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GLP-1 and Insulin Recruit Muscle Microvasculature and Dilate Conduit Artery Individually But Not Additively in Healthy Humans

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Context: Glucagon-like peptide-1 (GLP-1) and insulin increase muscle microvascular perfusion, thereby increasing tissue endothelial surface area and nutrient delivery.

Objective: To examine whether GLP-1 and insulin act additively on skeletal and cardiac microvasculature and conduit artery.

Design: Healthy adults underwent three study protocols in random order.

Setting: Clinical Research Unit at the University of Virginia.

Methods: Overnight-fasted participants received an intravenous infusion of GLP-1 (1.2 pmol/kg/min) or normal saline for 150 minutes with or without a 2-hour euglycemic insulin clamp (1 mU/kg/min) superimposed from 30 minutes onward. Skeletal and cardiac muscle microvascular blood volume (MBV), flow velocity, and flow; brachial artery diameter, flow velocity, and blood flow; and pulse wave velocity (PWV) were measured.

Results: GLP-1 significantly increased skeletal and cardiac muscle MBV and microvascular blood flow (MBF) after 30 minutes; these remained elevated at 150 minutes. Insulin also increased skeletal and cardiac muscle MBV and MBF. Addition of insulin to GLP-1 did not further increase skeletal and cardiac muscle MBV and MBF. GLP-1 and insulin increased brachial artery diameter and blood flow, but this effect was not additive. Neither GLP-1, insulin, nor GLP-1 and insulin altered PWV. Combined GLP-1 and insulin infusion did not result in higher whole-body glucose disposal.

Conclusion: GLP-1 and insulin at physiological concentrations acutely increase skeletal and cardiac muscle microvascular perfusion and dilate conduit artery in healthy adults; these effects are not additive. Thus, GLP-1 and insulin may regulate skeletal and cardiac muscle endothelial surface area and nutrient delivery under physiological conditions.

Abbreviations: CEU, contrast-enhanced ultrasound; eNOS, endothelial nitric oxide synthase; GLP-1, Glucagon-like peptide-1; HFD, high-fat diet; MBF, microvascular blood flow; MBV, microvascular blood volume; MCE, myocardial contrast echocardiography; MFV, microvascular flow velocity; NO, nitric oxide; PRCR, piecewise random coefficient regression; PWV, pulse wave velocity; RCR, random coefficient regression.

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Muscle is a primary site of insulin action. For insulin to exert its metabolic effects in muscle and for nutrients to be used by the myocytes, insulin and nutrients first have to be delivered to the capillaries bathing the myocytes and then transported through the vascular wall to reach muscle interstitium. An increase in total muscle blood flow leads to more insulin and nutrients delivered to the muscle, and an expansion of the muscle microvasculature represents an enlargement of the endothelial surface area available for the transport of insulin and nutrients into the muscle interstitium from plasma. Thus, total tissue blood flow and muscle microvascular volume are two major factors limiting the delivery of insulin and nutrients to muscle and, consequently, insulin action and nutrient availability in muscle.

Insulin regulates its own delivery to muscle by feed-forward actions on the vasculature [1]. In human and animal studies, insulin, at physiological concentrations, dilates conduit and resistance arteries to increase total blood flow to muscle [2–4], recruits microvasculature by relaxing terminal arterioles to expand the microvascular blood volume (MBV) [5, 6], and acts on its receptors on the endothelial cells to facilitate its own transport through the capillary wall [7]. These vascular effects are attenuated in clinical and experimental insulin resistant states such as obesity [3, 8] and during systemic lipid infusion to raise plasma free fatty acid concentrations [9, 10], further supporting insulin-mediated vasodilation as an important determinant of muscle insulin action [4, 11–13].

Glucagon-like peptide 1 (GLP-1), a hormone produced by intestinal L cells in response to nutrient ingestion, lowers postprandial plasma glucose via glucose-dependent stimulation of insulin secretion, suppression of glucagon secretion, and delayed gastric emptying [14]. Recent evidence confirms that GLP-1 exerts a potent vasodilatory effect on the conduit and resistance arteries as well as terminal arterioles to significantly increase total muscle blood flow and muscle microvascular perfusion in rats and in healthy humans [15–20]. This is not surprising, because GLP-1 receptors are abundantly expressed in the vascular endothelium [21].

Insulin and GLP-1 each cause vasodilation via a nitric oxide (NO)-dependent mechanism. Insulin acts through the phosphatidylinositol 3-kinase/protein kinase B/endothelial NO synthase (eNOS) pathway [5, 6, 22–25] and these actions may account for up to 40% of insulin's overall effect in stimulating muscle glucose uptake [4–6]. On the other hand, inhibition of eNOS with N^G-nitro-L-arginine methyl ester or removal of the endothelium each abolishes the vasorelaxant effect of GLP-1 [26, 27]. We have demonstrated in laboratory rodents that GLP-1 potently dilates the precapillary arterioles to recruit muscle microvasculature, likely via a protein kinase A-NO–mediated pathway [16, 17]. Though insulin and GLP-1 activate eNOS via distinctly different signal pathways and the vasodilatory actions of GLP-1 are preserved in the insulin-resistant states [15], it remains unclear whether they exert vasorelaxant effects additively.

The primary aims of this study were to evaluate the acute effects of insulin and GLP-1 infusions at physiological concentrations on skeletal and cardiac muscle microvasculature, as well as the vascular function of conduit arteries in young, healthy individuals, and to determine whether the vascular effects of these infusions influence insulin-mediated, whole-body glucose disposal. We also aimed to determine whether insulin and GLP-1 responses were additive.

1. Materials and Methods

A. Study Participants

A total of 22 healthy volunteers aged 18 to 35 years were included in this study. Participants had body mass index between 18 and 25 kg/m^2 and no chronic medical illness such as diabetes

mellitus; hypertension; hypotension (*i.e.*, blood pressure <100/60 mm Hg); hyperlipidemia; chronic or acute cardiac, pulmonary, liver, or kidney diseases; or intracardiac or intrapulmonary shunts. Current smokers (or those who had quit smoking within the past 6 months before study enrolment), individuals with first-degree relatives with type 2 diabetes, use of medications that may affect vascular function (*e.g.*, angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers, statins, fibrates, fish oil, aspirin, vitamin C, vitamin E), pregnant and/or breastfeeding women, or those with known hypersensitivity to perflutren were excluded from the study.

B. Study Protocol

All volunteers were screened for study eligibility at the University of Virginia Clinical Research Unit. Their vital signs, height, weight, and waist circumference were obtained; and fasting blood samples were drawn for complete blood cell count, lipid profile, and comprehensive metabolic testing. A pregnancy test was also performed for all female volunteers. Individuals with normal screening parameters (clinical and biochemical) and no exclusion criteria were invited to participate in the study.

The study comprised one outpatient visit and three study admissions. During the outpatient visit, the participants underwent a treadmill test for maximal oxygen consumption using the Bruce protocol, and body composition analysis using air displacement plethysmography (BOD POD; Life Management, Concorde, CA). They were then scheduled to return to the Clinical Research Unit for three study admissions, with at least a 2-week interval between the admissions. During these admissions, all participants underwent three different study protocols (one protocol per admission) in random order: protocol 1 was GLP-1 infusion alone; protocol 2 was insulin infusion alone; and protocol 3 was coinfusion of GLP-1 and insulin (Fig. 1).

For each study admission, the participants were admitted to the Clinical Research Unit at 7:00 AM, after having fasted from 20:00 PM onward the night before. They were also instructed to avoid caffeine intake and exercise for 24 hours before each admission. Venous access was obtained with two peripheral intravenous cannulas in the right upper arm, one at the antecubital fossa for infusion of GLP-1, insulin, glucose, saline, and microbubbles; and the other at the distal forearm for blood sampling. At baseline (0 minutes), blood samples were obtained for measurement of glucose, insulin, GLP-1 and glucagon levels. Brachial artery diameter, flow velocity, and blood flow were determined using ultrasonography with the patient in a supine position; and pulse wave velocity (PWV) was measured by applanation tonometry using the SphygmoCor system (AtCor Medical, Itasca, IL). A certified cardiac

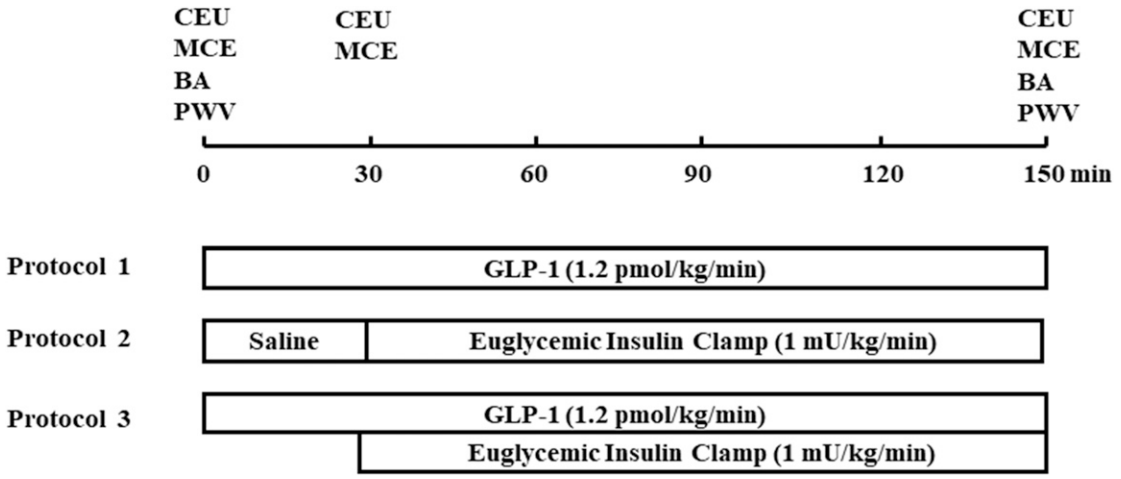

Figure 1. Study protocol. BA, brachial artery; CEU, contrast-enhanced ultrasound; MCE, myocardial contrast echocardiography; PWV, pulse wave velocity.

sonographer then performed Doppler color-flow imaging of the heart to evaluate for cardiac shunts. Once the presence of cardiac shunts had been excluded, contrast-enhanced ultrasound (CEU) and myocardial contrast echocardiography (MCE) were performed to determine cardiac and skeletal muscle MBV, microvascular flow velocity (MFV), and microvascular blood flow (MBF).

After the baseline blood samples and vascular measurements were obtained, GLP-1 and/or insulin infusions were commenced in accordance with the study protocol (Fig. 1). A systemic infusion of GLP-1 was administered at 1.2 pmol/kg/min for 150 minutes for protocols 1 and 3, with a 2-hour 1 mU/kg/min hyperinsulinemic euglycemic clamp superimposed from 30 minutes onward for protocol 3. For study protocol 2, only the 2-hour hyperinsulinemic euglycemic clamp was conducted between times 30 and 150 minutes. CEU and MCE of the skeletal and cardiac muscle were repeated at 30 and 150 minutes. Brachial artery diameter, flow velocity, and blood flow, as well as PWV were measured again at 150 minutes. Blood samples for glucose and insulin levels were obtained every 30 minutes, and obtained for glucagon and GLP-1 levels at 30 and 150 minutes. The participants remained supine throughout the study, and their blood pressure and pulse rate were measured every 30 minutes. During insulin infusion, plasma glucose concentrations were monitored every 5 minutes and maintained at ~10 mg/dL below the baseline level, using 20% dextrose infused at variable rates, to avoid arterial hyperglycemia [28, 29].

The study protocol was carried out in accordance with the 2013 Declaration of Helsinki of the World Medical Association and was approved by the University of Virginia Institutional Review Board. Written informed consent was obtained from each participant during the screening visit, before study enrolment. Data from protocol 1 have previously been published [20] and were reanalyzed together with data from protocols 2 and 3, with permission from the publisher.

B-1. Measurement of microvascular parameters in skeletal and cardiac muscle

CEU and MCE were performed with the participant in the left decubitus position, using the SONOS 7500 ultrasound system and a S3 phased array transducer (Philips Medical Systems, Andover, MA), as described previously [9, 28–30]. The contrast agent Definity (Lantheus Medical Imaging, North Billerica, MA), which contained octafluoropropane gas–filled lipid microbubbles, was infused continuously at a constant rate throughout the imaging process. When the concentration of the microbubbles reached steady state in the circulation (at ~2.5 minutes into the infusion), intermittent ultraharmonic images of the left forearm and myocardium were obtained. Transverse images of the left forearm skeletal muscle were obtained with pulse intervals of 1, 2, 3, 4, 5, 8, 12, 16, and 20 cardiac cycles. The cardiac images acquired included the apical two-, three-, and four-chamber views at progressively increasing pulse intervals of one, two, three, four, five, and eight cardiac cycles. The pulse interval is the time between successive destructive ultrasound pulses, with a longer pulse interval enabling greater replenishment of the microbubbles. At each pulsing interval, three images were captured digitally. All ultrasound images were analyzed using the QLAB software (Philips Medical System) to determine the skeletal and cardiac muscle MBV and MFV. MBV reflects the blood volume in the microvascular capillary bed, and MFV refers to the rate of blood flow through this capillary bed. MBF was calculated from MBV and MFV (*i.e.*, MBF = MBV × MFV). For cardiac muscle microvasculature, we reported the mean MBV, MFV, and MBF values from all three cardiac views. This was based on the assumption that these participants, being young and healthy with no chronic medical illness, should have homogenous perfusion throughout the myocardium.

B-2. Measurement of brachial artery diameter, flow velocity, and blood flow

Brachial artery diameter and flow velocity were measured with the participant in a supine position, using the SONOS 7500 ultrasound system, as described previously [9, 28–30]. A L11-3 linear array transducer (Philips Medical Systems) with a transmit frequency of 7.5 MHz was

used to obtain a two-dimensional longitudinal image of the participant's left brachial artery. The diameter was measured as the distance between the near- and far-wall lumen-intima boundaries during peak systole, and the time-averaged mean brachial artery flow velocity was determined using pulsed-wave Doppler. Brachial artery blood flow was calculated from the averages of three diameter and velocity measurements, using the formula $Q = v\pi(d/2)^2$, where Q stands for blood flow, v stands for velocity, and d stands for diameter.

B-3. Measurement of arterial stiffness

Carotid-femoral PWV was used as a marker of aortic arterial stiffness in this study. This was measured by applanation tonometry using the SphygmoCor system, which calculates PWV from the time taken for the arterial pulse to propagate from the carotid to the femoral artery. The stiffer the aorta, the shorter the PWV.

C. Biochemical Analysis

All biochemical investigations performed during screening were assayed at the University of Virginia Clinical Chemistry Laboratory. During the hyperinsulinemic euglycemic clamp, plasma glucose levels were determined using the YSI glucose analyzer (Yellow Spring Instruments). The Siemens Healthcare Diagnostics Immulite 2000 Random Access Analyzer was used to quantify plasma insulin levels. Plasma total GLP-1 concentrations were determined using the Millipore enzyme-linked immunosorbent assay kit. Plasma glucagon levels were measured using the Millipore Glucagon radioimmunoassay kit (catalog no. GL-32K) at the University of Virginia Center for Research in Reproduction Ligand Assay and Analysis Core Laboratory.

D. Statistical Analysis

D-1. Empirical data summarization

Continuous scale empirical data are summarized by the mean of the empirical distribution and by the standard error of the mean.

D-2. Piecewise random coefficient regression analyses

Changes in skeletal muscle and cardiac microvascular parameters from 0 to 30 minutes and from 30 to 150 minutes were analyzed via piecewise random coefficient regression (PRCR). The PRCR model specification was identical for all skeletal and cardiac muscle outcome parameters. As predictor variables, each PRCR model included a categorical variable that identified the study protocol (*i.e.*, protocol 1, 2, or 3) and a variable that identified the measurement assessment time (*i.e.*, 0, 30, and 150 minutes). A piecewise regression change point was introduced into the PRCR model to allow the slope of the relationship between the parameter of interest and measurement assessment time to differ between the 0- to 30-minute time interval and the 30- to 150-minute time interval. Study protocol by measurement assessment time interaction was also introduced into the PRCR model to allow the piecewise regression function to differ from one study protocol to the next. To account for intrasubject measurement correlation, the PRCR model was specified to include a subject-specific random intercept effect and subject-specific piecewise random slope effects. With regard to hypothesis testing, PRCR model–derived t tests were used to test for nonzero slope during the 0- to 30-minute time interval and nonzero slope during the 30- to 150-minute time interval. Similarly, PRCR model–derived t tests were used to conduct between–study-protocol slope parameter comparisons. All hypotheses tests were two sided, and a $P \leq 0.05$ decision rule was used as the null hypothesis rejection criterion.

D-3. Random coefficient regression analyses

Brachial artery diameter, flow velocity, and blood flow, and PWV were analyzed via random coefficient regression (RCR). The RCR model specification was identical for all the aforementioned outcome parameters. As predictor variables, each RCR model included a categorical variable that identified the study protocol and a variable that identified the measurement assessment time. Study protocol by measurement assessment time interaction was introduced into the RCR model to allow the regression function to differ from one study protocol to the next. To account for intrasubject measurement correlation, the RCR model was specified to include a subject-specific random intercept effect and a subject-specific random slope effect. With regard to hypothesis testing, RCR model–derived *t* tests were used to test for nonzero slope during the 0- to 150-minute time interval. Similarly, RCR model–derived *t* tests were used to conduct between–study-protocol slope parameter comparisons. All hypotheses tests were two sided, and a $P \leq 0.05$ decision rule was used as the null hypothesis rejection criterion.

D-4. Statistical software

The software package SAS, version 9.4 (SAS Institute Inc., Cary, NC), was used to conduct all statistical analyses.

2. Results

A. Participant Characteristics at Baseline and During Infusion Studies

Baseline participant characteristics are listed in Table 1. The participants were normotensive and had normal lipid profiles and good cardiovascular fitness. All participants completed the three study protocols. At the start of each study protocol, the mean systolic blood pressure, pulse rate, and plasma concentrations of insulin, GLP-1, and glucagon were similar for all three admissions (Table 2). The mean diastolic blood pressure at the start of study protocols 2 (insulin only) and 3 (GLP-1 and insulin) was slightly but significantly lower than that of participants at the start of protocol 1 (GLP-1 only; $P = 0.01$ and $P = 0.02$ respectively).

Systolic and diastolic blood pressure remained stable during the infusion studies throughout the three study protocols. On the contrary, mean pulse rate increased significantly in all three study protocols. The mean increases in pulse rate at the end of the study

Table 1. Participants' Characteristics at Baseline

Characteristic	Mean ± SEM
No. of participants	22
Sex, male: female	10: 12
Age, y	23.55 ± 0.62
Body mass index, kg/m^2	21.92 ± 0.43
Waist circumference, cm	74.05 ± 1.20
Body fat, %	20.26 ± 1.58
Systolic BP, mm Hg	115.55 ± 2.51
Diastolic BP, mm Hg	65.45 ± 2.40
Total cholesterol, mg/dL	166.32 ± 6.82
LDL cholesterol, mg/dL	93.32 ± 5.59
HDL cholesterol mg/dL	61.00 ± 2.59
Triglycerides, mg/dL	75.73 ± 6.51
VO_2 max, mL/kg of body weight per minute	44.92 ± 2.02

Abbreviations: BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SEM, standard error of the mean; VO_2 max, maximal oxygen consumption.

Table 2. Changes in Blood Pressure, Heart Rate, and Hormonal Concentrations During the Infusion Studies

Parameter Measured Over Time (min)	GLP-1	Insulin	GLP-1 Plus Insulin
Systolic blood pressure, mm Hg			
0	109.05 (1.89)	106.55 (2.66)	108.59 (2.37)
30	111.91 (2.36)	108.05 (2.64)	111.64 (2.15)
60	112.14 (2.27)	108.18 (3.00)	112.18 (2.46)
90	110.18 (2.11)	105.36 (2.68)	111.18 (2.92)
120	110.00 (2.27)	106.52 (3.22)	110.14 (2.47)
150	110.46 (2.26)	108.36 (2.87)	112.73 (2.46)
Diastolic blood pressure, mm Hg			
0	66.45 (1.14)	62.36 (1.75)	63.09 (1.54)
30	66.23 (1.34)	60.73 (1.53)	64.77 (1.35)
60	65.59 (1.42)	59.27 (1.59)	64.18 (1.50)
90	63.82 (1.39)	59.91 (1.41)	62.77 (1.96)
120	63.55 (1.69)	60.00 (1.66)	61.18 (1.58)
150	64.50 (0.93)	60.68 (1.73)	62.68 (1.18)
Pulse rate, bpm			
0	55.82 (1.74)	54.41 (1.45)	54.50 (1.75)
30	60.73 (1.99)	54.86 (1.59)	59.50 (1.53)
60	61.50 (2.15)	57.95 (1.72)	61.32 (1.89)
90	60.00 (1.79)	58.23 (2.29)	61.82 (2.10)
120	61.68 (1.82)	57.57 (1.90)	64.05 (2.01)
150	60.05 (1.73)	59.95 (2.05)	64.59 (2.53)
Plasma insulin, pmol/L			
0	17.48 (2.20)	19.05 (2.74)	21.94 (3.23)
10	71.35 (19.52)[a,b]	18.91 (5.12)[a]	54.61 (13.42)[c,d]
30	23.95 (2.88)[e]	37.43 (17.66)	32.76 (5.29)[b]
150	22.67 (3.33)[b]	219.43 (13.67)[f]	275.60 (20.07)[f]
Plasma GLP-1, pmol/L			
0	22.18 (1.66)	21.84 (1.54)	21.04 (1.83)
30	71.41 (3.47)[f]	20.03 (1.42)	74.96 (5.29)[f]
150	76.63 (2.70)[f]	14.49 (0.91)[f]	64.69 (4.27)[f]
Plasma glucagon, pg/mL			
0	67.19 (2.77)	67.77 (3.09)	67.85 (3.73)
30	62.00 (2.71)[b]	62.80 (3.09)[b]	60.82 (3.34)[e]
150	61.67 (3.22)[b]	51.74 (2.94)[f]	47.53 (2.72)[f]

Data given as mean (SEM).
Abbreviation: See Table 1 legend for expansion of abbreviation.
[a] n = 5.
[b] $P < 0.05$, compared with baseline (time, 0 minutes).
[c] n = 6.
[d] $P = 0.008$, compared with baseline (time, 0 minutes).
[e] $P < 0.01$, compared with baseline (time, 0 minutes).
[f] $P < 0.001$, compared with baseline (time, 0 minutes).

protocols compared with baseline were 4.2 ± 1.3 ($P < 0.01$), 5.6 ± 1.0 ($P < 0.001$), and 10.1 ± 1.5 ($P < 0.001$) beats per minute for the GLP-1 only, insulin only, and GLP-insulin protocols, respectively. The changes in plasma insulin, GLP-1, and glucagon levels during infusion of GLP-1 and/or insulin are summarized in Table 2. In protocols 1 and 3, after 30 minutes of GLP-1 infusion, plasma GLP-1 levels increased by approximately threefold to levels seen postprandially and remained elevated throughout. This was associated with a 40% to 50% rise in plasma insulin levels and decline in plasma glucagon levels, resulting in a significant reduction in venous plasma glucose concentrations at 30 minutes. In the GLP-1–only admission, plasma glucose levels decreased from 86.1 ± 1.0 mg/dL at baseline to 72.8 ± 1.2 mg/dL at 30 minutes ($P < 0.001$). The corresponding plasma glucose values for the GLP-insulin admission were 90.1 ± 1.3 mg/dL and 74.4 ± 1.7 mg/dL, respectively ($P < 0.001$). In addition, measurement of plasma insulin levels after 10 minutes of GLP-1 infusion in five to six

participants demonstrated a transient increase in plasma levels that remained significantly elevated at 30 minutes compared with baseline.

During GLP-1–alone infusion, plasma glucose levels gradually returned to baseline levels (82.6 ± 0.9 mg/dL). During the protocols 2 and 3, plasma glucose concentrations were clamped at ~10 mg/dL below the baseline levels, from 86.7 ± 0.93 mg/dL to 75.7 ± 1.4 mg/dL during the protocol 2 admission and from 90.1 ± 1.3 mg/dL to 82.1 ± 1.7 mg/dL during the protocol 3 admission [average differences: 11 and 8 mg/dL, respectively; Fig. 2(A)]. Insulin-mediated whole-body glucose disposal was determined during insulin infusion for study protocols 2 and 3 (Fig. 2). The steady-state glucose infusion rates were significantly higher when participants were infused with both GLP-1 and insulin, compared with insulin alone (7.65 ± 0.41 mg/kg/min vs 6.41 ± 0.35 mg/kg/min; $P < 0.01$). However, mean plasma insulin levels were also significantly higher in the GLP-1 and insulin group. The mean insulin levels during the last 30 minutes were 283.7 ± 19.7 pmol/L and 247.2 ± 12.5 pmol/L for protocols 3 and 2, respectively ($P = 0.02$). Insulin-mediated whole-body glucose disposal per unit of insulin, therefore, was not significantly different between these two protocols ($P = 0.30$).

B. Changes of Skeletal Muscle Microvascular Parameters

The changes in skeletal muscle microvascular parameters during GLP-1 and/or insulin infusion were analyzed for 21 participants (Fig. 3). One participant was excluded from the analysis because of poor CEU image quality. Systemic administration of GLP-1 and of insulin significantly increased skeletal muscle MBV and MBF. GLP-1 infusion alone significantly increased skeletal muscle MBV and MBF from 0 to 30 minutes ($P < 0.001$ for both). MBV was

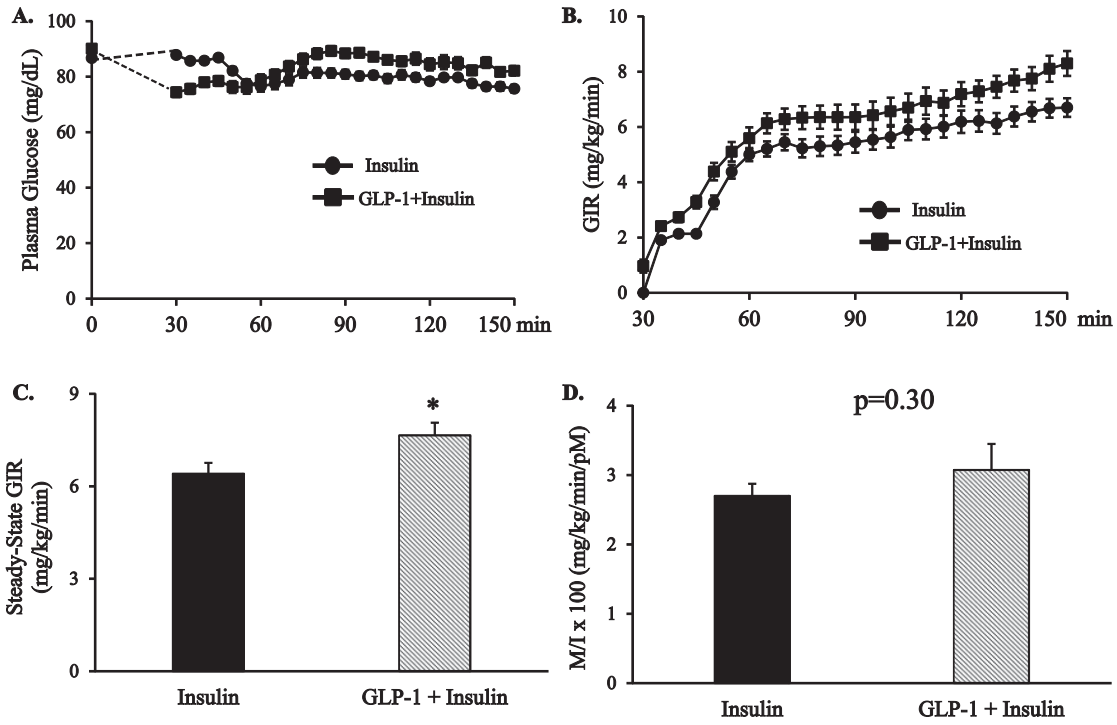

Figure 2. Plasma glucose concentrations and glucose infusion rates during insulin and GLP-1 plus insulin infusion. Each participant received a systemic infusion of either GLP-1 (1.2 pmol/kg/min) or saline for 150 minutes with or without a euglycemic insulin clamp superimposed for the last 120 minutes. For clarity, only insulin and GLP-1 plus insulin data are included. (A) Changes in plasma glucose concentrations. (B) Glucose infusion rates during insulin clamp. (C) Steady-state glucose infusion rates. (D) Steady-state glucose infusion rates corrected by plasma insulin concentrations. *$P < 0.01$ compared with insulin alone. GIR, glucose infusion rate; M/I, insulin-mediated whole-body glucose disposal per unit of insulin.

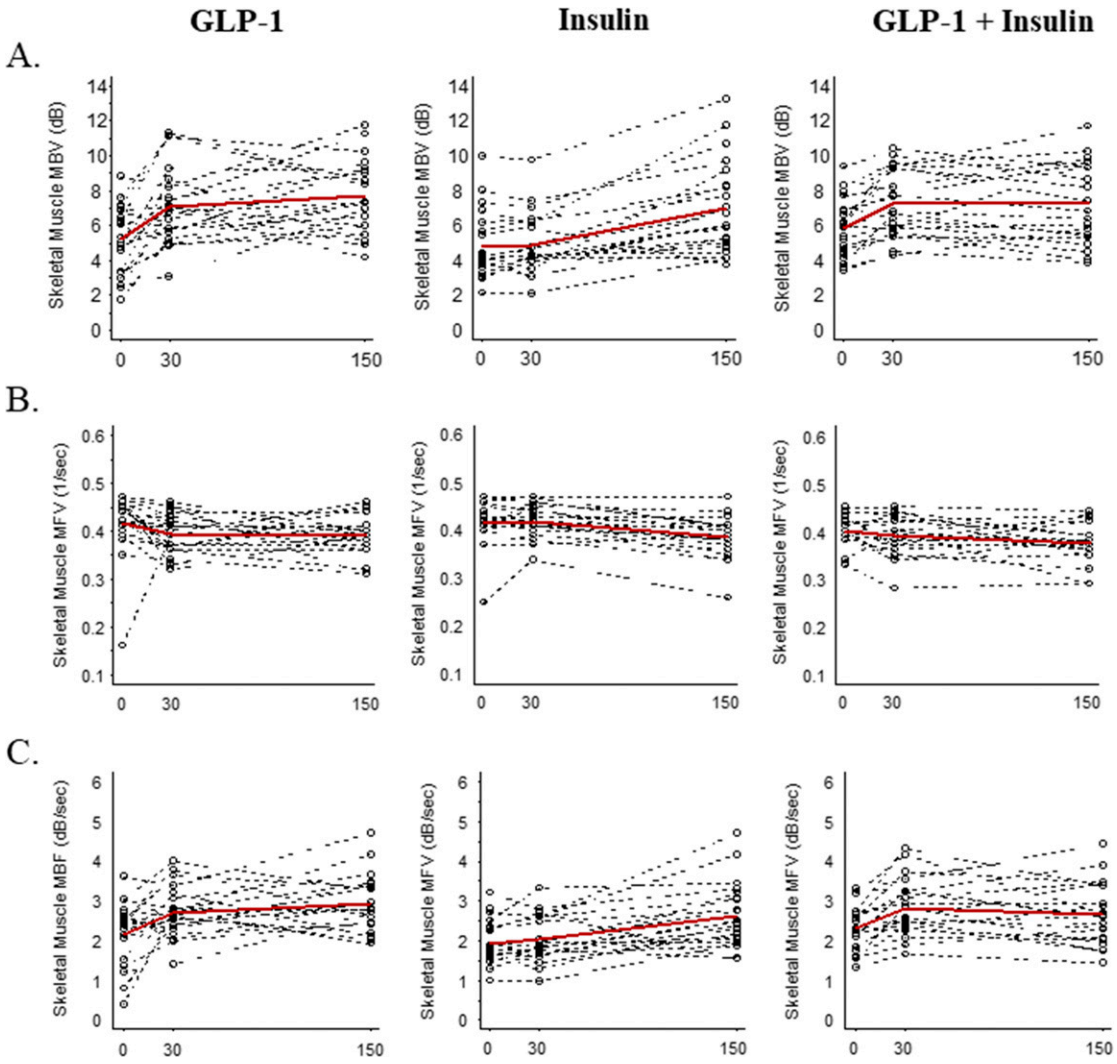

Figure 3. Changes of skeletal muscle microvascular parameters. Each participant received a systemic infusion of either GLP-1 (1.2 pmol/kg/min) or saline for 150 minutes with or without a euglycemic insulin clamp superimposed for the last 120 minutes. (A) MBV; (B) MFV; (C) MBF. Dotted lines indicate individual data; red lines indicate the mean. (D). Bar graph showing quantitative changes. *$P < 0.001$, compared with 0 minutes; #$P < 0.001$ and ##$P < 0.01$, compared with 30 minutes.

5.21 ± 0.42 dB at 0 minutes and 7.07 ± 0.49 dB at 30 minutes, whereas MBF was 2.16 ± 0.17 dB/sec at 0 minutes and 2.71 ± 0.14 dB/sec at 30 minutes, with no significant changes in MFV. Muscle MBV and MBF remained constant from 30 to 150 minutes. Intravenous insulin infusion also significantly increased skeletal muscle MBV from 4.91 ± 0.39 dB at 30 minutes to 6.97 ± 0.58 dB at 150 minutes ($P < 0.001$), resulting in an increase in MBF from 2.02 ± 0.13 dB/sec at 30 minutes to 2.62 ± 0.18 dB/sec at 150 minutes ($P < 0.001$). This was associated with a slight, but statistically significant, reduction in MFV (0.42 ± 0.01 per second and 0.39 ± 0.01 per second at 30 and 150 minutes, respectively; $P < 0.01$). The addition of insulin to GLP-1 in study protocol 3 did not result in further increase in skeletal muscle MBV and MBF, compared with GLP-1 or insulin alone.

C. Changes of Cardiac Muscle Microvascular Parameters

The MCE images of two participants in study protocol 1 were excluded from this analysis owing to poor image quality. The effects of systemic administration of GLP-1 and/or insulin on

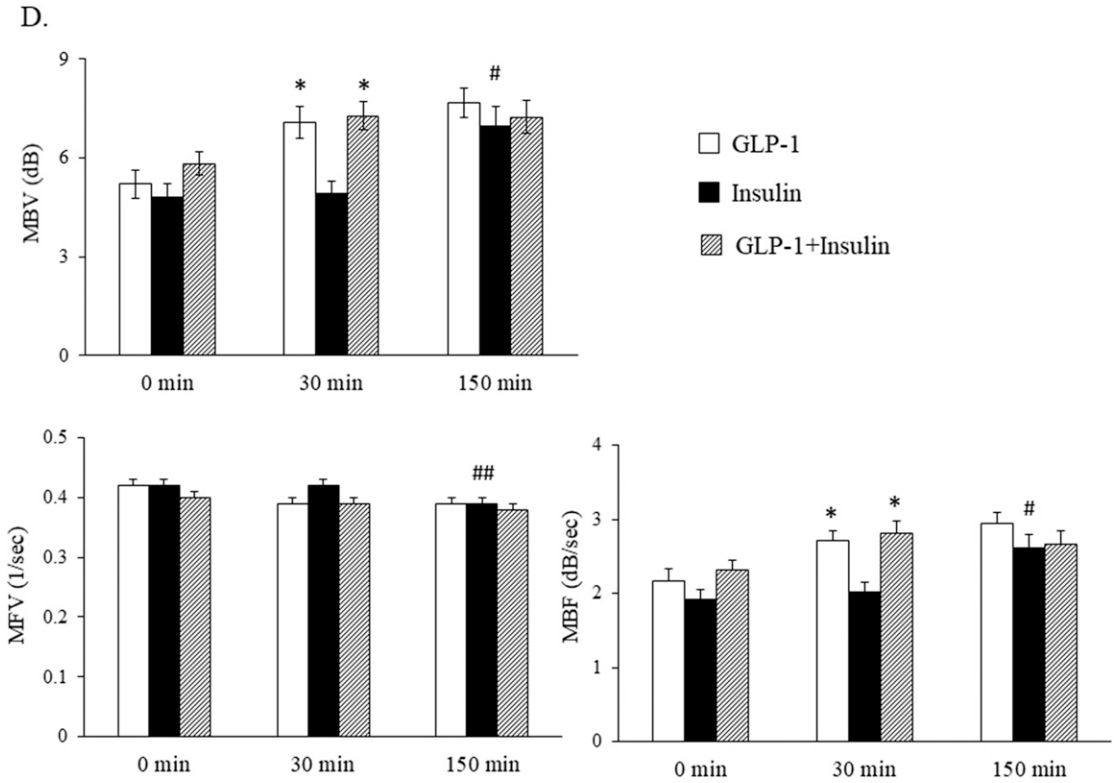

Figure 3. (Continued)

cardiac muscle microvascular parameters were similar to those on skeletal muscle (Fig. 4). GLP-1 infusion acutely increased cardiac MBV and MBF from 0 to 30 minutes ($P < 0.001$ for both), which was accompanied by a small, but statistically significant, decline in MFV, resulting in an increase in MBF from 1.47 ± 0.1 dB/sec at baseline to 2.26 ± 0.09 dB/sec at 30 minutes ($P < 0.001$). A significant increase in cardiac MBV and MBF, as well as a decrease in MFV, were also observed with intravenous insulin infusion (from 30 minutes to 150 minutes; $P < 0.001$ for all). Coinfusion of GLP-1 and insulin did not result in any significant changes in these cardiac muscle microvasculature parameters compared with separate systemic administration of GLP-1 and insulin.

D. Changes in Brachial Artery Diameter, Flow Velocity, And Blood Flow

The ultrasound images of one participant from study protocol 2 were excluded from analysis owing to poor image quality. As shown in Fig. 5, GLP-1 infusion significantly increased brachial artery diameter ($P < 0.01$) without affecting flow velocity, resulting in an ~30% increase in brachial artery blood flow ($P < 0.01$). Similarly, insulin increased brachial artery diameter and blood flow ($P < 0.01$ for both) without any significant effect on flow velocity. The effects of concomitant GLP-1 and insulin administration on brachial artery diameter, flow velocity, and blood flow were not significantly different from those induced by GLP-1 or insulin alone.

E. Changes in PWV

To assess the effects of GLP-1 and/or insulin on arterial stiffness, PWV was determined before and after the infusions (Fig. 6). GLP-1 and/or insulin infusions resulted in a small, statistically nonsignificant reduction in PWV. Compared with baseline, the mean reduction in PWV at 150 minutes induced by GLP-1 alone, insulin alone, and GLP-insulin coinfusion were 0.28 ± 0.19 m/s, 0.20 ± 0.18 m/s, and 0.28 ± 0.14 m/s, respectively.

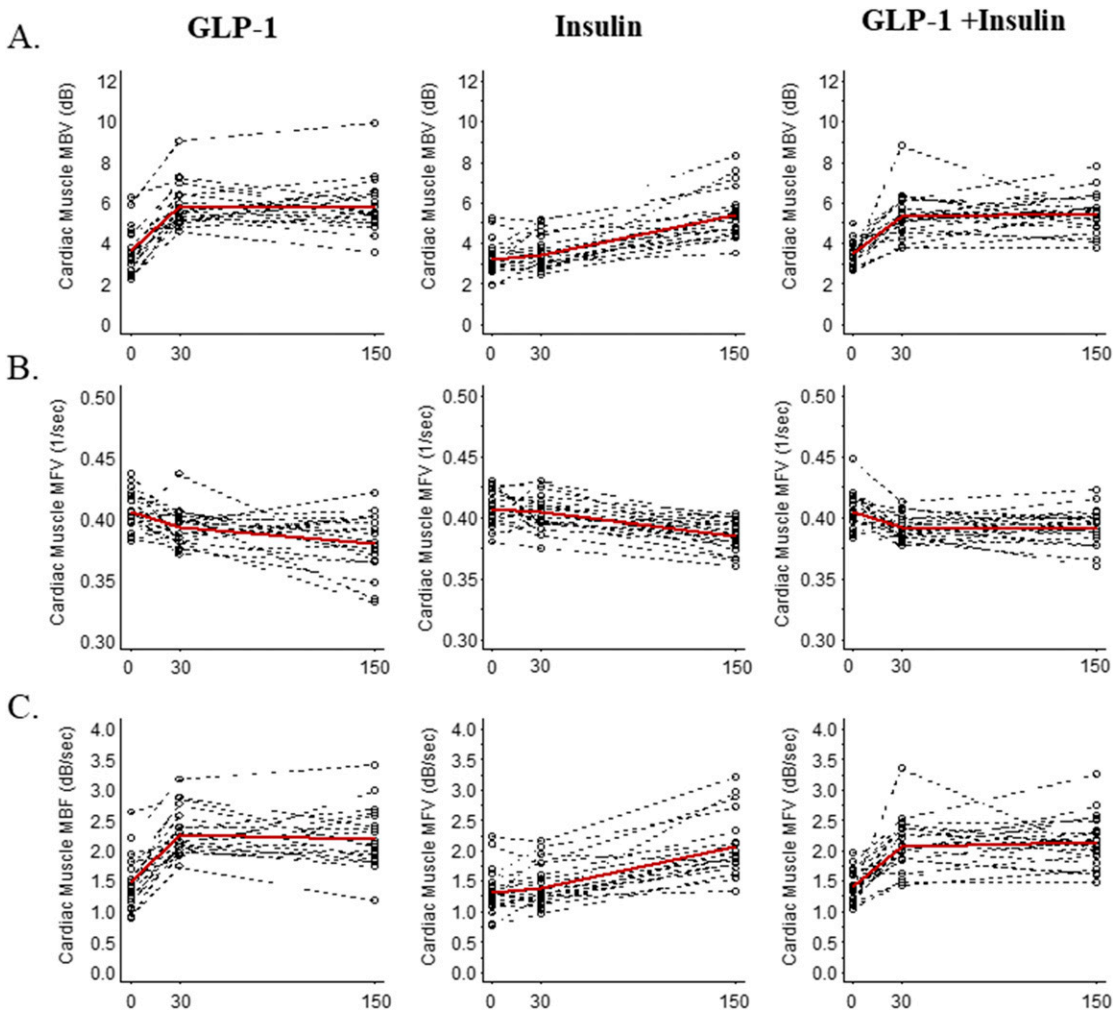

Figure 4. Changes in cardiac muscle microvascular parameters. Each participant received a systemic infusion of either GLP-1 (1.2 pmol/kg/min) or saline for 150 minutes with or without a euglycemic insulin clamp superimposed for the last 120 minutes. (A) MBV; (B) MFV; (C) MBF. Dotted lines indicate individual data; red lines indicate the mean. (D) Bar graph showing quantitative changes.*$P < 0.001$ and **$P < 0.01$, compared with 0 minutes; #$P < 0.001$ and ##$P < 0.05$ compared with 30 minutes.

3. Discussion

We have demonstrated in this study that GLP-1 and insulin recruit skeletal and cardiac muscle microvasculature and dilate conduit arteries at physiologically relevant concentrations and that these vascular effects are not additive in healthy humans. Because tissue microvasculature provides the needed endothelial surface area for substrate and hormone exchanges between the plasma compartment and tissue interstitium, conduit arteries regulate perfusion pressure and total tissue perfusion, and plasma concentrations of GLP-1 and insulin rise postprandially, our data suggest that tissue perfusion (particularly skeletal muscle, because of its large mass) plays a pivotal role in the regulation of substrate metabolism and that either GLP-1 or insulin can play physiologically important role in this process in humans.

We previously reported that mixed meals and insulin infusion recruit muscle microvasculature in healthy persons, with the former having a more potent effect despite achieving similar plasma insulin concentrations [10]. It is reasonable to speculate that GLP-1, secreted in response to meal ingestion, acted additively with insulin to increase microvascular

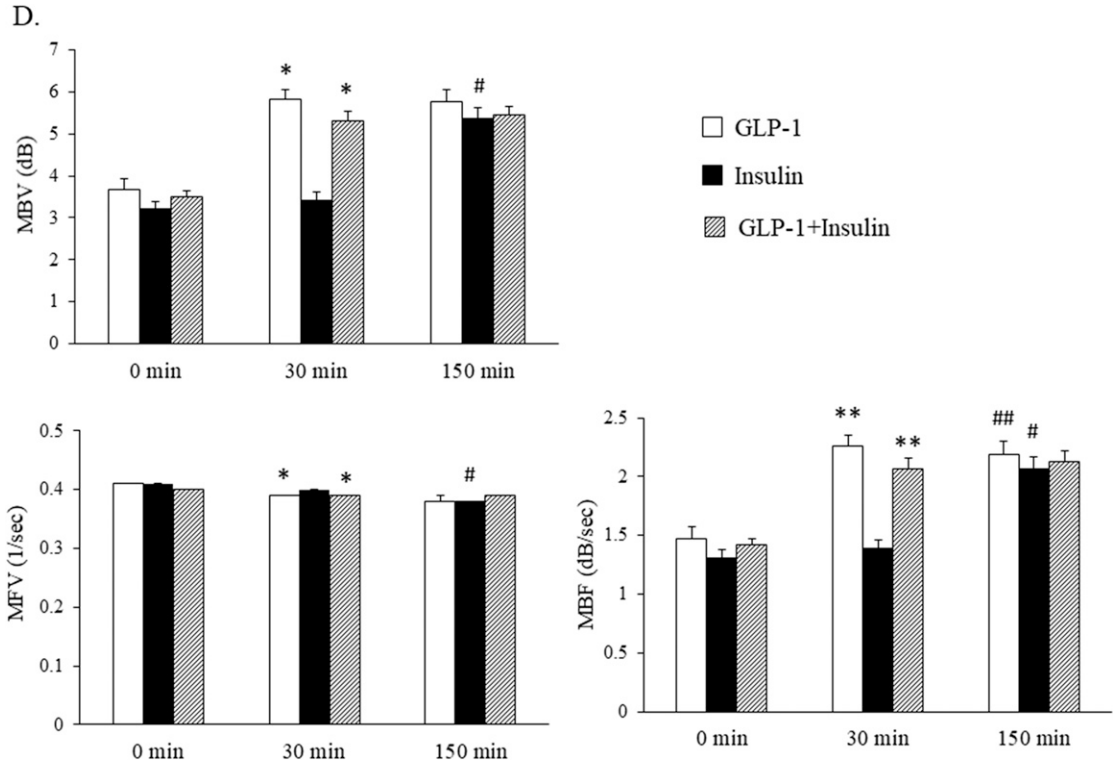

Figure 4. (Continued)

recruitment. However, in this current study, the effects of GLP-1 and insulin at physiologically relevant concentrations did not appear to be additive, either at the microvascular or conduit artery level. This is intriguing because prior evidence has confirmed that GLP-1 and insulin cause vasorelaxation through an NO-dependent mechanism [16, 25, 31] and that skeletal muscle MBV in rats increased by threefold after systemic infusion of GLP-1 at pharmacological concentrations (30 pmol/kg/min) [16]. The increases in MBV induced by insulin and GLP-1 are comparable in the current study (~30% to 40%) and this argues that at physiological condition, such as postprandial status, GLP-1 and insulin, acting either together or alone, induce a certain amount of microvascular recruitment to handle the postprandial fluxes of nutrients.

Expansion of muscle MBV, as seen with exercise [32, 33], GLP-1 at pharmacological concentrations [15, 16], losartan [34], angiotensin 1-7 [35], adiponectin [36, 37], and ranolazine [38], leads to increased muscle delivery of insulin and, thus, increased insulin action such as enhanced insulin-stimulated glucose disposal during insulin infusion. That insulin-mediated whole-body glucose disposal rates were similar between the insulin admission and the GLP-1 plus insulin admission on a per mole of insulin basis is of no surprise, because insulin, *per se*, rapidly promoted its own delivery to muscle within 5 to 10 minutes after the initiation of insulin infusion [1], and the degrees of muscle microvascular recruitment achieved were similar between the two admissions. These results are consistent with a prior report of a lack of additive action on both microvascular recruitment and insulin-mediated muscle glucose uptake when GLP-1 (100 pmol/L) and insulin were coinfused in normal control rodents [19].

Interestingly, GLP-1 reversed microvascular insulin resistance induced by both a 5-day and an 8-week high-fat diet (HFD) in rats. However, this increase in insulin sensitivity was only associated with a significant increase (90%) in skeletal muscle insulin-mediated glucose uptake in rats given 5 days of HFD but not 8 weeks of HFD [19]. This is quite similar to our prior findings that GLP-1 infusion completely rescued microvascular insulin responses in both acute (during lipid infusion) and chronic (4 weeks of HFD) insulin-resistant states,

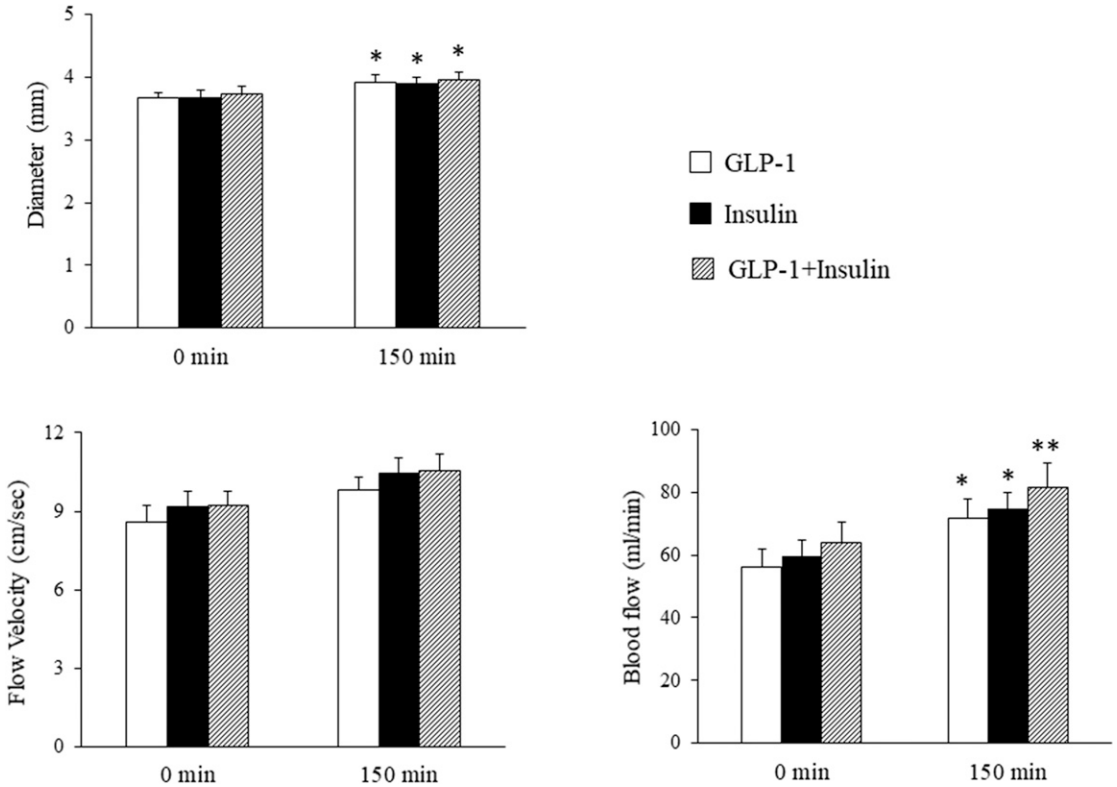

Figure 5. Effect of GLP-1, insulin, and GLP-1 plus insulin on brachial artery diameter, flow velocity, and blood flow. Each participant received a systemic infusion of either GLP-1 (1.2 pmol/kg/min) or saline for 150 minutes with or without a euglycemic insulin clamp superimposed for the last 120 minutes. (A) Brachial artery diameter; (B) brachial artery flow velocity; (C) brachial artery blood flow. *$P < 0.01$ and **$P < 0.001$, compared with 0 minutes.

completely restored insulin-stimulated glucose disposal in the acute insulin-resistant rats, and improved insulin action in muscle by 30% in chronically insulin-resistant animals [15]. Whether GLP-1 infusion enhances insulin's microvascular and/or metabolic actions in insulin resistant humans remains to be studied.

Inasmuch as, in animal studies, GLP-1 at high concentrations briefly but acutely stimulated insulin secretion [16] and insulin is capable of increasing muscle MBV [2, 6], we measured plasma insulin concentrations at 10 minutes after beginning GLP-1 infusion during the GLP-1 and the GLP-1 plus insulin admissions, respectively (n = 5 to 6 per admission). Indeed, plasma insulin concentrations increased briefly (by two- to threefold) during both study protocols. However, the insulin levels declined rapidly, and quickly returned to only slightly higher than baseline level. Given that during the insulin-alone admission, plasma insulin concentrations also increased from baseline to 30 minutes (Table 2) and neither cardiac nor skeletal muscle MBV changed, raising plasma insulin concentrations (from 30 minutes to 150 minutes) to >200 pM only recruited muscle MBV by ~30%, and, in rats, insulin-mediated microvascular recruitment persisted for only 15 to 30 minutes after insulin concentrations returned to basal levels [39], it is unlikely that this small and brief increase in plasma insulin concentrations contributed significantly to our current findings.

Insulin and GLP-1 have been shown to dilate conduit artery, which maintains adequate tissue perfusion via regulating tissue perfusion volume and pressure. We and others have shown that insulin at physiological concentrations dilates conduit arteries [3, 25, 30]. Basu *et al.* [40] demonstrated a clear vasodilatory action on the brachial artery by GLP-1 and this action was independent of endogenous insulin secretion. Our current results are consistent with these findings. Similar to the microvascular responses to GLP-1 and insulin coinfusion,

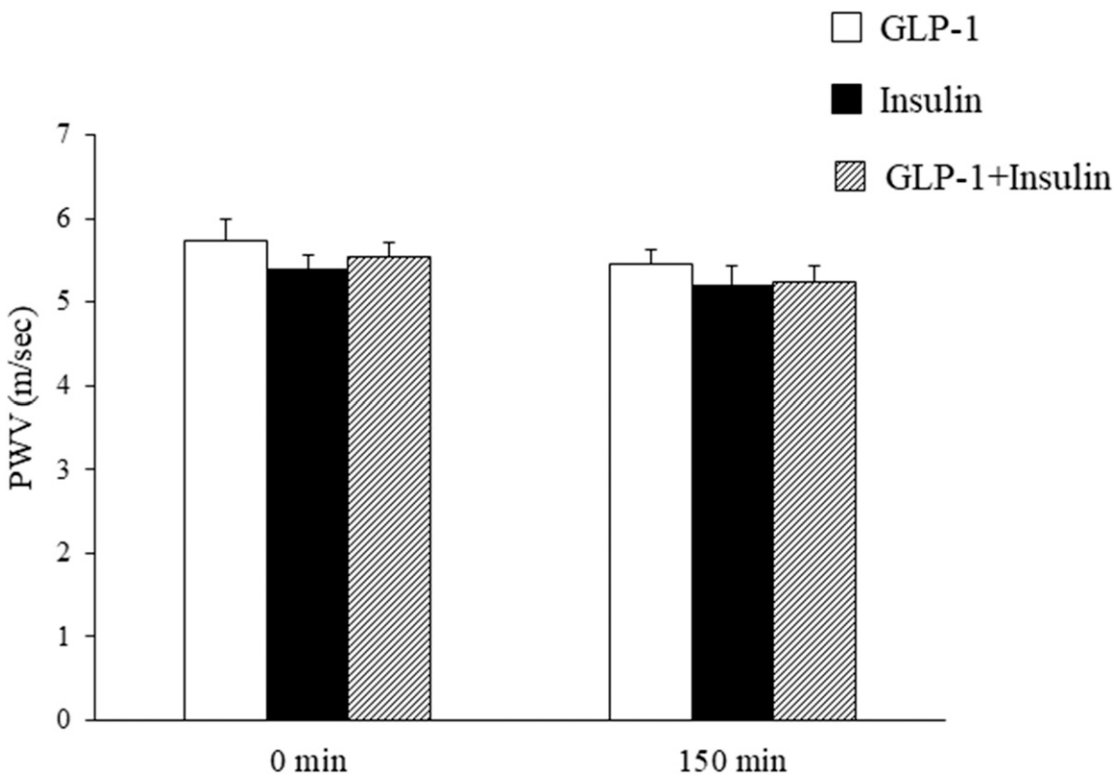

Figure 6. Effect of GLP-1, insulin, and GLP-1 plus insulin on PWV. Each participant received a systemic infusion of either GLP-1 (1.2 pmol/kg/min) or saline for 150 minutes with or without a euglycemic insulin clamp superimposed for the last 120 minutes.

the vasorelaxant effects of GLP-1 and insulin were not additive at the brachial artery level. The physiological significance remains to be defined.

PWV reflects arterial stiffness, which is associated with increased risk of cardiovascular events. Increased PWV has been observed consistently across all age groups in patients with diabetes and metabolic syndrome [41]. In the Slow the Adverse Effects of Vascular Aging (SAVE) trial, weight loss and reduction in fasting insulin levels were associated with an improvement in PWV [42]. On the other hand, treatment of early type 2 diabetes with dipeptidyl peptidase-4 inhibitor linagliptin for 26 weeks significantly improved PWV. This effect disappeared 4 weeks after stopping linagliptin [43]. Although PWV did not change acutely with either insulin or GLP-1 infusion alone or GLP-1 and insulin coinfusion in the current study, it remains possible that chronic use of GLP-1 receptor agonist may improve PWV in persons with diabetes and insulin resistance.

In conclusion, GLP-1 and insulin at physiological concentrations acutely increased skeletal and cardiac muscle microvascular perfusion and dilated conduit artery in lean healthy adults, but these effects were not additive. Because increased total blood flow and microvascular endothelial surface area increase skeletal and cardiac muscle delivery of oxygen, nutrients, and insulin, our results suggest that both GLP-1 and insulin regulate muscle endothelial surface area and nutrient and hormonal delivery under physiologic conditions. Although we have recently demonstrated that administration of liraglutide restored insulin's microvascular responses and improved insulin's metabolic responses in chronically insulin-resistant rodents [44], whether the current study results can be extrapolated to chronic insulin resistance in humans, such as occurs in those with obesity and diabetes or those with chronic exposure to GLP-1 receptor agonists, remains to be determined. Our results strongly suggest that GLP-1 receptor–mediated vascular actions, together with vascular insulin sensitization, may contribute to the glycemic effect of GLP-1 receptor agonists and may help decrease the cardiovascular morbidity and mortality associated with diabetes.

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Disclosure Summary: The authors have nothing to disclose.

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