The Effect of Recombinant Human Growth Hormone on Glucose and Leucine Metabolism in Cushing’s Syndrome*

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ABSTRACT

Cushing’s syndrome is characterized by central obesity and muscle wasting. As GH is anabolic, it may be able to counteract the loss of body protein. To evaluate the potential therapeutic use of GH preoperatively, eight patients with Cushing’s syndrome received sc injections of recombinant human GH (0.07 U/kg/day) for 7 days. Whole body leucine and glucose turnover were measured after an infusion of [1-13C]leucine and [6,6-2H2]glucose before (day 0) and after 2 and 7 days of GH treatment. Compared with the value on day 0, there was a significant increase on days 2 and 7 in insulin (P < 0.005 and P < 0.001), C peptide (P < 0.01 and P < 0.005), insulin-like growth factor I (P < 0.001), and glucose concentrations (P < 0.01 and P < 0.005) and a decrease in the leucine concentration (P < 0.005). There was no significant change in glucose production rate, glucose MCR, leucine production rate (a measure of protein degradation), or nonoxidative leucine disappearance rate (a measure of protein synthesis). The leucine MCR was increased after 7 days (P < 0.05), and the clearance of leucine into protein (nonoxidative leucine disappearance rate/leucine concentration) was increased (P < 0.05) after 2 and 7 days of GH treatment. This is consistent with GH stimulating the availability of amino acid transporters. GH may, therefore, have a therapeutic role in the preoperative treatment of Cushing’s syndrome. (J Clin Endocrinol Metab 82: 243–246, 1997)

THE CHRONIC glucocorticoid excess of Cushing’s syndrome is characterized by profoundly altered body composition. Muscle weakness (1) and reduced muscle mass (2) are common features and appear to be due to a reduction in whole body protein synthesis compared with that in normal subjects (3), whereas fat mass is increased (2).

Recent studies have shown that GH has an important role in regulating protein metabolism. GH-deficient adults treated with GH had increased lean body mass, due to stimulation of protein synthesis, and decreased fat mass (4). GH has also been shown to counteract the net catabolic effects on protein metabolism of short term excess glucocorticoids in normal subjects (5) and patients receiving chronic glucocorticoid treatment (6). There is thus some rationale for the possible use of exogenous GH to counteract the net catabolic effect of excess endogenous steroids in Cushing’s syndrome. However, as GH has been shown to decrease the insulin sensitivity of glucose metabolism (7), any beneficial effect of GH on protein anabolism may be offset by an increase in insulin resistance. To examine this further, we undertook isotopic studies to investigate the effect of short term GH administration to patients with Cushing’s syndrome on protein and glucose metabolism.

Subjects and Methods

Experimental subjects

Studies were performed in eight patients (aged 30–63 yr) 1 week before surgery for Cushing’s syndrome (Table 1). The diagnosis was established by the measurement of 0900 and 2400 h cortisol concentrations, the 0900 h cortisol level after the administration of 1 mg oral dexamethasone at 2300 h the previous day, cortisol secretion rate, and low and high dose (2 and 6 mg) dexamethasone suppression tests. In patients with pituitary-dependent Cushing’s syndrome, all other pituitary function was normal. All patients gave informed written consent, and the studies were approved by St. Thomas’s Hospital ethics committee.

Study design

Patients received sc injections of GH (0.07 U/kg-day) for 7 days and were studied at 0900 h after an overnight fast before treatment and then after 2 and 7 days of treatment. This dose of GH has previously been shown to increase protein synthesis in GH-deficient adults (4) and was chosen because Cushing’s syndrome patients were likely to be GH resistant. One patient (no. 3) received GH for only 2 days due to fluid retention, and, therefore, was studied before and after 2 days of treatment. All studies were performed in the metabolic research area of the Diabetes and Endocrinology Day Center at St. Thomas’ Hospital. A primed constant infusion of [1-13C]leucine (1 mg/kg; 1 mg/kg/h) and [6,6-2H2]glucose (170 mg; 102 mg/h; Mass Trace, Somerville, NJ) was administered iv for 180 min with a priming dose of sodium [13C]bicarbonate (0.2 mg/kg; mass trace). Blood and breath samples were taken at 0, 150, 155, 160, 165, 170, and 180 min to measure the isotopic enrichment and concentration of glucose, α-ketoisocaproic acid (αKIC) enrichment, leucine concentration, and enrichment of expired CO2. Baseline blood samples were also taken for the measurement of insulin, insulin-like growth factor I (IGF-I), and C peptide concentrations.
TABLE 1. Clinical details of patients

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>BW (kg)</th>
<th>BMI</th>
<th>LBM (kg)</th>
<th>CSR (μmol/day)</th>
<th>Diagnosis</th>
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<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>33</td>
<td>73.3</td>
<td>23.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>38</td>
<td>67.0</td>
<td>24.6</td>
<td>—</td>
<td>36.9</td>
<td>293 Pituitary dependent</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>63</td>
<td>73.5</td>
<td>27.8</td>
<td>—</td>
<td>46.8</td>
<td>109 Adrenal adenoma</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>49</td>
<td>91.0</td>
<td>34.0</td>
<td>—</td>
<td>50.6</td>
<td>909 Pituitary dependent</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>55</td>
<td>70.0</td>
<td>26.3</td>
<td>—</td>
<td>44.8</td>
<td>214 Pituitary dependent</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>39</td>
<td>61.0</td>
<td>24.1</td>
<td>—</td>
<td>33.8</td>
<td>117 Adrenal adenoma</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>31</td>
<td>48.0</td>
<td>20.9</td>
<td>—</td>
<td>41.6</td>
<td>85 Adrenal adenoma</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>30</td>
<td>68.9</td>
<td>25.5</td>
<td>—</td>
<td>42.4</td>
<td>260 Pituitary dependent</td>
</tr>
</tbody>
</table>

Mean ± SEM

<p>| | | | | | | | |</p>
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</thead>
<tbody>
<tr>
<td>42</td>
<td>69.2</td>
<td>25.5</td>
<td>42.4</td>
<td>260</td>
<td></td>
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<td></td>
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</tbody>
</table>

CSR, Cortisol secretion rate (normal, <83 μmol/day); —, data not available for this patient.

Analytical methods

Using gas chromatography-mass spectrometry (5971A MSD, Hewlett-Packard, Palo Alto, CA) and selected ion monitoring, oKIC enrichment was measured as the quinoxalinol-trimethylsilyl derivative at m/z 232 and 233 (8), and glucose enrichment was measured as the glucose acetate boronated derivative at m/z 297 and 299 (9) (within-assay coefficient of variation (CV) for both measurements, <2%). 13C enrichment of breath CO2 was measured on a VG SIRA series II isotope ratio mass spectrometer (VG Isotech, Cheshire, UK; within-assay CV, <1%). The CO2 production rate was measured at 0, 150, and 180 min with a Metabolic Measurement Cart (Medical Graphics Corp., Minneapolis, MN). Plasma leucine concentration was measured using an Alpha Plus II automated amino acid analyzer (within-assay CV, 4%; Pharmacia, Cambridge, UK). Insulin and C peptide were measured by double antibody RIA (within-assay CV, 6% and 9%, respectively) (10).

Measurements of leucine and glucose metabolism were calculated using standard isotope dilution equations for steady state conditions (13). The leucine production rate (Ra; a measure of protein degradation) was calculated using oKIC enrichment rather than plasma leucine enrichment, as it has been shown to give a better estimate of the intracellular enrichment of leucine (14). Leucine and glucose MCR were calculated as: MCR = Rd/plasma concentration, where Rd (rate of disappearance) = Ra, since subjects were studied in steady state.

The nonoxidative leucine disappearance rate (NOLD; a measure of protein synthesis) was calculated as the difference between the leucine production rate and the oxidation rate. The relative proportion of leucine cleared into the oxidative pathway and the protein synthetic pathway was determined by dividing the relevant flux by the plasma leucine concentration.

Results

GH treatment was tolerated by all patients except patient 3, in whom treatment was stopped due to fluid retention. Measurements of plasma IGF-I, insulin, C peptide, and glucose concentrations are shown in Fig. 1. Before treatment, the fasting total IGF-I concentration was within the normal range in all patients (31–40 yr old, 12.8–62.2 nmol/L; 41–60 yr old, 11.0–43.9 nmol/L; >60 yr old, 3.7–32.9 nmol/L).

Insulin, C peptide, and IGF-I were significantly increased after 2 days (P < 0.005, P < 0.01, and P < 0.001) and 7 days (P < 0.001, P < 0.005, and P < 0.001, respectively) of GH treatment (Fig. 1). The side-effects of GH treatment in patient 3 could not be explained in terms of increased sensitivity to GH, as IGF-I levels were similar to those in the other patients after 2 days of GH treatment. Mean plasma glucose concentrations significantly increased after GH treatment for 2 days (P < 0.01) and 7 days (P < 0.005; Fig. 1 and Table 2), and although the mean glucose Ra increased after 7 days of GH treatment, this failed to reach statistical significance. There was no significant change in the glucose MCR, although it fell after 7 days in five of the six patients.

Measurements of leucine metabolism are shown in Table 2. The mean leucine concentration significantly decreased (P < 0.005) after 2 and 7 days of GH treatment by 26% and 27%, respectively. The leucine MCR increased by 30% and 38% after 2 and 7 days. There was no significant change in leucine Ra, leucine oxidation, or NOLD, but the metabolic clearance of leucine attributable to protein synthesis (NOLD/leucine) was increased by 31% and 41% (P < 0.05) after 2 and 7 days of GH treatment.

Discussion

This study demonstrated for the first time that GH may be able to counteract the catabolic action of excess endogenous glucocorticoids in patients with Cushing’s syndrome.

Before treatment of the patients with GH, IGF-I levels were within the age-matched normal range, as reported in a previous study (15). Although short term glucocorticoid administration has been shown to stimulate GH secretion, long term glucocorticoid excess has been shown to inhibit spontaneous GH secretion (16) and GH secretion in response to GHRH (17). Hepatic production of IGF-I is dependent on both GH and insulin (18, 19); thus, the diabetogenic action of glucocorticoids with concomitant increased insulin secretion may explain why IGF-I levels remain within the normal range in Cushing’s syndrome in the face of reduced GH secretion. In the present study short term GH administration increased IGF-I significantly, and after 7 days, IGF-I concentrations were just outside the normal range in three of the patients.

There was an increase in fasting glucose, insulin, and C peptide in all patients in response to GH treatment. There was a trend for an increase in glucose Ra and a reduction in glucose MCR, but these did not reach statistical significance;
However, they may account for the increase in plasma glucose observed. These effects are in keeping with the known effects of GH on glucose metabolism (20).

The concentration of amino acids, in particular the branched chain amino acids, has been shown to be important in the control of protein synthesis both in vivo (21) and in vitro (22). An acute infusion of IGF-I in man (23) and dogs (24) has been shown to result in a marked decrease in amino acid concentrations due to a decrease in proteolysis, but these studies were unable to demonstrate an effect of IGF-I on protein synthesis. However, when an amino acid clamp technique was used to prevent a decrease in amino acid concentrations, IGF-I was shown to increase protein synthesis (25). In the present study the fasting leucine concentration decreased due to an increase in leucine metabolic clearance in response to GH treatment. Despite the marked fall in the leucine concentration, the rate of protein synthesis was maintained. It is possible that the decrease in the leucine concentration may be due to a change in leucine kinetics in the postprandial state. When the fraction of metabolic clearance of leucine from plasma attributable to pro-

**FIG. 1.** Plasma IGF-I, insulin, C peptide, and glucose concentrations in patients with Cushing’s syndrome before (0) and after 2 and 7 days of GH administration. +, $P < 0.05$; ++, $P < 0.005$; ***, $P < 0.001$ (significantly different from day 0).

**TABLE 2.** Measurements of glucose and leucine metabolism in patients with Cushing’s syndrome after 0, 2, and 7 days of GH treatment

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 2</th>
<th>Day 7$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>6.8 ± 1.0</td>
<td>7.8 ± 1.2$^b$</td>
<td>8.5 ± 1.6$^c$</td>
</tr>
<tr>
<td>Glucose MCR (mL/min · kg)</td>
<td>1.97 ± 0.28</td>
<td>1.75 ± 0.23</td>
<td>1.76 ± 0.17</td>
</tr>
<tr>
<td>Glucose Ra (μmol/min · kg)</td>
<td>13.89 ± 2.75</td>
<td>13.09 ± 1.16</td>
<td>14.36 ± 153</td>
</tr>
<tr>
<td>Leucine (μmol/L)</td>
<td>121 ± 10</td>
<td>90 ± 8$^a$</td>
<td>88 ± 7$^a$</td>
</tr>
<tr>
<td>Leucine Ra (μmol/min · kg)</td>
<td>1.82 ± 0.14</td>
<td>1.81 ± 0.14</td>
<td>1.84 ± 0.09</td>
</tr>
<tr>
<td>Leucine MCR (mL/min · kg)</td>
<td>15.90 ± 2.06</td>
<td>20.60 ± 1.69</td>
<td>21.96 ± 257$^d$</td>
</tr>
<tr>
<td>Leucine OX (μmol/min · kg)</td>
<td>0.28 ± 0.03</td>
<td>0.26 ± 0.03</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>Leu OX/Leu</td>
<td>2.38 ± 0.26</td>
<td>2.90 ± 0.33</td>
<td>2.88 ± 0.37</td>
</tr>
<tr>
<td>NOLD (μmol/min · kg)</td>
<td>1.54 ± 0.14</td>
<td>1.54 ± 0.14</td>
<td>1.60 ± 0.09</td>
</tr>
<tr>
<td>NOLD/Leu</td>
<td>13.52 ± 1.95</td>
<td>17.70 ± 1.92$^d$</td>
<td>19.08 ± 233$^d$</td>
</tr>
</tbody>
</table>

$^a$ n = 7, due to withdrawal of patient 3 from the study.
$^b$ Significantly different from day 0, $P < 0.01$.
$^c$ Significantly different from day 0, $P < 0.005$.
$^d$ Significantly different from day 0, $P < 0.05$. 

However, when an amino acid clamp technique was used to prevent a decrease in amino acid concentrations, IGF-I was shown to increase protein synthesis (25). In the present study the fasting leucine concentration decreased due to an increase in leucine metabolic clearance in response to GH treatment. Despite the marked fall in the leucine concentration, the rate of protein synthesis was maintained. It is possible that the decrease in the leucine concentration may be due to a change in leucine kinetics in the postprandial state. When the fraction of metabolic clearance of leucine from plasma attributable to pro-
tein synthesis was examined, this was found to markedly increase after GH administration. This rise in leucine metabolic clearance demonstrated that GH has an anabolic effect on protein metabolism in these patients mediated through increased efficiency in substrate extraction, possibly indicating an increase in amino acid transport. Inoue and co-workers (26) recently reported a stimulatory effect of GH on amino acid uptake from the human ileum and jejunum consistent with an increase in the number of functional carriers in the brush-border membrane. The increase in leucine MCR observed in the present study may be in keeping with this effect of GH stimulating the availability of amino acid transporters. This might indicate that GH has a role in regulating amino acid transporters, analogous to that of insulin with glucose transporters. These anabolic effects are probably mediated by GH rather than the concomitant rise in insulin, as previous studies have shown insulin to reduce whole body protein breakdown, with no effect on whole body protein synthesis (27, 28).

It has previously been demonstrated that GH has powerful protein anabolic effects in normal subjects and GH-deficient adults (4, 29). GH has been shown to promote positive nitrogen balance in postoperative patients (30), burn and trauma patients (31, 32), and patients with acquired immuno- deficiency syndrome (33). There have been no previous studies to determine the effects of GH treatment in patients with Cushing’s syndrome. However, in patients receiving chronic glucocorticoid treatment, GH treatment for 7 days has been shown to increase nitrogen balance (34) and protein synthesis (6), whereas in normal volunteers, GH has been shown to counteract the net catabolic effects of excess glucocorticoids on protein metabolism (5).

This study demonstrated that preoperative GH treatment of patients with Cushing’s syndrome decreased amino acid concentrations and increased the MCR of leucine. The increased clearance of leucine into the protein synthetic pathway provides evidence that GH treatment warrants further investigation as a possible therapeutic agent in Cushing’s syndrome. However, GH treatment was also associated with insulin resistance, as shown by the increases in glucose, insulin, and C peptide concentrations.

Acknowledgments

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References