GLP-1 increases microvascular recruitment but not glucose uptake in human and rat skeletal muscle

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GLP-1 increases microvascular recruitment but not glucose uptake in human and rat skeletal muscle. Am J Physiol Endocrinol Metab 306: E355–E362, 2014. First published December 3, 2013; doi:10.1152/ajpendo.00283.2013.—The insulinotropic gut hormone glucagon-like peptide-1 (GLP-1) has been proposed to have effects on vascular function and glucose disposal. However, whether GLP-1 is able to increase microvascular recruitment (MVR) in humans has not been investigated. GLP-1 was infused in the femoral artery in overnight-fasted, healthy young men. Microvascular recruitment was measured with real-time contrast-enhanced ultrasound and leg glucose uptake by the leg balance technique with and without inhibition of the insulinotropic response of GLP-1 by coinfusion of octreotide. As a positive control, MVR and leg glucose uptake were measured during a hyperinsulinemic-euglycemic clamp. Infusion of GLP-1 caused a rapid increase (P < 0.05) of 20 ± 12% (mean ± SE) in MVR in the vastus lateralis muscle of the infused leg after 5 min, and MVR further increased to 60 ± 8% above preinfusion levels by 60 min infusion. The effect was slightly slower but similar in magnitude in the noninfused contralateral leg, in which GLP-1 concentration was within the physiological range. Octreotide infusion did not prevent the GLP-1-induced increase in MVR. GLP-1 infusion did not increase leg glucose uptake with or without octreotide coinfusion. GLP-1 infusion in rats increased MVR by 28% (P < 0.05) but did not increase muscle glucose uptake. During the hyperinsulinemic clamp, MVR increased ~40%, and leg glucose uptake increased 35-fold. It is concluded that GLP-1 in physiological concentrations increases microvascular recruitment (MVR) and increase glucose utilization in muscle via a nitric oxide-dependent mechanism (7, 17), while others showed no effect (34). In addition, it has been repeatedly reported that GLP-1 increases glucose uptake in muscle cell culture in vitro (15, 16). Still, direct measures of the effect of GLP-1 on glucose uptake across a human leg has not been performed. Since the physiological effect of GLP-1 on microvascular flow is unknown and the effect on muscle glucose uptake in humans is controversial, the aim of the present study was to determine whether GLP-1 increases MVR and leg glucose uptake in humans independently of its ability to enhance insulin secretion. To this end, GLP-1 was infused in the femoral artery of healthy young men with and without inhibition of insulin secretion, and vastus lateralis MVR and leg glucose uptake were measured. To support these human findings, GLP-1 was also infused in anesthetized rats and muscle MVR and glucose uptake were measured.

RESEARCH DESIGN AND METHODS

Fifteen healthy male volunteers (age 26 ± 1 yr, BMI < 25 kg/m²) were enrolled in the study. The volunteers were allocated to four different protocols. Seven subjects participated in two infusion protocols. All subjects were blinded to the treatment they received. The study was approved by the Copenhagen Ethics Committee (H-4-2010-131) and conformed to the Code of Ethics of the World Medical Association. The volunteers each provided informed consent to participate in the study.

GLP-1 Femoral Arterial Infusion (n = 8)

In this and the other protocols, subjects refrained from any physical activity exceeding daily transportation for 48 h before the experiment. They consumed a standardized diet (60 E% carbohydrates, 15 E% protein, and 25 E% fats) 24 h prior to the experiment and arrived at the laboratory by public transportation at 7:30 AM after an overnight fast. Under local anesthesia (xylocaine 1%; AstraZeneca, Albertslund, DK) catheters were inserted into the femoral artery and vein of one leg below the inguinal ligament (Pediatric Jugular Catherization Set, Arrow Int.) for subsequent measurement of leg glucose uptake, infusion of GLP-1 and blood pressure monitoring using a pressure transducer interfaced to an IntelliVue MP5 monitor (Phillips Healthcare, Andover, NL). Polyethylene catheters were then placed in the dorsal hand vein for blood sampling and in antecubital veins for infusions. A heating pad was wrapped around the hand to arterialize the hand vein blood to oxygen saturation levels between 93 and 96%. GLP-1 (7–36 amide; Polypeptide Laboratories, Hillerød, DK) was diluted into 25 mL saline + 5 mL human albumin (5%) and administered as a constant femoral arterial infusion at 1 pmol·kg⁻¹·min⁻¹ for 60 min. Due to the expected insulinotropic effect of GLP-1, a slow, arm vein glucose (20% Fresenius Kabi, Upsala, SE) infusion (~0.5–1 mg·kg⁻¹·min⁻¹) was initiated after 15 min of GLP-1 infusion to
prevent hypoglycemia. The glucose infusion rate was guided by measurement of the arterialized plasma glucose concentration every 5–8 min. The aim was to allow a small drop (maximally 0.4 mM) in plasma glucose concentration during the initial 15-min GLP-1 infusion but then to clamp the plasma glucose concentration, since the insulinotropic effect of GLP-1 is minimized when the plasma glucose is allowed to decrease (22). The MVR was measured in the vastus lateralis muscle of the infused leg before, during the first 10 min, and after 60 min of GLP-1 infusion. Blood samples from the femoral vein and from the heated hand vein were obtained before and during the infusions. The sequence of events is depicted in Fig. 1A.

**GLP-1 Infusion with Pre- and Coinfusion of Octreotide (n = 5)**

To exclude the observed effects of GLP-1 infusion on MVR from being due to the small but significant increase in plasma insulin concentration, octreotide (Sandostatin, Novartis, DK) was infused in an antecubital vein at 0.5 μg/ml. The infusion was initiated 45 min prior to GLP-1 infusion and lasted throughout the 60 min of the following GLP-1 infusion to suppress the insulinotropic effect of GLP-1. Because octreotide also suppresses glucagon secretion, euglycemia was clamped by a slow glucose infusion in an arm vein (~0.5–1 mg·kg⁻¹·min⁻¹) after 15 min of octreotide infusion guided by measurements of plasma glucose concentration every 5–8 min.

Because infusion of GLP-1 of 1 pmol·kg⁻¹·min⁻¹ in the initial experiment described above did not increase leg glucose uptake, (see RESULTS) we in this experiment infused GLP-1 at 2 pmol·kg⁻¹·min⁻¹ rather than 1 pmol·kg⁻¹·min⁻¹ in order to investigate whether a higher concentration of GLP-1 was able to increase leg glucose uptake. In addition, an extra ultrasound transducer was fitted to the contralateral leg to observe the effect of any increase in systemic concentrations of GLP-1. See Fig. 1B for a timeline.

**Negative Control Experiments (n = 3)**

To exclude the observed effects of GLP-1 on MVR from being due to the local femoral infusion and elapsed time from the basal measurements to the GLP-1 stimulated measurements, control experiments were performed in which MVR was measured before, during the first 10 min, and after 60-min femoral arterial saline infusion [similar to GLP-1 experiments (Fig. 1A)].

**Positive Control Experiments**

Euglycemic hyperinsulinemic clamp (n = 6). To document the ability of the leg balance technique to measure increased glucose uptake when appropriately stimulated, six overnight-fasted subjects were enrolled. The femoral vein of one leg and a dorsal hand vein were catheterized as described above and the hand vein blood arterialized by wrapping a heating pad around the hand. Then MVR was measured, and blood samples from both catheters were obtained. Subsequently, a euglycemic hyperinsulinemic clamp was initiated. Insulin infusion rate in a forearm vein was 1.4 mU·kg⁻¹·min⁻¹, and the clamp was run for 60 min, after which MVR was measured again. Leg glucose uptake was measured every 15 min. Plasma glucose concentration was measured every 5–8 min, and a variable glucose infusion into a forearm vein was adjusted to clamp euglycemia as previously described (8). See Fig. 1C for a timeline.

**Rat experiments.** Male Sprague-Dawley rats (8–10 wk old) were obtained from Taconic Farms (Greve, DK). Animals were housed at 20–22°C on a 12:12 h light-dark cycle and were provided with a standard laboratory chow diet and water ad libitum for a minimum of 7 days before the experiments. The animals were fasted overnight before the experiment. On experimental days, animals were randomly assigned to saline (n = 5) or coinfusion of GLP-1 with saline (n = 5). The study was approved by the Danish Animal Experimental Inspectorate and complied with the European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes (Council of Europe 123, Strasbourg, FR).

**Experimental Procedure**

Rats were anesthetized with an intraperitoneal injection with pentobarbital sodium (60 mg/kg body wt). Polystyrene cannulae (PE-50, Intramedic) were inserted into the carotid artery and jugular veins for arterial blood sampling and continuous administration of anesthetics, saline ± GLP-1, and microbubbles, respectively. A tracheotomy was performed to assist spontaneous respiration during the experiment. Anesthesia was maintained during the experiment by a constant infusion of aqueous pentobarbital sodium (0.5 mg·min⁻¹·kg⁻¹ body wt⁻¹). Sixty minutes after completion of the surgery, basal blood samples for determination of plasma glucose and insulin concentrations were drawn, and the basal measurements of the MVR were performed in the hindlimb muscles. Thereafter, a constant intravenous infusion of saline ± GLP-1 (100 pmol·min⁻¹·kg⁻¹) (Bachem, Bubendorf, Switzerland) was initiated. After 60 min of saline ± GLP-1 stimulation, blood was sampled for plasma glucose and insulin concentrations. After ~75 min, the saline ± GLP-1-stimulated MVR was measured. At 80 min, a 37-MBq bolus of 2-deoxy-[¹⁴C]glucose (2-DG) in isotonic saline was administered via the jugular vein. Immediately following the 2-DG injection, an arterial blood sample was drawn by an automated syringe pump at 50 μl/min over 10 min to determine the average plasma specific radioactivity of 2-DG. At 90 min, the combined gastrocnemius (red and white) and soleus muscles were immediately dissected out and freeze-clamped for later determination of muscle 2-DG uptake.

**Measurement of MVR in Humans and Rats**

The MVR in the vastus lateralis muscle was measured with a real-time contrast-enhanced ultrasound technique (CEU) using an ultrasound system (L9–3 transducer, iU22; Philips Ultrasound, Santa Ana, CA) combined with infusion of Definity microbubbles, as described previously (32). In brief, the transducer was fixed to the thigh using an in-house manufactured strap-on device and keeping it in the same place throughout the experiment, allowing for cross-sectional imaging of...
the vastus lateralis muscle. During the GLP-1 and saline infusions the transducer was placed on the thigh of the infused leg, whereas during GLP-1 and octreotide co-infusion an additional transducer was fixed to the contralateral thigh. During the euclidean clamp experiment, only one transducer was fixed to one of the thighs. In rats the transducer was positioned over the left hindlimb and secured for the course of the experiment to image a cross section of the adductor magnus and semimembranosus muscles. Perflutren lipid microspheres (Definity; Lantheus Medical Imaging) were activated by a vial mixer (Lantheus Medical Imaging) at 4,500 oscillations/min for 45 s. Microbubbles (3 \times 1.5 \text{ ml suspension}) were diluted to 20 ml in the human experiments and (1.5 ml suspension) to 60 ml in the rat experiments with sterile saline and infused intravenously at a rate of 0.4 to 0.5 ml/min (humans) and 60 ml/min using a rotating syringe pump (Vuje Ject, BR-inf 100; Bracco, Geneva, Switzerland) to ensure a homogenous microbubble solution. Microbubbles were infused for 10 min before MVR recordings were performed to ensure steady state as described previously (32). Real-time imaging was performed using a low mechanical index (MI) of 0.08, thereby allowing the microbubbles in the ultrasound beam to resonate without destruction. A high MI of 1.20 was used at the beginning of each recording to destroy the microbubbles, thereby allowing recording of the replenishment of the microbubbles in the vasculature within the ultrasound beam. The acoustic intensities (AI) obtained during the first 0.5 s in the basal and GLP-1-stimulated state were averaged and subtracted from the AI recorded during the remaining seconds, thereby eliminating background noise and the contribution from rapid filling vessels (i.e., arteries, veins, and large arterioles or venules). Calculations were made in accordance with Wei et al. (38), where AI vs. time curves were fitted to the exponential function: 
\[ y = A[1 - e^{-[\beta t - r]}], \]
where \( r \) is time (s), \( B \) the time used for background subtraction, \( y \) the AI at any given \( t \), \( A \) the plateau AI defined as MVR, and \( \beta \) the flow rate constant (1/s) that determines the rate of rise of AI (38).

Leg bulk flow was measured using a high-frequency 17–5 MHz linear array transducer in Power Doppler mode interfaced to an IU22 ultrasound machine (Phillips Ultrasound). Diameter of the femoral artery was measured using 2-D imaging as the distance between inner arterial walls. Velocity was determined using pulse-wave Doppler, and leg bulk flow was calculated from the diameter and the velocity measurements by the system. As reported previously, the contrast agent distorts the Doppler signal used for leg bulk flow measurements during the microbubble infusion and for \( \sim 20–30 \text{ min} \) thereafter (32). Leg bulk flow reported during or after microbubble infusion is therefore calculated from the basal preinfusion leg bulk flow by applying Poiseuille’s law, thereby using the change in femoral artery diameter to calculate changes in leg bulk flow, because mean arterial pressure was constant during all infusions compared with the basal state (Tables 1 and 2).

\[
\text{leg bulk flow (ml/min)} = \frac{(P_a - P_v) \cdot \pi \cdot r^4}{1 - \eta \cdot 8}
\]

where \( P_a \) is mean femoral arterial blood pressure and \( P_v \) is mean femoral venous blood pressure (mmHg), \( r \) is the radius (cm), \( l \) the length of the artery, and \( \eta \) is the blood viscosity constant. We previously calculated a 1:6,000 microbubble-to-erythrocyte ratio during steady-state conditions (32); therefore, microbubble contribution to blood viscosity is negligible. It is assumed that blood viscosity and vessel length are constant. \( P_v \) was not measured, but it is unlikely that this changed during any of the interventions, since heart rate and mean arterial pressure, body position, and respiration were unchanged during infusions. Thus, because of the unchanged pressure gradient during the experiment, changes in leg bulk flow are only possible if the femoral artery diameter is changed.

Leg glucose uptake was calculated as the glucose concentration difference between the arterialized and the femoral venous blood multiplied by the bulk leg flow.

**Blood Analysis**

**Plasma glucose.** Plasma glucose concentrations during the experiments were measured on an ABL 800 FLEX (Radiometer Medical, CPH, Denmark) but subsequently analyzed by spectrophotometric analysis using an ANC 767 kit (Roche) on a Hitachi 912 analyzer (Boehringer, Manheim, Germany) to obtain improved accuracy of the small arteriovenous differences, which are reported in Tables 3 and 4.

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**Table 1. Heart rate, mean arterial blood pressure, femoral artery diameter, and total femoral blood flow before and during infusion of GLP-1 and GLP-1-Oct**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Heart Rate, beats/min</th>
<th>Mean Arterial Pressure, mmHg</th>
<th>Femoral Artery Diameter, mm</th>
<th>Total Femoral Artery Flow, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GLP-1</td>
<td>GLP-1 + Oct</td>
<td>GLP-1</td>
<td>GLP-1 + Oct</td>
</tr>
<tr>
<td>0</td>
<td>60 ± 4</td>
<td>54 ± 3</td>
<td>89 ± 2</td>
<td>94 ± 2</td>
</tr>
<tr>
<td>15</td>
<td>63 ± 4</td>
<td>55 ± 3</td>
<td>88 ± 3</td>
<td>94 ± 2</td>
</tr>
<tr>
<td>30</td>
<td>63 ± 3</td>
<td>56 ± 3</td>
<td>89 ± 2</td>
<td>96 ± 2</td>
</tr>
<tr>
<td>45</td>
<td>65 ± 3</td>
<td>57 ± 3</td>
<td>89 ± 3</td>
<td>96 ± 2</td>
</tr>
<tr>
<td>60</td>
<td>64 ± 3</td>
<td>58 ± 4</td>
<td>89 ± 3</td>
<td>92 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE; GLP-1, n = 8; GLP-1-Oct, n = 5. Different from basal: *\( P < 0.05 \).

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**Table 2. Heart rate, mean arterial blood pressure, femoral artery diameter, and total femoral blood flow before and during infusion of saline (negative control) and insulin (positive control)**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Heart Rate, beats/min</th>
<th>Mean Arterial Pressure, mmHg</th>
<th>Femoral Artery Diameter, mm</th>
<th>Total Femoral Artery Flow, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>Insulin</td>
<td>Saline</td>
<td>Insulin</td>
</tr>
<tr>
<td>0</td>
<td>66 ± 8</td>
<td>59 ± 4</td>
<td>94 ± 7</td>
<td>94 ± 5</td>
</tr>
<tr>
<td>15</td>
<td>64 ± 5</td>
<td>59 ± 3</td>
<td>93 ± 4</td>
<td>93 ± 7</td>
</tr>
<tr>
<td>30</td>
<td>65 ± 6</td>
<td>62 ± 2</td>
<td>91 ± 7</td>
<td>94 ± 7</td>
</tr>
<tr>
<td>45</td>
<td>65 ± 4</td>
<td>63 ± 4</td>
<td>93 ± 6</td>
<td>92 ± 7</td>
</tr>
<tr>
<td>60</td>
<td>69 ± 6</td>
<td>59 ± 7</td>
<td>93 ± 7</td>
<td>95 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE; saline experiments (negative control), n = 3; insulin experiments (positive control), n = 6.

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RESULTS

MVR

GLP-1 femoral arterial infusion of 1 pmol·kg⁻¹·min⁻¹ resulted in a rapid increase in MVR by ~20%, ~30%, and ~60% at 5, 10, and 60 min, respectively, compared with baseline in the vastus lateralis muscle, respectively (Fig. 2A). In overnight-fasted healthy subjects, leg glucose uptake is very low, with varying arteriovenous differences around 0.05–0.1 mM and occasionally even negative (Tables 3 and 4). Leg glucose uptake did not increase at any time (Table 3), although plasma insulin concentration increased by 80% (still a small increase in absolute numbers in plasma insulin concentration) after 30 min compared with baseline, with a return to baseline levels at 60 min (Table 3). Even though the increase in plasma insulin concentration was small and insufficient to increase leg glucose uptake during GLP-1 infusion, it could not be excluded that the effect of GLP-1 on MVR was caused by insulin, since insulin has a well-known effect on MVR (24, 32). Furthermore, we speculated that a higher GLP-1 concentration might be able to increase leg glucose uptake. We therefore, in the next experiment, doubled the infusion rate of GLP-1 but at the same time combined it with a pre- and coinfusion of octreotide to prevent the insulinotropic effect of GLP-1 (Fig. 1B). Following octreotide forearm infusion of 45 min, femoral arterial GLP-1 infusion of 2 pmol·kg⁻¹·min⁻¹ with a continued infusion of octreotide resulted in a rapid increase in MVR (filled bars in Fig. 2B) by ~35%, ~40%, and ~50% at 5, 10, and 60 min, respectively, compared with baseline in the vastus lateralis muscle of the infused leg (Fig. 2B). The changes in MVR were similar to the changes observed in the GLP-1 experiments without octreotide infusion (Fig. 2A). Plasma insulin concentrations were decreased below fasting levels by octreotide infusion and did not increase with GLP-1 infusion (Table 3).

Whereas the arterial GLP-1 concentration in the infused leg likely was higher than physiological levels judged by the concentration in the femoral venous blood of the infused leg (Table 5), in this series of experiments also measured

Table 3. Plasma glucose and insulin concentrations, arteriovenous plasma glucose difference, and leg glucose uptake before and during infusion of GLP-1 and GLP-1 + Oct

<table>
<thead>
<tr>
<th>Time, (min)</th>
<th>Arterial Glucose, mmol/l</th>
<th>Arteriovenous Difference, mmol/l</th>
<th>Glucose Uptake, μmol min⁻¹ kg leg⁻¹</th>
<th>Plasma Insulin, pmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GLP-1</td>
<td>GLP-1 + Oct</td>
<td>GLP-1</td>
<td>GLP-1 + Oct</td>
</tr>
<tr>
<td>0</td>
<td>5.46 ± 0.14</td>
<td>5.50 ± 0.32</td>
<td>0.14 ± 0.03</td>
<td>0.08 ± 0.06</td>
</tr>
<tr>
<td>15</td>
<td>5.26 ± 0.13</td>
<td>5.21 ± 0.20</td>
<td>0.04 ± 0.05</td>
<td>0.07 ± 0.04</td>
</tr>
<tr>
<td>30</td>
<td>5.15 ± 0.16</td>
<td>5.18 ± 0.14</td>
<td>0.14 ± 0.09</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>45</td>
<td>5.08 ± 0.11</td>
<td>5.12 ± 0.23</td>
<td>0.07 ± 0.04</td>
<td>0.01 ± 0.03</td>
</tr>
<tr>
<td>60</td>
<td>5.07 ± 0.13</td>
<td>5.23 ± 0.10</td>
<td>0.04 ± 0.08</td>
<td>0.05 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE; GLP-1 experiments (GLP-1), n = 8; GLP-1 with coinfusion of octreotide (GLP-1+Oct), n = 5. Different from basal: *P ≤ 0.05 and †P ≤ 0.01.

Table 4. Plasma glucose and insulin concentrations, arteriovenous plasma glucose difference, and leg glucose uptake before and during infusion of saline (negative control) and insulin (positive control)

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Arterial Glucose, mmol/l</th>
<th>Arteriovenous Difference, mmol/l</th>
<th>Glucose Uptake, μmol min⁻¹ kg leg⁻¹</th>
<th>Plasma Insulin, pmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>Insulin</td>
<td>Saline</td>
<td>Insulin</td>
</tr>
<tr>
<td>0</td>
<td>5.35 ± 0.17</td>
<td>5.43 ± 0.11</td>
<td>0.10 ± 0.04</td>
<td>0.05 ± 0.04</td>
</tr>
<tr>
<td>15</td>
<td>5.29 ± 0.15</td>
<td>5.12 ± 0.21</td>
<td>0.01 ± 0.06</td>
<td>1.13 ± 0.20</td>
</tr>
<tr>
<td>30</td>
<td>5.03 ± 0.08</td>
<td>5.65 ± 0.18</td>
<td>0.03 ± 0.09</td>
<td>1.52 ± 0.36</td>
</tr>
<tr>
<td>45</td>
<td>5.09 ± 0.04</td>
<td>5.63 ± 0.15</td>
<td>0.07 ± 0.07</td>
<td>1.45 ± 0.23</td>
</tr>
<tr>
<td>60</td>
<td>5.10 ± 0.04</td>
<td>5.55 ± 0.18</td>
<td>0.02 ± 0.04</td>
<td>1.58 ± 0.24</td>
</tr>
</tbody>
</table>

Values are means ± SE; saline experiments (negative control), n = 3; insulin experiments (positive control), n = 6. Different from basal: ‡P ≤ 0.001.
changes in MVR in the contralateral noninfused leg. In this leg, the arterial GLP-1 concentration was similar to the systemic concentration measured in the arterialized blood sampled from the heated hand vein. As can be seen from the values in Table 5, the concentration of GLP-1 during infusion of GLP-1 and octreotide was ~100 pmol/l, which is within the physiological range (25). The increase in MVR within the contralateral leg (open bars in Fig. 2B) was not significant at 5 min \(P = 0.14\) but was increased by ~30% and ~40% compared with baseline in the vastus lateralis muscle at 10 and 60 min, respectively, and similar to values in the infused leg (filled bars in Fig. 2B). As previously shown (32), negative control experiments with saline infusion did not induce any changes in MVR or leg glucose uptake (Fig. 2C and Table 4).

Finally, as demonstrated before (12, 21, 30), during the euglycemic hyperinsulinemic clamp, leg glucose uptake was markedly increased already after 15-min insulin infusion (Table 4) and remained increased during the 60-min clamp. In addition, as shown previously (24, 31, 32), insulin was able to increase MVR in muscle (Fig. 2D). After 60 min of insulin infusion, the glucose infusion rate was on average 7.2 ± 0.3 mg·min\(^{-1}\)·kg body mass\(^{-1}\).

Collectively, these data show that GLP-1 in both physiological and supraphysiological concentrations increases MVR in skeletal muscle independently of insulin, but GLP-1 does not affect muscle glucose uptake in the fasted euglycemic state. In contrast to GLP-1, insulin increased both MVR by ~40% and leg glucose uptake 35-fold (Table 4).

**Leg Bulk Flow**

Baseline femoral artery diameter was similar between the GLP-1 and GLP-1 + octreotide protocols (Table 1). GLP-1 infusion of 1 or 2 pmol·kg\(^{-1}\)·min\(^{-1}\) increased the femoral artery diameter equally by ~3% throughout the 60 min, which resulted in an equally increased calculated change in total femoral blood flow by ~12% or ~30 ml/min (Table 1). Femoral artery diameter and calculated leg blood flow were unchanged both during the saline and the insulin infusions (Table 2).

**Heart Rate and Mean Arterial Blood Pressure**

Basal heart rate (HR) and blood pressure remained at baseline levels throughout the experiments (Tables 1 and 2). Infusion of microbubbles did not affect HR and blood pressure in the basal or in the stimulated state.

**GLP-1**

Local GLP-1 infusion of 1 and 2 pmol·kg\(^{-1}\)·min\(^{-1}\) in the femoral artery increased the plasma concentration of GLP-1 in

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**Table 5** Plasma GLP-1 concentrations (pmol/l) sampled in an arterialized hand vein for systemic concentrations and in the femoral vein of the locally infused leg for local concentrations

<table>
<thead>
<tr>
<th>Experiment/Time, min</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLP-1 infusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ArterIALIZED blood</td>
<td>8 ± 1</td>
<td>50 ± 7(^i)</td>
<td>52 ± 7(^i)</td>
<td>67 ± 5(^i)</td>
</tr>
<tr>
<td>Femoral venous blood</td>
<td>5 ± 1</td>
<td>194 ± 31(^i)</td>
<td>216 ± 18(^i)</td>
<td>232 ± 38(^i)</td>
</tr>
<tr>
<td>GLP-1 + Oct infusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ArterIALIZED blood</td>
<td>12 ± 1</td>
<td>90 ± 5(^i)</td>
<td>106 ± 9(^i)</td>
<td>120 ± 11(^i)</td>
</tr>
<tr>
<td>Femoral venous blood</td>
<td>10 ± 2</td>
<td>340 ± 27(^i)</td>
<td>389 ± 31(^i)</td>
<td>392 ± 44(^i)</td>
</tr>
<tr>
<td>Saline infusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ArterIALIZED blood</td>
<td>16 ± 4</td>
<td>10 ± 4</td>
<td>12 ± 3</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Femoral venous blood</td>
<td>9 ± 2</td>
<td>9 ± 3</td>
<td>8 ± 2</td>
<td>9 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE; GLP-1, \(n = 8\); GLP-1+Oct, \(n = 5\). Saline experiments (negative control), \(n = 3\). Different from basal: \(\ddagger P < 0.001\).
the femoral vein of the infused leg ∼20- to 30-fold compared with baseline in the GLP-1 and the GLP-1 + octreotide experiments, respectively (Table 5). The arterialized hand vein plasma concentration of GLP-1 (which represents the systemic GLP-1 concentration) increased ∼7- to 10-fold compared with baseline in the GLP-1 and the GLP-1 + octreotide experiments, respectively (Table 5). The plasma concentration of GLP-1 remained at baseline levels throughout the saline experiment (Table 5).

**Rat Experiments**

Intravenous coinfusion of GLP-1 (100 pmol·min⁻¹·kg⁻¹) with saline increased the MVR by ∼28%, whereas saline infusion did not induce any changes in MVR in the hindlimb of anesthetized rats (Fig. 3A). The increase in MVR occurred without any change in plasma insulin concentration and despite of a slight drop in blood glucose concentration after 30 min, which returned to baseline levels at 60 min (Table 6) this was not accompanied by an increased muscle 2-DG uptake compared with saline (Fig. 3B).

**DISCUSSION**

In the present study, we demonstrate that GLP-1(7–36 amide) in humans rapidly and potently increases the MVR in skeletal muscle. Furthermore, similar results were obtained in rat skeletal muscle. Importantly, the effect on MVR was independent of insulin and was obtained at physiological as well as supraphysiological concentrations of GLP-1 (22). In contrast to what has been published in cell culture (16) and recent rodent studies (4), we found no effect of GLP-1 on human leg glucose uptake even at supraphysiological concentrations. This shows the importance of performing studies in humans when studying clinically relevant physiology. Furthermore, in our hands, infusion of GLP-1 also did not increase rat muscle glucose uptake. In contrast, infuson of insulin during a euglycemic hyperinsulinemic clamp increased both MVR and leg glucose uptake in humans.

The GLP-1-induced increase in MVR is a novel finding in humans. However, GLP-1 has been reported to have a direct vasorelaxant effect in vitro in large vessels including the rat pulmonary artery (14), femoral artery (28), and in pig (19) and mouse mesenteric arteries (1). A growing body of evidence indicates that the vasorelaxant effects of GLP-1 are endothelial dependent, although one study did not support this conclusion (28). In a recent study, Chai et al. (4) showed in the rat that GLP-1 acts via a NO-dependent mechanism similar to that utilized by insulin (37) and that the GLP-1-induced increase in MVR could be blunted by a preinfusion of the NO inhibitor L-NAME (4). These findings are further substantiated by cell culture experiments performed with human umbilical vein endothelial cells (10) and bovine aortic endothelial cells (4). In those studies, GLP-1 increased eNOS phosphorylation in a dose-dependent manner, which was attenuated when cells were incubated with either L-NAME (10) or exendin(9–39), a specific GLP-1 receptor antagonist (4). In our experiments where octreotide was coinfused with GLP-1 to suppress endogenous insulin production, MVR was measured both in the infused leg with supraphysiological GLP-1 concentration and in the contralateral leg, in which the vasculature was exposed to the systemic GLP-1 concentration, which was raised to be within the physiological range and equivalent to postmeal concentrations (36). The supraphysiological dose in the locally infused leg is in fact similar to that found in patients who have had Roux-en-Y gastric bypass surgery where meal-stimulated increases in endogenous GLP-1 concentration have been observed above 300 pmol/l both 3 and 12 mo after surgery (11).

There is considerable controversy with respect to GLP-1 tissue-specific receptor localization. GLP-1 receptors have been reported to be expressed in heart and vasculature of both humans and rodents, with a specific localization in vascular smooth muscle, cardiomyocytes, endocardium, and coronary endothelial cells (3, 39). These findings are in accord with the vasodilatory effects of GLP-1. Some groups have demonstrated GLP-1 receptors in rat muscle (9) and have reported clear effects of GLP-1 on muscle glucose uptake in isolated incubated rat soleus muscle (35). Recently, Chai et al. (4) demonstrated GLP-1-stimulated changes in MVR, muscle 125I-insulin clearance, and muscle glucose uptake expressed as a-v difference in rats. In contrast to these findings, isolated incubated rat soleus muscle with either GLP-1 or insulin did not result in any glycogenic or glycolytic effect (13) or additive effect of GLP-1 to the effect of insulin on glucose transport (18). Recently, it was shown in type 2 diabetics that a local infusion of insulin with a coinfusion of GLP-1 did not change fractional forearm glucose extraction or forearm glucose uptake compared with infusion of insulin alone despite improved total forearm blood flow (33). In the present study, a direct infusion of GLP-1 into the femoral artery, resulting in a

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**Table 6. Plasma glucose and insulin concentrations in rats**

<table>
<thead>
<tr>
<th>Time, min</th>
<th>0</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>5.2 ± 0.2</td>
<td>5.1 ± 0.2</td>
<td>5.2 ± 0.2</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>88 ± 32</td>
<td>78 ± 34</td>
<td>94 ± 41</td>
</tr>
<tr>
<td>Saline+GLP-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>4.9 ± 0.1</td>
<td>4.1 ± 0.1†</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>103 ± 27</td>
<td>85 ± 16</td>
<td>113 ± 26</td>
</tr>
</tbody>
</table>

Values are means ± SE; saline experiments, n = 5; GLP-1 with coinfusion of saline, n = 5. Different from basal: †P ≤ 0.01.

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Fig. 3. A: MVR presented as the mean plateau value of the AI in rat hindlimb muscles at basal and after 75-min infusion of saline or saline + GLP-1. B: 2-deoxyglucose uptake in mixed gastrocnemius-soleus muscle after saline or saline + GLP-1 infusion. Different from basal, †P < 0.01. Bar graph values are means of n = 5 per experiment; error bars are SE. All stimulated AI values are normalized to the individual basal (A).
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supraphysiological GLP-1 plasma concentration, also did not increase leg glucose uptake. In accordance, raising the systemic GLP-1 levels during a 25-g intravenous glucose tolerance test has also been reported not to affect insulin action in humans (34). Therefore, whereas GLP-1 in some rat and cell culture studies has been reported to increase glucose uptake in muscle, this does not seem to be the case in humans, even at supraphysiological concentrations. However, it cannot be excluded that small increases in leg glucose uptake may have been missed in the present experiment, if increases were smaller than the accuracy of the leg balance technique. Since in the fasting state arterial-femoral venous differences of glucose in humans are not constant, but usually vary between 0.05–0.1 mM and are occasionally even negative (Tables 3 and 4), also a 50% increase might have been difficult to detect. However, this would still be a very small and physiologically insignificant leg glucose uptake compared with e.g., effects of insulin, where arterial-femoral venous differences and leg glucose uptake increase ~35-fold during physiological hyperinsulinemia (Table 4).

It has previously been demonstrated that increased muscle glucose uptake is associated with the increased MVR that occurs upon insulin stimulation in humans (24) and rats (37). It might be expected that the substantial GLP-1-stimulated increase in muscle MVR in the present study would be accompanied by an increase in femoral arterial flow and systemic cardiovascular activation. In fact, GLP-1 caused a very small increase in estimated femoral arterial blood flow of about 12% or ~30 ml/min, which was too small to elicit measurable changes in blood pressure or heart rate. It might seem surprising that microvascular recruitment can increase by about 60% when total leg flow as measured in the femoral artery only increased by 12%. However, it is thought that a local redistribution of microvascular flow from so-called nonnutritive to nutritive flow takes place without necessarily increasing total flow (2, 5).

Both insulin-mediated increases in MVR and glucose uptake are diminished in obese and type 2 diabetic subjects (6, 24), and when changes in MVR are attenuated by either an intralipid infusion (26) or an infusion of l-NAME (37), there is a decrease in insulin-mediated glucose uptake. In the present study, although acute GLP-1 treatment induced rapid changes in the MVR, there was no change in muscle glucose disposal. The reason why microvascular recruitment and thus greater glucose delivery does not result in greater glucose uptake during GLP-1 infusion is likely related to the fact that GLP-1, unlike insulin, has very little, if any, direct effect on muscle cells. Thus, increasing capillary recruitment is of no consequence for glucose uptake in the absence of direct effects on the muscle cells. As GLP-1 is usually secreted together with insulin after a meal, then it may augment the effect of insulin in peripheral tissues by increasing MVR and thus delivery of insulin, perhaps especially in states of insulin resistance. Thus, the physiological role of the increased capillary recruitment by GLP-1 may be related more to vascular rather than direct metabolic effects, or it may also be of importance for other metabolic pathways than glucose metabolism.

In the present study a slow glucose infusion was applied in both GLP-1 protocols to avoid hypoglycemia. This was necessary because GLP-1 infusions of ~1 pmol·kg$^{-1}$·min$^{-1}$ decrease hepatic glucose output in healthy male subjects (23) and in type 2 diabetic patients (20) due to the insulinitropic effect combined with decreasing plasma glucagon levels. In the present study it was thought to be important to avoid hypoglycemia and the ensuing activation of the autonomic nervous system, as this would probably affect blood flow and MVR and would therefore invalidate our measurements.

In conclusion, the present study shows that GLP-1 rapidly increases microvascular recruitment in human and rat skeletal muscle but does not increase muscle glucose uptake in the fasting state. Thus, like insulin, GLP-1 increases microvascular recruitment but, unlike insulin, GLP-1 has no direct effect on muscle glucose uptake.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES

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