Acute peripheral tissue effects of ghrelin on interstitial levels of glucose, glycerol, and lactate: a microdialysis study in healthy human subjects

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Vestergaard ET, Möller N, Jørgensen JO. Acute peripheral tissue effects of ghrelin on interstitial levels of glucose, glycerol, and lactate: a microdialysis study in healthy human subjects. Am J Physiol Endocrinol Metab 304: E1273–E1280, 2013. First published April 16, 2013; doi:10.1152/ajpendo.00662.2012.—Ghrelin is a gut-derived peptide and an endogenous ligand for the ghrelin receptor. Intravenous infusion of ghrelin induces insulin resistance and hyperglycaemia and increases circulating levels of nonesterified free fatty acids. Our objective was to investigate whether the metabolic effects are mediated directly by ghrelin in skeletal muscle and adipose (peripheral and central) tissues. Ten healthy men (24.9 ± 1.3 yr) received 300 min of supraphysiological ghrelin administration by microdialysis catheters in skeletal muscle and adipose tissues in a randomized, single-blind, and placebo-controlled study. Microdialysis perfusates were analyzed every 30 min for glucose, glycerol, and lactate during both a basal period and a hyperinsulinaemic euglycaemic clamp. The primary outcome measures were interstitial concentrations of glucose, glycerol, and lactate in skeletal muscle and adipose tissues. Interstitial concentrations of glucose were similar in skeletal muscle, peripheral, and central adipose tissue in the basal period. During hyperinsulinaemia, interstitial concentrations of glucose in skeletal muscle decreased in response to ghrelin exposure [2.84 ± 0.25 (ghrelin) vs. 3.06 ± 0.26 mmol/l (placebo), P = 0.04]. Ghrelin exposure did not impact on interstitial concentrations of glycerol and lactate. We conclude that ghrelin administration into skeletal muscle decreases interstitial concentrations of glucose during euglycaemic hyperinsulinaemia, which is indicative of increased insulin sensitivity without any effects on interstitial glycerol levels in either muscle or adipose tissue. These data contrast with the metabolic effects of ghrelin observed after systemic exposure and suggest the existence of a second messenger that remains to be identified.

acetyl-glyceryl; microdialysis; glucose metabolism; lipolysis

Ghrelin has widespread direct and indirect hormonal and metabolic effects (7, 26–29). Activation of ghrelin receptors in the hypothalamus and the pituitary gland stimulates the secretion of growth hormone (GH), adrenocorticotropic hormone (ACTH), and prolactin (1, 12, 18, 24, 30). In addition, the presence of ghrelin receptor in peripheral tissues (9, 20) suggests that ghrelin also exerts peripheral effects. Exogenous ghrelin suppresses insulin secretion, whereas anti-ghrelin antiserum enhances glucose-induced insulin secretion (5). Ghrelin knockout mice are insulin sensitive and exhibit improved glycemic control during a glucose tolerance test (5, 22). Conversely, in a ghrelin gain-of-function rodent model, the opposite effects on glucose metabolism are observed (3).

The effect of ghrelin on insulin sensitivity and glucose tolerance appears also to be of importance during calorie restriction, during which knockout models in mice exhibit low blood glucose levels (23). During an isocaloric diet, ghrelin receptor knockout mice also become hypoglycaemic (31), suggesting ghrelin to be an agent with counterregulatory actions. Ghrelin infusion in human subjects results in dose-dependent insulin suppression (25) and insulin resistance (27, 29). However, it is difficult to dissect direct effects of ghrelin from those caused by its stimulatory effects on growth hormone (GH) and ACTH secretion even during concomitant somatostatin infusion (27). However, in hypopituitary subjects without endogenous GH and cortisol secretion, intravenous ghrelin infusion still induces insulin resistance (28). Intravenous ghrelin infusion also induces lipolysis and increases interstitial concentrations of glycerol in femoral subcutaneous adipose tissue and counteracts the antilipolytic effect of insulin in abdominal subcutaneous adipose tissue, as determined by the microdialysis technique (27). In skeletal muscle, interstitial concentrations of glucose fluctuate in parallel with those in the circulation during intravenous ghrelin infusion (28). However, it remains to be determined whether glycerol and glucose concentrations in interstitial tissue of adipose tissue and skeletal muscle increase due to direct effects of ghrelin in the tissues or indirect systemic effects. We recently infused ghrelin regionally into the femoral artery and observed stimulated lipolysis in situ together with systemic GH stimulation, which may have obscured the peripheral effects of ghrelin (26).

Therefore, the aim of the present study was to investigate the direct peripheral effects of ghrelin on interstitial levels of glucose, glycerol, and lactate in skeletal muscle and adipose tissues in humans by microdialysis. This technique can be used as a real-time biosensor and also as a mean to increase interstitial concentrations of low-molecular-weight compounds such as ghrelin (10). Tissue concentrations of glucose, glycerol, and lactate reflect tissue glucose uptake, lipolysis, and glycolysis, respectively, and we hypothesized that infusion of ghrelin directly into muscle and fat tissues using microdialysis catheters would increase interstitial concentrations of glycerol in adipose tissue and increase interstitial concentrations of glucose in skeletal muscle.

MATERIALS AND METHODS

The study was conducted at an Aarhus University Hospital research unit. The study protocol was approved by the local ethics committee (Central Denmark Region Ethics Committee No. 2012-28512), it was registered at ClinicalTrials.gov (ID NCT01595373), and the study...
was performed according to the Declaration of Helsinki. The subjects were informed about the possible risks before giving oral and written consent to participate in the trial. Ten healthy young male volunteers completed a medical interview, received a full physical examination, and underwent an initial laboratory screening before the study. None of the participants revealed any symptoms or signs of disease and received no medications known or suspected to affect insulin sensitivity or metabolism. The isometric strengths of both quadriceps muscles were assessed by means of an electronic dynamometer (Good Strength; Metitut, Jyväskylä, Finland). The strength was calculated as the mean of four voluntary maximum isometric contractions separated by 30-s intervals. Body fat content was estimated using a Lange skinfold caliper (Beta Technology, Santa Cruz, CA) involving four skinfold sites: triceps, biceps, subscapular, and suprailiac locations on the right side. The sum of the skinfolds was converted to body fat percentage (6). Leg circumferences were measured 10 cm proximal to the patella.

Study protocol. All subjects were examined on a single occasion after 12 h of fasting. They reported to the laboratory at 0640. One intravenous cannula was inserted in the anteceubital region for intravenous infusions, and one intravenous cannula was inserted into a dorsal hand vein for blood sampling. The latter was heated, allowing for arterialized blood samples to be drawn. Blood samples were drawn every 30 min and analyzed for acyl and desacyl ghrelin, GH, cortisol, insulin, glucose, and nonesterified fatty acids (NEFA).

The bidirectional fluxes through a microdialysis membrane allow for administration of water-soluble compounds to locally increase tissue concentrations of a specific compound (given that the cutoff limit of the membrane exceeds the molecular weight of the compound) and simultaneous sampling of metabolites to detect local metabolic effects of the compound in question (10, 14–16). Acyl ghrelin reconstitutes rapidly in water solutions and has a molecular mass of 3.3 kDa. After applying local a 0.1% solution of lidocaine (10 mg/ml Xylocain; AstraZeneca, Albertslund, Denmark) superficially to the fascia of the right and left lateral vastus muscles, two microdialysis catheters (CMA-63; CMA, Stockholm, Sweden) were inserted into the muscles 14 cm above the patella at t = 60 min. Subsequently, four microdialysis catheters (CMA-60) were positioned in the subcutaneous (sc) adipose tissue; two catheters were inserted 5 cm to the right and to the left side of the umbilicus (hereafter referred to as central adipose tissue), and two catheters were inserted in the femoral sc adipose tissue (hereafter referred to as peripheral adipose tissue) also on the right and left sides after 1 ml of lidocaine sc placement. All microdialysis catheters had a molecular cutoff of 20 kDa and a membrane length of 30 mm. Prior to insertion, the catheters were flushed manually with perfusion fluid (ringer chloride, CMA T1, 147 mmol/l Na+, 4 mmol/l K+, 2.3 mmol/l Ca2+, and 156 mmol/l Cl−, pH 6; osmolality: 290 mosmol/kg), as recommended by the manufacturer. The microdialysis systems were perfused with a flow rate of 2 μl/min using CMA-107 perfusion pumps (CMA). Sampling of the interstitial fluid was commenced at t = 0 min after 60 min of equilibration (from t = −60 to 0 min) to minimize the influence of local edema and hemorrhage. The sampling was performed every 30 min and continued until t = 300 min.

At t = 0 min, acyl ghrelin (6.25 mg/l, GMP-grade human acyl ghrelin; Bachem, Weil am Rhein, Germany) was added to the microdialysis perfusion fluid at either the right or the left side of the body according to a single-blind randomization. This dose was chosen as the maximum dose of acyl ghrelin that has previously been demonstrated to induce systemic effects, including GH release (19).

The study consisted of a basal period (120 min) followed by a 180-min hyperinsulinemic euglycemic clamp period [insulin (Actrapid) 0.6 mU·kg−1·min−1; Novo Nordisk, Bagsvaerd, Denmark]. Plasma glucose was clamped at 5 mmol/l by adjusting the rate of infusion of 20% glucose according to arterialized plasma glucose measurements every 10 min. Insulin sensitivity was calculated from the glucose infusion rate during the last 30 min of the clamp.

Leg blood flow was estimated before acyl ghrelin was added to the microdialysis liquid and during the last 20 min of both the basal and clamp period by using the ultrasonic Doppler technique (Vivid c; GE, Milwaukee, WI).

Blood samples and measurements. Plasma glucose and lactate were analyzed in duplicate using the glucose oxidase and lactate oxidase methods, respectively, (YSI 2300 STAT Plus; Yellow Springs Instruments, Yellow Springs, OH). Serum samples were frozen immediately and stored at −20°C. Serum NEFAs were analyzed by a commercial kit (Wako Chemicals, Neuss, Germany). Plasma samples for acyl and desacyl ghrelin were pretreated with 4-(2-aminoethyl)benzenesulfonyl fluoride buffer and acidified before freezing and measured using a commercial two-site sandwich ELISA assay with intra-assay coefficients of <5.5% (acyl ghrelin assay) and <4.7% (desacyl ghrelin assay) (Sceti, Tokyo, Japan). Serum GH was analyzed using chemiluminescence technology (IDS-ISYS Multi-Discipline Automated Analyzer; Immunodiagnostics Systems Nordic, Herlev, Denmark).

Serum insulin was analyzed using time-resolved fluororimmunoassay assay (AutoDELFIA Insulin kit, catalog no. B080-101; PerkinElmer, Turku, Finland). Serum cortisol was measured using a DRG ELISA kit (DRG Instruments, Marburg, Germany). Glucose, lactate, and acetate in the microdialysis dialysate were measured in duplicate by an automated spectrophotometric kinetic enzymatic analyzer (CMA 600). The relative recovery of interstitial glycerol was assessed by the internal reference method using [2-3H(N)]-glycerol (PerkinElmer, Skovlund, Denmark) (21). Glycerol and lactate were counted using a Wallac 1450 Microbeta liquid scintillation counter, applying Ultima Gold scintillation fluid (Packard BioScience, Meriden, CT).

Statistical analysis. Results are expressed as means ± SE. The effect of ghrelin compared with placebo was analyzed by ANOVA for repeated measurements. The differences between the ghrelin- and placebo-exposed tissues were calculated for the interstitial concentrations of glucose, glyceral, and lactate for skeletal muscle, peripheral, and central adipose tissues, respectively. The time series of interest was the basal (30–120 min) and clamp (120–300 min) periods, and the statistical null hypothesis was as follows: no difference in interstitial concentrations of the metabolites in tissues exposed to ghrelin compared with placebo (difference between ghrelin and placebo exposed tissues equal to zero).

At baseline, the P values refer to comparisons by a Student two-tailed paired t-test for normal distributed data. Regarding P values for the basal and the clamp periods: 1) changes in interstitial concentrations of glucose, glyceral, and lactate were tested with ANOVA for repeated measurements, where the repeated measurement was the concentration of the specific metabolite of interest and the P values refer to the effect of the time factor; and 2) the effect of the treatment factor was tested with ANOVA for repeated measurements, where the repeated measurement was the difference in interstitial concentrations of glucose, glyceral, and lactate between the ghrelin- and placebo-exposed tissues, respectively, and the P values refer to the effect of the time factor. A P value of <0.05 was considered significant. Statistical analysis was performed using IBM SPSS Statistics 20 for Mac (IBM SPSS, Armonk, NY).

RESULTS

Characterization of the subjects. Data on blood pressure, pulse, isometric strength of the quadriceps muscles, leg circumferences, and percent body fat are summarized in Table 1. Muscle strength and leg circumferences were similar on the two legs.

Interstitial concentrations of glucose. Baseline concentrations of interstitial glucose (mmol/l) in skeletal muscle did not differ between ghrelin and placebo [3.74 ± 0.17 (ghrelin) vs. 3.86 ± 0.85 (placebo), P = 0.90]. A significant and steady decline in interstitial glucose concentrations in skeletal muscle

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with time was observed after both ghrelin and placebo exposure ($P < 0.01$) (Fig. 1A). However, the interstitial glucose concentration was lower during the clamp period, when ghrelin was infused ($P = 0.04$), and post hoc statistics revealed relatively lower glucose concentrations at time 240 min ($P = 0.03$). This difference between ghrelin and placebo remained when mean glucose levels obtained during the clamp period were compared [2.84 ± 0.25 (ghrelin) vs. 3.06 ± 0.26 (placebo), $P = 0.04$; Table 2]. Baseline concentrations of interstitial glucose (mmol/l) in adipose tissue did not differ between ghrelin and placebo (peripheral adipose tissue: 3.35 ± 0.25 (ghrelin) vs. 4.18 ± 0.33 (placebo), $P = 0.06$; central adipose tissue: 4.26 ± 0.23 (ghrelin) vs. 4.37 ± 0.70 (placebo), $P = 0.85$). In ghrelin-exposed peripheral adipose tissue there was a significant increase in interstitial glucose concentrations with time during the basal period ($P < 0.01$) and a significant decrease in interstitial glucose concentrations with time after placebo exposure ($P < 0.01$; Fig. 1B), but the differences did not translate into a treatment effect ($P = 0.90$), and the difference in the direction of change between ghrelin and placebo infusion appears to be driven by the baseline interstitial glucose levels in peripheral adipose tissue at time of study onset. Concentrations of interstitial glucose in peripheral adipose tissue increased in response to both ghrelin and placebo during the clamp ($P < 0.01$), without a treatment effect of ghrelin ($P = 0.85$). A significant increase in interstitial glucose concentrations in central adipose tissue with time was observed after both ghrelin and placebo exposure during the basal period ($P < 0.01$; Fig. 1C), whereas a significant decrease with time was observed after both ghrelin and placebo exposure during the clamp period ($P < 0.01$), still without treatment effects of ghrelin (basal period $P = 0.87$ and clamp period $P = 0.91$, respectively). 

**Table 1.**

<table>
<thead>
<tr>
<th>Subject Characterization</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>24.9 ± 1.3</td>
</tr>
<tr>
<td>Height, cm</td>
<td>181 ± 1.5</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.7 ± 0.4</td>
</tr>
<tr>
<td>Blood pressure systolic/diastolic, mmHg</td>
<td>133 ± 2.8/77 ± 2.3</td>
</tr>
<tr>
<td>Pulse, beats/min</td>
<td>62 ± 2.2</td>
</tr>
<tr>
<td>Muscle strength, quadriceps muscle (N)</td>
<td></td>
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<tr>
<td>Ghrelin</td>
<td>732 ± 35</td>
</tr>
<tr>
<td>Placebo</td>
<td>734 ± 40</td>
</tr>
<tr>
<td>Leg circumference, cm</td>
<td>42.3 ± 0.5</td>
</tr>
<tr>
<td>Ghrelin</td>
<td>42.2 ± 0.6</td>
</tr>
<tr>
<td>Placebo</td>
<td>15.9 ± 0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. N, newton.

**Interstitial concentrations of glycerol.** Baseline concentrations of interstitial glycerol concentrations (µmol/l) in skeletal muscle did not differ between ghrelin and placebo [$61.9 ± 16.3$ (ghrelin) vs. $89.7 ± 8.1$ (placebo), $P = 0.46$]. In ghrelin-exposed skeletal muscle, there was a significant increase in interstitial glycerol concentrations with time during the basal period ($P < 0.01$) and a significant decrease in interstitial glycerol concentrations with time after placebo exposure ($P < 0.01$) (Fig. 2A) but no treatment effect of ghrelin ($P = 0.55$). Concentrations of interstitial glycerol declined with time in response to both ghrelin and placebo exposure during the clamp ($P < 0.01$), without a treatment effect of ghrelin being

Fig. 1. Interstitial concentrations of glucose in different interstitial tissues during ghrelin (black lines) and placebo (gray lines) infusions. Vertical punctuated lines separate the basal from the clamp period. A: interstitial concentrations of glucose in skeletal muscle were similar in the basal period but significantly suppressed in response to ghrelin in the clamp period. B and C: interstitial concentrations of glucose were similar in peripheral (B) and abdominal adipose tissue (C) in the basal and clamp periods. All data are presented as means ± SE. *Significant difference between ghrelin- and placebo-exposed skeletal muscle at $t = 240$ min.
observed \((P = 0.96)\). Baseline concentrations \((\mu\text{mol/l})\) of interstitial glycerol in adipose tissue did not differ between ghrelin and placebo \([\text{peripheral adipose tissue: } 118.8 \pm 44.1 \text{ (ghrelin) vs. } 158.7 \pm 15.4 \text{ (placebo), } P = 0.40; \text{central adipose tissue: } 197.4 \pm 50.0 \text{ (ghrelin) vs. } 176.8 \pm 35.0 \text{ (placebo), } P = 0.85; \text{Fig. 2, B and C}]\). A significant and steady incline in interstitial glycerol concentrations with time was observed during the basal period in both peripheral and central adipose tissue and after both ghrelin and placebo \((P < 0.01)\), without treatment effects of ghrelin \((\text{peripheral adipose tissue } P = 0.61 \text{ and central adipose tissue } P = 0.17)\). The concentrations of interstitial glycerol were suppressed during the clamp period with time in both peripheral and central adipose tissues \((P < 0.01)\) in both ghrelin- and placebo-exposed tissues and without treatment effects of ghrelin \((\text{peripheral adipose tissue } P = 0.79 \text{ and central adipose tissue } P = 0.39)\).

**Interstitial concentrations of lactate**. Baseline concentrations of interstitial lactate \((\text{mmol/l})\) in skeletal muscle did not differ between ghrelin and placebo \([1.00 \pm 0.58 \text{ (ghrelin) vs. } 0.83 \pm 0.06 \text{ (placebo), } P = 0.83]\). A significant incline in interstitial lactate concentrations in skeletal muscle with time was observed after both ghrelin and placebo exposure \((P < 0.01)\) \((\text{Fig. 3A})\), with no treatment effect of ghrelin \((P = 0.70)\). The incline continued with time during the clamp period \((P < 0.01)\) still without a treatment effect of ghrelin \((P = 0.31)\). Baseline concentrations \((\text{mmol/l})\) of interstitial lactate concentrations in adipose tissues were not different between ghrelin and placebo \([\text{peripheral adipose tissue: } 1.12 \pm 0.07 \text{ (ghrelin) vs. } 0.65 \pm 0.25 \text{ (placebo), } P = 0.39; \text{central adipose tissue: } 1.12 \pm 0.07 \text{ (ghrelin) vs. } 0.65 \pm 0.25 \text{ (placebo), } P = 0.39]\). In peripheral adipose tissue, interstitial lactate concentrations of lactate increased with time after both ghrelin and placebo \((P < 0.01; \text{Fig. 3B})\) still without a treatment effect of ghrelin \((P = 0.60)\), and the incline continued with time during the clamp period \((P < 0.01)\) still without a treatment effect of ghrelin \((P = 0.81)\). By contrast, interstitial concentrations of lactate decreased with time in central adipose tissue \((P = 0.02 \text{ (ghrelin) and } P < 0.01 \text{ (placebo); Fig. 3C})\) without a treatment effect of ghrelin \((P = 0.83)\). During the clamp a significant increase in interstitial concentrations of lactate was recorded with time after both ghrelin and placebo \((P < 0.01)\), but there was no treatment effect of ghrelin \((P = 0.43)\).

**Plasma glucose levels**. At \(t = 0\), plasma glucose was \(5.0 \pm 0.1 \text{ mmol/l and remained between } 5.1 \pm 0.1 \text{ mmol/l at } t = 0 \text{ min and } 4.9 \pm 0.1 \text{ mmol/l at } t = 90 \text{ min in the basal period (Fig. 4A). After the basal period, plasma glucose was clamped to } -5.0 \text{ mmol/l and reached a near plateau between } t = 270 \text{ and } 300 \text{ min of } 4.9 \pm 0.1 \text{ mmol/l.}$$

**Insulin sensitivity**. The glucose infusion rate increased gradually during the clamp period, and insulin sensitivity \((M \text{ value})\), calculated as the average glucose infusion rate from \(t = 270\) to \(300 \text{ min, was } 7.75 \text{ mg·kg}^{-1}\text{·min}^{-1} \text{ (Fig. 4B).}$$

**Serum nonesterified free fatty acid levels**. At \(t = -60\), NEFA levels were \(0.615 \pm 0.100 \text{ mmol/l. NEFA levels decreased before the basal period and were further suppressed during the clamp period (ANOVA, } P < 0.001)\) to a nadir of \(0.018 \pm 0.005 \text{ mmol/l at } t = 300 \text{ min (Fig. 4C).}$$

**Plasma lactate levels**. At \(t = 0\), lactate levels were \(0.80 \pm 0.14 \text{ mmol/l. Plasma lactate increased during the clamp and reached a maximal value at } t = 300 \text{ min of } 1.12 \pm 0.06 \text{ mmol/l (} P = 0.046; \text{Fig. 4D).}$$

**Plasma ghrelin levels**. At \(t = -60\), plasma concentrations of acyl and desacyl ghrelin were \(13.75 \pm 2.85 \text{ and } 12.54 \pm 1.91 \text{ fmol/ml, respectively, corresponding to a acyl/desacyl ghrelin ratio of } 1.35 \pm 0.32 \text{ (Fig. 5A). Basal mean concentrations of plasma acyl ghrelin were } 13.97 \pm 2.24 \text{ fmol/ml and were suppressed during hyperinsulinemia to (on average) } 10.02 \pm 1.36 \text{ fmol/ml (} P = 0.008). Basal mean concentrations of plasma desacyl ghrelin were } 10.70 \pm 1.74 \text{ fmol/ml and were not suppressed by the clamp: } 10.99 \pm 2.43 \text{ fmol/ml (} P = 0.87).$$

**Systemic levels of insulin, GH, and cortisol**. Serum concentrations of insulin \((\text{Fig. 5B})\) were \(35.3 \pm 3.6 \text{ pmol/l at } t = 0.\) During the clamp, serum insulin concentrations increased to \(223.3 \pm 11.9 \text{ pmol/l. Serum concentrations of GH were } 1.3 \pm 0.6 \text{ mg/ml at } t = 0 \text{ (Fig. 5C), and ghrelin infusion did not stimulate endogenous GH secretion. Serum concentrations of cortisol (Fig. 5D) were } 165.5 \pm 15.4 \text{ ng/ml at } t = -60 \text{ and were not increased in response to ghrelin administration (ANOVA, } P > 0.05).$$
Fig. 2. Interstitial concentrations of glycerol in different interstitial tissues during ghrelin (black lines) and placebo (gray lines) infusions. Vertical punctuated lines separate the basal from the clamp period. Interstitial concentrations of glycerol were similar in skeletal muscle (A), peripheral (B), and abdominal adipose tissue (C) in the basal and clamp periods. All data are presented as means ± SE.

Fig. 3. Interstitial concentrations of lactate in different interstitial tissues during ghrelin (black lines) and placebo (gray lines) infusions. Vertical punctuated lines separate the basal from the clamp period. Interstitial concentrations of lactate were similar in skeletal muscle (A), peripheral (B), and abdominal adipose tissue (C) in the basal and clamp periods. All data are presented as means ± SE.
Leg blood flow. Blood flow was similar at all time points (ml/min) \( t = 0: 249 \pm 22 \) (ghrelin) vs. \( 266 \pm 29 \) (placebo), \( P = 0.65; t = 120 \) min: \( 332 \pm 38 \) (ghrelin) vs. \( 328 \pm 31 \) (placebo), \( P = 0.91; t = 300 \) min: \( 403 \pm 48 \) (ghrelin) vs. \( 406 \pm 49 \) (placebo), \( P = 0.94 \).

DISCUSSION
The metabolic effects of ghrelin in human subjects have been the subject of several studies involving intravenous and intra-arterial administration. Studies suggest that ghrelin binds...
directly to metabolically active tissues such as skeletal muscle, adipose tissue, and the liver (20). Intravenous ghrelin administration in human subjects causes hyperglycemia, insulin resistance, and lipolysis (27, 29). These effects have subsequently been replicated in hypopituitary patients (7, 28), suggesting a mechanism independent of GH and cortisol release. In support of a peripheral mechanism, direct intra-arterial infusion of ghrelin in the femoral artery also elicits in situ lipolysis, but that study was hampered by overflow of ghrelin to the systemic circulation and a concomitant stimulation of GH secretion (26). Therefore, it still remains to be established whether the peripheral effects are mediated by ghrelin itself or via known or unrecognized secondary messengers.

Therefore, in the present study, we further investigated direct peripheral effects of ghrelin in muscle and fat tissues in vivo by means of microdialysis catheters. The basis for using microdialysis catheters for both administration and sampling is that 1) changes in interstitial glycerol concentration reflect regional lipolysis (11) and are inducible by known lipolytic agents such as epinephrine (10) and 2) increased interstitial glucose concentrations in skeletal muscle have been shown to parallel hyperglycemia during ghrelin infusion (28). The expected ghrelin-induced increase in interstitial concentrations of glycerol in adipose tissue and glucose in skeletal muscle was not observed. By contrast, we observed no effects on interstitial glycerol levels and a decrease in interstitial concentrations of glucose in muscle, indicating that ghrelin increases glucose uptake in this tissue.

The strength of our study is that all interstitial spaces were exposed to the same substrate and hormonal background, except for the exposure to ghrelin. Moreover, local administration of ghrelin excluded systemic effects, including those related to the metabolic effects of GH and cortisol at the whole body level as well as locally in muscle and adipose tissue. In addition, there was no day-to-day variability because each subject was examined only once, eliminating the intra-individual and analytical variation encountered in crossover trials.

However, there are also certain limitations to our study. Although pilot in vitro experiments showed that interstitial ghrelin levels increased during administration by microdialysis catheters (data not shown), we had no methods to verify or quantify the increase in interstitial ghrelin concentrations in vivo. However, the in vitro experiments indicated that tissue ghrelin concentrations were supraphysiological or even pharmacological. Blood flow was similar in the femoral arteries during placebo and ghrelin administration, but we had no measurements to detect the exact blood flow in the vicinity of the microdialysis catheters in muscle or in adipose tissue. Thus, there could have been subtle differences in tissue blood flow impacting on the interstitial concentrations of different metabolites, although this is not a feature that has been described previously during ghrelin exposure.

A number of reports point toward ghrelin playing an important role in protecting against hypoglycemia by suppressing insulin secretion and promoting insulin resistance (7, 25–29). In our previous study with systemic ghrelin administration and microdialysis (28), skeletal muscle interstitial glucose concentration was ~78% of plasma glucose at baseline and increased during the basal period to ~90% of plasma glucose levels independently of GH and cortisol, which indicated that ghrelin directly induced insulin resistance. In the present study, with local administration of ghrelin directly into skeletal muscle, interstitial glucose concentration was ~3.7 mmol/l, corresponding to ~73% of plasma glucose at baseline, but interstitial glucose concentration remained just above 70% during the basal period and was reduced to ~55% during the clamp. The purpose of using the hyperinsulinemic euglycemic clamp technique was not to mimic a physiological condition but rather to test whether ghrelin exhibited insulin-antagonistic effects [which may be difficult to disclose in the basal (unstimulated) state]. Thus, the present study indicates that ghrelin-induced insulin resistance is not attributable to a direct effect of ghrelin in skeletal muscle but rather by a systemic secondary messenger. Ghrelin binds to the adrenal gland (20) and also increases systemic levels of epinephrine (17) as well as skeletal muscle sympathetic nervous activity in healthy volunteers (13). Since epinephrine stimulates lipolysis (2, 8) and induces insulin resistance (4, 8), we speculate that it could be the secondary messenger, but experimental studies in human models are warranted to pursue this hypothesis. Theoretically, the current design with ghrelin administration directly into metabolic active tissues does not exclude that the observed metabolic effects of ghrelin during the clamp are mediated by local neural regulation.

In conclusion, supraphysiological ghrelin administration into skeletal muscle decreases interstitial concentrations of glucose during euglycemic hyperinsulinemia, indicating enhanced glucose uptake without any effects on interstitial glycerol or lactate levels in either muscle or adipose tissue. It remains to be investigated whether these effects have any physiological implications. In the basal postabsorptive state, ghrelin did not directly impact on skeletal muscle and adipose tissue metabolism. These data contrast with the metabolic effects of ghrelin observed after systemic exposure and suggest the existence of a second messenger that remains to be identified.

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DISCLOSURES

The authors have nothing to declare.

AUTHOR CONTRIBUTIONS

E.T.V., N.M., and J.O.L.J. contributed to the conception and design of the research; E.T.V. performed the experiments; E.T.V. analyzed the data; E.T.V., N.M., and J.O.L.J. interpreted the results of the experiments; E.T.V. prepared the figures; E.T.V. drafted the manuscript; E.T.V., N.M., and J.O.L.J. edited and revised the manuscript; E.T.V., N.M., and J.O.L.J. approved the final version of the manuscript.

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