Glucose flux is normalized by compensatory hyperinsulinaemia in growth hormone-induced insulin resistance in healthy subjects, while skeletal muscle protein synthesis remains unchanged

Jonas NYGREN*, Anders THORELL*, Kerstin BRISMAR†, Pia ESSÉN‡, Jan WERNERMAN‡, Margaret A. McNURLAN§, Peter J. GARLICK§ and Olle LJUNGQVIST*

*Centre for Gastrointestinal Disease, Ersta Hospital, Karolinska Institute, 116 91 Stockholm, Sweden, †Department of Endocrinology, Karolinska Hospital, Karolinska Institute, 171 76 Stockholm, Sweden, ‡Department of Anaesthesia, Huddinge University Hospital, Karolinska Institute, 141 86 Stockholm, Sweden, and §Department of Surgery, State University of New York, Stony Brook, NY 11794, U.S.A.

ABSTRACT

The aim of this present investigation was to study the relationship between the reduction in insulin sensitivity accompanying 5 days of treatment with growth hormone (GH; 0.05 mg · 24 h⁻¹ · kg⁻¹) and intracellular substrate oxidation rates in six healthy subjects, while maintaining glucose flux by a constant glucose infusion and adjusting insulin infusion rates to achieve normoglycaemia (feedback clamp). Protein synthesis rates in skeletal muscle (flooding dose of L-[²H₅]phenylalanine) were determined under these conditions. We also compared changes in insulin sensitivity after GH treatment with simultaneous changes in energy requirements, protein synthesis rates, nitrogen balance, 3-methylhistidine excretion in urine, body composition and the hormonal milieu. After GH treatment, 70% more insulin was required to maintain normoglycaemia (P < 0.01). The ratio between glucose infusion rate and serum insulin levels decreased by 34% at the two levels of glucose infusion tested (P < 0.05). Basal levels of C-peptide, insulin-like growth factor (IGF)-I and IGF-binding protein-3 increased almost 2-fold, while levels of glucose, insulin, glucagon, GH and IGF-binding protein-1 remained unchanged. Non-esterified fatty acid levels decreased (P < 0.05). In addition, 24 h urinary nitrogen excretion decreased by 26% (P < 0.01) after GH treatment, while skeletal muscle protein synthesis and 3-methylhistidine excretion in urine remained unchanged. Energy expenditure increased by 5% (P < 0.05) after treatment, whereas fat and carbohydrate oxidation were unaltered. In conclusion, when glucose flux was normalized by compensatory hyperinsulinaemia under conditions of GH-induced insulin resistance, intracellular rates of oxidation of glucose and fat remained unchanged. The nitrogen retention accompanying GH treatment seems to be due largely to improved nitrogen balance in non-muscle tissue.

Key words: glucose clamp technique, glucose metabolism, growth hormone, insulin resistance, protein metabolism.

Abbreviations: GH, growth hormone; IGF, insulin-like growth factor; IGFBP, IGF-binding protein; M/I ratio, mean level of glucose infusion/mean serum insulin level; NEFA, non-esterified fatty acids.

Correspondence: Dr Jonas Nygren, Centre for Gastrointestinal Disease, Ersta Hospital, Box 4622, 116 91 Stockholm, Sweden (e-mail jonas.nygren@ersta.se).
INTRODUCTION

It is generally believed that most of the anabolic actions of growth hormone (GH) are mediated by insulin-like growth factor-I (IGF-I). However, GH also exerts metabolic effects directly on tissues, while IGF-I may enhance or counteract the direct metabolic effects of GH. In addition, the effect of IGF-I is dependent on its binding to specific binding proteins, since mainly the free form of IGF-I is metabolically active [1]. This makes the effect of the GH/IGF-I axis on metabolism difficult to study, to some extent explaining the contradictory results found in some previous studies. Thus intracellular rates of oxidation of glucose and fat, as well as protein synthesis rates in skeletal muscle, were reported to be affected by GH in some studies [2–4], but not in others [5–8].

An important difference between the direct and indirect (through IGF-I) effects of GH relates to insulin sensitivity. GH is known to induce insulin resistance, whereas IGF-I has insulin-like effects on glucose and protein metabolism. Although GH-induced insulin resistance has been studied over the years, the mechanism responsible for this condition is still not clear. Inconsistencies with regard to the effects of GH treatment on glucose and protein metabolism may be attributed to the relationship between the direct and indirect effects of GH, as well as to methodological issues. Insulin resistance has been studied mainly by the use of the hyperinsulinaemic normoglycaemic clamp [9]. However, since glucose flux is reduced in states of insulin resistance, this will also automatically affect intracellular substrate utilization and reduce glucose oxidation. If insulin rather than glucose infusion rates (as used in the classical clamp technique) were adjusted during the clamp, this alternative technique would test whether the normalization of glucose flux could also correct other insulin-sensitive metabolic pathways.

The aim of the present study was to investigate the relationship between the decrease in insulin sensitivity that accompanies treatment with GH and intracellular substrate oxidation rates measured while maintaining glucose flux by a constant glucose infusion and adjusting insulin infusion rates to achieve normoglycaemia (feedback clamp). Thus similar glucose flux was maintained before and after GH treatment, and protein synthesis rates in skeletal muscle were determined under these conditions. We also compared changes in insulin sensitivity after GH treatment with simultaneous changes in substrate oxidation, energy requirements, protein synthesis rates, nitrogen balance, urinary 3-methylhistidine excretion, body composition and the hormonal milieu.

METHODS

Subjects
Six healthy male volunteers (age 22 ± 1 years; body mass index 23 ± 1 kg/m²) participated in the study. None had a history or clinical evidence of medical illness, and none was taking any medication. Informed consent was obtained from each volunteer before participation in the study, which was reviewed and approved by the Institutional Ethical Committee.

Experimental design
The subjects underwent the same study protocol twice. The first study represents a control situation, before treatment. Then, 2–4 weeks later, the same hyperinsulinaemic normoglycaemic clamp protocol was repeated after a treatment period of 5 daily subcutaneous injections of human recombinant GH (0.05 mg · 24 h⁻¹ · kg⁻¹; Genotropin*, kindly donated by Kabi Vitrum, Stockholm, Sweden), administered at 08.00 hours. The second hyperinsulinaemic normoglycaemic clamp was performed on the sixth day. Before entering the study, the resting energy expenditure of each subject was determined using indirect calorimetry (Deltatrac®; Dansjö, Stockholm, Sweden). For the 3 days preceding each clamp study, each subject was given a standardized diet (46% carbohydrate, 41% fat and 15% protein) with an energy content of 135% of their measured resting energy expenditure. The diet provided a fixed 10 460 kJ (2500 kcal)/24 h, and the remaining energy was provided as a drink [Semper standard®; 502 kJ (120 kcal)/100 ml; 53% carbohydrate, 30% fat and 17% protein]. During the 3 days of controlled food intake, urine was collected in 24 h portions for analyses of total nitrogen excretion and 3-methylhistidine excretion. Total urinary nitrogen excretion was determined using a chemoluminescence nitrogen analyser [ANTEK 720®; Edect (Scientific) Ltd, Harrogate, Northhamptonshire, U.K.]. Nitrogen balance was calculated as:

\[
\text{Nitrogen balance} = \text{nitrogen intake per 24 h} - [\text{nitrogen excretion + 2 g (for losses other than in urine)}].
\]

Urinary 3-methylhistidine excretion was measured by HPLC [10].

Euglucaemic clamp
At 08.00 hours, after the subject had fasted overnight, a hyperinsulinaemic normoglycaemic clamp was performed using an artificial pancreas (Biostator®; Life Science Instruments, Elkhart, IN, U.S.A.) as described previously [11]. Arterialization of venous blood was attained using a thermoregulated sleeve (Kanthal; Medical Heating ASB, Stockholm, Sweden) on both arms, set at
45°C [12]. The subjects were given a continuous infusion of 20% (w/v) glucose (Kabi Pharmacia, Uppsala, Sweden) in a two-step manner (2 and 5 mg ⋅ min⁻¹ ⋅ kg⁻¹) for 120 min at each rate. Normoglycaemia was maintained by a variable infusion of insulin (Actrapid Human®; Novo, Copenhagen, Denmark). Steady-state conditions were attained after approx. 60 min of insulin and glucose infusion, and the glucose clamp was maintained for another 60 min at each level of glucose infusion. During the two periods of steady state, the mean insulin infusion rate required to maintain normoglycaemia was calculated (m-units ⋅ min⁻¹ ⋅ kg⁻¹). The mean level of glucose infusion divided by the mean serum insulin level (M/I ratio) at each level of glucose infusion was used as a measurement of whole-body insulin sensitivity.

Blood sampling and analysis
Blood samples were taken for substrate and hormone determinations in the basal state prior to glucose infusion. Sampling was also performed during the normoglycaemic clamps at 30 min intervals during the last 60 min of each level of glucose infusion (i.e. at 60, 90, 120, 180, 210 and 240 min). All samples were placed on ice; serum samples were allowed to clot before separation, while plasma samples were centrifuged immediately for 10 min at 4°C at 2010 g and the aliquots were stored at −20°C until batch analysis. Blood glucose was determined by a glucose analyser (model 23 AM; Yellow Springs Instruments) using the glucose peroxidase method [13]. Determinations of insulin [14], glucagon [15], IGF-I [16], IGF-binding protein-1 (IGFBP-1) [17] and IGFBP-3 [18] were performed using RIA techniques. C-peptide was analysed using a commercially available kit (Hoechst, Frankfurt, Germany). GH was analysed using a fluorophotometric assay (model 23 AM; Yellow Springs Instruments) using the glucose peroxidase method [13].

Indirect calorimetry
This was performed with a hood calorimeter (Deltatrac®) [22] before glucose infusion and during the last 30 min of steady state at both levels of glucose infusion. After excluding the part required for protein oxidation (calculated from urinary nitrogen excretion), respiratory quotients, energy expenditure and oxidation rates for glucose and fat were calculated [23].

Protein synthesis
The determination of protein synthesis rates in human skeletal muscle using the flooding technique has been described previously [24,25]. The protein synthesis measurements, with a study protocol of 90 min, were performed at the end of the glucose clamp measurement (i.e. 240–330 min), while maintaining a glucose infusion rate of 5 mg ⋅ min⁻¹ ⋅ kg⁻¹ and a variable insulin infusion to obtain normoglycaemia. Measurements were obtained following a 10 min intravenous injection of [1H₄]phenylalanine (45 mg/kg; 7.5 mol% excess at the first measurement and 15 mol% excess at the second measurement; Mass Trace Inc., Somerville, MA, U.S.A.). Blood samples were taken from a venous line in the opposite arm to that used for glucose and insulin infusion [11] at 0, 5, 10, 15, 30, 60 and 90 min after the phenylalanine injection, for determination of the isotope enrichment of phenylalanine in plasma. At 90 min after the isotope injection, a muscle biopsy was taken percutaneously from the lateral portion of the quadriceps femoris using a Bergström needle [26] and was frozen immediately in liquid nitrogen.

The determination of 1-[1H₄]phenylalanine enrichment in plasma samples, as well as in samples of hydrolysed muscle protein, was carried out by GC-MS with electron-impact ionization and selective ion monitoring. The enrichment in plasma was measured by monitoring the ions at m/z 336 and 341 of the tertiary butyldimethylsilyl derivative [27]. The enrichment of phenylalanine from protein hydrolysates was measured by enzymic decarboxylation to phenylethylamine, followed by GC-MS of the heptafluorobutyryl derivative at m/z 106 and 109 [27]. The determinations were performed on a Fisons MD800 quadrupole mass spectrometer. The rates of protein synthesis were calculated from the enrichments of phenylalanine in protein and the free phenylalanine in plasma, as described previously [24,25].

Whole-body impedance measurements
Immediately before the first injection of GH and again 24 h after the last injection of GH, before beginning the second clamp, whole-body impedance measurements were performed using a body impedance analyser (BIA-101/S; AKERN srl, Florence, Italy) [28]. Both measurements were performed at 08.00 hours after an overnight fast, with the subject in a relaxed supine position with arms and legs apart in a slightly abducted manner. The right hand and right foot were used, and the sensing electrodes were placed on an imaginary line bisecting the hand and foot articulations, while the active electrodes were placed at the root of the fingers and toes respectively. After reading values for resistance and reactance, body composition was calculated using software (RJL Systems, Detroit, MI, U.S.A.) connected to a PC-compatible computer.

Statistics
Results are presented as means ± S.E.M. Statistical significance was accepted at P < 0.05 using two-way ANOVA (entering mean levels obtained during baseline, low and high clamp levels, and before and after treatment into the equation) and least significant difference as post-hoc testing. Wilcoxon’s signed rank test (two tailed) was used.
for analysis of whole-body impedance data. Correlations between data were calculated using simple regression analysis.

RESULTS

Substrates and hormones
Basal blood glucose levels remained unchanged, whereas NEFA levels decreased, after GH treatment ($P < 0.001$) (Table 1). Basal insulin levels tended to be elevated after GH treatment ($P = 0.06$), while C-peptide levels increased significantly ($P < 0.01$). Basal levels of IGF-I increased 2-fold ($P < 0.001$) and IGFBP-3 displayed an 84% increase after GH treatment ($P < 0.001$). Basal levels of GH, IGFBP-1 and glucagon did not change after treatment (Table 1). Levels of adrenaline and noradrenaline did not change in response to GH treatment (results not shown).

Normoglycaemia was maintained during clamp measurements, with a mean intra-individual coefficient of variation for glucose of 6.5%. During clamps, insulin, C-peptide, IGF-I and IGFBP-3 levels were higher, while NEFA levels were lower, after treatment when compared with the control clamp. No change was found in clamp levels of blood glucose or IGFBP-1 after treatment when compared with the control clamp. Apart from the change in insulin levels ($P < 0.01$) in response to glucose and insulin infusion, GH treatment did not affect the responses of substrates and hormones during glucose and insulin infusion when compared with basal levels (Table 1).

Insulin sensitivity measurements and indirect calorimetry
Insulin infusion rates were required to be 40–50% higher in order to maintain normoglycaemia after GH treatment, and the M/I ratio decreased by 30–40% (Table 2) compared with control conditions. The relative changes in the M/I ratio (M/I%) at high glucose infusion rates after treatment, measuring the change in insulin sensitivity, correlated with the change in basal levels of serum insulin ($r = -0.86$, $P = 0.03$) and IGF-I ($r = -0.85$, $P = 0.03$) and the insulin/glucagon ratio ($r = -0.92$, $P = 0.009$). Energy expenditure increased after GH treatment ($P < 0.05$; Table 2), whereas no change was found in respiratory quotients or in glucose or fat oxidation rates.

Nitrogen balance, urinary nitrogen excretion, urinary 3-methylhistidine excretion and protein synthesis rates
There were no changes in 24 h urinary nitrogen excretion, nitrogen balance or urinary 3-methylhistidine excretion, urinary 3-methylhistidine excretion, and protein synthesis rates.

Table 1  Effects of GH treatment on substrate and hormone levels in healthy subjects

<table>
<thead>
<tr>
<th>Substrate/hormone</th>
<th>Glucose infusion</th>
<th>$2 \text{ mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$</th>
<th>$5 \text{ mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (mmol/l)</td>
<td>Control</td>
<td>4.5 (0.1)</td>
<td>4.5 (0.2)</td>
</tr>
<tr>
<td></td>
<td>GH</td>
<td>4.7 (0.1)</td>
<td>4.6 (0.1)</td>
</tr>
<tr>
<td>Plasma NEFA (\mu mol/l)</td>
<td>Control</td>
<td>557 (33)</td>
<td>289 (26)</td>
</tr>
<tr>
<td></td>
<td>GH</td>
<td>461 (48)**</td>
<td>162 (16)**</td>
</tr>
<tr>
<td>Serum insulin (\mu-units/ml)</td>
<td>Control</td>
<td>10 (1)</td>
<td>14 (1)</td>
</tr>
<tr>
<td></td>
<td>GH</td>
<td>13 (3)</td>
<td>22 (4)**</td>
</tr>
<tr>
<td>Serum C-peptide (pmol/ml)</td>
<td>Control</td>
<td>0.26 (0.05)</td>
<td>27 (0.06)</td>
</tr>
<tr>
<td></td>
<td>GH</td>
<td>0.47 (0.04)**</td>
<td>0.41 (0.04)**</td>
</tr>
<tr>
<td>Serum GH (m-units/l)</td>
<td>Control</td>
<td>2.6 (1.6)</td>
<td>4.2 (1.7)</td>
</tr>
<tr>
<td></td>
<td>GH</td>
<td>1.3 (0.7)</td>
<td>5.1 (2.4)</td>
</tr>
<tr>
<td>Plasma glucagon (pg/ml)</td>
<td>Control</td>
<td>126 (14)</td>
<td>90 (9)</td>
</tr>
<tr>
<td></td>
<td>GH</td>
<td>140 (23)</td>
<td>112 (28)</td>
</tr>
<tr>
<td>Serum IGF-I (ng/ml)</td>
<td>Control</td>
<td>191 (7)</td>
<td>180 (7)</td>
</tr>
<tr>
<td></td>
<td>GH</td>
<td>376 (44)**</td>
<td>369 (20)**</td>
</tr>
<tr>
<td>Serum IGFBP-1 (ng/ml)</td>
<td>Control</td>
<td>45 (5)</td>
<td>39 (3)</td>
</tr>
<tr>
<td></td>
<td>GH</td>
<td>40 (6)</td>
<td>34 (4)</td>
</tr>
<tr>
<td>Serum IGFBP-3 (mg/l)</td>
<td>Control</td>
<td>3.2 (0.2)</td>
<td>3.3 (0.2)</td>
</tr>
<tr>
<td></td>
<td>GH</td>
<td>5.9 (0.4)**</td>
<td>5.4 (0.2)**</td>
</tr>
</tbody>
</table>

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The subjects (*n* = 6) underwent a clamp study twice, before and after 5 days of single subcutaneous injections of GH (0.05 mg · 24 h⁻¹ · kg⁻¹). The clamp involved predetermined glucose infusion rates of 2 and 5 mg · min⁻¹ · kg⁻¹, with a variable infusion of insulin given in order to maintain normoglycaemia. Values are given as means (S.E.M.). Significance of differences: *P* < 0.05, **P** < 0.01, ***P** < 0.001 compared with control, using two-way ANOVA for repeated measurements, and least significant difference as post hoc test. The respiratory quotients is calculated as (CO₂ output)/(O₂ consumption). Note that 1 kcal = 4.184 kJ.

Table 2  Effects of GH treatment on insulin sensitivity and indirect calorimetry measurements in healthy volunteers
The subjects (*n* = 6) underwent a clamp study twice, before and after 5 days of single subcutaneous injections of GH (0.05 mg · 24 h⁻¹ · kg⁻¹). The clamp involved predetermined glucose infusion rates of 2 and 5 mg · min⁻¹ · kg⁻¹, with a variable infusion of insulin given in order to maintain normoglycaemia. Values are given as means (S.E.M.). Significance of differences: *P* < 0.05, **P** < 0.01, ***P** < 0.001 compared with control, using two-way ANOVA for repeated measurements, and least significant difference as post hoc test. The respiratory quotients is calculated as (CO₂ output)/(O₂ consumption). Note that 1 kcal = 4.184 kJ.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Basal</th>
<th>2 mg · min⁻¹ · kg⁻¹</th>
<th>5 mg · min⁻¹ · kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin infusion rate (m-units · min⁻¹ · kg⁻¹)</td>
<td>Control 0 (0)</td>
<td>0.15 (0.003)</td>
<td>0.21 (0.004)</td>
</tr>
<tr>
<td></td>
<td>GH 0 (0)</td>
<td>0.25 (0.002)*</td>
<td>0.36 (0.004)**</td>
</tr>
<tr>
<td>M/I ratio</td>
<td>Control 0 (0)</td>
<td>0.15 (0.01)</td>
<td>0.32 (0.02)</td>
</tr>
<tr>
<td></td>
<td>GH 0 (0)</td>
<td>0.10 (0.02)*</td>
<td>0.21 (0.04)**</td>
</tr>
<tr>
<td>Energy expenditure (kJ · 24 h⁻¹ · kg⁻¹)</td>
<td>Control 102.5 (3.3)</td>
<td>102.5 (3.8)</td>
<td>104.6 (3.3)</td>
</tr>
<tr>
<td></td>
<td>GH 107.9 (2.1)*</td>
<td>107.9 (3.3)*</td>
<td>110.9 (2.9)**</td>
</tr>
<tr>
<td>Respiratory quotient</td>
<td>Control 0.82 (0.02)</td>
<td>0.86 (0.02)</td>
<td>0.92 (0.02)</td>
</tr>
<tr>
<td></td>
<td>GH 0.82 (0.02)</td>
<td>0.88 (0.02)</td>
<td>0.94 (0.02)</td>
</tr>
<tr>
<td>Glucose oxidation (mg · min⁻¹ · kg⁻¹)</td>
<td>Control 1.5 (0.2)</td>
<td>1.9 (0.2)</td>
<td>3.2 (0.4)</td>
</tr>
<tr>
<td></td>
<td>GH 1.7 (0.2)</td>
<td>2.5 (0.2)</td>
<td>3.5 (0.4)</td>
</tr>
<tr>
<td>Fat oxidation (mg · min⁻¹ · kg⁻¹)</td>
<td>Control 0.9 (0.1)</td>
<td>0.7 (0.1)</td>
<td>0.4 (0.1)</td>
</tr>
<tr>
<td></td>
<td>GH 0.7 (0.2)</td>
<td>0.6 (0.1)</td>
<td>0.3 (0.1)</td>
</tr>
</tbody>
</table>

Whole-body impedance measurements
GH treatment for 5 days resulted in slight but significant changes in body composition (Table 3). Body fat was reduced, while lean body weight, lean/fat ratio and total body water increased after treatment (*P* < 0.05).

DISCUSSION
As anticipated, GH treatment reduced insulin sensitivity. However, when normalizing glucose flux with additional insulin, intracellular oxidation rates of glucose and fat were also normalized. The improved nitrogen economy after GH treatment seems to be due largely to an improved nitrogen balance in non-muscle tissues.

In the present study, we did not measure hepatic glucose production, and an increase in glucose production after GH treatment could possibly interfere with the interpretation of our results [9,29]. However, several studies have shown that there is only a minimal, if any, change in glucose production after brief treatment with moderate doses of GH [3,5,30–32]. Healthy subjects given an infusion of GH after consumption of a standardized mixed meal displayed a similar decrease in hepatic glucose production postprandially as those given saline [31], possibly as a result of a compensatory increase in endogenous insulin secretion in the GH-treated subjects. Thus, since glucose flux was also normalized by compensatory hyperinsulinaemia in the present study, we believe that the contribution of glucose production to whole-body glucose disposal was not markedly different after GH treatment when compared with during the control period. Assuming that glucose production was unchanged by treatment, non-oxidative glucose disposal would also be unaffected by GH during glucose and insulin infusion, since glucose oxidation was unchanged and glucose infusion rates were the same after treatment.
The observed reduction in insulin sensitivity after treatment with GH in the present study was directly related to the increase in basal levels of serum IGF-I. This relationship may suggest that insulin resistance in response to GH treatment is induced to counteract the increased insulin-like activity contributed by IGF-I, in order to avoid side effects such as hypoglycaemia. This hypothesis remains to be tested in future studies. However, in postoperative patients with insulin resistance where insulin sensitivity is acutely reduced by surgery, insulin infusion increases levels of free IGF-I, via increased proteolytic activity directed against IGFBP-3 [33,34]. In contrast, preliminary data have shown that, in a situation where insulin sensitivity was acutely increased, insulin infusion immediately after exercise was associated with a reduction in the levels of free IGF-I, through reduced proteolysis of IGFBP-3 and increased levels of IGFBP-1 [35]. Together, these data indicate that the effects of insulin and IGF-I are balanced in situations where insulin sensitivity is acutely increased or decreased, possibly to reduce the risk of systemic side effects such as hypoglycaemia.

Notably, the decreased insulin sensitivity after GH treatment was not related to a decreased insulin/glucagon ratio, which has been demonstrated in insulin-resistant states such as diabetes mellitus [36], starvation [36] and after burn trauma [37]. In contrast, a direct relationship was found between the decreased insulin sensitivity and an increased insulin/glucagon ratio. This suggests that mechanisms behind GH-induced insulin resistance differ from those reported in other conditions of insulin resistance.

GH promotes lipolysis directly by stimulating the activity of hormone-sensitive lipase. Administration of IGF-I has been shown both to inhibit [39] and to stimulate [40] lipolysis. The effects of IGF-I on lipolysis may be mediated directly by IGF-I through the insulin receptor, since virtually no IGF-I receptors are found in adipose tissue [41], or by modulation of serum insulin levels. Similar to what was found after 3 days of treatment with GH in rats [8], NEFA levels actually decreased in our subjects after treatment with GH. In addition, no change was found in fat oxidation rates after GH treatment. Altogether, these data do not support an involvement of the glucose/fatty acid cycle [42] in the development of insulin resistance after GH treatment. However, since increased lipolysis may be an early effect of GH treatment [8], contributions of the glucose/fatty acid cycle to the reduced insulin sensitivity in the early phase after GH administration, even in the present study, cannot be excluded.

There may be a difference between the direct and indirect (through IGF-I) effects of GH on protein metabolism. Effects of GH on protein metabolism after short-term infusions are probably due to GH directly, since IGF-I concentrations increase only after 6–12 h [4]. Furthermore, when GH was infused in healthy subjects and IGF-I and insulin were maintained using an infusion of somatostatin, protein oxidation was decreased and protein synthesis in non-muscular tissues was increased, whereas no change was found in skeletal muscle protein synthesis [43]. In contrast, GH infused into the brachial artery for a longer period of time was shown to stimulate muscle protein synthesis, possibly due to paracrine production of IGF-I [4]. In addition, IGF-I given as an arterial infusion was shown to both increase protein synthesis and reduce proteolysis in skeletal muscle [44]. In the present study, no effect on skeletal muscle protein synthesis was found after treatment with GH, which is in accordance with some previous reports [43], but not others [4]. McNurlan et al. [45] demonstrated a 27% increase in protein synthesis rates in healthy subjects after 2 weeks of treatment with GH (6 mg/day as daily subcutaneous injections), as compared with the non-significant 11% increase observed in the present study using a dose of 3–4 mg/day (0.05 mg · 24 h^{-1} · kg^{-1}). In the study of McNurlan et al. [45], the IGF-I response was more pronounced (4-fold) than in the present study (2-fold), due to the higher dose of GH given, while energy intake was similar (1.5 × basal energy expenditure; 20% of energy from protein). Protein synthesis rates were measured in the present study with subjects in a ‘pseudofed’ state during insulin and glucose infusion, as glucose flux was maintained at the control level after GH treatment by compensatory hyperinsulinaemia. Thus protein synthesis rates may have been influenced not only by GH and IGF-I, but also by the prevailing insulin levels, which are the common conditions for ‘daytime’ metabolism. However, insulin infusion has repeatedly been reported not to stimulate protein synthesis rates in skeletal muscle when amino acids are not administered along with the insulin and glucose infusion [25,46,47], and may have little impact in the present study.

Similar to what has been reported previously [48], nitrogen excretion in urine was reduced after treatment with GH, indicating a decrease in whole-body protein oxidation [43] and urea synthesis [49]. Protein synthesis in skeletal muscle and urinary 3-methylhistidine excretion, a measure of muscle protein breakdown [10], were unchanged by GH treatment. Thus the present data suggest that the nitrogen-sparing effects of GH may be due largely to effects on protein synthesis and/or protein breakdown in tissues other than skeletal muscle (i.e. liver). This was not measured. However, the reduction in liver protein synthesis found in otherwise healthy subjects during laparoscopic cholecystectomy was shown to be prevented by 5 days’ administration of GH (4 mg/24 h) [50], a dose similar to the one used in the present study.

The increases in energy expenditure, lean body mass and water retention, as well as the reduced fat content as
measured by indirect calorimetry and whole-body impedance analysis, are in accordance with previous studies and have been discussed elsewhere [2,51].

In summary, hyperinsulinemia normalizes the glucose utilization rate, despite the presence of insulin resistance accompanying GH treatment, thereby normalizing intracellular oxidation rates of glucose and fat. Improved nitrogen economy after GH treatment seems to be due largely to an improved nitrogen balance in non-muscle tissues.

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