in only the one subject who had the highest baseline values for each. Moreover, the change above baseline was significantly greater for AH compared to EU with both MBV (RGM: 1.50; 95% CI: 1.15, 1.95; $P = 0.008$) and MBF (RGM: 1.68; 95% CI: 1.06, 2.64; $P = 0.031$; Bonferroni-adjusted $P = 0.062$).

The response of cardiac muscle to 4 h of EU or AH is shown in Fig. 5. As seen in skeletal muscle, EU during OCT infusion did not alter cardiac MBV (RGM: 1.08; 95% CI: 0.91, 1.29; $P = 0.356$), MFV (RGM: 1.16; 95% CI: 0.93, 1.45; $P = 0.181$), or MBF (RGM: 1.25; 95% CI: 0.94, 1.67; $P = 0.113$). By contrast, AH significantly increased cardiac MBV (RGM: 1.34; 95% CI: 1.07, 1.67; $P = 0.014$) (Table 3). MFV did not change significantly (RGM: 0.89; 95% CI: 0.67, 1.18; $P = 0.415$) and MBF increased in 7 of 9 subjects, but this trend was not significant (RGM: 1.24; 95% CI: 0.86, 1.78; $P = 0.235$).

Vascular responses by sex

Our study was neither designed nor powered to determine vascular effects by sex, but we did conduct a purely exploratory analysis to determine if any sex-by-protocol interactions existed. There were no such interactions identified for any vascular measure assessed. Sex-by-protocol interaction testing (adjusted for individual baseline responses) identified P-values of 0.760 for cfPWV; 0.804 for FMD; 0.147 for PIFV; 0.605, 0.952 and 0.596 for skeletal muscle MBV, MFV and MBF, respectively; and 0.718, 0.448 and 0.380 for cardiac muscle MBV, MFV and MBF, respectively.

Discussion

The current study provides several novel findings regarding the acute effects of AH on vascular function. First, compared to EU, AH significantly enhanced brachial artery flow-mediated dilatation. This has not been previously reported and contrasts with the consistently reported vascular dysfunction provoked by oral glucose or high carbohydrate meals in healthy young adults (Loader *et al.* 2015). Almost certainly our use of I.V. glucose delivery and of OCT to block insulin and incretin responses during AH each contributed to the observed result. As OCT infusion during euglycaemia did not affect FMD, we cannot attribute the enhanced FMD in protocol B to a vascular action of OCT. We also note that van Gurp *et al.* (2005) previously demonstrated that I.V.-induced hyperglycaemia during somatostatin infusion did not alter sympathetic nerve output. Therefore, altered sympathetic tone is unlikely to account for the enhanced FMD seen here. The unaltered ICAM-1 levels, coupled with enhanced FMD, suggests that 4 h of AH did not interfere with the

ability of shear stress to stimulate nitric oxide production by brachial artery endothelium. Two prior studies utilized similar pancreatic clamp methodology to assess the effects of I.V. glucose-induced AH on vascular inflammatory biomarkers and endothelial function in non-diabetic (Joy *et al.* 2016) and overweight and obese (Perkins *et al.* 2015) humans. Both studies found that 4 h of I.V. glucose-induced AH increased ICAM-1 (and several other inflammatory biomarkers) and decreased brachial artery FMD, neither of which were seen in the current study. However, the recruited populations for these studies had mean BMI of 29 kg m^{-2} (with range of 23–36 kg m^{-2}) (Joy *et al.* 2016) and 30.1 kg m^{-2} (Perkins *et al.* 2015). Ceriello *et al.* also used pancreatic clamp methodology to assess the effects of I.V. glucose-induced AH on nitrotyrosine (a biomarker of oxidative stress) and brachial artery FMD in healthy subjects (Ceriello *et al.* 2008a,b). Their results showed that AH increased nitrotyrosine and reduced FMD in healthy subjects, and that these negative effects were completely prevented with vitamin C administration. These studies, however, also had healthy cohorts with mean age and BMI of 50.3 years and 27.5 kg m^{-2} (Ceriello *et al.* 2008*a*) and 50.5 years and 28.5 kg m^{-2} (Ceriello *et al.* 2008*b*). Obesity has been clearly linked to reduced brachial artery FMD (Ne *et al.* 2017), and weight loss itself coincides with increased brachial artery FMD (Joris *et al.* 2015). Brachial artery FMD is also impaired in older adults compared to young, healthy adults (Celermajer *et al.* 1994; Donato *et al.* 2007; Gates *et al.* 2007), and a steady age-related decline begins at age 30 years in men and age 45 years in women (Skaug *et al.* 2013). These findings underscore the contrast between our testing the effect of isolated AH from I.V. glucose in young, healthy adults *versus* the effect of oral glucose (with accompanying hormonal and autonomic changes) or the effect of I.V. glucose in overweight and obese or older populations.

We also note that OCT infusion during euglycaemia affected neither cardiac nor skeletal muscle microvascular perfusion. To our knowledge, these results are the first to demonstrate that OCT does not alter cardiac or skeletal muscle microvascular perfusion in humans. This finding is consistent with a prior canine microsphere study that reported no change in total blood flow to either tissue after a high-dose somatostatin infusion (Becker *et al.* 1982). Likewise, human studies using a similar OCT dose reported no change in large vessel endothelial function (Moller *et al.* 1995; Beckman *et al.* 2001; Beckman *et al.* 2002; Joy *et al.* 2016), but did not examine the effect on cardiac or skeletal muscle microvascular perfusion. Recent reports have employed similar hyperglycaemic clamp methods with OCT infusion to assess vascular function and inflammatory biomarker levels in overweight and obese humans without DM (Perkins *et al.* 2015; Joy *et al.* 2016). Thus, use of OCT provided a useful control for the current study. Inasmuch as OCT did not alter any vascular parameter evaluated in our study, its use to test the specific vascular effect of AH independent of changes in insulin, incretins and autonomic activity that accompany oral glucose or mixed meal ingestion was justified.

The present study also provides the first report of acute cardiac and skeletal muscle microvascular responses to isolated AH. Our use of contrast-enhanced ultrasound allowed specific focus on the muscle compartment at both sites while excluding contributions from skin, subcutaneous adipose and bone that contribute particularly to measures of forearm flow. Our study design unexpectedly revealed that 4 h of isolated AH from I.V. glucose significantly increased MBV in both skeletal and cardiac muscle. The increased MBV

indicates an increased endothelial surface area available for nutrient and gas exchange even in the absence of increased total flow. Overall flow (MBV × MFV) likewise increased in skeletal muscle and trended towards increase in cardiac muscle. Thus, AH *per se* enhanced rather than inhibited microvascular perfusion. Insulin secretion was effectively blocked by OCT infusion, discounting any role for insulin-induced vasodilatation. Additionally, our use of I.V. glucose loading (together with OCT infusion) removed possible contributions of incretin hormones on the vasculature. We note that AH has been reported to increase both retinal (Burgansky-Eliash *et al.* 2012; Klefter *et al.* 2015) and renal resting blood flow (Woods *et al.* 1987; Marre *et al.* 1999); however, insulin secretion was not controlled in those studies. Accumulating data have also demonstrated increased resting coronary blood flow in DM subjects compared to healthy controls (Meyer & Schwaiger, 1997; Picchi *et al.* 2011; Sorensen *et al.* 2020), possibly due to increased resting cardiac metabolic demand (Haas *et al.* 2019). This flow increase in the resting heart does not appear benign, as studies have confirmed it as an independent predictor of cardiovascular mortality (Gupta *et al.* 2017) and shown an association with diastolic dysfunction (Haas *et al.* 2019). Future studies could examine pathophysiological mechanisms linking hyperglycaemia, microvascular function and resting MBF in both health and DM.

A fourth finding is that AH did not adversely affect cfPWV or PIFV. Our cfPWV result confirms findings from other studies demonstrating no cfPWV response to AH in healthy humans (Kobayashi *et al.* 2015; Williams *et al.* 2020). PIFV, which principally reflects resistance arteriolar tone, was also not impaired by AH. However, PIFV (like FMD, MBV and MFV) is sensitive to local nitric oxide production and might have been expected to change in concert with these other variables. We cannot at present explain this difference, but considered that PIFV (which like forearm blood flow sums contributions from multiple tissues) might be less sensitive to AH stimulation.

Taken together, neither micro- nor macrovascular function appeared adversely affected by 4 h of isolated hyperglycaemia in the current study. While we are not suggesting that hyperglycaemia has no adverse vascular effects over time, our results do suggest that it is incorrect to conclude that during brief periods of AH, glucose *per se* inhibits macro- and microvascular function. Our findings also suggest that the widely reported adverse vascular responses to oral glucose may reflect contributions of endocrine and/or neural factors beyond AH itself. I.V. glucose delivery bypasses the gut and subsequent release of many gut-derived vasoactive hormones (Holst, 2019). In contrast, mixed meal ingestion (Alsalim *et al.* 2015) and oral glucose (Faerch *et al.* 2015) stimulate incretin and islet hormone release in healthy humans. Recent studies have shown that oral glucose (Russell *et al.* 2018) and high-glucose mixed meals (Parker *et al.* 2020) impair, while low-glucose mixed meal ingestion increases (Russell *et al.* 2018), skeletal muscle microvascular perfusion. As such, our findings support the hypothesis that vascular functional and microvascular perfusion responses may differ by glucose delivery method (Loader *et al.* 2015) and/or nutritional composition of a meal (Roberts-Thomson *et al.* 2020; Alvares, 2020). In many of the studies reviewed by Loader *et al.* (2015), the oral glucose ingestion may confound results by increasing concentrations of both glucose and insulin. For example, insulin is a potent vasodilator that acts through endothelial stimulation (Steinberg *et al.* 1994; Baron *et al.* 1995; Barrett *et al.* 2011; Hoffman, 2015). Beyond this, hyperglycaemia without

accompanying changes in plasma insulin has been shown to markedly increase baseline forearm blood flow in both healthy adults (Hoffman *et al.* 1999) and adolescents with type 1 DM (Dye *et al.* 2012). Further study is needed to determine specific mechanisms by which AH induces different vascular responses by glucose delivery method and/or meal composition.

There are both strengths and limitations of this study that should be noted. Strengths include: (1) the use of OCT to block endogenous insulin (D'Alessio *et al.* 1989) and incretin(s) (Plockinger *et al.* 1999) secretion during AH, which is important given that these hormones have vasodilatory effects (Steinberg *et al.* 1994; Beckman *et al.* 2002; Ban *et al.* 2008; Wang *et al.* 2020); (2) induction of hyperglycaemia by I.V. glucose, which avoided the sympathetic haemodynamic changes that accompany oral glucose or meal ingestion (Young & Landsberg, 1977; Welle *et al.* 1981); (3) a study design that allowed us to characterize the microvascular effects of OCT and AH in cardiac and skeletal muscle, which had heretofore been unreported; and (4) enhancement of scientific rigor by blinding study personnel to subject and protocol during analysis of key vascular parameters.

There are several limitations to our study that also warrant consideration. By design, all study participants were healthy and lean with intact vascular function. Those who are older and/or less healthy might respond differently, and we suggest that this be a focus of future investigation. Second, it is possible that the use of OCT has in some unknown manner skewed the vascular response to AH. We recognize that this possibility should not be discounted, though we do note that previous studies using a similar dose of OCT reported no changes in endothelium-dependent vasodilatation (Beckman *et al.* 2001, 2002; Joy *et al.* 2016) or forearm perfusion (Moller *et al.* 1995). Prior work has also reported that OCT infusion does not alter the haemodynamic effects of AH (Marfella *et al.* 2000). Third, we chose to study the vascular effects of AH over 4 h instead of shorter time periods. Two key considerations led to using a 4-h duration for glucose infusion: (1) increasing plasma free fatty acid concentrations for 4 h impairs vascular function (Steinberg *et al.* 2000) and vascular insulin sensitivity (Liu *et al.* 2011), yet we recently observed that 4 h of AH did not inhibit insulin's microvascular vasodilatory action, suggesting that the acute vascular responses to glucose *versus* fat nutrient excess diverge (Horton *et al.* 2020); and (2) the United States Food and Drug Administration limits the allowable daily dose of Definity® microbubble infusion to one vial per day (allowing collection of only a before-and-after treatment image set), and a 4-h duration ensured adequate time for any AH effect(s) to develop. We cannot exclude that a shorter AH duration may yield different results.

Conclusions

We provide the first evidence that AH *per se* enhanced skeletal muscle microvascular perfusion, cardiac muscle MBV and brachial artery vascular function in the same subjects. We also found that OCT altered neither micro- nor macrovascular function during euglycaemia. Compared to other published findings, our results suggest that vascular responses to AH differ based on the study population (i.e. normal weight *vs.* overweight/obese) and/or glucose delivery method (i.e. I.V. *vs.* oral glucose).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding

This work was supported in part by NIH research grants F32HL14230401, KL2TR003016, and UL1TR003015 (to W.B.H.) and RO1DK073059 and RO1HL142250 (to E.J.B.).

Biography

William Horton is an Assistant Professor of Medicine in the University of Virginia Division of Endocrinology and Metabolism. He completed postdoctoral fellowship training under Eugene Barrett, MD, PhD, where he studied the acute effects of insulin and glucose on micro- and macrovascular function. He lives with type 1 diabetes and seeks to extend this training into new areas of investigation, including the effects of glycaemic variability on cardiovascular disease in persons with type 1 diabetes.

Data availability statement

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

References

Akbari CM, Saouaf R, Barnhill DF, Newman PA, LoGerfo FW & Veves A (1998). Endothelium-dependent vasodilatation is impaired in both microcirculation and macrocirculation during acute hyperglycemia. J Vasc Surg 28, 687–694. [PubMed: 9786265]

Alsalim W, Omar B, Pacini G, Bizzotto R, Mari A & Ahren B (2015). Incretin and islet hormone responses to meals of increasing size in healthy subjects. J Clin Endocrinol Metab 100, 561–568. [PubMed: 25375983]

Alvares TS (2020). High-glucose mixed meals impair microvascular function: the attenuating effect of exercise. J Physiol 599, 11–12. [PubMed: 33241858]

Bagg W, Whalley GA, Sathu A, Gamble G, Sharpe N & Braatvedt GD (2000). The effect of acute hyperglycaemia on brachial artery flow mediated dilatation in normal volunteers. Aust N Z J Med 30, 344–350. [PubMed: 10914752]

Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Drucker DJ & Husain M (2008). Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. Circulation 117, 2340–2350. [PubMed: 18427132]

Baron AD, Steinberg HO, Chaker H, Leaming R, Johnson A & Brechtel G (1995). Insulin-mediated skeletal muscle vasodilation contributes to both insulin sensitivity and responsiveness in lean humans. J Clin Invest 96, 786–792. [PubMed: 7635973]

Barrett EJ, Liu Z, Khamaisi M, King GL, Klein R, Klein BEK, Hughes TM, Craft S, Freedman BI, Bowden DW, Vinik AI & Casellini CM (2017). Diabetic microvascular disease: an endocrine society scientific statement. J Clin Endocrinol Metab 102, 4343–4410. [PubMed: 29126250]

Barrett EJ, Wang H, Upchurch CT & Liu Z (2011). Insulin regulates its own delivery to skeletal muscle by feed-forward actions on the vasculature. Am J Physiol Endocrinol Metab 301, E252–E263. [PubMed: 21610226]

Becker RH, Scholtholt J, Scholkens BA, Jung W & Speth O (1982). A microsphere study on the effects of somatostatin and secretin on regional blood flow in anesthetized dogs. Regul Pept 4, 341–351. [PubMed: 6129683]

Beckman JA, Goldfine AB, Gordon MB & Creager MA (2001). Ascorbate restores endothelium-dependent vasodilation impaired by acute hyperglycemia in humans. Circulation 103, 1618–1623. [PubMed: 11273987]

Beckman JA, Goldfine AB, Gordon MB, Garrett LA & Creager MA (2002). Inhibition of protein kinase Cβ prevents impaired endothelium-dependent vasodilation caused by hyperglycemia in humans. Circ Res 90, 107–111. [PubMed: 11786526]

Brownlee M (2001). Biochemistry and molecular cell biology of diabetic complications. Nature 414, 813–820. [PubMed: 11742414]

Burgansky-Eliash Z, Barak A, Barash H, Nelson DA, Pupko O, Lowenstein A, Grinvald A & Rubinstein A (2012). Increased retinal blood flow velocity in patients with early diabetes mellitus. Retina 32, 112–119. [PubMed: 21878846]

Celermajer DS, Sorensen KE, Spiegelhalter DJ, Georgakopoulos D, Robinson J & Deanfield JE (1994). Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. J Am Coll Cardiol 24, 471–476. [PubMed: 8034885]

Ceriello A, Esposito K, Piconi L, Ihnat M, Thorpe J, Testa R, Bonfigli AR & Giugliano D (2008a). Glucose “peak” and glucose “spike”: Impact on endothelial function and oxidative stress. Diabetes Res Clin Pract 82, 262–267. [PubMed: 18950890]

Ceriello A, Esposito K, Piconi L, Ihnat MA, Thorpe JE, Testa R, Boemi M & Giugliano D (2008b). Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. Diabetes 57, 1349–1354. [PubMed: 18299315]

Charkoudian N, Vella A, Reed AS, Minson CT, Shah P, Rizza RA & Joyner MJ (2002). Cutaneous vascular function during acute hyperglycemia in healthy young adults. J Appl Physiol 93, 1243–1250. [PubMed: 12235021]

D’Alessio DA, Sieber C, Beglinger C & Ensinck JW (1989). A physiologic role for somatostatin 28 as a regulator of insulin secretion. J Clin Invest 84, 857–862. [PubMed: 2569481]

DECODE study group; European Diabetes Epidemiology Group (1999). Glucose tolerance and mortality: comparison of WHO and American Diabetes Association diagnostic criteria. Lancet 354, 617–621. [PubMed: 10466661]

Deedwania P, Kosiborod M, Barrett E, Ceriello A, Isley W, Mazzone T & Raskin P (2008). Hyperglycemia and acute coronary syndrome: a scientific statement from the American Heart Association Diabetes Committee of the Council on Nutrition, Physical Activity, and Metabolism. Circulation 117, 1610–1619. [PubMed: 18299505]

DeFronzo RA, Tobin JD & Andres R (1979). Glucose clamp technique: A method for quantifying insulin secretion and resistance. Am J Physiol 237, E214–E223. [PubMed: 382871]

Donato AJ, Eskurza I, Silver AE, Levy AS, Pierce GL, Gates PE & Seals DR (2007). Direct evidence of endothelial oxidative stress with aging in humans: relation to impaired endothelium-dependent dilation and upregulation of nuclear factor-κB. Circ Res 100, 1659–1666. [PubMed: 17478731]

Dye AS, Huang H, Bauer JA & Hoffman RP (2012). Hyperglycemia increases muscle blood flow and alters endothelial function in adolescents with type 1 diabetes. Exp Diabetes Res 2012, 170380. [PubMed: 22701470]

Faerch K, Torekov SS, Vistisen D, Johansen NB, Witte DR, Jonsson A, Pedersen O, Hansen T, Lauritzen T, Sandbaek A, Holst JJ & Jorgensen ME (2015). GLP-1 response to oral glucose is reduced in prediabetes, screen-detected type 2 diabetes, and obesity and influenced by sex: The ADDITION-PRO study. Diabetes 64, 2513–2525. [PubMed: 25677912]

Gates PE, Boucher ML, Silver AE, Monahan KD & Seals DR (2007). Impaired flow-mediated dilation with age is not explained by L-arginine bioavailability or endothelial asymmetric dimethylarginine protein expression. J Appl Physiol 102, 63–71. [PubMed: 16946027]

Grasser EK, Yepuri G, Dulloo AG & Montani JP (2014). Cardio- and cerebrovascular responses to the energy drink Red Bull in young adults: A randomized cross-over study. Eur J Nutr 53, 1561–1571. [PubMed: 24474552]

Gupta A, Taqueti VR, van de Hoef TP, Bajaj NS, Bravo PE, Murthy VL, Osborne MT, Seidelmann SB, Vita T, Bibbo CF, Harrington M, Hainer J, Rimoldi O, Dorbala S, Bhatt DL, Blankstein R, Camici PG & Di Carli MF (2017). Integrated noninvasive physiological assessment of coronary circulatory function and impact on cardiovascular mortality in patients with stable coronary artery disease. Circulation 136, 2325–2336. [PubMed: 28864442]

Haas AV, Rosner BA, Kwong RY, Rao AD, Garg R, Di Carli MF & Adler GK (2019). Sex differences in coronary microvascular function in individuals with type 2 diabetes. Diabetes 68, 631–636. [PubMed: 30409780]

Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, McLeod L, Delacqua G, Delacqua F, Kirby J, Duda SN & Consortium RE (2019). The REDCap consortium: Building an international community of software platform partners. J Biomed Inform 95, 103208. [PubMed: 31078660]

Hoffman RP (2015). Hyperglycemic endothelial dysfunction: does it happen and does it matter? J Thorac Dis 7, 1693–1695. [PubMed: 26623085]

Hoffman RP, Hausberg M, Sinkey CA & Anderson EA (1999). Hyperglycemia without hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. J Diabetes Complications 13, 17–22. [PubMed: 10232705]

Holst JJ (2007). The physiology of glucagon-like peptide 1. Physiol Rev 87, 1409–1439. [PubMed: 17928588]

Holst JJ (2019). The incretin system in healthy humans: the role of GIP and GLP-1. Metabolism 96, 46–55. [PubMed: 31029770]

Horton WB, Jahn LA, Hartline LM, Aylor KW, Patrie JT & Barrett EJ (2020). Hyperglycemia does not inhibit insulin's effects on microvascular perfusion in healthy humans: A randomized crossover study. Am J Physiol Endocrinol Metab 319, E753–E762,

Houben AJ, Schaper NC, de Haan CH, Huvers FC, Slaaf DW, de Leeuw PW & Nieuwenhuijzen Kruseman C (1996). Local 24-h hyperglycemia does not affect endothelium-dependent or -independent vasoreactivity in humans. Am J Physiol 270, H2014–H2020. [PubMed: 8764252]

Ishihara M, Inoue I, Kawagoe T, Shimatani Y, Kurisu S, Nishioka K, Umemura T, Nakamura S & Yoshida M (2003). Impact of acute hyperglycemia on left ventricular function after reperfusion therapy in patients with a first anterior wall acute myocardial infarction. Am Heart J 146, 674–678. [PubMed: 14564322]

Jahn LA, Hartline L, Rao N, Logan B, Kim JJ, Aylor K, Gan LM, Westergren HU & Barrett EJ (2016). Insulin enhances endothelial function throughout the arterial tree in healthy but not metabolic syndrome subjects. J Clin Endocrinol Metab 101, 1198–1206. [PubMed: 26756115]

Joris PJ, Zeegers MP & Mensink RP (2015). Weight loss improves fasting flow-mediated vasodilation in adults: a meta-analysis of intervention studies. Atherosclerosis 239, 21–30. [PubMed: 25568949]

Joy NG, Perkins JM, Mikeladze M, Younk L, Tate DB & Davis SN (2016). Comparative effects of acute hypoglycemia and hyperglycemia on pro-atherothrombotic biomarkers and endothelial function in non-diabetic humans. J Diabetes Complications 30, 1275–1281. [PubMed: 27445005]

Klefter ON, Vilsboll T, Knop FK & Larsen M (2015). Retinal vascular and structural dynamics during acute hyperglycaemia. Acta Ophthalmol 93, 697–705. [PubMed: 26146858]

Kobayashi R, Yoshida S & Okamoto T (2015). Arterial stiffness after glucose ingestion in exercise-trained versus untrained men. Appl Physiol Nutr Metab 40, 1151–1156. [PubMed: 26444929]

Lipsitz LA, Ryan SM, Parker JA, Freeman R, Wei JY & Goldberger AL (1993). Hemodynamic and autonomic nervous system responses to mixed meal ingestion in healthy young and old subjects and dysautonomic patients with postprandial hypotension. Circulation 87, 391–400. [PubMed: 8425288]

Liu J, Jahn LA, Fowler DE, Barrett EJ, Cao W & Liu Z (2011). Free fatty acids induce insulin resistance in both cardiac and skeletal muscle microvasculature in humans. J Clin Endocrinol Metab 96, 438–446. [PubMed: 21047922]

Loader J, Montero D, Lorenzen C, Watts R, Meziat C, Reboul C, Stewart S & Walther G (2015). Acute hyperglycemia impairs vascular function in healthy and cardiometabolic diseased subjects: systematic review and meta-analysis. Arterioscler Thromb Vasc Biol 35, 2060–2072. [PubMed: 26112007]

Marfella R, Nappo F, De Angelis L, Paolisso G, Tagliamonte MR & Giugliano D (2000). Hemodynamic effects of acute hyperglycemia in type 2 diabetic patients. Diabetes Care 23, 658–663. [PubMed: 10834426]

Marre M, Bouhanick B, Berrut G, Gallois Y, Le Jeune JJ, Chatellier G, Menard J & Alhenc-Gelas F (1999). Renal changes on hyperglycemia and angiotensin-converting enzyme in type 1 diabetes. Hypertension 33, 775–780. [PubMed: 10082486]

Meyer C & Schwaiger M (1997). Myocardial blood flow and glucose metabolism in diabetes mellitus. Am J Cardiol 80, 94A–101A.

Moller N, Bagger JP, Schmitz O, Jorgensen JO, Ovesen P, Moller J, Alberti KG & Orskov H (1995). Somatostatin enhances insulin-stimulated glucose uptake in the perfused human forearm. J Clin Endocrinol Metab 80, 1789–1793. [PubMed: 7775624]

Ne JYA, Cai TY, Celermajer DS, Caterson ID, Gill T, Lee CMY & Skilton MR (2017). Obesity, arterial function and arterial structure – a systematic review and meta-analysis. Obes Sci Pract 3, 171–184. [PubMed: 28702212]

Parker L, Morrison DJ, Betik AC, Roberts-Thomson K, Kaur G, Wadley GD, Shaw CS & Keske MA (2020). High-glucose mixed nutrient meal ingestion impairs skeletal muscle microvascular blood flow in healthy young males. Am J Physiol Endocrinol Metab 318, E1014–E1021, [PubMed: 32286881]

Perkins JM, Joy NG, Tate DB & Davis SN (2015). Acute effects of hyperinsulinemia and hyperglycemia on vascular inflammatory biomarkers and endothelial function in overweight and obese humans. Am J Physiol Endocrinol Metab 309, E168–E176. [PubMed: 26015434]

Picchi A, Limbruno U, Focardi M, Cortese B, Micheli A, Boschi L, Severi S & De Caterina R (2011). Increased basal coronary blood flow as a cause of reduced coronary flow reserve in diabetic patients. Am J Physiol Heart Circ Physiol 301, H2279–H2284. [PubMed: 21984541]

Plockinger U, Holst JJ, Messerschmidt D, Hopfenmuller W & Quabbe HJ (1999). Octreotide suppresses the incretin glucagon-like peptide (7–36) amide in patients with acromegaly or clinically nonfunctioning pituitary tumors and in healthy subjects. Eur J Endocrinol 140, 538–544. [PubMed: 10377503]

Roberts-Thomson KM, Betik AC, Premilovac D, Rattigan S, Richards SM, Ross RM, Russell RD, Kaur G, Parker L & Keske MA (2020). Postprandial microvascular blood flow in skeletal muscle: similarities and disparities to the hyperinsulinaemic euglycaemic clamp. Clin Exp Pharmacol Physiol 47, 725–737. [PubMed: 31868941]

Russell RD, Hu D, Greenaway T, Sharman JE, Rattigan S, Richards SM & Keske MA (2018). Oral glucose challenge impairs skeletal muscle microvascular blood flow in healthy people. Am J Physiol Endocrinol Metab 315, E307–E315. [PubMed: 29763373]

Sauder KA, Johnston ER, Skulas-Ray AC, Campbell TS & West SG (2012). Effect of meal content on heart rate variability and cardiovascular reactivity to mental stress. Psychophysiology 49, 470–477. [PubMed: 22236402]

Shah MS & Brownlee M (2016). Molecular and cellular mechanisms of cardiovascular disorders in diabetes. Circ Res 118, 1808–1829. [PubMed: 27230643]

Shechtman O (2013). The coefficient of variation as an index of measurement reliability. In Methods of Clinical Epidemiology, ed. Doi SAR & Williams GM, pp. 39–49.Springer, Berlin, Heidelberg.

Skaug EA, Aspenes ST, Oldervoll L, Morkedal B, Vatten L, Wisloff U & Ellingsen O (2013). Age and gender differences of endothelial function in 4739 healthy adults: the HUNT3 Fitness Study. Eur J Prev Cardiol 20, 531–540. [PubMed: 22456692]

Sorensen MH, Bojer AS, Pontoppidan JRN, Broadbent DA, Plein S, Madsen PL & Gaede P (2020). Reduced myocardial perfusion reserve in type 2 diabetes is caused by increased perfusion at rest and decreased maximal perfusion during stress. Diabetes Care 43, 1285–1292. [PubMed: 32193248]

Steinberg HO, Brechtel G, Johnson A, Fineberg N & Baron AD (1994). Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. J Clin Invest 94, 1172–1179. [PubMed: 8083357]

Steinberg HO, Paradisi G, Hook G, Crowder K, Cronin J & Baron AD (2000). Free fatty acid elevation impairs insulin-mediated vasodilation and nitric oxide production. Diabetes 49, 1231–1238. [PubMed: 10909983]

Thijssen DHJ, Bruno RM, van Mil A, Holder SM, Faita F, Greyling A, Zock PL, Taddei S, Deanfield JE, Luscher T, Green DJ & Ghiadoni L (2019). Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. Eur Heart J 40, 2534–2547. [PubMed: 31211361]

Townsend RR, Wilkinson IB, Schiffrin EL, Avolio AP, Chirinos JA, Cockcroft JR, Heffernan KS, Lakatta EG, McEniery CM, Mitchell GF, Najjar SS, Nichols WW, Urbina EM & Weber T (2015). Recommendations for improving and standardizing vascular research on arterial stiffness. Hypertension, 66, 698–722. [PubMed: 26160955]

Urbaniak GC & Plous S (2013). Research Randomizer (Version 4.0) [Computer software]. Retrieved on August 22, 2020, from http://www.randomizer.org/.

Van den Berghe G, Schetz M, Vlasselaers D, Hermans G, Wilmer A, Bouillon R & Mesotten D (2009). Clinical review: intensive insulin therapy in critically ill patients: NICE-SUGAR or Leuven blood glucose target? J Clin Endocrinol Metab 94, 3163–3170. [PubMed: 19531590]

van Gurp PJ, Rongen GA, Lenders JW, Al Nabawy AK, Timmers HJ & Tack CJ (2005). Sustained hyperglycaemia increases muscle blood flow but does not affect sympathetic activity in resting humans. Eur J Appl Physiol 93, 648–654. [PubMed: 15778893]

Wang N, Tan AWK, Jahn LA, Hartline L, Patrie JT, Lin S, Barrett EJ, Aylor KW & Liu Z (2020). Vasodilatory actions of glucagon-like peptide 1 are preserved in skeletal and cardiac muscle microvasculature but not in conduit artery in obese humans with vascular insulin resistance. Diabetes Care 43, 634–642. [PubMed: 31888883]

Welle S, Lilavivat U & Campbell RG (1981). Thermic effect of feeding in man: increased plasma norepinephrine levels following glucose but not protein or fat consumption. Metabolism 30, 953–958. [PubMed: 7024722]

Williams JS, Stimpson TV, Tremblay JC, Fenuta AM & Pyke KE (2020). No impact of acute hyperglycaemia on arterial stiffness in the early and late follicular phases of the menstrual cycle in young females. Exp Physiol 105, 174–183. [PubMed: 31628691]

Williams SB, Goldfine AB, Timimi FK, Ting HH, Roddy MA, Simonson DC & Creager MA (1998). Acute hyperglycemia attenuates endothelium-dependent vasodilation in humans in vivo. Circulation 97, 1695–1701. [PubMed: 9591763]

Woods LL, Mizelle HL & Hall JE (1987). Control of renal hemodynamics in hyperglycemia: possible role of tubuloglomerular feedback. Am J Physiol 252, F65–F73. [PubMed: 3812702]

Young JB & Landsberg L (1977). Stimulation of the sympathetic nervous system during sucrose feeding. Nature 269, 615–617. [PubMed: 917110]

Key points

- Multiple clinical studies report that acute hyperglycaemia (induced by mixed meal or oral glucose) decreases arterial vascular function in healthy humans. Feeding, however, impacts autonomic output, blood pressure, and insulin and incretin secretion, which may themselves alter vascular function.
- No prior studies have examined the effect of acute hyperglycaemia on both macro- and microvascular function while controlling plasma insulin concentrations.
- Macrovascular and microvascular functional responses to euglycaemia and hyperglycaemia were compared. Octreotide was infused throughout both protocols to prevent endogenous insulin release.
- Acute hyperglycaemia (induced by intravenous glucose) enhanced brachial artery flow-mediated dilatation, increased skeletal muscle microvascular blood volume and flow, and expanded cardiac muscle microvascular blood volume.
- Compared to other published findings, the results suggest that vascular responses to acute hyperglycaemia differ based on the study population (i.e. normal weight *vs.* overweight/obese) and/or glucose delivery method (i.e. intravenous *vs.* oral glucose).

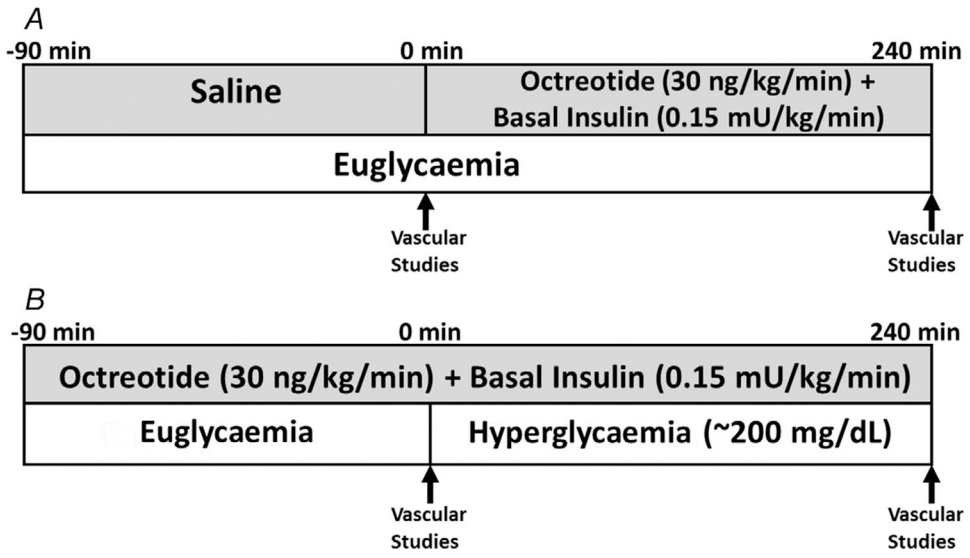

Figure 1.
Experimental protocols

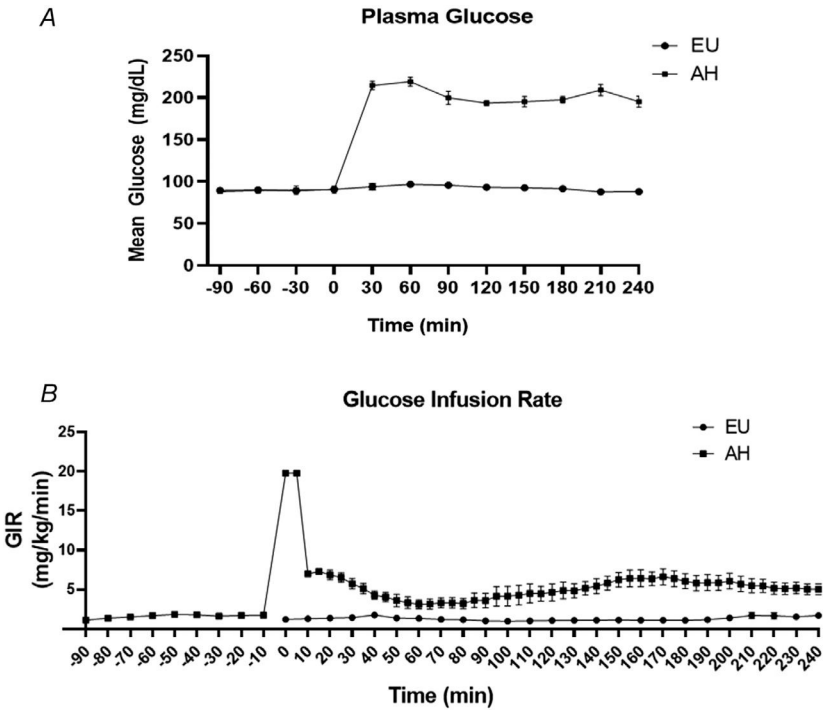

Figure 2. Time course for mean plasma glucose

(*A*) and mean glucose infusion rate (*B*) throughout each infusion protocol

AH, acute hyperglycaemia; EU, euglycaemia; GIR, glucose infusion rate.

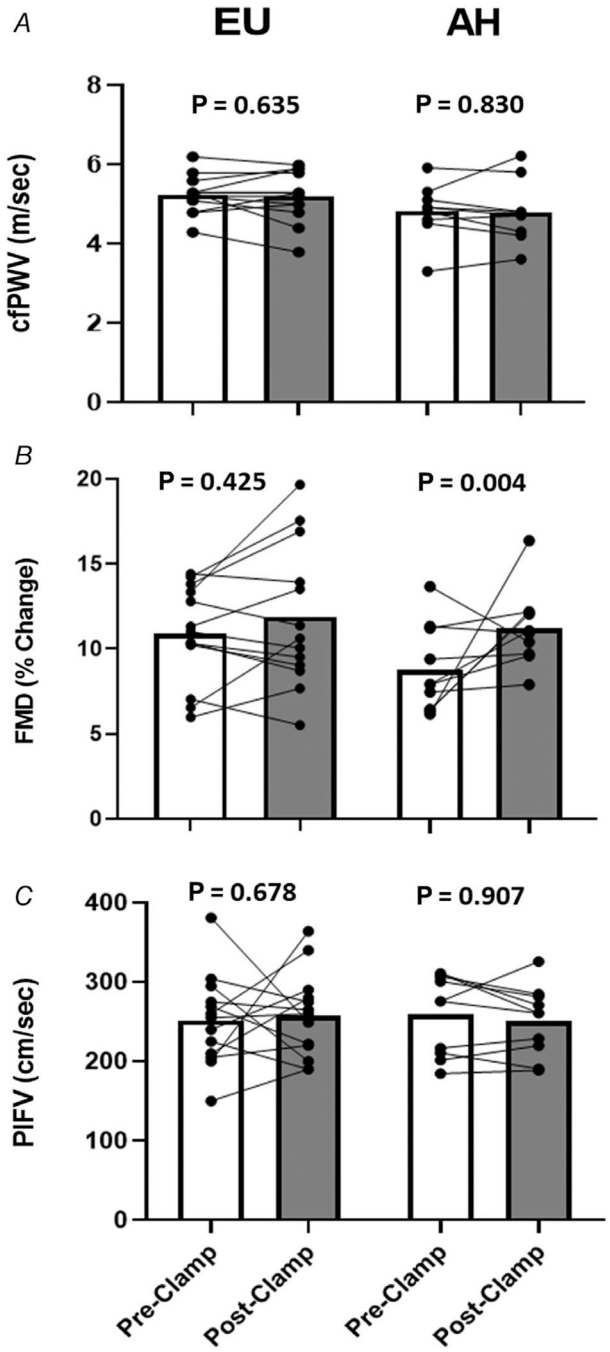

Figure 3. Paired individual trend lines and group mean bar graphs (pre- and post-insulin clamp) detailing cfPWV (*A*), FMD (*B*), and PIFV (*C*) responses to both the EU and AH protocols

Data are presented as raw (i.e. arithmetic) values. AH, acute hyperglycaemia; cfPWV, carotid-femoral pulse wave velocity; EU, euglylcaemia; FMD, flow-mediated dilatation; PIFV, post-ischaemic flow velocity.

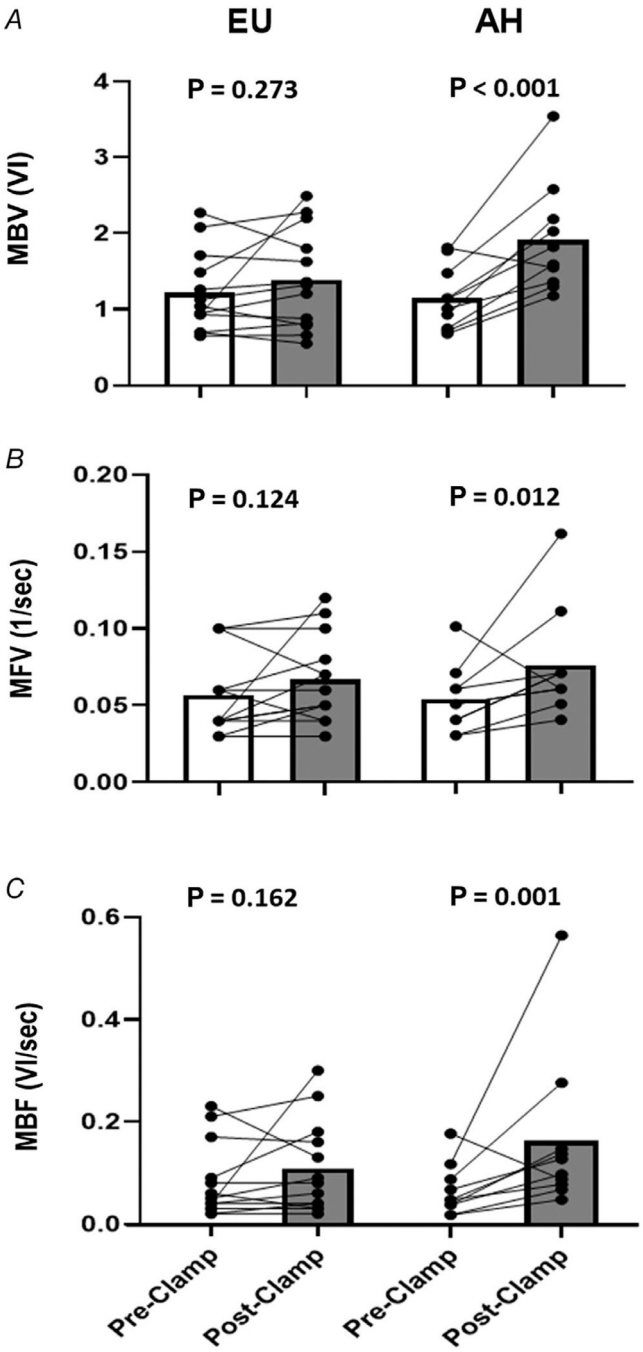

Figure 4. Paired individual trend lines and group mean bar graphs (pre- and post-insulin clamp) detailing skeletal muscle MBV (*A*), MFV (*B*), and MBF (*C*) responses to both the EU and AH protocols

Data are presented as raw (i.e. arithmetic) values. AH, acute hyperglycaemia; EU, euglycaemia; MBF, microvascular blood flow; MBV, microvascular blood volume; MFV, microvascular flow velocity; VI, video intensity.

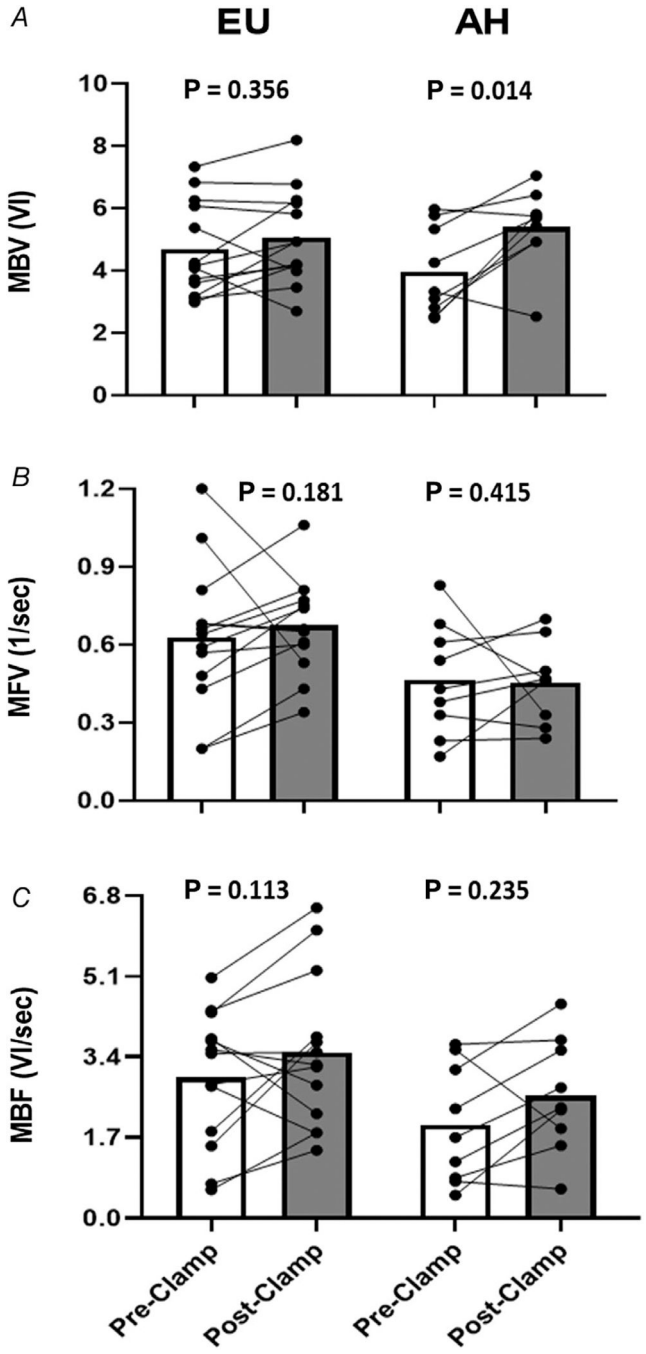

Figure 5. Paired individual trend lines and group mean bar graphs (pre- and post-insulin clamp) detailing cardiac muscle MBV (*A*), MFV (*B*), and MBF (*C*) responses to both the EU and AH protocols

Data are presented as raw (i.e. arithmetic) values. AH, acute hyperglycaemia; EU, euglycaemia; MBV, microvascular blood volume; MBF, microvascular blood flow; MFV, microvascular flow velocity; VI, video intensity.

Table 1.

Baseline subject characteristics and demographics

Variable	Value
Sex	7 female; 6 male
Age (years)	25.15 (4.39)
Body mass index (kg m^{-2})	22.00 (2.08)
Systolic blood pressure (mmHg)	112.15 (10.29)
Diastolic blood pressure (mmHg)	64.85 (6.39)
Fasting plasma glucose (mg dl^{-1})	87.69 (6.59)
Total cholesterol (mg dl^{-1})	167.92 (25.18)
LDL cholesterol (mg dl^{-1})	97.00 (21.68)
HDL cholesterol (mg dl^{-1})	60.77 (13.02)
Triglycerides (mg dl^{-1})	62.00 (19.38)

Data are presented as mean (SD). HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 2.

Summary statistics for pre- and post-intervention plasma insulin (μIU ml^{-1}) and ICAM-1 (ng ml^{-1}) levels

Variable	Protocol	Assessment	*n* [a]	Mean	SD	CV	GM	Within-protocol *P*-value[b]	Between-protocol *P*-value[c]
Insulin	EU	Pre	7	6.06	4.37	0.72	4.70	0.439	0.316
		Post	7	8.55	5.91	0.69	6.79		
	AH	Pre	6	14.24	14.04	0.98	7.16	0.510	
		Post	6	11.48	7.29	0.64	10.04		
ICAM-1	EU	Pre	13	234.88	59.53	0.25	228.44	0.766	0.173
		Post	13	232.06	49.59	0.21	227.32		
	AH	Pre	10	222.36	43.50	0.20	218.48	0.192	
		Post	10	217.04	42.98	0.20	213.08		

[a] Six subjects in the euglycaemia protocol and4 subjects in the acute hyperglycaemia protocol had plasma insulin levels below the limits of detection.

[b] Within-protocol *P*-values reflect the point estimate for the mean pre- to post-intervention change in plasma insulin after conversion to the geometric mean ratio scale.

[c] Between-protocol *P*-values represent the baseline-corrected ratio of geometric means (RGM) between protocols (i.e. RGM acute hyperglycaemia: RGM euglycaemia). AH, acute hyperglycaemia; CV, coefficient of variation; EU, euglycaemia; GM, geometric mean; ICAM-1, intercellular adhesion molecule-1.

J Physiol. Author manuscript; available in PMC 2022 February 16.

Table 3.

Within admission and baseline-adjusted between admission post- to pre-intervention geometric mean outcome measure ratios

Outcome measure	Protocol	Estimate ratio of GMs (RGMs)	95% CI	*P*-value
FMD	EU	1.07	0.90, 1.26	0.425 (0.850)
	AH	1.34	1.11, 1.62	0.004 (0.008)
	RGM AH: RGM EU[a]	1.15	0.93, 1.42	0.178 (0.357)
PIFV	EU	1.03	0.90, 1.18	0.678 (1.000)
	AH	1.01	0.87, 1.18	0.907 (1.000)
	RGM AH: RGM EU[a]	1.03	0.93, 1.15	0.470 (0.939)
cfPWV	EU	0.99	0.94, 1.04	0.635 (1.000)
	AH	0.99	0.94, 1.05	0.830 (1.000)
	RGM AH: RGM EU[a]	1.00	0.92, 1.09	0.971 (1.000)
Skeletal MBV	EU	1.10	0.92, 1.32	0.273 (0.545)
	AH	1.68	1.37, 2.06	<0.001 (<0.001)
	RGM AH: RGM EU[a]	1.50	1.15, 1.95	0.008 (0.016)
Skeletal MFV	EU	1.19	0.95, 1.48	0.124 (0.247)
	AH	1.39	1.08, 1.79	0.012 (0.024)
	RGM AH: RGM EU[a]	1.13	0.89, 1.44	0.274 (0.549)
Skeletal MBF	EU	1.31	0.89, 1.93	0.162 (0.324)
	AH	2.34	1.51, 3.63	0.001 (0.001)
	RGM AH: RGM EU[a]	1.68	1.06, 2.64	0.031 (0.062)
Cardiac MBV	EU	1.08	0.91, 1.29	0.356 (0.712)
	AH	1.34	1.07, 1.67	0.014(0.027)
	RGM AH: RGM EU[a]	1.14	0.91, 1.41	0.211 (0.423)
Cardiac MFV	EU	1.16	0.93, 1.45	0.181 (0.362)
	AH	0.89	0.67, 1.18	0.415 (0.830)
	RGM AH: RGM EU[a]	0.70	0.55, 0.88	0.007 (0.014)
Cardiac MBF	EU	1.25	0.94, 1.67	0.113 (0.226)
	AH	1.24	0.86, 1.78	0.235 (0.469)

Outcome measure	Protocol	Estimate ratio of GMs (RGMs)	95% CI	*P*-value
	RGM AH: RGM EU[a]	0.79	0.57, 1.10	0.137 (0.274)

[a]Baseline corrected comparison (Bonferroni-adjusted *P*-value). AH, acute hyperglycaemia; cfPWV, carotid femoral pulse wave velocity; CI, confidence interval; EU, euglycaemia; FMD, flow-mediated dilatation; GM, geometric mean; MBF, microvascular blood flow; MBV, microvascular blood volume; MFV, microvascular flow velocity; RGM, ratio of geometric mean; PIFV, post-ischaemic flow velocity.