The role of leptin in human lipid and glucose metabolism: the effects of acute recombinant human leptin infusion in young healthy males

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ABSTRACT

Background: Obese and lean humans treated with leptin have not experienced convincing weight-loss results compared with the dramatic weight losses observed in obese rodents.

Objective: We sought to investigate the effect of acutely elevating leptin to concentrations observed in obese individuals on muscle and adipose tissue metabolism and muscle signaling in healthy lean males.

Design: Healthy, lean, postabsorptive males were infused with either recombinant human leptin (rhleptin; n = 8) or saline (control; n = 8) for 4 h, which elicited leptin concentrations of ~20 and ~1 ng/mL, respectively. Systemic, skeletal muscle, and adipose tissue fat and glucose metabolism in vivo were assessed before, during, and 2 h after cessation of the infusion. Skeletal muscle biopsy specimens were obtained to quantify changes in signal transducers and activators of transcription–5′AMP-activated protein kinase (STAT-AMPK) signaling.

Results: During the infusion of rhleptin, no differences in either systemic, skeletal muscle, or adipose tissue glucose or fat metabolism were observed. These observations were made despite increased activation of STAT (~17-fold) and AMPK (1.43-fold) after 1 h of rhleptin infusion. After the rhleptin infusion, an increase in systemic palmitate and fat oxidation was observed (P < 0.0003), which likely was caused by a concomitant increase in skeletal muscle palmitate oxidation (P < 0.02). This was observed despite lowered leptin concentrations and basal skeletal muscle STAT-AMPK signaling.

Conclusions: Elevating circulating leptin concentrations to concentrations comparable with those of obese individuals increases human in vivo skeletal muscle signaling through the AMPK pathway and causes an increase in skeletal muscle fatty acid oxidation. Abdominal adipose tissue was unaffected by the acute physiologic increase of extracted rat muscle (6). Although Prieur et al (7) were not able to demonstrate that leptin has a role in peripheral metabolism in rodents, most cell culture and rodent studies suggest that leptin has a direct effect on peripheral metabolism in addition to centrally mediated effects on satiety and food-seeking behavior.

Whereas rodent studies with exogenous leptin administration have shown dramatic weight losses (8, 9), human equivalents have not been so dramatic; weight losses in the 1–7-kg range were observed during 24 wk of treatment (10). However, human interventional studies also show opposing results. When obese humans were subjected to dietary restriction and given either recombinant leptin or placebo, no difference in weight loss, body composition, or energy expenditure was observed between interventions (11). In contrast, energy expenditure in weight-reduced subjects increased to preweight loss levels during leptin supplementation, which indicates that leptin may increase metabolism in humans (12).

A common feature of human interventional studies with rhleptin is that the aim has been to observe changes brought on by chronically elevating systemic leptin. In this context, all...
of these studies used subcutaneous deposition of leptin to ensure a slow and prolonged release. For the study of acute leptin-induced changes in human peripheral metabolism, intravenous administration may provide a faster and hence clearer picture of the kinetics behind any metabolic changes brought on by leptin.

We infused rhleptin for 4 h in healthy humans with the aim of increasing leptin to concentrations observed in obese individuals (10, 11, 13). We used a human in vivo model with arteriovenous differences across the leg and subcutaneous adipose tissue to quantify metabolic changes in skeletal muscle, in subcutaneous adipose tissue, and systemically. Biopsy specimens were obtained from skeletal muscle to determine whether rhleptin affected known metabolic pathways. We hypothesized that acute rhleptin infusion in physiologic concentrations comparable with those observed in obese individuals would stimulate lipolysis and fatty acid oxidation and increase glucose uptake in human skeletal muscle through activation of STAT3 and AMPK in skeletal muscle.

Subjects and methods

Subjects

The relevant ethics committee approved the protocol (H-D-2007-0012), and all aspects of the Helsinki Declaration were respected. Enrollment began in June 2007. After giving their written and oral consent, subjects underwent a physical examination, health indicators were examined in blood, and an electrocardiogram was performed. We recruited 8 nonsmoking male subjects for each intervention; the characteristics of these subjects are shown in Table 1. Subjects were enrolled if they had been weight stable for 3 mo, were moderately physically active, were medication free, had a balanced diet, and did not have acute or chronic illness. Furthermore, erythrocyte and leukocyte concentrations, indicators of immune activation, and results of an oral-glucose-tolerance test and of thyroid, renal, and liver function all had to be within normal ranges. Body composition of fat and fat-free mass was measured by using a dual-energy X-ray absorptiometry scanner (Lunar Prodigy; GE Medical Systems LUNAR; software version 8.8). Body-composition data are provided in Table 1.

Experimental design

After having refrained from meal ingestion after 2200 and from exercise the previous day, the subjects reported to the laboratory at 0700. Subjects voided and remained supine and fasting for the entire experimental day. Water was allowed ad libitum. The superficial epigastric vein and the femoral vein and artery were catheterized according to the methods described by Frayn et al (14) and van Hall et al (15), respectively. The catheter in the femoral vein was positioned distally to the effluence of the saphenous vein to ensure that the sampled blood represented skeletal muscle with minimal contamination from subcutaneous adipose tissue, as described by van Hall et al (15). The femoral artery catheter was placed antegradely, positioning the tip of the catheter 10 cm from the iliac ligament. An antecubital vein was used for the infusion of stable isotopes. A priming bolus of Na[13CO3](1.5 μmol/kg), a primed continuous infusion of [6,6-D2]glucose (17.6 μmol/kg, 0.4–1.5 μmol·kg⁻¹·min⁻¹) and [1,2,3,3-D4]glycerol (1.5 μmol/kg, 0.1 μmol·kg⁻¹·min⁻¹), and a continuous infusion of potassium [U-13C18]palmitate (0.05 μmol·kg⁻¹·min⁻¹) were provided. All stable isotopes (Cambridge Isotope Laboratories) were prepared under sterile conditions on the morning of the experiment. After 2 h of tracer infusion to establish steady state in metabolite concentrations and enrichment of the stable isotopes,
A 4-h infusion of either saline or recombinant human leptin (Sigma-Aldrich) was started, and changes were followed for another 2 h (recovery) after cessation of the infusions. LPS concentrations were within approved ranges. Recombinant human leptin (Sigma-Aldrich) was sterilized and tested at the hospital pharmacy. Blood pressure and pulse were monitored continuously. Auricular temperature was monitored every hour. Blood was drawn every 30 min the first 3 h, after which blood was drawn every hour. Because of the invasive nature of the study and the technical challenges of placing the superficial epigastric venous catheter, a noncrossover design was chosen.

Skeletal muscle biopsy specimens from the vastus lateralis were obtained by the percutaneous needle biopsy method at baseline, 1 h into the infusion and 2 h after cessation of the infusion. To minimize any influence from the stress of biopsy sampling on the humoral indicators measured, baseline sampling was commenced 15 min after the first biopsy specimen was collected, and subsequent sampling was carried out before biopsy sampling. Blood and visible connective tissue were quickly removed from the biopsy specimens before being frozen in liquid nitrogen and stored at −80°C until analyzed. During the experimental day, the subjects’ blood pressure and pulse were monitored continuously. Auricular temperature was monitored every hour. Blood was drawn every 30 min the first 3 h, after which blood was drawn every hour. Because of the invasive nature of the study and the technical challenges of placing the superficial epigastric venous catheter, a noncrossover design was chosen.

**Blood flow**

Leg blood flow in the femoral artery was measured by using the Doppler ultrasound method (CFM-800; Wingmed A/S) as described by Rådegran (16) and corrected with leg oxygen extraction data. Abdominal adipose tissue flow was measured by using the
133-Xenon washout method as described by Karpe et al (17) and Bülow et al (18). A tissue/blood partition coefficient (lambda) for xenon was set at 8 (19). Data were analyzed by using GammaScan software (version 1.20; FBJ Engineering). Plasma flow for both methods was obtained by using hematocrit values:

\[
\text{Plasma flow} = \frac{\text{blood flow}}{3} \left(1 - \text{Hct}\right)
\]  

\[ (I) \]

Calculations

**Palmitate and glucose kinetics**

\( V^{O_2} \) and \( V^{CO_2} \) were measured with a breath-by-breath pulmonary exchange system (Quark b^2 by Cosmed).

The systemic \( R_a \) and \( R_d \) of palmitate, glycerol, and glucose were calculated as follows:

\[ R_a = R_d = \frac{F}{E_a} \]  

where \( F \) is the rate of tracer infusion, and \( E_a \) is the arterial enrichment at plateau in TTR. In view of the relative small changes in concentration and enrichment over time, steady state equations have been applied for the calculation of systemic \( R_a \), which under those conditions is equal to the \( R_d \). Similar calculations were applied to glycerol and glucose kinetics.

Palmitate oxidation rates were calculated as follows:

The repeated-measures ANOVA was applied as the statistical model with a post hoc t test with Tukey’s correction for time points of statistical significance. A: Leg palmitate net uptake (uptake – release). There was a borderline significant difference between the groups (treatment effect: \( P < 0.06 \)). B: Leg palmitate uptake. There was a significant difference between the groups (treatment effect: \( P < 0.04 \)). C: Leg palmitate release. There were no significant changes over time or between groups. D: Leg palmitate oxidation. Oxidation was affected by the rleptin infusion (time x treatment interaction: \( P < 0.02 \)). Significantly different from baseline, \( P < 0.03 \). E: Leg palmitate re-esterification. There was a significant difference between groups (treatment effect: \( P < 0.01 \)) and a borderline significant effect of rleptin infusion (interaction: \( P < 0.08 \)). F: Unidirectional palmitate:glycerol release ratio. There were no significant changes over time or between groups. rleptin, recombinant human leptin.

**FIGURE 3.** Leg palmitate kinetics in response to a 4-h infusion of either rleptin (n = 8) or saline (control; n = 6). Values are means ± SEMs. The repeated-measures ANOVA was applied as the statistical model with a post hoc t test with Tukey’s correction for time points of statistical significance. A: Leg palmitate net uptake (uptake – release). There was a borderline significant difference between the groups (treatment effect: \( P < 0.06 \)). B: Leg palmitate uptake. There was a significant difference between the groups (treatment effect: \( P < 0.04 \)). C: Leg palmitate release. There were no significant changes over time or between groups. D: Leg palmitate oxidation. Oxidation was affected by the rleptin infusion (time x treatment interaction: \( P < 0.02 \)). Significantly different from baseline, \( P < 0.03 \). E: Leg palmitate re-esterification. There was a significant difference between groups (treatment effect: \( P < 0.01 \)) and a borderline significant effect of rleptin infusion (interaction: \( P < 0.08 \)). F: Unidirectional palmitate:glycerol release ratio. There were no significant changes over time or between groups. rleptin, recombinant human leptin.
TABLE 2
Systemic hormonal concentrations and blood flow in subjects infused with either saline (control) or rhleptin.1

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1–2 h of infusion</th>
<th>3–4 h of infusion</th>
<th>Second hour of recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adrenaline (ng/mL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0.06 ± 0.01a</td>
<td>0.05 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>rhleptin</td>
<td>0.04 ± 0.01a</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td><strong>Cortisol (ng/mL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>33.6 ± 5.5b</td>
<td>49.8 ± 11.1</td>
<td>24.2 ± 2.6</td>
<td>26.7 ± 3.3</td>
</tr>
<tr>
<td>rhleptin</td>
<td>48.8 ± 10.2b</td>
<td>39.7 ± 6.9</td>
<td>28.8 ± 3.5</td>
<td>38.5 ± 3.6</td>
</tr>
<tr>
<td><strong>Glucagon (pg/mL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>52.7 ± 11.9c</td>
<td>56.3 ± 14.3</td>
<td>55.5 ± 16.9</td>
<td>55.4 ± 17.4</td>
</tr>
<tr>
<td>rhleptin</td>
<td>50.7 ± 4.3c</td>
<td>57.1 ± 4.5</td>
<td>53.9 ± 4.7</td>
<td>57.3 ± 6.6</td>
</tr>
<tr>
<td><strong>Leg blood flow (mL/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>350 ± 19a</td>
<td>374 ± 53</td>
<td>375 ± 51</td>
<td>356 ± 44</td>
</tr>
<tr>
<td>rhleptin</td>
<td>269 ± 43a</td>
<td>237 ± 32</td>
<td>230 ± 34</td>
<td>282 ± 56</td>
</tr>
<tr>
<td><strong>Adipose tissue blood flow (mL · 100 g tissue−1 · min−1)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>2.7 ± 0.4d</td>
<td>2.3 ± 0.4</td>
<td>2.6 ± 0.4</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>rhleptin</td>
<td>2.3 ± 0.3d</td>
<td>2.4 ± 0.3</td>
<td>2.5 ± 0.3</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td><strong>Thyroxine (nmol/L)</strong></td>
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<td></td>
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<tr>
<td>Saline</td>
<td>75.6 ± 3.5e</td>
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<td>NA</td>
<td>72.9 ± 3.5</td>
</tr>
<tr>
<td>rhleptin</td>
<td>80.4 ± 3.8e</td>
<td>NA</td>
<td>NA</td>
<td>78.4 ± 3.8</td>
</tr>
<tr>
<td><strong>Tri-iodothyronine (nmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>1.6 ± 0.05f</td>
<td>NA</td>
<td>NA</td>
<td>1.5 ± 0.05</td>
</tr>
<tr>
<td>rhleptin</td>
<td>1.5 ± 0.08f</td>
<td>NA</td>
<td>NA</td>
<td>1.4 ± 0.07</td>
</tr>
</tbody>
</table>

1 All values are means ± SEMs; n = 8 in both groups. There were no significant differences at baseline between the groups in any of the variables. Significant changes were observed over time (repeated-measures ANOVA): *P < 0.004, † P < 0.0008, ‡ P < 0.03, § P < 0.0001, ¶ P < 0.03, ‰ P < 0.0004.

where \( E_{CO_2} \) is the enrichment of carbon dioxide in the expired air; \( V_{CO_2} \) is the exhaled volume of carbon dioxide, which is divided by 16, because this is the number of \( ^{13}C \) atoms released as carbon dioxide from the oxidation of one molecule of \( [U-^{13}C] \) palmitate; \( E_a \) is the enrichment of palmitate in the blood; and \( a \) is the acetate correction factor.

The acetate correction factor was obtained from young healthy males at rest from a study by van Hall et al (20).

Whole body palmitate \( R_d \) being re-esterified was calculated as:

\[
\text{Whole body palmitate re-esterification (%) = (} R_d \text{ palmitate-palmitate oxidation) \times 100\%}
\]

**Tissue palmitate, glycerol, and glucose kinetics**

Blood was sampled from the femoral vein to measure palmitate and glycerol release from skeletal muscle, whereas blood was sampled from the superficial epigastric vein to measure palmitate or glycerol release from adipose tissue. The same calculations were used with glucose kinetics. We assumed that by bypassing the effluence of the saphenous vein, our sampling from the femoral vein primarily represented leg skeletal muscle (15), that the venous return equaled the arterial blood flow measured, and that the blood flow measured with \(^{133}\text{Xenon} \) was representative of the area drained by our superficial epigastric vein catheter.

Tissue palmitate, glycerol, and glucose kinetics calculations:

\[
\text{net leg balance} = (C_a - C_v) \times \text{plasma flow (blood flow)}
\]

Plasma flow is used in the case of palmitate, and blood flow is used in the case of glycerol and glucose calculations. Tissue unidirectional palmitate or glycerol uptake and release were calculated as follows:

\[
\text{Fractional extraction} = \frac{(C_a \times E_a) - (C_v \times E_v)}{C_a \times E_a}
\]

Unidirectional palmitate or glycerol uptake:

\[
\text{Uptake} = \text{Fractional extraction} \times C_a \times \text{plasma/blood flow}
\]

Unidirectional palmitate or glycerol release:

\[
\text{Release} = \text{uptake} - \text{net balance}
\]

where \( C_a \) and \( C_v \) and \( E_a \) and \( E_v \) are the palmitate or glycerol concentrations and enrichments in TTR in the femoral artery, femoral vein, or superficial epigastric vein, respectively.

The equations used to calculate palmitate oxidation and re-esterification are as follows:

\[
\text{Uptake} = \frac{(ECO_2 \times VCO_2)/16}{E_a \times a}
\]
Leg palmitate re-esterification equation

\[ \text{Tissue intracellular palmitate re-esterification} = \frac{\text{unidirectional tissue palmitate release}}{\text{unidirectional tissue glycerol release}} \]

\[ (11) \]

Statistical methods

The statistical design was conferred with a biostatistician. All values are given as means ± SEMs, except for subject characteristics, which are presented as means ± SDs. The data were tested for normality graphically and by using formal tests. Changes between treatments and over time were tested with a repeated-measures ANOVA. A time × treatment interaction was used to test for an effect of rhlreptin infusion compared with saline infusion. A post hoc test with Tukey’s correction was applied to identify time points of statistical significance. A probability level of 5% was chosen as being significant. SAS statistical software was used to analyze the data (SAS 9.1; SAS Institute Inc).

Supplemental data

The methods pertaining to hormone, cytokine, and metabolite concentrations; isotopic enrichments; and skeletal muscle signal analyses are described in detail elsewhere (see “Supplemental data” in the online issue).
RESULTS

In response to the rhleptin infusion, plasma leptin concentrations increased continuously in plasma until a steady state level was reached after 3 h. A significant difference in systemic rhleptin concentrations was observed between the saline and rhleptin groups (time \( \times \) treatment interaction: \( P < 0.0001 \)).

During the final hour of the infusion, we achieved a steady state concentration of 22.3 \( \pm \) 2.3 ng/mL, which did not cause any changes in blood pressure, temperature, or heart rate (data not shown). The subjects did not report any malaise or other adverse reactions and were unaffected by both infusions (Figure 1).

Whole-body fat oxidation and lipolysis

Whole-body glycerol and palmitate concentrations (time effect: \( P < 0.01 \) and \( P < 0.0001 \), respectively) and \( R_a \) (time effect: \( P < 0.001 \) and \( P < 0.0001 \), respectively) increased during the day of the experiment, indicative of an increase in whole-body lipolysis over time (Figure 2, A and B). In the rhleptin group, systemic palmitate oxidation increased (time \( \times \) treatment interaction: \( P < 0.0001 \); Figure 2C) during the recovery period (\( P < 0.05 \)). Similar to palmitate oxidation measurements, whole-body fat oxidation assessed by indirect calorimetry showed a statistically significant increase in oxidation (time \( \times \) treatment interaction: \( P < 0.0001 \)) during the recovery period in response to rhleptin infusion (\( P < 0.05 \); Figure 2D). Any changes in palmitate re-esterification were not subjected to statistical analysis, because palmitate re-esterification is calculated from palmitate \( R_a \) and oxidation rather than from direct measurements (Figure 2E).

Leg skeletal muscle lipid kinetics

No changes over time or due to the interventions were observed in palmitate net uptake in the leg (Figure 3A); hence, no changes in unidirectional uptake (Figure 3B) or release of palmitate (Figure 3C) were found. A consistent group difference in all leg palmitate flux measurements was observed. This was most likely caused by differences in leg blood flow (Table 2).

During the rhleptin infusion, leg palmitate oxidation rates increased in every subject in the rhleptin group (time \( \times \) treatment interaction: \( P < 0.02 \); Figure 3D); however, numerically, the increase was statistically significant only during the second hour of recovery (\( P < 0.03 \)). No statistically significant changes in leg palmitate re-esterification rates were observed (Figure 3E).

The unidirectional palmitate:glycerol release ratio was consistently \( \sim 0.6 \) during the entire experiment (Figure 3F). The palmitate:fatty acid ratio was consistently \( \sim 0.22 \) in humans (21). This meant that the fatty acid:glycerol ratio was consistently \( \sim 2.7 \) (0.6/0.22), which implied near complete hydrolysis of triacylglycerol, with no effect of rhleptin on intracellular fatty acid re-esterification.

Subcutaneous adipose tissue lipid kinetics

A continuous increase in net and unidirectional glycerol was observed in abdominal adipose tissue (Table 3) and palmitate
release during the day (Figure 4, A and C). No effect of rhleptin on adipose tissue lipid kinetics was observed.

**Glucose metabolism**

Endogenous glucose production (glucose Ra), systemic blood glucose, and leg glucose uptake were not affected by rhleptin (Figure 5). A group difference in saline- and rhleptin-infused glucose uptake over the leg was observed (treatment effect: \( P < 0.02 \)). Insulin remained stable in the control group but decreased during the rhleptin infusion (time \( \times \) treatment interaction: \( P < 0.03 \)). Significantly different from baseline, \( P < 0.08 \). R\( a \), rate of appearance; rhleptin, recombinant human leptin.

**Skeletal muscle metabolic signaling**

The ratio of phosphorylated STAT3 to STAT3 increased 17-fold after 1 h of infusion compared with baseline (\( P < 0.009 \)) and returned to baseline values within 2 h after cessation of the infusion. Likewise, an increased activation of AMPK was observed after 1 h, as the p-AMPK:AMPK ratio increased 1.43-fold above baseline (\( P < 0.05 \)). p-ACC was not affected by rhleptin (Figure 6).

**Hormones**

Adrenaline gradually increased during the day in both groups, probably in response to fasting (\( P < 0.004 \)), but no significant differences were observed between the 2 groups. Cortisol and glucagon were similar between the 2 groups throughout the day and decreased slightly during the day (time effect: \( P < 0.0008 \) and \( P < 0.03 \), respectively). Thyroxine and triiodothyronine decreased equally in both groups throughout the day (time effect: \( P < 0.03 \) and \( P < 0.0004 \), respectively). Hormone data are listed in Table 2.

**Blood flow**

Neither adipose tissue nor leg blood flow was affected by the rhleptin infusion (Table 2). A significant group difference in leg blood flow was observed between the 2 interventions (treatment effect: \( P < 0.05 \)). The cause of this group difference is unknown, because the method of quantification was the same. A numerical difference of \( \approx 15\% \) in leg fat-free mass between the groups may have been a contributing factor (Table 1). Adipose tissue blood flow consistently increased throughout the day (time effect: \( P < 0.0001 \)).

**Tracer measurements**

Tracer equilibrium was evident, because the glycerol and palmitate TTRs did not increase from 30 min before baseline (\( -30 \) min) until the baseline sampling point (Table 4).

**DISCUSSION**

The major and novel finding of the current study is that when leptin concentrations were raised acutely in healthy humans to concentrations comparable with those in obese individuals (10, 11, 13), fat metabolism was affected. Systemic palmitate oxidation and fat oxidation (Figure 2, C and D) increased during the
recovery period after rhleptin infusion. Although leg palmitate oxidation (Figure 3D) seemed to correlate well with systemic oxidation measurements, the apparent decrease in leg palmitate release (Figure 3C) and palmitate re-esterification (Figure 3E) during the infusion suggests that leg palmitate oxidation was already stimulated during the rhleptin infusion. Numerically, this could well be the case when viewing the leg palmitate oxidation graph (Figure 3D). This suggests that rhleptin may acutely shift the fate of plasma fatty acids taken up by skeletal muscle from storage to oxidation. Equally interesting is that abdominal adipose tissue fat metabolism was unaffected in any way during and after rhleptin infusion. Our steady tissue palmitate:glycerol unidirectional release ratios throughout the study are further validation that the changes measured in fat metabolism were correct (Figure 3F, Figure 4D) and that there was an unchanged and near complete hydrolysis of fat and, hence, little intracellular re-esterification.

A temporal delay between increases in systemic leptin concentration and fat oxidation is likely due to signaling events; however, it could also be due to secondary mediators eliciting the effects. Leptin supplementation to weight-reduced subjects is reported to increase both thyroid hormone concentrations and sympathetic nervous system activity back to levels before weight loss (12). Hence, it is conceivable that they could also be involved in the current metabolic changes noted. Thyrroxine and triiodothyronine concentrations were not significantly different between interventions. This indicates that thyroid hormones were not involved in the acute metabolic effects of rhleptin in the current study.

Acutely elevating the systemic leptin concentration activated STAT3 and AMPK signaling in human skeletal muscle after 1 h
of infusion (Figure 6). STAT3 phosphorylation increased 17-fold. This was accompanied by a 1.43-fold increase in the p-AMPK:AMPK ratio. The signaling events leading to an increase in lipid metabolism in vitro have been identified as an increase in AMPK phosphorylation. Thus far, this has only been addressed in vitro (3) or in rodents ex vivo (4).

The temporal delay between the increases in leptin, p-AMPK, and systemic fat oxidation observed in the current study was also observed in rodent studies. Data from acute leptin infusion studies in rodents also show a late-onset increase in fat oxidation that is dissociated with the systemic leptin concentration. When Minokoshi et al. (3) injected mice with an intravenous leptin bolus, they observed an acute elevation in systemic leptin concentrations that was followed by a biphasic activation of AMPK that was dissociated from systemic leptin concentrations. Leptin returned to baseline concentrations after 6 h. In contrast, fat oxidation increased significantly 6 h after the injection (3). Hence, the changes in systemic fat metabolism from the rodent studies appeared to be comparable with our data.

The activation of AMPK without changes in p-ACC in our study may suggest that other pathways were activated or simply that our design failed to sample when p-ACC was increased. The dramatic increase in p-STAT3 does not contradict this notion. STAT3 is phosphorylated on leptin binding to its receptor and translocates to the nucleus, where it is involved in regulating the expression of several metabolic genes (eg, CPT-1 and PGC-1) (22). In rodents, leptin stimulated cardiac fatty acid oxidation via a p38-MAPK pathway without changes in AMPK activity or ACC, which indicated that leptin may stimulate metabolism through AMPK-independent pathways (23, 24).

The systemic oxidation of fat and fatty acids observed in our study can principally take place in either skeletal muscle and/or the liver. Other studies examining the effect of leptin on peripheral metabolism have found that leptin stimulates oxidation in muscle (2, 25), whereas the evidence pertaining to leptin-induced oxidation in liver is less clear (7, 26, 27). We did not measure hepatic metabolism, but our increase in skeletal muscle palmitate oxidation data are well in line with previous animal and cell culture studies.

We observed no significant changes in skeletal muscle or adipose tissue fatty acid or glycerol release, which indicated that rhleptin did not affect lipolysis. Cell culture studies have shown that leptin stimulates lipolysis in adipocytes (28). When leptin was administered chronically to rats, an increase in skeletal muscle lipolysis was observed (6). These results contrast with our findings on lipid metabolism of an acute elevation in leptin concentrations. Our results seem more in line with the decreases in lipolysis observed in rhleptin-treated HIV-positive lipoatrophy patients (26) and the unchanged uptake of palmitate in human muscle after leptin incubation, as previously reported by Steinberg et al (25). The discrepancy may have been a result of our in vivo measurements, as opposed to the in vitro methods that have been applied in previous studies (6, 28). Furthermore, differences in species and in the supraphysiologic concentrations of leptin often used in vitro may explain the differences in findings.

Of the major metabolic hormones measured (Table 2), only insulin was different between interventions (Figure 5D). Insulin was significantly lower in the rhleptin group than in the saline group, and insulin decreased by 35% from baseline with rhleptin infusion. This could be explained by a transient rhleptin-induced increase in insulin sensitivity, but the current study was not designed to investigate this. Another possibility is that rhleptin suppressed insulin release (26, 29). This scenario fails to explain why no statistically significant change in endogenous glucose production, systemic glucose concentration, or skeletal muscle glucose uptake occurred during the rhleptin infusion. It cannot be excluded that the changes in fat metabolism brought on by rhleptin were mediated in part by the decrease in insulin. It is difficult to assess the effect of the noted decrease in insulin on fat metabolism, but it is clear that changes in insulin did not affect any indicators of glucose metabolism measured.

Limitations of the current study included our short steady state period of elevated leptin in the rhleptin group, which lasted only 1 h. Furthermore, the major changes in fat oxidation seemed to be delayed over time, which made our recovery period too short to observe a return to baseline of the metabolic variables. A future similar experiment would perhaps benefit from a longer rhleptin infusion period. Also evident was the group difference in leg blood flow, which could affect flux calculations. Last, we cannot be certain that our biopsy sampling time points were at the times...
of maximal changes in protein expression. A final, but important limitation, was that our results pertain to young, healthy, lean males; therefore, extrapolation to other populations should be done with great caution.

In attempts to determine the role of leptin in normal human physiology, it is important to recognize the limitations of results derived from studies distinct from human physiologic conditions. Acute and chronic supplemental studies may not reflect normal physiology, because endogenous secretion of leptin is pulsatile and is affected by many factors, including food intake and fasting (30), exercise (31, 32), disease (33), and menstrual cycle (34). Our aim was to gain knowledge on the physiologic role of leptin in human metabolism more so than the pharmacologic role. Acute short-term rhleptin infusion in young healthy males in the postabsorptive state stimulated palmitate oxidation systemically and in skeletal muscle but did not affect glucose metabolism systemically or in skeletal muscle. These changes were preceded by activation of skeletal muscle STAT3-AMPK signaling, which suggests a possible link.

We acknowledge the invaluable help of Fredrik Karpe with the abdominal subcutaneous fat vein catheter (Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Churchill Hospital, Oxford, United Kingdom) and Nina Pluszek, Noemi Galicki, Ruth Rousing, and Hanne Villumsen for their technical assistance.

The authors’ responsibilities were as follows—EW: designed and conducted the study, analyzed the data, and prepared the manuscript; HM: designed and conducted the study; TSG: conducted the study and analyzed data; GvH: designed the study, analyzed the data, and prepared the manuscript; and BKP: designed the study and prepared the manuscript. The authors declared that there was no duality of interest associated with this manuscript. Funders had no role in the planning, execution, analyses, writing, or submission of the manuscript.

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