The Journal of Clinical Endocrinology & Metabolism, 2022, **00**, 1–8
https://doi.org/10.1210/clinem/dgac613
Advance access publication 19 October 2022
Clinical Research Article

Effects of GLP-1 Infusion Upon Whole-body Glucose Uptake and Skeletal Muscle Perfusion During Fed-state in Older Men

Haitham Abdulla,[1,3] Bethan Phillips,[1,2] Daniel Wilkinson,[1,2] Amanda Gates,[1] Marie Limb,[1] Tereza Jandova,[1,4] Joseph Bass,[1] Johnathan Lewis,[1] John Williams,[1,2,5] Kenneth Smith,[1,2,*] Iskandar Idris,[1,2,6,*] and Philip Atherton[1,2]

[1]MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research, Centre of Metabolism, Ageing and Physiology (COMAP), Academic Unit of Injury, Recovery and Inflammation Sciences (IRIS), School of Medicine, University of Nottingham, Royal Derby Hospital, Derby DE22 3DT, UK
[2]NIHR, Nottingham BRC, University of Nottingham, Nottingham NG7 2UH, UK
[3]Diabetes and Endocrinology Centre, University Hospitals Birmingham NHS Foundation Trust, Heartlands Hospitals, Birmingham B9 5SS, UK
[4]Department of Physiology and Biochemistry, Faculty of Physical Education and Sport, Charles University, Prague 6, Czech Republic
[5]Department of Anaesthesia, University Hospitals Derby and Burton NHS Foundation Trust, Derby DE22 3NE, UK
[6]Department of Endocrinology and Diabetes, University Hospitals Derby and Burton NHS Foundation Trust, Derby DE22 3NE, UK

Correspondence: Philip J. Atherton, PhD, University of Nottingham School of Medicine, Royal Derby Hospital, Uttoxeter Road, Derby, DE22 3DT, UK. Email: Philip.atherton@nottingham.ac.uk
*Denotes equal senior authors.

Abstract

Introduction: Ageing skeletal muscles become both insulin resistant and atrophic. The hormone glucagon-like peptide 1 (GLP-1) facilitates postprandial glucose uptake as well as augmenting muscle perfusion, independent of insulin action. We thus hypothesized exogenous GLP-1 infusions would enhance muscle perfusion and positively affect glucose metabolism during fed-state clamps in older people.

Methods: Eight men (71 ± 1 years) were studied in a randomized crossover trial. Basal blood samples were taken before postprandial (fed-state) insulin and glucose clamps, accompanied by amino acid infusions, for 3 hours. Reflecting this, following insertions of peripheral and femoral vessels cannulae and baseline measurements, peripheral IV infusions of octreotide, insulin (Actrapid), 20% glucose, and mixed amino acids; Vamin 14-EF with or without a femoral arterial GLP-1 infusion were started. GLP-1, insulin, and C-peptide were measured by ELISA. Muscle microvascular blood flow was assessed via contrast enhanced ultrasound. Whole-body glucose handling was assayed by assessing glucose infusion rate parameters.

Results: Skeletal muscle microvascular blood flow significantly increased in response to GLP-1 vs feeding alone (5.0 ± 2.1 vs 1.9 ± 0.7 fold-change from basal, respectively; $P = 0.008$), while also increasing whole-body glucose uptake (area under the curve 16.9 ± 1.7 vs 11.4 ± 1.8 mg/kg^{-1}/180 minutes^{-1}, $P = 0.02$ ± GLP, respectively).

Conclusions: The beneficial effects of GLP-1 on whole-body glycemic control are evident with insulin clamped at fed-state levels. GLP-1 further enhances the effects of insulin on whole-body glucose uptake in older men, underlining its role as a therapeutic target. The effects of GLP-1 in enhancing microvascular flow likely also affects other glucose-regulatory organs, reflected by greater whole-body glucose uptake.

Key Words: glucagon like peptide 1, extrapancreatic effects, muscle glucose uptake, muscle glucose metabolism, microvascular blood flow, microvascular recruitment, microcirculation

Abbreviations: AA, amino acid; AI, acoustic intensity; AUC, area under the curve; CEUS, contrast-enhanced ultrasound; CV, coefficient of variation; GIR, glucose infusion rate; GLP-1, glucagon like peptide 1; LBF, leg blood flow; M, glucose metabolized; MBF, microvascular blood flow; MBV, microvascular blood volume; MFV, microvascular flow velocity; MVR, microvascular recruitment; NO, nitric oxide; ROI, region of interest; SEM, standard error of the mean.

The metabolic effects of glucagon-like peptide 1 (GLP-1), an incretin hormone released from the gut, are largely understood within the context of its ability to stimulate pancreatic β cells to secrete insulin in response to oral carbohydrate ingestions in healthy individuals and in diabetes (1-6). The extrapancreatic properties of GLP-1 in relation to insulin sensitivity and skeletal muscle glucose uptake and disposal remain unclear, however. Although some reports question the relevance of GLP-1's extrapancreatic properties in healthy adults (7, 8) and in diabetes (9); others describe improved glucose tolerance in healthy individuals (10-12), whole body glucose uptake in type 1 diabetes (13), and hind-limb glucose uptake in rats (14). The latter effects are thought to be facilitated by insulin-induced postprandial hyperemia mediated by nitric oxide (NO)-dependent vasodilatation of terminal arterioles (in addition to nutrients) and delivery to terminal capillaries within muscle beds (15). Thereafter, glucose uptake into muscle tissue follows insulin receptor/IRS/PI3-K-stimulated

Received: 15 June 2022. **Editorial Decision:** 16 October 2022. **Corrected and Typeset:** 24 November 2022
© The Author(s) 2022. Published by Oxford University Press on behalf of the Endocrine Society.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

Downloaded from https://academic.oup.com/jcem/advance-article/doi/10.1210/clinem/dgac613/6763659 by guest on 02 December 2022

GLUT-4 translocation to the cell membrane (15, 16). Interestingly, GLP-1 receptors have been shown to be expressed in rats myocytes (17) and in vitro data support its ability to activate glycogen synthase and increase glucose metabolism in human myocytes (18, 19).

In addition to potential extrapancreatic effects of GLP-1 in regulating glucose metabolism, insulin-independent properties for GLP-1 in the vasculature have also been described in relation to skeletal muscle microvascular recruitment (MVR) under hyperinsulinemic euglycemic clamp conditions in younger individuals (20, 21). Its physiological effects on older individuals, on the other hand, who would normally show reduced microvascular response to feeding (22, 23), has not been explored. Because GLP-1 has been shown to increase vascular blood flow independent of insulin (20, 21), it is likely that GLP-1 may also enhance muscle MVR, insulin delivery, and glucose disposal. However, the link between human aging and impaired glucose disposal remains controversial, with some data supporting this (24) and others reporting little difference in comparison to younger people (25, 26).

Although data showing a potently positive effect of GLP-1 on peripheral tissue MVR is encouraging, to date, no study has explored the extrapancreatic potentials of GLP-1 on muscle glucose disposal and MVR under postprandial conditions in the context of older age. To address this, we hypothesized that GLP-1 upregulates postprandial skeletal muscle microcirculation that may reflect positively on glucose use in older individuals. Therefore, the aims of the present study were to test whether exogenous GLP-1 would have added benefits on skeletal muscle glucose uptake and MVR in older individuals, beyond the impact of postprandial insulin and glucose.

Materials and Methods

Subjects and Design

Subjects

This study was approved by The University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee (reference: G12122013 MSGEM) and conducted in line with the Declaration of Helsinki and prospectively registered at www.clinicaltrials.gov (NCT02370745). Eight healthy male volunteers (65-75 years of age; see Table 1 for subject characteristics) were recruited to the study via targeted (for age) postal invites to those residing close to the study site. Before enrollment, all participants underwent a comprehensive clinical examination and metabolic screening at the University of Nottingham Royal Derby Hospital Centre to confirm eligibility. Subjects with metabolic disease, lower limb musculoskeletal abnormalities, acute cerebrovascular or cardiovascular disease, active malignancy, uncontrolled hypertension, body mass index <18 or >28 kg/m^{-2}, on medications that impact glucose metabolism or modulate vascular tone, or those with known allergy to any of the study infusions were excluded. All volunteers were studied following overnight fast (water ad libitum) of 10 to 12 hours. Each volunteer was studied on 2 occasions, approximately 3 weeks apart. Volunteers were randomly assigned to receive either GLP-1 infusion or placebo into the femoral artery of one leg on each visit. Volunteers were blinded to which visit they would receive the GLP-1 infusion.

Table 1. Characteristics of study participants (n = 8)

Parameter	
Age (y)	71 ± 1.04
Height (m)	1.77 ± 0.03
Weight (kg)	83 ± 4.25
BMI (kg/m^2)	26.2 ± 0.63
Leg mass (g)	9201.5 ± 28
Sarcopenic index (ASM kg/m^2)	8.13 ± 0.18
Fasting plasma glucose (mM)	5.65 ± 0.18

Data are presented as mean ± SEM. BMI defined as weight in kg/height in meters^2. Sarcopenic index corresponds to the ASM mass index, which was calculated as ASM in kg/height in meters^2.
ASM, appendicular skeletal muscle; BMI, body mass index.

Conduct of the Study

Reporting and preparation

On the morning of study days, volunteers reported to the laboratories at 0800 hours. Following a dual-energy x-ray absorptiometry scan (first study only), volunteers lay supine on a bed for the duration of the study day. Three polyethylene cannulae (two 20G and one 18G sizes) for IV infusions were inserted in the 2 forearms. This was followed by the insertion of femoral arterial cannula in the femoral artery of the leg designated for study. The area below the inguinal ligament was anesthetized before the introduction of wire-guided femoral catheters under ultrasound scan guidance (Philips iU22 Ultrasound, Bothell, WA, USA).

Postprandial clamp

Following insertion of peripheral and femoral vessel cannulae and baseline blood acquisition, peripheral IV infusions of octreotide (Novartis, Surry, UK), insulin Actrapid (Novo Nordisk, Gatwick, UK), 20% glucose (Baxter, UK), and mixed amino acids (AA); Vamin 14-EF (Fresenius Kabi Ltd, Runcorn, UK) with or without a femoral arterial GLP-1 infusion (Bachem AG, Bubendorf, Switzerland) were started. Glucose and insulin were infused as previously described (27) aiming to clamp glucose at 7 to 7.5 mM and insulin at a postprandial level of approximately 30 µIU/mL^{-1}. Octreotide was infused at a rate of 30 ng/kg^{-1}/min^{-1} (28), with Vamin 14-EF started at a prime rate of 34 mg/kg^{-1} AA followed by a constant infusion rate of 102 mg/kg^{-1}/h^{-1}, with all infusions being 3 hours. GLP-1 was obtained as a powder and stored at −20°C. On the day of the study, GLP-1 was dissolved in 2 mL of 0.9% saline, diluted, and infused into the femoral artery at a constant rate of 1.2 pmol/kg^{-1}/min^{-1} (21). The placebo infusion contained 0.9% saline only.

Contrast-enhanced ultrasound

A baseline measurement of microvascular blood flow parameters was conducted using contrast-enhanced ultrasound (CEUS; Philips iU22 Ultrasound, Bothell, USA). Using our previously published protocol (23), in brief, Sonovue (Bracco, Courcouronnes, France) was infused via a peripheral vein at an initial rate of 2 mL/min^{-1}for 1 minute and 1 mL/min^{-1}for a further 2 minutes. During the last 90 seconds of

Downloaded from https://academic.oup.com/jcem/advance-article/doi/10.1210/clinem/dgac613/6763659 by guest on 02 December 2022

this 3-minute infusion, three 30-second cycles of flash/replenishment videos were recorded and analyzed using Q-Lab software (Philips, Andover, MA, USA). A further CEUS assessment of muscle microvascular parameters was made 120 minutes after the start of the postprandial clamp.

Blood sampling and leg blood flow

Blood samples were taken at baseline and at regular intervals throughout the study. Following the start of the postprandial clamp, venous samples were taken every 5 to 10 minutes to allow adjustment of the glucose infusion rate to maintain blood glucose between 7 and 7.5 mM, measured using a Yellow Springs Instrument (UK Ltd, Hampshire, UK). At baseline, 3 measures of whole-leg (femoral artery) blood flow (LBF) were also taken using our published protocol (29), with further LBF assessments between 40 and 80 minutes and 130 and 170 minutes after the start of the clamp. At the end of the study day, participants were provided a meal and monitored for 60 minutes before leaving.

Figure 1 shows schematic representation of preparatory phase (−60 to 0 minutes) and clamp phase (0-180 minutes) during the acute study day.

Laboratory Analysis and Measurements

Plasma insulin, C-peptide, and GLP-1 concentrations

ELISA kits (Milliplex Map Kit, EMS Millipore, Germany) were used to determine insulin, C-peptide, and GLP-1. For GLP-1 analysis, samples were collected in P800 tubes, which stabilize GLP-1 on collection. The kit used was the Milliplex Map Kit—human metabolic hormone magnetic bead panel (catalog #HMHEMAG-34K, PRID:AB_2910198), measured on a Luminex-Magpix (Thermo Fisher Scientific, UK).

Micro- and macrovascular blood flow parameters

Two sets of 3 video recordings were obtained (baseline and after intervention) for each study day for the assessment of microvascular blood flow. Videos were exported to quantification software (QLab, Philips, Andover, MA, USA) for analysis. Regions of interest (ROIs) were manually selected avoiding areas of connective tissue and rapid filling vessels and copied into each file to ensure that regions were identical for each recording within a study day. The period immediately following each flash (0.57 seconds) was used to calculate background acoustic intensity (AI), an arbitrary unit, attributable to rapidly filing larger nonexchange vessels and tissue echogenicity. The mean AI during this period was calculated and subtracted from all subsequent values during the associated replenishment period. The mean AI across all 3 flash/replenishment recording cycles at each timepoint was calculated after background correction and curtailed at 24 seconds. Then, AI vs time curves were generated and fitted to the exponential function: $y = A\,[1 - e^{-\beta(t - Bt)}]$, where t is time in seconds, Bt the time used for background subtraction, y is the AI at any given t, A is the plateau AI defined as microvascular volume (MBV) in milliliters in the ROI, and β is the flow rate constant (liters/s^{-1}) that determines the rate of rise of AI and corresponds to the mean microvascular flow velocity (MFV) or perfusion rate in milliliters per second of all vessels in the ROI. Using data derived from this equation, microvascular blood flow (MBF) in milliliters per second is defined as MBV × MFV (30). Fold change from baseline was calculated as the difference between intervention value and baseline value relative to baseline value. Net incremental area under response curve (AUC) for AI was calculated for each individual separately and presented as grouped analysis.

Plasma AA (Phenylalanine)

For measurement of phenylalanine concentrations, internal standards were added to plasma samples before addition of urease solution and incubation at room temperature for 20 minutes. Samples were then deproteinized with ice-cold ethanol for 20 minutes at 4°C, before centrifugation at 13 000*g*. Dried AA were vortex mixed in 0.5 M HCl, and lipids extracted in ethyl acetate before evaporation to dryness of the aqueous fraction at 90°C under N_2. Then, AA were derivatized through the addition of equal volumes of acetonitrile and N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide and heated to 90°C for 60 minutes. Samples were allowed

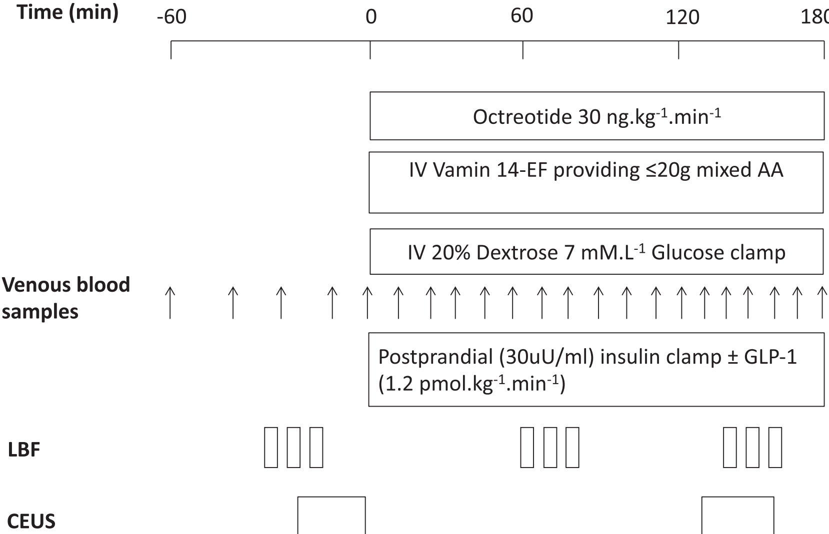

Figure 1. Schematic representation of study protocol. Eight older men were studied on 2 occasions under fed-state insulin clamp conditions for 3 hours with and without GLP-1 infusion. AA, amino acids; CEUS, contrast-enhanced ultrasound; LBF, leg blood flow.

Downloaded from https://academic.oup.com/jcem/advance-article/doi/10.1210/clinem/dgac613/6763659 by guest on 02 December 2022

to cool before being transferred to autosampler vials. Phenylalanine concentrations were finally quantified against a standard curve of known concentrations, using gas chromatography mass spectrometry (31).

Calculation of Glucose Uptake

Glucose metabolized (M) was calculated in accordance with DeFronzo et al (27), where M is equal to glucose infusion rate (GIR) minus urinary losses of glucose (which averages 0.2 $mg/kg^{-1}/min^{-1}$) and a space correction. In this study, M was determined over 15-minute intervals using glucose concentration values. The M for each study visit was calculated as the mean of twelve 15-minute intervals. The net incremental AUC was calculated for each individual separately and presented as a 2-group comparison.

Statistical Analysis

The sample size was prospectively determined based on previous studies to detect differences in MVR, glucose, and AA metabolism in response to feeding (23, 32). For repeated measures of AA and glucose concentrations of the same blood sample, the coefficient of variation (CV) is ~3.8%. The population CVs are ~10% to 12% for young and older men. With a population CV of 15% (based on previous laboratory data) and CV of laboratory techniques also of 15% (propagated error ~21%), a difference of ±21% (ie, 1 SD) could be detected (with 80% confidence, 5% significance level) in the fed state. Given these facts, the smallest number of subjects needed to detect (with 80% confidence, 5% significance level) a cross-sectional difference between groups, or a 1-way difference on a paired basis, of 20%, is 8. Analysis was conducted using Prism 7 (GraphPad, San Diego, CA, USA). Data are presented as means ± standard error of the mean (SEM). Normality of distribution was tested using D'Agostino and Pearson Omnibus normality tests. Comparison between values was made via Student *t* test or repeated measures ANOVA with Bonferroni post hoc, as appropriate.

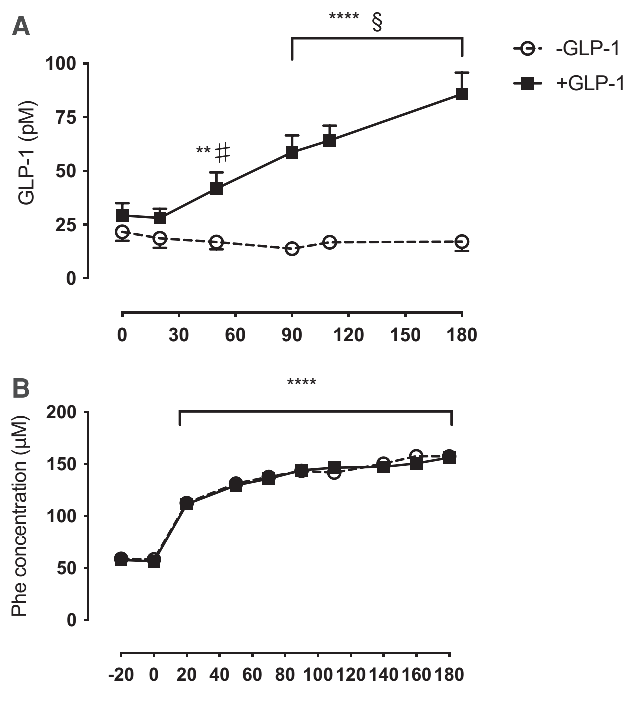

Figure 2. Plasma GLP-1 (A) and phenylalanine (B) concentrations. Analysis via 2-way ANOVA. $**P < 0.01$, $****P = 0.0001$ vs fasted value in the same condition. $\#P < 0.01$, $§P < 0.0001$ between conditions. Data presented as means ± SEM.

Results

Subject Characteristics

The physical and demographic characteristics of participants are shown in Table 1.

GLP-1 and Phenylalanine Concentrations

Total GLP-1 levels at baseline were comparable between the 2 experiments (21 ± 4 and 29 ± 6 pM, with or without GLP-1 infusion, respectively). GLP-1 infusions caused a gradual rise in concentrations over the postprandial clamp period to a peak at 180 minutes (85.7 ± 10 compared with 17.1 ± 4.3 pM, at the same time point in the experiment where it was not infused). Mean GLP-1 concentration over the 180-minute feeding period was 58 ± 8 and 17.1 ± 1 pM, with and without GLP-1 infusions, respectively (Fig. 2A). Phenylalanine concentrations, as a proxy for feeding efficacy were comparable between the 2 experiments (basal: 57 ± 0.7 vs 58 ± 0.2 μM, with and without GLP-1, respectively), rising to a mean of 140 ± 5.1 vs 141 ± 5.3 μM with and without GLP-1, respectively (Fig. 2B).

Leg Blood Flow

LBF was not significantly different between the groups at baseline (0.341 ± 0.025 vs 0.328 ± 0.022 L/min^{-1}, $P > 0.05$), nor in response to a fed-state insulin clamp (0.385 ± 0.03 vs 0.372 ± 0.031 L/min^{-1}, $P > 0.05$, both with and without GLP-1, respectively).

Microvascular Recruitment

AI rose as expected following feeding in both experiments (Fig. 3A and B). Although GLP-1 infusions caused a 1.8-fold increase in replenishment curve AUC (corresponds to MBV) compared with a 0.8-fold increase without GLP-1 ($P = 0.03$) (Fig. 3C), calculated increments in MBV were not significantly different between the 2 experiments, however (0.7 ± 0.2 vs 0.4 ± 0.2-fold, $P > 0.05$, with and without GLP-1, respectively) (Fig. 3D). Increase in MFV was numerically but not significantly higher with GLP-1 (1.1 ± 0.4 vs 2.6 ± 1.3-fold increment from fasted, $P = 0.09$) (Fig. 3E). Increase in MBF, calculated as the product of MBV and MFV, however, was significantly greater with GLP-1 (5.0 ± 2.1 vs 1.9 ± 0.73-fold increment from fasted respectively, $P = 0.008$) (Fig. 3F).

Plasma Insulin, C Peptide, Glucose, GIR, and Glucose Uptake

Mean insulin concentrations were similar at baseline (fasted state) in both groups (5.1 ± 0.5 and 5.6 ± 0.9 $μIU/mL^{-1}$, with and without GLP-1, respectively). Infusion of insulin in the postprandial state led to levels rising to 25 ± 0.4 $μIU/mL^{-1}$ and to 31 ± 1.3 $μIU/mL^{-1}$ when GLP-1 was co-infused ($P < 0.001$ vs baseline in both experiments), with no between groups differences at any time points (Fig. 4A). C-peptide concentrations were not significantly different at baseline between

Downloaded from https://academic.oup.com/jcem/advance-article/doi/10.1210/clinem/dgac613/6763659 by guest on 02 December 2022

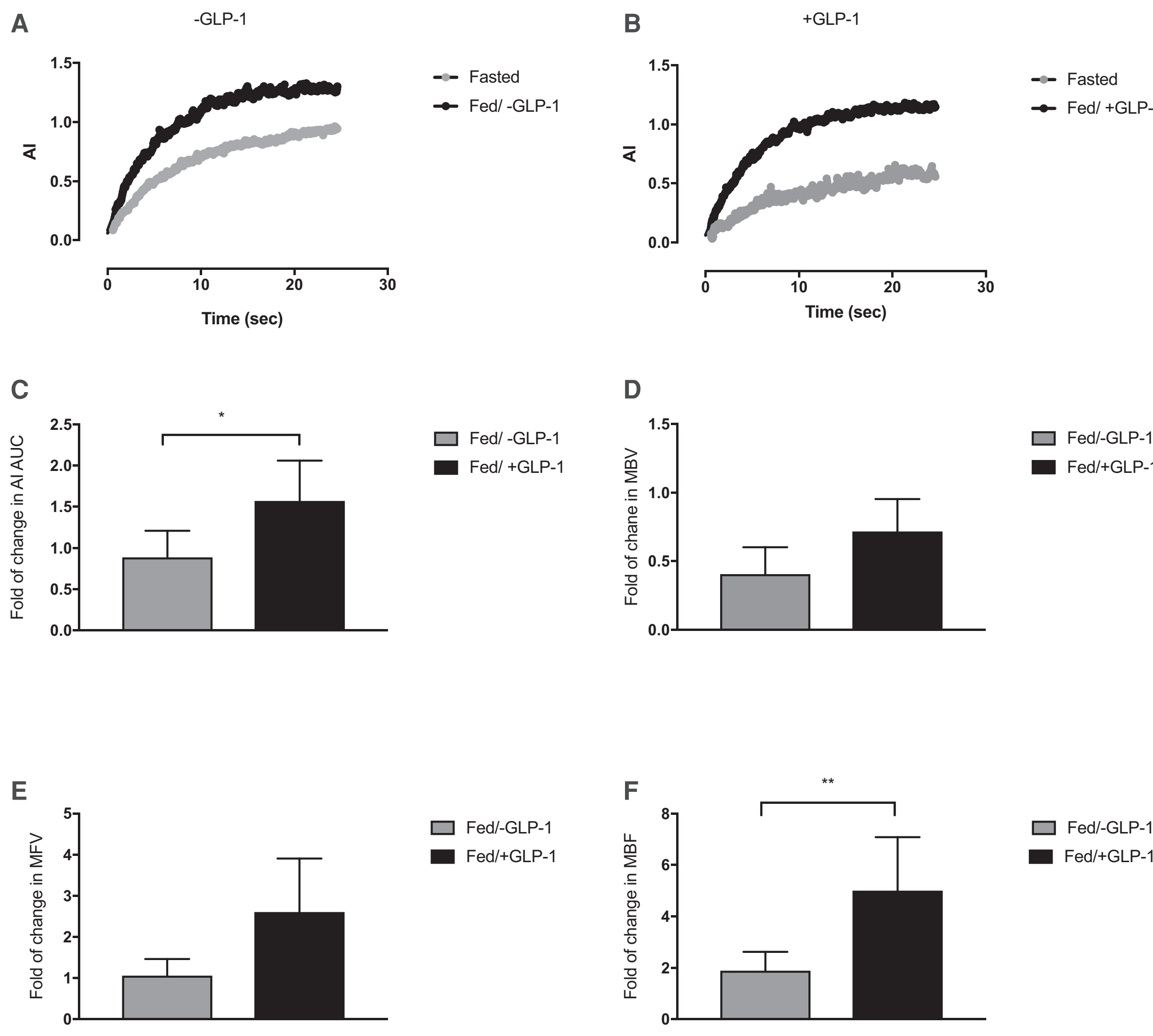

Figure 3. Microvascular responses to feeding with and without GLP-1 as assessed by contrast-enhanced ultrasound (CEUS). A and B show the acoustic index generated from microvascular microbubble contrast refilling (corresponds to MBV) plotted against time following application of a high mechanical index ultrasonic signal to destroy microbubbles. Fold change in AI AUC from fasted to fed state with and without GLP-1 infusion is shown in panel C, with fold changes in microvascular blood volume (MBV), microvascular flow velocity (MFV), and microvascular blood flow (MBF) from fasted to fed-state insulin clamp, with and without GLP-1 infusion, shown in panels D, E, and F, respectively. Analysis via 2-way ANOVA. $^*P < 0.05$, $^{**}P < 0.01$ between conditions. Data presented as means ± SEM.

the 2 groups (1.7 ± 0.2 vs 1.6 ± 0.3 ng/mL^{-1}, with and without GLP-1, respectively). C-peptide levels dropped numerically but not significantly following octreotide infusion at the start of the postprandial clamp (0.93 ± 0.23 vs 0.91 ± 0.18 ng/mL^{-1}, with and without GLP-1, respectively). C-peptide remained similar between groups throughout the clamp, not significantly different to baseline (Fig. 4B).

The time course of leg blood glucose levels during the fasted and postprandial periods in both experiments is shown in Fig. 4C. Mean blood glucose in the fasted state was 5.8 ± 0.2 vs 5.7 ± 0 mM in the experiments with and without GLP-1, respectively. Following the start of feeding, mean femoral glucose over the entire of feeding period was 7.5 ± 0.14 vs 7.9 ± 0.17 mM with and without GLP-1, respectively. GIR is shown in Fig. 5A. Rates of glucose infusion started high, guided by individual's surface area per the Defronzo protocol and followed a reducing pattern in the first 15 minutes, when glucose levels are expected to rise to the desired postprandial range of 7.0 to 7.5 mM. GIR was significantly higher at 150 to 165 minutes (2.3 ± 0.08 vs 0.5 ± 0.10 mg/kg^{-1}/min^{-1}, $P = 0.02$ with and without GLP-1, respectively) and 165 to 180 minutes (2.4 ± 0.09 vs 0.6 ± 0.08 mg/kg^{-1}/min^{-1}, $P = 0.009$ with and without GLP-1, respectively) with GLP-1. Whole body glucose uptake (M) calculated from the glucose infusion rate is presented as a comparison of AUC between the 2 groups in Fig. 5B. Whole body glucose uptake was significantly higher with GLP-1 (M, AUC 16.9 ± 1.7 vs 11.4 ± 1.8 mg/kg^{-1}/180 minutes^{-1}, $P = 0.02$, with and without GLP, respectively).

Discussion

It is important when testing the metabolic impact of GLP-1 on glucose metabolism to control insulin and nutrients (glucose and AA). We chose to mimic a physiological postprandial, hyperaminoacidemic fed state because insulin and glucose stimulate skeletal muscle glucose uptake via different mechanisms. In our study, 8 individuals studied twice with and without

Downloaded from https://academic.oup.com/jcem/advance-article/doi/10.1210/clinem/dgac613/6763659 by guest on 02 December 2022

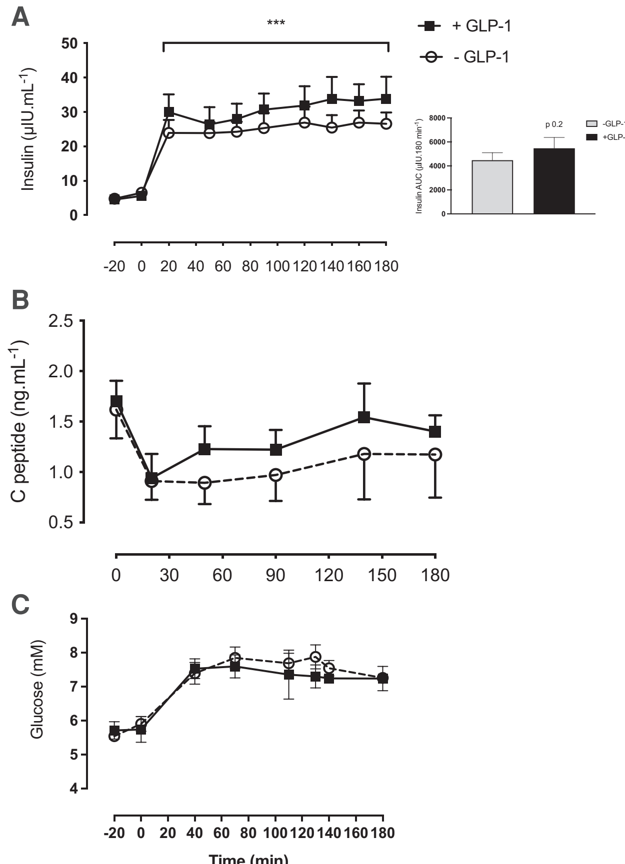

Figure 4. Insulin and AUC above baseline (inset) (A), C-peptide (B), and glucose (C) plasma concentrations at baseline and during fed-state insulin clamp. Analysis via 2-way ANOVA. $P = <0.05$, $P = <0.01$, $***P < 0.001$ vs fasted value in the same condition. Data presented as mean ± SEM. AUC, area under the curve.

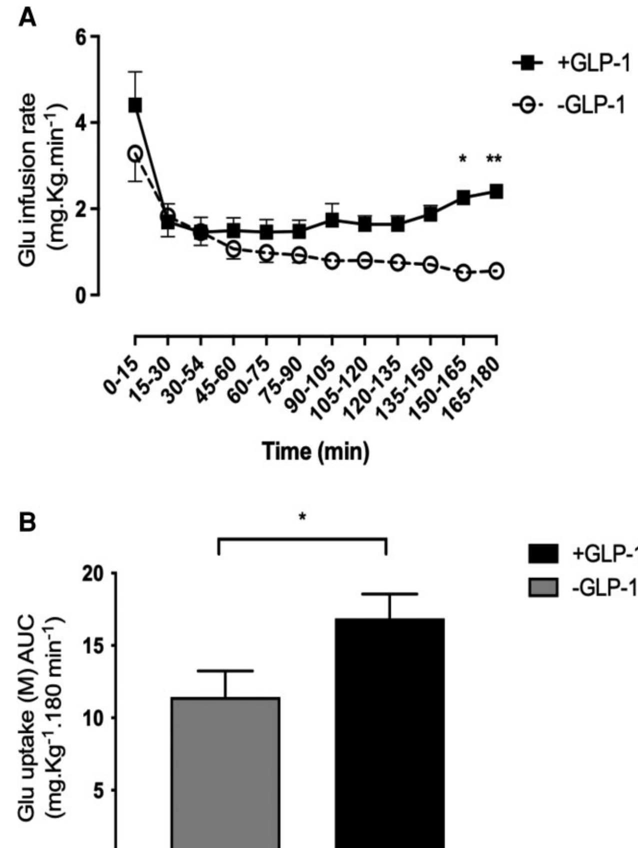

Figure 5. Glucose infusion rate (A) and whole body glucose uptake, M (B). $*P < 0.05$, $**P < 0.01$ between groups. Analysis via 2-way ANOVA. Data presented as means ± SEM.

GLP-1, in a model that minimized variability between subjects. In this study, the clamp protocol successfully achieved target levels of glucose and insulin. In addition, phenylalanine concentration (Fig. 2B) showed the expected 2- to 3-fold increase in phenylalanine to a steady state, demonstrating successful intravenous infusion of AA to achieve postprandial AA concentrations, as in previous feeding studies giving AA orally (33) or IV (28). Both groups received identical infusions of glucose and mixed AA to generate a postprandial insulinemic, hyperaminoacidemic clamp, mimicking feeding. AA infusions have been reported to increase insulin resistance (34); however, because we have studied the same individuals on 2 occasions, wherein the only difference is the GLP-1 infusion, we therefore believe the impact on glucose metabolism is from the presence of GLP-1. To our knowledge, this study is the first to report the effects of GLP-1 infusion on skeletal muscle MVR and glucose uptake in older individuals under fed conditions. In sum, we demonstrated that: (1) infusion of GLP-1 improves skeletal muscle MVR beyond increases seen with insulin alone and (2) postprandial GLP-1 infusion significantly enhances whole body glucose uptake.

We have shown that GLP-1 significantly improves MVR through enhancement of both MFV and MBV responses to a fed-state insulin clamp. Moreover, this positive effect on MVR is shown for the first time in the context of aging, and complements previous observations in young individuals (20, 21). This novel observation is crucial because aging has been associated with blunted insulin-mediated vasodilatory stimulation (33). For instance, Skilton et al demonstrated that postprandial microvascular dilatation is impaired with advancing age even after adjusting for potentially confounding baseline parameters (35). Although the response to NO is thought to be intact in older age, the underexpression of endothelial nitric oxide synthase in response to insulin is thought to play an important role in the blunted microvascular vasodilatory responses to insulin observed with aging (36-37). Beyond this, several other mechanisms have also been proposed as potential reasons for this blunted response, including a decline in total limb blood flow (38), insulin resistance, increased degradation of NO by oxygen-derived free radicals, increased release of vasoconstrictors (35), a reduction in vascular responsiveness (39), reduced capillary density (40), and endothelial dysfunction (41). All of these factors may jeopardize the ability of skeletal muscles to respond to vasodilatory stimuli such as insulin (exogenously infused or in response to feeding) and contractile activity (ie, exercise).

Our MVR results are of importance in the context of resistance to insulin action at the microcirculatory level, as seen in aging and diabetes, with this resistance thought to be due to reduced rate of transcapillary insulin movement to the interstitial space across vascular endothelial cells (42). Further, insulin uptake by endothelial cells is also impaired in these conditions, possibly because of raised levels of cytokines (43). These facts are supported by the observation that insulin

Downloaded from https://academic.oup.com/jcem/advance-article/doi/10.1210/clinem/dgac613/6763659 by guest on 02 December 2022

concentrations in the interstitium is significantly lower than in plasma, with a consequent delayed insulin action at the myocyte level (42). It is therefore speculated that increased MVR would lead to increased endothelial surface area and thus to improved insulin and glucose delivery to skeletal muscle cells (14, 44). Indeed, improvements in glucose delivery to muscle has been demonstrated using pharmacological preparations that are known to reduce peripheral microvascular tone such as losartan, an angiotensin II type 1 receptor blocker (45). Although the increments in whole body glucose disposal shown in this study do not exclude the possibility that GLP-1 might also being acting on other tissues, skeletal muscle is likely a major contributor as the predominant site for insulin-mediated glucose disposal (46, 47). Finally, both insulin and GLP-1 mediate their microvascular action through NO-dependent mechanisms (14, 15). Our data suggest GLP-1 appears to have additive effects beyond that of insulin alone in recruiting skeletal muscle microvasculature in older individuals and may have a role in the insulin resistance associated with aging and chronic disease such as type 2 diabetes, which is linked to endothelial dysfunction.

It is noteworthy that GLP-1 infusions resulted in numerically higher concentrations of insulin during the clamp, but with the C-peptide profiles indicating this marginal difference seems to be related to endogenous insulin production rather than disparities in exogenous insulin concentrations. This may be related to the dose of octreotide used in this study, which is lower than in previous similar experimental studies (9, 13), and was designed to reduce side effects given the age-related characteristics of our cohort. This dose has been effective, however, in completely blocking endogenous insulin production in other studies aimed at achieving experimental postprandial insulin levels, albeit without GLP-1 infusion (28). This disparity may be explained by a presumed "β-cell escape phenomenon" in relation to GLP-1 infusion, in which higher doses of octreotide are needed to completely block endogenous insulin production if GLP-1 is being co-infused. A potential limitation of our study includes not including lipid co-infusion (to mimic meal feeding). We reasoned that because rises in lipid levels following oral ingestion are delayed, the impact of lipid on blood flow and glucose utilization during the early feeding response when insulin and GLP-1 rise transiently is likely negligible.

In conclusion, compared with hyperinsulinemia alone, this study demonstrates that co-infusion of GLP-1 improves skeletal muscle MVR and whole body glucose uptake/use during a hyperglycemic, hyperaminoacidemic clamp. These extrapancreatic benefits of GLP-1 require further exploration in relation to the use of existing incretin therapeutics that may be able to enhance microvascular and metabolic responses to food intake in older age. This thesis is further underlined by our recent report of GLP-1 infusions enhancing muscle protein synthetic responses to a fed-state insulin clamp in older men (48).

Acknowledgments

The authors thank Margaret Baker, Jessica Cejielski, and Imran Ramzan for technical and clinical assistance. Great appreciation goes to all our study participants for the time they devoted.

Funding

This work was supported by the Medical Research Council (grant number MR/K00414X/1) and Versus Arthritis (grant number 19891).

Author Contributions

P.J.A., I.I., K.S., B.E.P., D.J.W., and H.A. conceptualized and planned the study. H.A., J.P.W., and I.I. performed clinical studies. A.G. performed vascular studies. B.E.P., M.L., T.J., D.J.W., J.L., and J.J.B. performed laboratory samples analyses, which were revised and approved by K.S. H.A. and B.E.P. analyzed microvascular blood flow data. H.A. drafted the initial manuscript, which was further edited by B.E.P., P.J.A., I.I., and K.S. All authors approved the final version of the manuscript.

Conflict of Interest

All authors declare no conflict of interest in relation to this work.

Data Availability Statement

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

Clinical Trial Registration

NCT02370745.

References

1. Weir GC, Mojsov S, Hendrick GK, Habener JF. Glucagonlike peptide I (7-37) actions on endocrine pancreas. *Diabetes*. 1989;38(3):338-342.
2. Nathan DM, Schreiber E, Fogel H, Mojsov S, Habener JF. Insulinotropic action of glucagonlike peptide-I-(7–37) in diabetic and nondiabetic subjects. *Diabetes Care*. 1992;15(2):270-276.
3. Montrose-Rafizadeh C, Egan JM, Roth J. Incretin hormones regulate glucose-dependent insulin secretion in RIN 1046-38 cells: mechanisms of action. *Endocrinology*. 1994;135(2):589-594.
4. Elahi D, McAloon-Dyke M, Fukagawa NK, *et al*. The insulinotropic actions of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (7-37) in normal and diabetic subjects. *Regul Pept*. 1994;51(1):63-74.
5. Larsson H, Holst JJ, Ahren B. Glucagon-like peptide-1 reduces hepatic glucose production indirectly through insulin and glucagon in humans. *Acta Physiol Scand*. 1997;160(4):413-422.
6. Todd JF, Wilding JP, Edwards CM, Khan FA, Ghatei MA, Bloom SR. Glucagon-like peptide-1 (GLP-1): a trial of treatment in non-insulin-dependent diabetes mellitus. *Eur J Clin Invest*. 1997;27(6):533-536.
7. Ryan AS, Egan JM, Habener JF, Elahi D. Insulinotropic hormone glucagon-like peptide-1-(7–37) appears not to augment insulin-mediated glucose uptake in young men during euglycemia. *J Clin Endocrinol Metab*. 1998;83(7):2399-2404.
8. Ørskov L, Holst JJ, Møller J, *et al*. GLP-1 does not acutely affect insulin sensitivity in healthy man. *Diabetologia*. 1996;39(10):1227-1232.
9. Vella A, Shah P, Basu R, Basu A, Holst JJ, Rizza RA. Effect of glucagon-like peptide 1 (7-36) amide on glucose effectiveness and insulin action in people with type 2 diabetes. *Diabetes*. 2000;49(4):611-617.
10. Gutniak M, Orskov C, Holst JJ, Ahrén B, Efendic S. Antidiabetogenic effect of glucagon-like peptide-1 (7-36) amide in normal subjects and patients with diabetes mellitus. *N Engl J Med*. 1992;326(20):1316-1322.
11. D'Alessio DA, Kahn SE, Leusner CR, Ensinck JW. Glucagon-like peptide 1 enhances glucose tolerance both by stimulation of insulin release and by increasing insulin-independent glucose disposal. *J Clin Invest*. 1994;93(5):2263-2266.

Downloaded from https://academic.oup.com/jcem/advance-article/doi/10.1210/clinem/dgac613/6763659 by guest on 02 December 2022

12. D'Alessio DA, Prigeon RL, Ensinck JW. Enteral enhancement of glucose disposition by both insulin-dependent and insulin-independent processes. A physiological role of glucagon-like peptide I. *Diabetes*. 1995;44(12):1433-1437.
13. Vella A, Shah P, Basu R, *et al.* Effect of glucagon-like peptide-1(7-36)-amide on initial splanchnic glucose uptake and insulin action in humans with type 1 diabetes. *Diabetes*. 2001;50(3):565-572.
14. Chai W, Dong Z, Wang N, *et al.* Glucagon-like peptide 1 recruits microvasculature and increases glucose use in muscle via a nitric oxide-dependent mechanism. *Diabetes*. 2012;61(4):888-896.
15. Barrett EJ, Eggleston EM, Inyard AC, *et al.* The vascular actions of insulin control its delivery to muscle and regulate the rate-limiting step in skeletal muscle insulin action. *Diabetologia*. 2009;52(5):752-764.
16. Ijuin T, Hatano N, Hosooka T, Takenawa T. Regulation of insulin signaling in skeletal muscle by PIP3 phosphatase, SKIP, and endoplasmic reticulum molecular chaperone glucose-regulated protein 78. *Biochim Biophys Acta*. 2015;1853(12):3192-3201.
17. Delgado E, Luque MA, Alcantara A, *et al.* Glucagon-like peptide-1 binding to rat skeletal muscle. *Peptides*. 1995;16(2):225-229.
18. Villanueva-Penacarrillo ML, Alcantara AI, Clemente F, Delgado E, Valverde I. Potent glycogenic effect of GLP-1(7-36) amide in rat skeletal muscle. *Diabetologia*. 1994;37(11):1163-1166.
19. Luque MA, Gonzalez N, Marquez L, *et al.* Glucagon-like peptide-1 (GLP-1) and glucose metabolism in human myocytes. *J Endocrinol*. 2002;173(3):465-473.
20. Sjøberg KA, Holst JJ, Rattigan S, Richter EA, Kiens B. GLP-1 increases microvascular recruitment but not glucose uptake in human and rat skeletal muscle. *Am J Physiol Endocrinol Metab*. 2014;306(4):E355-E362.
21. Subaran SC, Sauder MA, Chai W, *et al.* GLP-1 at physiological concentrations recruits skeletal and cardiac muscle microvasculature in healthy humans. *Clin Sci (Lond)*. 2014;127(3):163-170.
22. Phillips BE, Atherton PJ, Varadhan K, Limb MC, Williams JP, Smith K. Acute cocoa flavanol supplementation improves muscle macro- and microvascular but not anabolic responses to amino acids in older men. *Appl Physiol Nutr Metab*. 2016;41(5):548-556.
23. Mitchell WK, Phillips BE, Williams JP, *et al.* Development of a new Sonovue™ contrast-enhanced ultrasound approach reveals temporal and age-related features of muscle microvascular responses to feeding. *Physiol Rep*. 2013;1(5):e00119.
24. Luzi L, Giordano M, Caloni M, Castellino P. Effects of insulin and amino acids on leucine metabolism in young and middle-aged humans. *Eur J Nutr*. 2001;40(3):106-112.
25. Rasmussen BB, Fujita S, Wolfe RR, *et al.* Insulin resistance of muscle protein metabolism in aging. *FASEB J*. 2006;20(6):768-769.
26. Chevalier S, Goulet EDB, Burgos SA, Wykes LJ, Morais JA. Protein anabolic responses to a fed steady state in healthy aging. *J Gerontol A Biol Sci Med Sci*. 2011;66(6):681-688.
27. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol*. 1979;237(3):E214-E223.
28. Greenhaff PL, Karagounis LG, Peirce N, *et al.* Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. *Am J Physiol Endocrinol Metab*. 2008;295(3):595-604.
29. Phillips B, Williams J, Atherton P, *et al.* Resistance exercise training improves age-related declines in leg vascular conductance and rejuvenates acute leg blood flow responses to feeding and exercise. *J Appl Physiol*. 2012;112(3):347-353.
30. Weber M-A, Krakowski-Roosen H, Delorme S, *et al.* Relationship of skeletal muscle perfusion measured by contrast-enhanced ultrasonography to histologic microvascular density. *J Ultrasound Med*. 2006;25(5):583-591.
31. Wilkinson DJ, Bukhari SSI, Phillips BE, *et al.* Effects of leucine-enriched essential amino acid and whey protein bolus dosing upon skeletal muscle protein synthesis at rest and after exercise in older women. *Clin Nutr*. 2018;37(6):2011-2021.
32. Bukhari SSI, Phillips BE, Wilkinson DJ, *et al.* Intake of low-dose leucine-rich essential amino acids stimulates muscle anabolism equivalently to bolus whey protein in older women at rest and after exercise. *Am J Physiol Endocrinol Metab*. 2015;308(12):E1056-E1065.
33. Volpi E, Mittendorfer B, Wolf SE, Wolfe RR. Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction. *Am J Physiol*. 1999;277(3 Pt 1):E513-E520.
34. Flakoll PJ, Kulaylat M, Frexes-Steed M, Hill JO, Abumrad NN. Amino acids enhance insulin resistance to exogenous glucose infusion in overnight-fasted humans. *JPEN J Parenter Enteral Nutr*. 1991;15(2):123-127.
35. Skilton MR, Lai NT, Griffiths KA, *et al.* Meal-related increases in vascular reactivity are impaired in older and diabetic adults: insights into roles of aging and insulin in vascular flow. *Am J Physiol Heart Circ Physiol*. 2005;288(3):H1404-H1410.
36. Bearden SE. Effect of aging on the structure and function of skeletal muscle microvascular networks. *Microcirculation*. 2006;13(4):279-288.
37. Samengo G, Avik A, Fedor B, *et al.* Age-related loss of nitric oxide synthase in skeletal muscle causes reductions in calpain S-nitrosylation that increase myofibril degradation and sarcopenia. *Aging Cell*. 2012;11(6):1036-1045.
38. Donato AJ, Uberoi A, Wray DW, Nishiyama S, Lawrenson L, Richardson RS. Differential effects of aging on limb blood flow in humans. *Am J Physiol Heart Circ Physiol*. 2006;290(1):H272-H278.
39. Celermajer DS, Sorensen KE, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Deanfield JE. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. *J Am Coll Cardiol*. 1994;24(2):471-476.
40. Coggan AR, Spina RJ, King DS, *et al.* Histochemical and enzymatic comparison of the gastrocnemius muscle of young and elderly men and women. *J Gerontol*. 1992;47(3):B71-B76.
41. Hamilton CA, Brosnan MJ, McIntyre M, Graham D, Dominiczak AF. Superoxide excess in hypertension and aging: a common cause of endothelial dysfunction. *Hypertension*. 2001;37(2 Pt 2):529-534.
42. Keske MA, Premilovac D, Bradley EA, Dwyer RM, Richards SM, Rattigan S. Muscle microvascular blood flow responses in insulin resistance and ageing. *J Physiol*. 2016;594(8):2223-2231.
43. Wang H, Wang AX, Barrett EJ. Caveolin-1 is required for vascular endothelial insulin uptake. *Am J Physiol Endocrinol Metab*. 2011;300(1):E134-E144.
44. Moore MC, Cherrington AD, Wasserman DH. Regulation of hepatic and peripheral glucose disposal. *Best Pract Res Clin Endocrinol Metab*. 2003;17(3):343-364.
45. Chai W, Wang W, Liu J, *et al.* Angiotensin II type 1 and type 2 receptors regulate basal skeletal muscle microvascular volume and glucose use. *Hypertension*. 2010;55(2):523-530.
46. Thiebaud D, Jacot E, DeFronzo RA, Maeder E, Jequier E, Felber JP. The effect of graded doses of insulin on total glucose uptake, glucose oxidation, and glucose storage in man. *Diabetes*. 1982;31-(11):957-963.
47. DeFronzo RA. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes*. 1988;37(6):667-687.
48. Abdulla H, Phillips BE, Wilkinson DJ, *et al.* Glucagon-like peptide 1 infusions overcome anabolic resistance to feeding in older human muscle. *Aging Cell*. 2020;19(9):e13202.

Downloaded from https://academic.oup.com/jcem/advance-article/doi/10.1210/clinem/dgac613/6763659 by guest on 02 December 2022