Additive effects of cortisol and growth hormone on regional and systemic lipolysis in humans

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Djurhuus, C. B., C. H. Gravholt, S. Nielsen, S. B. Pedersen, N. Møller, and O. Schmitz. Additive effects of cortisol and growth hormone on regional and systemic lipolysis in humans. Am J Physiol Endocrinol Metab 286: E488–E494, 2004. First published November 4, 2003; 10.1152/ajpendo.00199.2003.—Growth hormone (GH) and cortisol are important to ensure energy supplies during fasting and stress. In vitro experiments have raised the question whether GH and cortisol mutually potentiate lipolysis. In the present study, combined in vivo effects of GH and cortisol on adipose tissue were explored. Seven lean males were examined four times over 510 min. Microdialysis catheters were inserted in the vastus lateralis muscle and in the subcutaneous adipose tissue of the thigh and abdomen. A pancreatic-pituitary clamp was maintained with somatostatin infusion and replacement of GH, insulin, and glucagon at baseline levels. At $t = 150$ min, administration was performed of NaCl (I), a 2 $\mu$g/kg/min hydrocortisone infusion (II), a 200-µg bolus of GH (III), or a combination of II and III (IV). Systemic free fatty acid (FFA) turnover was estimated by [9,10-3H]palmitate appearance. Circulating levels of glucose, insulin, and glucagon were comparable in I–IV. GH levels were similar in I and II (0.50 ± 0.08 µg/l, mean ± SE). Peak levels during III and IV were ~9 µg/l. Cortisol levels rose to ~900 nmol/l in II and IV. Systemic (i.e., palmitate fluxes, s-FFA, s-glycerol) and regional (interstitial adipose tissue and skeletal muscle) markers of lipolysis increased in response to both II and III. In IV, they were higher and equal to the isolated additive effects of the two hormones. In conclusion, we find that GH and cortisol stimulate systemic and regional lipolysis independently and in an additive manner when coadministered. On the basis of previous studies, we speculate that the mode of action is mediated through different pathways.

Microdialysis; corticosteroids

ADIPOSE TISSUE CONSTITUTES the largest fuel reservoir in the body, vastly exceeding available glycogen and protein stores (5). It has been estimated that the fat depots provide energy for ~2 mo of living in lean subjects compared with the energy stored as glycogen, lasting one day (37). Free fatty acids (FFA) from triacylglycerol (TAG) breakdown are a major energy source for heart and skeletal muscle (40).

Regulation of FFA release from adipocytes involves the enzyme hormone-sensitive lipase (HSL) (16, 21). Upon activation, HSL is translocated to the periphery of the intracellular fat droplet (4). There, the enzyme hydrolyzes TAG to FFA and glycerol. Studies on postabsorptive skeletal muscle fuel metabolism have until recently focused primarily on carbohydrates or FFA derived from the adipose tissue as sources of energy. However, it has become increasingly clear that TAG located in the muscle contributes as well, especially at rest and during moderate exercise (25, 42).

Both growth hormone (GH) and cortisol, among other effects, ensure substrate supplies, postabsorptively, during prolonged fasting (3, 33) and stress (43). Previous studies have demonstrated isolated effects of physiological levels of GH on lipolysis (17, 32, 34), and recently we (10) have demonstrated acute lipolytic effects of cortisol as assessed by interstitial glycerol levels, serum FFA, and glycerol and systemic FFA turnover.

In vitro studies have revealed lipolytic actions of GH and an inhibitory effect of glucocorticoids, whereas a combined effect of GH and cortisol exceeding the GH-induced lipolysis has been described (36). Another in vitro study found glycerol release to be increased with isolated and concomitant stimulation of lipolysis with GH and dexamethasone (DEX) (44). The overall explanation for this finding could be that GH in the presence of DEX downregulated the G-coupled $\alpha_2$-receptors and, hence, increased lipolysis.

Because GH and cortisol are cosecreted during stress conditions, it is conceivable that both hormones are involved in the regulation of adipose tissue metabolism during fasting and stress. The present study was undertaken to gain further insight into the isolated and combined impact of GH and cortisol on overall and interstitial subcutaneous adipose tissue and skeletal muscle TAG metabolism by applying systemic tracer dilution and regional microdialysis techniques and utilizing glycerol as a marker of interstitial lipolysis (13, 19).

MATERIALS AND METHODS

Before the study, a power analysis was performed on the basis of findings by Divertie et al. (9) and Gravholt et al. (17). On that basis we included seven healthy young male subjects [26.6 ± 0.2 yr and body mass index 22.9 ± 0.5 kg/m² (mean ± SE)]. All gave their informed consent after receiving oral and written information according to the Declaration of Helsinki II. The study was approved by the Scientific Ethics Committee of Aarhus County.

Experimental protocol. The study was carried out as a single-blinded, placebo-controlled, randomized trial.

The subjects were admitted to the research laboratory at 0700 ($t = 150$ min) after an overnight fast (~10 h). The participants were instructed not to perform any physical exercise or ingest alcohol 1 day before the start of the study and to consume a weight-maintaining, carbohydrate-rich diet for 3 days before examination.

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Upon arrival at the research laboratory, subjects were placed in the supine position in bed wearing light hospital clothing in a room with an ambient temperature of 22–24°C, and they remained in that position throughout the study. Two intravenous catheters (Venflon; Viggo, Helsingborg, Sweden) were inserted in the antecubital vein of the left arm and in a dorsal vein of the left hand. The latter was placed in a heated box at 65°C, allowing for arterialized blood samples to be drawn (1). Blood samples were drawn every 30 min and analyzed for plasma glucose (PG), FFA, glycerol, cortisol, GH, glucagon, insulin, C-peptide, and catecholamines.

At $t = -120$ min, infusion of somatostatin (330 μg/h; Ferring Pharmaceuticals), insulin (0.08 mU·kg$^{-1}$·min$^{-1}$ Actrapid; Novo Nordisk, Bagsvaerd, Denmark), and GH (2 ng·kg$^{-1}$·min$^{-1}$ Norditropin; Novo Nordisk) was commenced. Infusion of glucagon (0.5 ng·kg$^{-1}$·min$^{-1}$ Glucagen; Novo Nordisk) was not added until $t = 90$ min.

Euglycemia was intended and isotonic glucose was infused to ensure PG >80 mg/dl to prevent hypoglycemia-induced breakthrough of the pancreatic clamp.

At $t = 0$ min, an infusion in a randomized manner of either 0.9% NaCl (I), infusion of hydrocortisone sodium succinate 2 μg·kg$^{-1}$·min$^{-1}$ (Solu-Cortef, Pharmacia Upjohn; II), a bolus of 200 μg GH (Norditropin; III), or a combined bolus of GH and hydrocortisone succinate infusion (IV) was initiated. Infusions were continued throughout the duration of the study ($t = 360$ min).

Microdialysis. After application of a local analgesic, 0.25 ml of lidocaine, a microdialysis catheter (CMA-60; CMA, Stockholm, Sweden) was inserted at $t = -150$ min in the subcutaneous adipose tissue ~5 cm dextralateral of the umbilicus and in the right femoral subcutaneous adipose tissue. Lidocaine (1 ml) was injected superficial to the fascia of the lateral vastus muscle 10 and 14 cm above the patella before insertion. Correct placement of the microdialysis catheters in the muscle was confirmed by the presence of muscle twitches during insertion. Two catheters were used to ensure survival of at least one, as muscle twitches can disrupt the microdialysis membrane. The microdialysis catheters have a molecular cut-off of 20 kDa and a membrane length of 30 mm.

Before insertion, the catheters were manually flushed with perfusion fluid (Ringer Chloride T1, CMA: 147 mmol/l Na$^+$, 1.4 mmol/l K$^+$, 2.3 mmol/l Ca$^{2+}$, 156 mmol/l Cl$^-$, pH 6, osmolality 290 mosmol/kg) to allow for clearance of air bubbles from the microdialysis membranes. Additionally, dipping of the membranes in the perfusate medium was performed as recommended by CMA. The microdialysis systems were perfused at a flow rate of 1 μl/min using the CMA-107 perfusion pump (CMA). The relative recovery of interstitial glycerol was assessed by the internal reference method with $[^3]$Hglycerol (28, 41).

$[^3]$Hglycerol was added to the perfusate to obtain ~1.000 cm²/μl. Perfusate and dialysate were counted using a Wallac 1450 liquid scintillation counter applying the Optiphase supermix scintillation fluid. Changes in interstitial glycerol concentration can be seen as an index of lipolysis (2, 19, 22).

Sampling of the interstitial fluid commenced at $t = -90$ min, allowing for 60 min of equilibration to minimize the influence of local edema and hemorrhage. The sampling was performed every 30 min and continued until $t = 360$ min.

Palmitate turnover. Systemic palmitate fluxes were measured at $t = 240–270$ min by use of the isotope dilution technique and steady-state equations. [9,10-3H]palmitate (Laegemiddelstyrelsen, Copenhagen, Denmark) was infused continuously at 0.3 μCi/min from 210 to 270 min, and blood samples were drawn for analysis of palmitate concentration and specific activity (SA). Steady state of SA was verified ($t = 240, 255$, and 270 min) for each individual.

### Table 1. Time-averaged values during baseline and stimulation

<table>
<thead>
<tr>
<th>Hormones</th>
<th>I Placebo</th>
<th>II Hydrocortisone</th>
<th>III GH Pulse</th>
<th>IV GH Pulse + Hydrocortisone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, pmol/l</td>
<td>37[19;82]</td>
<td>34[13;69]</td>
<td>36[29;91]</td>
<td>30[16;82]</td>
</tr>
<tr>
<td>Stimulated</td>
<td>33[21;69]</td>
<td>33[27;71]</td>
<td>38[30;75]</td>
<td>31[24;70]</td>
</tr>
<tr>
<td>C-peptide, pmol/l</td>
<td>332[56;1,042]</td>
<td>223[20;592]</td>
<td>417[98;1,082]</td>
<td>231[72;671]</td>
</tr>
<tr>
<td>Stimulated</td>
<td>56[0;342]</td>
<td>10[0;97]</td>
<td>60[0;350]*</td>
<td>50[0;157]</td>
</tr>
<tr>
<td>Glucagon, ng/l</td>
<td>16[4;78]</td>
<td>22[6;88]</td>
<td>18[4;64]</td>
<td>20[1;88]</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>68[50;170]</td>
<td>51[50;190]</td>
<td>50[25;392]</td>
<td>50[50;469]</td>
</tr>
<tr>
<td>Stimulated</td>
<td>50[50;210]</td>
<td>50[50;208]</td>
<td>53[31;340]</td>
<td>54[50;146]</td>
</tr>
</tbody>
</table>

Nonparametric data are expressed as median[interquartile range]; parametric data are means ± SE. GH, growth hormone. Baseline, $t = -120–0$ min; stimulation, $t = 0–360$ min [analysis of area under curve (AUC) during baseline and stimulation], I–IV, treatments (see Experimental protocol in text.) *$P < 0.05$. 

Fig. 1. Serum concentration of administered hormones. A: growth hormone (GH), B: cortisol. Symbols denote the 4 treatment groups: placebo, I; hydrocortisone, II; GH pulse, III; GH + hydrocortisone, IV. Time, in min.
stable FFA concentrations at 240 min. To account for this, we analyzed FFA and detected (dpm/H9262 mol).

Table 2.

<table>
<thead>
<tr>
<th>Circulating Metabolites</th>
<th>I Placebo</th>
<th>II Hydrocortisone</th>
<th>III GH Pulse</th>
<th>IV GH Pulse + Hydrocortisone</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFA, mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC 150–0 min</td>
<td>0.310±0.05</td>
<td>0.386±0.05</td>
<td>0.316±0.05</td>
<td>0.357±0.06</td>
</tr>
<tr>
<td>AUC 150–360 min</td>
<td>0.300±0.04</td>
<td>0.519±0.10</td>
<td>0.580±0.08</td>
<td>0.719±0.08</td>
</tr>
<tr>
<td>Glycerol, µmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC 150–0 min</td>
<td>39.4±6.9</td>
<td>44.1±6.7</td>
<td>40.0±7.4</td>
<td>41.9±6.1</td>
</tr>
<tr>
<td>AUC 150–360 min</td>
<td>40.0±5.3</td>
<td>52.9±8.1</td>
<td>61.2±7.0</td>
<td>74.2±6.7</td>
</tr>
<tr>
<td>[9,10-3H]palmitate, µmol/min</td>
<td>240–270 min</td>
<td>94.8±8.1</td>
<td>160.0±19.2</td>
<td>148.1±12.9</td>
</tr>
<tr>
<td>II &gt; I*(P = 0.07); III &gt; I*; IV &gt; II*; IV &gt; III*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis of AUC during baseline and stimulation. FFA, free fatty acids. *P < 0.05.

Fig. 2. A: serum concentration free fatty acids (FFA) during exposures. II > I*, III > I*, IV > III and II* and IV > I*. B: serum glycerol during exposures. II > I (P = 0.07), III > I* IV > III and II* and IV > I*. *P < 0.05.

Plasma palmitate concentration and SA were determined by HPLC (31) using [3H2]palmitate as internal standard (24). Systemic palmitate flux (µmol/min) was calculated using the [9,10-3H]palmitate infusion rate (dpm/min) divided by the steady-state palmitate SA (dpm/µmol).

The bolus administration of GH induces a non-steady-state appearance of FFA. To account for this, we analyzed FFA and detected stable FFA concentrations at 240–270 min. Further analysis of palmitate SA confirmed that palmitate SA was stable throughout this period during the four treatments (see Fig. 4, inset). Linear regression showed that the slope of the curves did not differ from zero in any of the treatments (I, P = 0.86; II, P = 0.37; III, P = 0.71; and IV, P = 0.38). Likewise, no difference was observed between treatments (P = 0.30). On this basis, we applied steady-state equations for subsequent calculation of palmitate fluxes.

Skeletal muscle blood flow. Skeletal muscle blood flow (SMBF) was assessed by plethysmography applied to the thigh contralateral to the microdialysis probes. A cuff was inflated to 300 mmHg at the level of the patella to measure flow exclusively in the thigh. Although this method includes skin and bone as well as muscle tissue, the majority of the tissue perfused is skeletal muscle. Measurements were performed at t = 0, 120, 240, and 360 min in duplicate.

Adipose tissue blood flow. We have previously assessed the impact of similar GH and cortisol exposures on adipose tissue blood flow (ATBF) with the xenon washout technique (10, 17). Here, we did not detect any changes in ATBF after GH or cortisol administration. We therefore assume that coadministration of GH and cortisol does not influence ATBF.

Assays. Plasma glucose was analyzed in duplicate using the glucose oxidase method (Beckman Coulter, Palo Alto, CA). Measurements were performed immediately to determine the isotopic glucose infusion rate.

Serum GH was analyzed with a double monoclonal immunofluorometric assay (Delfia, Wallac Oy, Turku, Finland). Serum C-peptide and insulin were measured with an immunonanassay (DAKO, Glostrup, Denmark). Plasma glucagon was measured by an in-house radioimmunoassay modified from that in Ref. 35. Serum cortisol was measured with a solid-phase, time-resolved fluoroimmunoreassay (Delfia). Serum FFA was determined by a colorimetric method employing a commercial kit (Wako Pure Chemical Industries, Neuss, Germany). Blood levels of glycerol were assayed with an automated fluoroimmetric method (27). Epinephrine and norepinephrine were measured by HPLC (6). Glycerol in the microdialysis dialysate was measured in duplicate by an automated spectrophotometric kinetic enzyme analyzer (CMA-600).

Statistical analysis. Results are expressed as time-averaged values during baseline (t = −150 to 0) and the stimulated period (t = 0–360 min). Statistical difference is based on area under the curve (AUC)0–360min. Because of the difference in the administration of GH (bolus) and hydrocortisone succinate (infusion), subanalysis based on AUC was performed in the intervals 0–150 and 240–360 min where stated. AUC was calculated using ICU/PI version 1.0 (CM) using the trapezoid method. Statistical analysis was performed using SPSS for Windows version 11.0 (SPSS, Chicago, IL). Normality of the data was tested with the Kolmogorov-Smirnov test of normal distribution. Where P > 0.20, the data were considered to be normally distributed.

Equality among the four treatments was assessed by repeated measures or Friedman’s test for k-related samples where appropriate. Between-treatment differences were assessed by Student’s t-test for
related samples or Wilcoxon signed rank test (for related samples). \( P \) values < 0.05 were considered significant.

Parametric data are presented as means ± SE and nonparametric data as medians [min;max].

RESULTS

Circulating hormones. Infusion of hydrocortisone succinate resulted in steady-state cortisol levels of II: 829 ± 118 vs. IV: 907 ± 56 nmol/l, [not significant (NS)]. During GH bolus infusion, GH levels rose to peak values of III: 7.91 ± 1.59 vs. IV: 9.80 ± 1.21 μg/l at \( t = 30 \) min (NS) (Fig. 1).

No differences were detected in circulating levels of insulin, glucagon, and C-peptide in I vs. II, III, or IV, with the exception of C-peptide (see Table 1) in III being higher (III: 60[0;350] pmol/l, \( P < 0.05 \), vs. I: 56[0;342], II: 10[0;97], and IV: 50[0;157] pmol/l). The insulin levels did not differ (\( P = 0.48 \)).

Circulating metabolites. Serum FFA rose to the same magnitude and within the same time frame as seen previously with GH (17) and hydrocortisone (10), with a peak value of III: 0.748 ± 0.07 mmol/l after 150 min and II: 0.779 ± 0.131 mmol/l at \( t = 360 \) min (Fig. 2 and Table 2). The combined administration of GH and hydrocortisone succinate resulted in a maximal value of 0.906 ± 0.08 mmol/l at \( t = 360 \) min, being higher than both II and III (\( P < 0.05 \)).

Serum glycerol revealed corresponding changes.

Interstitial glycerol concentrations. Interstitial skeletal muscle glycerol (ISMG) showed changes mimicking the ones observed in serum (Fig. 3 and Table 3). There was no difference between the two insertion sites (10 and 14 cm above patella), and for this reason combined results are presented. At baseline, the ISMG was ~50% higher than serum glycerol (68 ± 6 vs. 42 ± 4 μmol/l, \( P < 0.01 \)). During the entire duration of the experiment, ISMG declined to a level of 34 ± 4 μmol/l at \( t = 360 \) min. During hydrocortisone infusion (II), the decline was dampened significantly, with levels at \( t = 360 \) min of 50 ± 3 μmol/l (\( P < 0.01 \)). GH administration resulted in a glycerol surge with peak values at \( t = 150 \) min of 63 ± 7 μmol/l, being significantly different from placebo in the interval 0–150 min (\( P < 0.01 \)). The combined effect of GH and hydrocortisone (IV) resulted in peak levels at \( t = 150 \) min of 79 ± 9 μmol/l, whereas the plateau at \( t = 360 \) min was 64 ± 7 μmol/l, both being respectively different from III and II, when the intervals 0–150 min (\( P < 0.01 \)) and 240–360 min (\( P = 0.11 \)) were analyzed, although the latter did not reach statistical significance.

We did not observe any disruptions of the microdialysis membranes, probably because we studied resting muscle.

Results in interstitial subcutaneous adipose tissue measurements were blemished with an error when relative recovery was corrected for. When the uncorrected values were analyzed, similar changes were seen as in serum or interstitial muscle.

We tested the dialysate for autoscintillation, which was not found, and, since the uncorrected interstitial adipose tissue values were more trustworthy, we suspect that quenching introduced the error. Because of the limited amount of dialysate (~30 μl), we were not able to test another scintillation fluid. Because recovery increases over time (Fig. 3A), we present the uncorrected values corrected for mean adipose tissue recovery during the intervals ~90–0, 0–150, and 240–360 min in Table 3.
The relative recoveries, measured as $C_{\text{perfsate}} - C_{\text{dialysate}} / C_{\text{perfsate}}$ [C being counts per minute (cpm) for [2-$^3$H]glycerol], were comparable to the ones obtained previously with a flow rate of 1 $\mu$l/min (39) (Fig. 3A).

**Palmitate metabolism.** Palmitate fluxes increased due to both GH and hydrocortisone administration ($P < 0.05$ vs. placebo; Fig. 4). No difference was detected between GH and hydrocortisone fluxes, and both fluxes were significantly lower than the palmitate fluxes observed in the combined administration of GH ($P < 0.01$) and hydrocortisone ($P < 0.05$).

The interindividual coefficients of variation (CV) of SA during this interval were I, 9.2; II, 8.0; III, 6.3; and IV, 5.8%. No difference was found between them ($P = 0.76$). The magnitudes of these CVs are below those obtained under very controlled circumstances (30).

**Glucose, glucose infusion rate, and blood flow.** Plasma glucose tended to increase upon institution of the pancreatic clamp and tended to be higher in III (120 ± 11 mg/dl) and IV (110 ± 9 mg/dl) than in I (93 ± 11 mg/dl) and II (94 ± 6 mg/dl); however, no statistical difference was observed. The amount of glucose needed to maintain plasma glucose above 80 mg/dl was very modest and identical in either of the study arms ($P = 0.75$).

SMBF did not change over time during the experiments, and no difference was observed between treatments. The average blood flow was 3.2 ± 0.5 ml·100 ml tissue$^{-1}$·min$^{-1}$.

**Additive effects of GH and cortisol on lipolysis.** Any additive effects (E) of GH and cortisol on lipolysis can be evaluated by comparing the theoretically deduced additive product with the observed

$$(E_{\text{cortisol + GH}}) - E_{\text{placebo}} = E_{\text{cortisol+GH}}$$

When these theoretically deduced additive parameters for FFA $R_{{fas}}$, systemic FFA, serum glycerol, ISMG, and femoral/abdominal interstitial glycerol were compared with the observed findings during IV (Fig. 5), we found no difference, indicating that cortisol potentiates GH-induced lipolysis.

**DISCUSSION**

The aim of the present study was to assess acute lipolytic actions of GH and cortisol at the whole body level and in subcutaneous adipose tissue and skeletal muscle, since these hormones are cosecreted during fasting (3, 33), exercise (11, 12, 26, 29), and other kinds of perceived stress (43).

In line with our previous studies involving GH and cortisol, we observed an increase in the circulating lipolytic parameters FFA and glycerol upon stimulation. Interstitial adipose tissue lipolysis, although technical difficulties prevailed, also showed similar results to those found previously. The novelty of this study clearly lies in the additive effects of the two hormones.

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**Table 3. Time-averaged values during baseline and stimulation**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>I Placebo</th>
<th>II Hydrocortisone</th>
<th>III GH Pulse</th>
<th>IV GH Pulse + Hydrocortisone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal muscle</td>
<td>61±8</td>
<td>66±6</td>
<td>65±11</td>
<td>65±10</td>
</tr>
<tr>
<td>$\text{AUC}_{0-150\text{min}}$</td>
<td>43±6</td>
<td>47±6</td>
<td>61±7*</td>
<td>71±9*</td>
</tr>
<tr>
<td></td>
<td>III &gt; I*·IV &gt; I*·IV &gt; II*·IV &gt; III*</td>
<td>II &gt; I*·III &gt; I*·IV &gt; I*·IV &gt; II*·IV &gt; III*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{AUC}_{240-360\text{min}}$</td>
<td>35±4</td>
<td>50±3</td>
<td>47±6*</td>
<td>65±8*</td>
</tr>
<tr>
<td>sc Abdominal adipose tissue</td>
<td>237±31</td>
<td>214±48</td>
<td>230±32</td>
<td>278±30</td>
</tr>
<tr>
<td>$\text{AUC}_{0-150\text{min}}$</td>
<td>197±17</td>
<td>191±47</td>
<td>255±24</td>
<td>313±41</td>
</tr>
<tr>
<td></td>
<td>III &gt; I (P = 0.06)·IV &gt; I (P = 0.07)</td>
<td>II &gt; I*·IV &gt; I*·IV &gt; III*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{AUC}_{240-360\text{min}}$</td>
<td>194±13</td>
<td>233±49*</td>
<td>231±22</td>
<td>363±41*</td>
</tr>
<tr>
<td>sc Femoral adipose tissue</td>
<td>244±43</td>
<td>189±25</td>
<td>264±28</td>
<td>214±27</td>
</tr>
<tr>
<td>$\text{AUC}_{0-150\text{min}}$</td>
<td>180±31</td>
<td>180±27</td>
<td>248±30</td>
<td>244±22</td>
</tr>
<tr>
<td></td>
<td>III &gt; I (P = 0.06)·IV &gt; I (P = 0.07)</td>
<td>II &gt; I*·IV &gt; I*·IV &gt; III*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{AUC}_{240-360\text{min}}$</td>
<td>138±16</td>
<td>202±35*</td>
<td>197±26</td>
<td>265±31*</td>
</tr>
</tbody>
</table>

*Values are means ± SE. Baseline, t = 0; stimulation, t = 150 and 240–360 min (paired analysis of treatments). Analysis of interstitial glycerol uncorrected for recovery is performed for subcutaneous (sc) parameters. Average time-related recovery correction is made for the time-averaged values. *P < 0.05.

Fig. 4. Systemic palmitate fluxes at t = 240–270 min. IV > III, and II > I, $P < 0.05$. *Inset: palmitate specific activities during the 4 treatment arms in the interval 240–270 min (see text for statistics).
As early as the 1960s, Fain and coworkers (14, 15) showed additive lipolytic effects of GH coincubated with DEX after 2 h. More recently, this has been described by Yip and Goodman (44), further emphasizing the possible additive mode of action.

As of now, the exact mechanisms of the lipolytic properties of GH and cortisol are not known. It has been the general perception that GH enhances the effects of catecholamines, and the aforementioned in vitro studies have to some extent confirmed this.

Recently, we (20) have shown that GH-mediated lipolysis is maximally induced at a dose of 3 μg/kg. This dose is comparable to the dose chosen in the present study. Our finding that markers of lipolysis appear to be increased in an additive manner during combined stimulation with GH and cortisol suggests that the mechanisms of action for these two hormones are via two distinct pathways. This corresponds favorably with the findings of Yip and Goodman (44), wherein GH and DEX reduced the inhibitory G-protein α₂-receptors, thereby increasing sensitivity to adrenergic β-receptor-mediated lipolysis. Whether the isolated stimulations of GH or cortisol have any effect on the α₂-receptors was not addressed. In the same study, selective blockade of cAMP production reduced the lipolytic potential of GH plus DEX, indicating that the lipolytic pathways involved are mediated by increased cAMP production. The authors, however, found that lipolysis induced by GH or DEX given alone was unaffected by inhibition of cAMP production. These results are difficult to reconcile but may indicate either that GH and DEX (cortisol) work via more than one pathway or that only combined GH plus DEX increases cAMP production, whereas when given independently they work via different pathways.

Intramuscular TAG breakdown has been suggested to be an important source of fuel in skeletal muscle. Increased interstitial glycerol has been interpreted by many authors as a marker of intramuscular lipolysis (13, 18, 39). However, the presence of glycerol kinase in skeletal muscle, enabling the use of glycerol derived from TAG to undergo reacylation, rather than glucose, may distort results. Until recently, there has been no indication of its presence. Coppock et al. (8) and Jensen et al. (23) have, however, challenged this conception. In addition, glycerol observed in interstitial skeletal muscle could be interpreted as glycerol derived from the general circulation, although this seems unlikely in our study because the ratio of serum glycerol to interstitial muscle glycerol was not constant even though no change in skeletal muscle blood flow was observed. Furthermore, the concentrations observed in interstitial muscle glycerol were ~50% higher during baseline than those in serum glycerol, a finding hardly explained by assay differences.

We did not observe any changes in glucose infusion in either of the arms, indicating that no, or very minute, insulin resistance was instituted with the short duration of these stimulations, in line with previous observations (38). This corresponds to previous findings with GH (32) and cortisol (10), whereas prolonged exposure to both hormones is known to generate insulin resistance. Clearly, the exact pathways through which these two key metabolic hormones influence lipid metabolism during normal physiological excursions deserves further exploration. The finding that GH causes translocation of perilipin in rat adipocytes (7), and therefore renders the lipid droplet more accessible to HSL, could very well be an important regulatory mechanism.

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