Effects of GH on urea, glucose and lipid metabolism, and insulin sensitivity during fasting in GH-deficient patients

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Nørrelund, Helene, Christian Djurhuus, Jens Otto Lunde Jørgensen, Søren Nielsen, K. Sreekumar Nair, Ole Schmitz, Jens Sandahl Christiansen, and Niels Møller. Effects of GH on urea, glucose and lipid metabolism, and insulin sensitivity during fasting in GH-deficient patients. Am J Physiol Endocrinol Metab 285: E737–E743, 2003. First published June 10, 2003; 10.1152/ajpendo.00092.2003.—Fasting-related states of distress pose major health problems, and growth hormone (GH) plays a key role in this context. The present study was designed to assess the effects of GH on substrate metabolism and insulin sensitivity during short-term fasting. Six GH-deficient adults underwent 42.5 h of fasting on two occasions, with and without concomitant GH replacement. Palmitate and urea fluxes were measured with the steady-state isotope dilution technique after infusion of [9,10-3H]palmitate and [13C]urea. During fasting with GH replacement, palmitate concentrations and fluxes increased by 50% [palmitate: 378 ± 42 (GH) vs. 244 ± 12 μmol/l, P < 0.05; palmitate: 412 ± 58 (GH) vs. 276 ± 42 μM, P = 0.05], and urea turnover and excretion decreased by 30–35% [urea rate of appearance: 336 ± 22 (GH) vs. 439 ± 43 μmol·kg−1·h−1, P < 0.01; urea excretion: 445 ± 43 (GH) vs. 602 ± 74 mmol/24 h, P < 0.05]. Insulin sensitivity (determined by a euglycemic hyperinsulinemic clamp) was significantly decreased [M value: 1.26 ± 0.06 (GH) vs. 2.07 ± 0.22 mg·kg−1·min−1, P < 0.01] during fasting with GH replacement. In conclusion, continued GH replacement during fasting in GH-deficient adults decreases insulin sensitivity, increases lipid utilization, and conserves protein.

fasting; growth hormone; substrate metabolism

WORLDWIDE ILLNESSES related to fasting continue to pose enormous health problems. The disease states include hunger, malnutrition, and wasting syndromes associated with chronic diseases such as acquired immunodeficiency syndrome.

During fasting and stress, growth hormone (GH) secretion is augmented, and these conditions may be viewed as the natural metabolic domain for GH action. A vast number of investigations have coherently demonstrated that GH exerts important metabolic actions in humans, and several studies have shown that GH postabsorptively antagonizes the effects of insulin on glucose and lipid metabolism, although the underlying mechanisms remain to be fully understood. After an overnight fast, physiological elevations in plasma GH concentrations in humans cause resistance to the actions of insulin on glucose metabolism both at the hepatic site and peripherally (4, 34), together with increased (or inadequately suppressed) lipid oxidation.

Studies of the effect of GH on substrate metabolism during more prolonged fasting are, however, sparse. In fasted dogs, administration of GH was associated with an increase in plasma insulin levels and unchanged glucose turnover but resistance to the effects of administered insulin (33). Hypoglycemia during fasting is a common occurrence in untreated, GH-deficient children (17), and hypopituitary children have been shown to have decreased fasting glucose production and utilization (3).

In a controlled study of the metabolic impact of GH during fasting, we (27) observed in GH-deficient subjects increased whole body protein synthesis, increased lipid oxidation, unaltered glucose turnover and insulin levels, together with a significant increase in fasting glucose during GH replacement. However, direct measurements of urea turnover, lipid turnover, or insulin sensitivity (hyperinsulinemic euglycemic clamp) were not performed in that study. The finding of unchanged glucose turnover and oxidation rates, despite a substantial increase in glucose level during GH replacement, suggests that GH leads to a relative decrease in glucose oxidation. The ability to spare glucose from oxidation during fasting is potentially important, because it prevents a rapid decline in glucose levels and reduces the need for gluconeogenic precursors from muscle protein.

In the present study, we used GH deficiency as a model to define the physiological role of GH in the regulation of metabolism during fasting. In particular, we focused on the effects of GH on turnover rates of urea, free fatty acids (FFA), and glucose and on insulin sensitivity. The patients underwent 42.5 h of fasting on
two occasions, with and without concomitant GH replacement, and the methods employed included isotopic determination of whole body glucose, urea, and palmitate fluxes in the fasting state and subsequent measurements of insulin sensitivity by the glucose clamp technique.

**METHODS**

**Subjects.** Six hypopituitary GH-deficient adults (1 female and 5 males) with a mean age of 44.7 ± 5.9 yr and a mean body mass index of 31.1 ± 2.9 kg/m² participated. All patients had been on stable replacement therapy, including GH (1.5 ± 0.2 IU/day), for ≥1 yr and remained so before, between, and after the studies. The patients had severe GH deficiency, defined by a peak GH response to hypoglycemia and arginine of <3 μg/l. All patients had multiple pituitary hormone deficiencies and were receiving hormone replacement (thyroid, adrenal, and gonadal steroid [testosterone] therapy) when appropriate. None of the patients had acromegaly, Cushing’s disease, or diabetes. All patients gave informed consent to participate in the study, which had been approved by the Ethics Committee of Aarhus County.

The patients were each studied twice during 42.5 h of fasting with I) GH replacement (Norditropin; Novo Nordisk, Copenhagen, Denmark), partly as subcutaneous injections [after 12 h (0.7 ± 0.1 IU) and after 26 h (2.2 ± 0.3 IU)] of fasting) and partly as a continuous intravenous infusion (0.005 IU·kg⁻¹·h⁻¹ during the last 6.5 h, or 2) discontinuation of the regular evening GH injections, respectively (Fig. 1). The two studies were done in random order. During fasting, only tap water was allowed.

**Measurements.** Substrate metabolism was investigated during the last 6.5 h. After baseline blood sampling, a priming dose of [¹³C]urea (390.6 mg; Cambridge Isotopes Laboratories, Andover, MA) was given over 20 min to accomplish an early plateau and to minimize tracer loss in the urine, immediately followed by a continuous infusion of the tracer at 42 mg of [¹³C]urea/h for 4 h (15, 20). At 0900, a priming dose of [³H]glucose (20 μCi; NEN Life Science Products, Boston, MA) was given, followed by a continuous infusion of [³H]glucose (20 μCi/h) for 5.5 h. Infusion of [9,10-³H]palmitate (0.3 μCi/min; Laegemiddelstyrelsen, Bronshoj, DK) was started at 1100 and maintained for 1 h. Enrichment of plasma urea was measured in its bistrimethylsilyl derivative by gas chromatography-mass spectrometry (GC-MS) as previously described (15, 20). We used a Hewlett-Packard GC-MS and monitored signal intensities of mass-charge ratios 231.1 and 232.1 (M + 1). Whole body urea flux (Q_u) was calculated as

\[ Q_u = I[(E_i/E_p) - 1] \]

in which I is the rate of tracer infusion (μmol·kg⁻¹·h⁻¹) and E_i and E_p are enrichment of the tracer infused and plasma enrichment of the tracer at isotopic plateau, respectively. Isotopic plateau was observed from 120 to 240 min, indicating the occurrence of steady-state conditions. This was assessed on the basis of the observation that, when isotopic enrichment values of urea were plotted against time, the ensuing slopes were not different from zero.

The specific activity (SA) of tritiated glucose was assayed as previously described (24). Systemic palmitate flux was measured with the isotope dilution technique and steady-state equations. Blood samples for measurements of palmitate concentration and SA were drawn before the infusion and after 30, 45, and 60 min of the infusion period. Plasma palmitate concentration and SA were determined by HPLC (22) by use of [³H]palmitate as internal standard (21). Systemic palmitate flux was calculated using the [9,10-³H]palmitate infusion rate divided by the steady-state palmitate SA.

Plasma glucose was measured in duplicate immediately after sampling on a glucose analyzer (Beckman Coulter, Palo Alto, CA). A double monoclonal immunofluorometric assay (Delfia, Wallac, Turku, Finland) was used to measure serum GH. Plasma glucagon (30) and serum total and free insulin-like growth factor I (IGF-I) (13, 14) were measured by RIAs. Insulin was determined by a commercial enzyme-linked immunosorbent assay (DAKO, Glostrup, DK), and cortisol was measured with a solid-phase time-resolved fluorimunoasay (Delfia). FFA were determined by a colorimetric method employing a commercial kit (Wako Chemicals, Neuss, Germany), and glycerol, 3-hydroxybutyrate (3-OHB), lactate, and alanine were analyzed by automatic enzymatic assay (Laesegmiddelstyrelsen, Bronshoj, DK). On the basis of the rate of change of 3-OHB, lactate, and alanine, the amount of fatty acids was calculated and monitored as previously described (24).

**Blood sampling.** Blood samples for measurements of insulin sensitivity by the glucose clamp technique and systemic palmitate flux were drawn before the infusion and after 30, 45, and 60 min of the infusion period. Plasma palmitate concentration and SA were determined by HPLC (22) by use of [³H]palmitate as internal standard (21).

**Fig. 1.** Experimental design. See text for details.

![Fig. 1.](http://ajpendo.physiology.org/)
Table 1. Basal serum concentrations of hormones in 6 GH-deficient patients during fasting with and without GH replacement

<table>
<thead>
<tr>
<th>Hormone</th>
<th>With GH Replacement</th>
<th>Without GH Replacement</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dl</td>
<td>89.5 (66–124)</td>
<td>89.5 (67–231)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>209 ± 23</td>
<td>175 ± 24</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>GIP, pmol/l</td>
<td>5.5 ± 0.11</td>
<td>3.0 ± 0.11</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cortisol, nmol/l</td>
<td>175 ± 8</td>
<td>176 ± 16</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>59 ± 7</td>
<td>97 ± 12</td>
<td>0.01</td>
</tr>
<tr>
<td>Norepinephrine, pg/ml</td>
<td>259 ± 33</td>
<td>244 ± 17</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Data are means ± SE or median (interquartile range). GH, growth hormone; GIP, free GIP.

Indirect calorimetry (Deltatrac monitor; Datex Instrumentarium, Helsinki, Finland) was performed for 30 min at the end of the basal period and at the end of the clamp period, allowing measurements of energy expenditure (EE) and the respiratory exchange ratio (RER). The initial 5 min of calorimetry were used for acclimatization, and calculations were based on mean values of 25 1-min measurements. Net lipid and glucose oxidation rates (Rd, ox) were calculated from the above measurements, and protein oxidation rates were estimated from the urinary excretion of urea (12). Net nonoxidative glucose disposal (Rd nonox) was calculated by subtracting oxidative glucose disposal (Rd ox) from total glucose Rd measured isotopically. Basal hepatic glucose production (HGP) was calculated by dividing the [3-13C]glucose infusion rate by the steady-state plateau of [3-13C]glucose SA in plasma during the last 30 min of the basal tracer infusion period.

Euglycemic hyperinsulinemic glucose clamp. Insulin sensitivity was estimated by means of a hyperinsulinemic euglycemic clamp. From 1200–1430, a constant amount (0.6 nmol/kg⁻¹·min⁻¹) of insulin (Actrapid; Novo Nordisk, Copenhagen, DK) was infused. On the basis of the measurements every 5 min, plasma glucose was clamped at 5.0 mmol/l by infusion of variable rates of a 20% glucose solution. During the clamp, HGP was calculated by subtracting the amount of exogenous glucose necessary to maintain euglycemia (M value) from the isotopically determined overall appearance rate (r0) for glucose.

During administration of insulin and glucose, a nonsteady-state condition in plasma [3-13C]glucose SA exists. At high rates of glucose uptake, the classical model of Steele is known to produce negative estimates of HGP. When [3-13C]glucose is added to the variable exogenous glucose infusion, the plasma [3-13C]glucose SA was maintained constant.

Statistics. Data on hormones and metabolites are based on duplicate/triplicate measurements within the last 30 min of the basal and the clamp periods. Values are presented as means ± SE or as the median (interquartile range) if the variable was not normally distributed as tested by Kolmogorov-Smirnov. Differences in the total areas under the curves and data based on means of duplicate/triplicate measurements were analyzed by the Wilcoxon signed rank matched pairs test. A P value of <0.05 was considered significant.

RESULTS

Circulating hormones and metabolites. During the basal period, circulating levels of GH, total and free IGF-I, insulin, and C-peptide were significantly increased during fasting with GH replacement (Table 1). Glucagon and cortisol concentrations did not change during fasting with or without GH substitution. Epinephrine concentrations were increased during fasting without GH replacement, whereas norepinephrine concentrations remained comparable in the two settings.

Plasma concentrations of FFA, glycerol, and 3-OHB were significantly increased during fasting with GH (Table 2). Fasting without GH substitution was associated with increased plasma concentrations of lactate and alanine.

Glucose metabolism. Insulin-stimulated glucose uptake was significantly greater during fasting without GH replacement [M value: 1.26 ± 0.06 (GH) vs. 2.07 ± 0.22 mg·kg⁻¹·min⁻¹, P < 0.01; Fig. 2]; this was partly due to an increased rate of glucose oxidation [Rd ox: 0.49 ± 0.12 (GH) vs. 0.82 ± 0.12 mg·kg⁻¹·min⁻¹, P < 0.05]. The basal rate of endogenous glucose production (EGP) was similar in both situations [EGP: 1.41 ± 0.08 (GH) vs. 1.39 ± 0.1 mg·kg⁻¹·min⁻¹, P > 0.05], although EGP was slightly more suppressed by insulin during fasting without GH, this difference did not reach statistical significance [0.44 ± 0.07 (GH) vs. 0.41 ± 0.06 mg·kg⁻¹·min⁻¹, P > 0.05].

GH did not affect circulating levels of glucose (Table 2).

Lipid metabolism and indirect calorimetry. FFA concentrations were significantly decreased during fasting without GH replacement [FFA: 1.43 ± 0.01 (GH) vs. 0.82 ± 0.02 mM, P < 0.01], and palmitate concentrations and fluxes were significantly decreased [palmitate: 378 ± 42 (GH) vs. 244 ± 12 µM, P < 0.05; 9,10-13C]palmitate: 412 ± 58 (GH) vs. 276 ± 42 µmol/min, P = 0.05; Fig. 3]. The ability of insulin to suppress lipid oxidation was significantly decreased after GH administration. The basal rates of EE and RER were comparable, whereas insulin-stimulated EE decreased during fasting without GH [EE: 1,950 ± 122 (GH) vs. 1,790 ± 95 kcal/24 h, P = 0.05], and RER increased [0.76 ± 0.02 (GH) vs. 0.82 ± 0.01, P < 0.05].

Urea metabolism. The primed continuous urea tracer infusion allowed a plateau in urea enrichment to be accomplished after 2 h and be maintained throughout. During fasting without GH replacement, urea turnover and excretion increased by 30–35% [R, urea: 336 ± 22 (GH) vs. 439 ± 43 µmol·kg⁻¹·h⁻¹, P < 0.01; urea

Table 2. Circulating concentration of metabolites at end of basal period

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>With GH Replacement</th>
<th>Without GH Replacement</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mM</td>
<td>4.1 ± 0.1</td>
<td>4.2 ± 0.1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>FFA, mM</td>
<td>1.43 ± 0.1</td>
<td>0.82 ± 0.02</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Glycerol, µM</td>
<td>117 ± 7</td>
<td>65 (40–140)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>3-OHB, µM</td>
<td>1,614 ± 223</td>
<td>673 ± 72</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lactate, µM</td>
<td>650 (595–835)</td>
<td>790 (600–885)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Alanine, µM</td>
<td>175 (125–295)</td>
<td>234 ± 11</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Data are means ± SE or median (interquartile range). FFA, free fatty acids; 3-OHB, 3-hydroxybutyrate.
We (28) have previously published data on the role of GH during fasting in healthy subjects, which showed that urea excretion and forearm protein breakdown increased substantially during somatostatin-induced GH deficiency. In this context, it should be noted that GH-deficient and healthy subjects are not directly comparable; in the present study, patients with GH deficiency had higher concentrations of GH, insulin, and C-peptide and lower concentrations of IGF-I and cortisol compared with healthy control subjects. These dif-

DISCUSSION

The results of our study establish that GH plays a pivotal role in the regulation of intermediary metabolism during fasting. The main findings are that GH deficiency results in a 30% increase in $R_a$ urea, reflecting increased, irreversible protein loss and a 50% decrease in palmitate turnover, reflecting decreased lipolysis. To our knowledge, these results are novel and have never been reported previously. In addition, we found that lack of GH increased the $M$ value during a glucose clamp by 60%, reflecting increased insulin sensitivity to glucose metabolism.

Fig. 2. Insulin sensitivity (determined by a euglycemic hyperinsulinemic clamp) after 42.5 h of fasting with and without growth hormone (GH) replacement, respectively. Basal and insulin-stimulated oxidative and nonoxidative glucose turnover during fasting with and without GH substitution.

Fig. 3. Isotopic determination of lipid turnover at end of basal period. Basal and insulin-stimulated lipid oxidation as measured by indirect calorimetry.
ferences probably reflect increased fat mass, decreased physical fitness, and hormonal replacement in the GH-deficient patients. It should also be noted that, in the present study, GH was administered as a continuous intravenous infusion during the metabolic study to accomplish steady-state conditions; in healthy subjects, GH is secreted in a pulsatile manner during fasting (19).

Fasting is characterized by progressive fuel depletion, and successful metabolic adaptation to fasting depends on the organism’s ability to partition substrate metabolism to a more selective use of fat as fuel and sparing of glucose and nitrogen (6, 9). Fasting constitutes a very robust stimulus for pituitary GH release (17, 19), and low levels of GH in fasting GH-deficient adults have been shown to decrease lipid oxidation and accelerate protein loss (27). We studied a group of adult GH-deficient patients, which made it possible to compare GH levels in the physiological range with low or absent GH concentrations. With the present design and mode of GH administration, we accomplished GH levels of ~6 µg/l and IGF-I levels of ~210 µg/l; compared with a previous study (28) comprising healthy subjects fasted for 40 h, in which we observed spontaneous GH levels of ~2.5 µg/l and IGF-I levels of ~275 µg/l, GH concentrations were thus somewhat greater and IGF-I concentrations lower in the present study. It may therefore be argued that, although GH levels were elevated in GH-deficient subjects, they were still in the physiological range in the sense that they failed to maintain appropriately high IGF-I levels.

A key observation in our study is that lack of GH in the fasting state increases production rates for urea determined with isotope dilution by more than 30%. This is in line with earlier studies showing 45–50% increased urinary urea-N excretion rates during fasting without GH in GH-deficient and healthy adults (27, 28). Most of the nitrogen released as a result of protein catabolism is incorporated in the ornithine cycle to form urea, which is considered to be the final product of the nitrogen disposal pathways. Most urea is excreted in the urine, and measurement of urinary urea excretion has been used to estimate net protein catabolism. The technique, however, does not allow precise determination of whole body urea synthesis, and the shortcomings of the method have previously been documented (15). An accurate estimate of the entire net protein catabolism is best obtained from the direct measurement of urea production, which can only be made by tracer experiments employing labeled urea. In the present study, we used a primed continuous tracer infusion, and, in accord with previous studies (15, 20), steady state was reached after 2 h. Urinary urea excretion was found to underestimate the actual urea synthetic rate by 30%. This is in line with earlier investigations (15).

GH may promote protein conservation and nitrogen retention in a number of ways. Apart from direct effects on target cells, e.g., in muscle and splanchnic tissue, GH increases circulating concentrations of several compounds with anabolic properties, including FFA, ketones, IGF-I, and insulin (7, 25, 37, 40). It is thus likely that elevated levels of IGF-I and insulin may contribute to the protein-sparing effects of GH and at the same time restrain the effects of GH on glucose and lipid metabolism. Our study employing labeled palmitate dilution provides evidence to show that GH actively stimulates lipolysis under fasting conditions.

Several lines of evidence support our finding that GH reduces insulin sensitivity after short-term fasting. In the postabsorptive state, administration of a physiolog-
Growth hormone (GH) has been shown to stimulate lipolysis after a time lag of 2–3 h, whereas it induced only minimal fluctuations in plasma glucose and no changes in serum insulin and C-peptide levels. More sustained exposure to high GH levels has been shown to induce both peripheral and hepatic insulin resistance (5, 11, 23, 38). Moreover, during GH exposure, circulating levels of FFAs and lipid oxidation rates were elevated, whereas glucose oxidation was suppressed. In addition, it has been shown that GH-induced insulin resistance is accompanied by reduced muscle glycogen synthase activity (1) and diminished glucose-dependent glucose disposal (29).

The mechanism by which GH induces insulin resistance during fasting remains to be precisely defined. Randle et al. (32) proposed the existence of a glucose fatty acid cycle in 1963, and the concept of substrate competition has been supported by numerous studies. Suppression of circulating levels of FFA, induced by pharmacological antilipolysis, increased insulin sensitivity (31, 35, 41), and fat infusion has been observed to inhibit glucose uptake and carbohydrate oxidation (2, 42). In GH deficiency, the insulin resistance induced by GH administration was nearly abolished with concomitant suppression of FFA (26, 36). On the other hand, some studies of forearm glucose uptake have shown an acute (i.e., within minutes) decrement in forearm glucose uptake after administration of GH (10, 25, 43), implying that GH-induced insulin resistance preceded the increase in circulating levels and forearm uptake of lipid intermediates, as the lipolytic action of GH is seen only after 2–3 h (25). Reduced glucose disposal in skeletal muscle after fasting will spare glucose for the brain. This important adaption reduces the need for gluconeogenesis from amino acids from protein breakdown. The reduced nitrogen loss (urea) reflects the resulting sparing of gluconeogenic and other amino acids.

Insulin secretion tended to be higher during GH exposure. Elevated insulin secretion could be a compensatory phenomenon related to insulin resistance, or it could be attributed to a direct insulinotropic effect of GH (39, 40). There was no difference between circulating insulin levels during the final 30 min of the clamp in the two settings.

The finding that epinephrine concentrations were increased during lack of GH is unexpected and its significance uncertain. It is unlikely that the observation relates to hypoglycemia, because we recorded identical plasma glucose concentrations in the two experimental situations.

In conclusion, our study in GH-deficient subjects provides clear evidence that GH is a key hormone in the metabolic adaptations to fasting. In the absence of GH we observed a substantial loss of protein assessed by urea isotope dilution and an inappropriately low rate of lipolysis assessed by a reduced palmitate R. Finally, deficiency of GH during fasting is associated with an increase in insulin sensitivity, thus resulting in increased glucose oxidation in insulin-sensitive tissues. A critical role of GH during fasting seems to be sparing of glucose and thus reduced gluconeogenesis and sparing of gluconeogenic amino acids.

We are grateful to Lone Svendsen and Joan Hansen for excellent technical assistance. GH preparations were generously supplied by Novo Nordisk (Copenhagen, Denmark).

DISCLOSURES

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