Effects of morning cortisol replacement on glucose and lipid metabolism in GH-treated subjects

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Abstract

Objective: Insulin resistance is a frequent consequence of GH replacement therapy but patients on GH replacement therapy often also have replacement of other hormone deficiencies which theoretically could modify the metabolic effects of GH. In particular, cortisol replacement if given in supra physiologic doses immediately before the evaluation of insulin sensitivity could influence insulin sensitivity. The aim of this study was thus to evaluate the effect of morning cortisol replacement given prior to a euglycaemic clamp combined with infusion of [3-3H]glucose and indirect calorimetry on glucose and lipid metabolism.

Methods: Ten GH/ACTH-deficient adults received, in a double-blind manner, either cortisol (A) or placebo (B) before the clamp whereas five GH-deficient–ACTH-sufficient adults participated in a control (C) clamp experiment. All subjects received GH replacement therapy.

Results: Serum cortisol levels were significantly higher after cortisol than after placebo (324 ± 156 vs 132 ± 136 nmol/l; P = 0.006) and similar to controls (177 ± 104 nmol/l). As a measure of the biological effect of cortisol, eosinophil leukocyte counts in peripheral blood decreased (164 ± 91 × 10^9/l vs 216 ± 94 × 10^9/l; P = 0.04). Cortisol replacement had no significant effect on insulin-stimulated glucose uptake (11.8 ± 1.8 vs 13.2 ± 3.9 mmol/kg min), either on glucose oxidation or on glucose storage. There was also no significant effect of cortisol on fasting endogenous glucose production and no effect was seen on serum free fatty acid concentrations.

Conclusion: Administration of cortisol in the morning before a clamp cannot explain the insulin resistance seen with GH replacement therapy.

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Introduction

Insulin resistance is a frequent corollary of growth hormone (GH) replacement therapy but its mechanisms are not fully understood. The most likely explanation is a GH-induced increase in lipolysis and activation of the free fatty acid (FFA)–glucose cycle (1–4). However, patients on GH replacement therapy often also have replacement of other hormone deficiencies which theoretically could modify the metabolic effects of GH. In particular, cortisol replacement if given in supra physiologic doses immediately before the evaluation of insulin sensitivity could influence the insulin sensitivity. Cortisol not only causes insulin resistance but also stimulates lipolysis and could thus mimic the effects of GH (5). Cortisol is usually given twice daily with peak concentrations within 2 h after administration and the nadir after about 8 h (6). Therefore, diurnal changes in insulin sensitivity could be possible during cortisol replacement therapy.

The present study was designed to evaluate whether administration of cortisol prior to a euglycaemic clamp influences insulin sensitivity in GH/adrenocorticotrophin (ACTH)-deficient adults (groups A and B) receiving GH replacement therapy. We also included a control group of GH-deficient/ACTH-sufficient adults (group C) receiving only GH replacement. This allowed us also to compare metabolic effects between patients with isolated GH deficiency (GHD) and patients with multiple pituitary deficiencies.

Materials and methods

Subjects

Fifteen GH-deficient patients, five of whom had an isolated GHD (ACTH-sufficient, group C) and ten with multiple pituitary deficiencies including ACTH deficiency (groups A and B) were included in the study. The subjects with isolated GHD were used as a control group to...
exclude the confounding effects of other hormonal deficiencies. They were younger than those with multiple deficiencies (29.2±9.9 vs 43.2±6.0 years) but there were no differences in body weight or body composition (Tables 1 and 2). None of the patients had treatment interfering with glucose metabolism. One patient had mild hypercholesterolaemia and had been treated with a stable low-dose simvastatin for the last 6 months before inclusion in the study. The patients gave their written consent before participating. The study protocol was approved by the Ethics Committee of the Medical Faculty of Lund University and the Isotope Committee at the Malmö University Hospital MAS.

The patients included in the study had severe GHD as defined by a peak plasma growth hormone concentration of <9 mU/l after insulin-induced hypoglycaemia or clonidine (7). The clonidine test was mainly performed in patients with multiple pituitary hormonal deficiencies due to a pituitary tumour or surgical operation and radiation treatment of a pituitary tumour. All these patients had a low insulin-like growth factor-I (IGF-I) value and/or low or no diurnal variation in GH. The clonidine test was performed in these patients before the consensus guidelines for diagnosis in 1998 and only in patients with contraindications for the insulin tolerance test (ITT). A 2 day metyrapone test or insulin-induced hypoglycaemia was used to evaluate ACTH function. Adequacy of the hypothalamo-pituitary–adrenal (HPA) axis was defined as a serum 11-deoxycortisol response greater than 200 nmol/l in the metyrapone test, and a cortisol response greater than 550 nmol/l during insulin-induced hypoglycaemia. The control patients with isolated GHD had a significantly higher GH peak during stimulation than the patients with multiple hormone deficiencies (4.4±2 vs 1.4±2 mU/l), but there were no differences in their daily GH dose (both 0.4±0.2 mg/day). The ACTH-insufficient patients had received GH replacement therapy for a mean duration of 57.9±13.3 months compared with 44.4±22.4 months for the ACTH-sufficient patients. The patients in groups A and B had a mean IGF-I level of 29.3±6.7 nmol/l and the group C patients 41.3±13.3 nmol/l on GH replacement therapy. This corresponded to a mean IGF-I standard deviation score of 0.4 (~0.2 to 0.7) and 0.4 (0.4–0.6) respectively. Any additional pituitary replacement therapy was stable and monitored for at least 6 months before inclusion in the study and was kept unchanged during the study period.

**Experimental design**

The GH-deficient patients with ACTH insufficiency participated in two euglycaemic clamps with prior administration of cortisol or placebo with at least a 1-week interval while the patients with isolated GHD participated in one euglycaemic clamp without prior administration of cortisol. The daily GH dose (Genotropin, Pfizer AB, Stockholm, Sweden or Humatrope, Eli Lilly) was similar for both groups (0.4±0.2 mg) and was administered s.c. at 22 h the night before the study. All subjects were admitted to a metabolic ward at 0700 h after a 10 h overnight fast. All studies were performed with the patient in a supine position. Prior to the study a catheter was inserted anterogradely into an antecubital vein for infusions, another catheter was positioned retrogradely into a wrist vein for blood sampling. This hand was then enclosed in a heated box (70°C) to achieve arterialization of venous blood, and the wrist catheter was kept open by a saline infusion. Before the clamps, GH-deficient patients with ACTH insufficiency received, in a double-blind manner, either a tablet of cortisone acetate (mean dose 16±2 mg, Cortal, Organon AB, Gothenburg, Sweden) or a placebo. Thereafter, a 2 h euglycaemic hyperinsulinaemic insulin clamp with a variable infusion of 20% glucose labeled with [3-3H]glucose in combination with indirect calorimetry and constant infusion of [3-3H]glucose was performed (Fig. 1).

**Methods**

Glucose metabolism was assessed by a euglycaemic hyperinsulinaemic clamp (8) and substrate oxidation by indirect calorimetry (9). Endogenous glucose production (EGP) was measured by infusion of [3-3H]glucose. A primed continuous infusion of human insulin (Actrapid Human, Novo Industri, Copenhagen, Denmark), at a dose of 1110 pmol/m²/min over a 10 min period, was administered to raise the plasma insulin concentration acutely. To maintain plasma insulin concentrations at the desired level throughout the rest of the insulin clamp the patient received a continuous insulin infusion of 70 pmol/m²·min. A variable infusion of 20% glucose was begun and was periodically adjusted (clamped) to maintain the plasma glucose concentration constant at 5.5 mmol/l. Plasma glucose was measured at 5 min intervals.

**Table 1 Clinical characteristics of the subjects.**

<table>
<thead>
<tr>
<th>ACTH deficient</th>
<th>Control subjects</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>81.2±15.4</td>
<td>85±25.4</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.3±3.7</td>
<td>29.2±6.1</td>
</tr>
<tr>
<td>Weight to height ratio</td>
<td>0.9±0.06</td>
<td>0.9±0.04</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>60.7±14</td>
<td>63.1±14</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>20.6±7.3</td>
<td>21.7±13.8</td>
</tr>
<tr>
<td>GH dose (mg/day)</td>
<td>0.4±0.2</td>
<td>0.4±0.2</td>
</tr>
<tr>
<td>IGF-I (nmol/l)</td>
<td>29.3±6.7</td>
<td>41.3±13.3</td>
</tr>
<tr>
<td>IGF-I s.d. score</td>
<td>0.4 (~0.2–0.7)</td>
<td>0.4 (0.4–0.6)</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.6±1.2</td>
<td>4.6±0.7</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.2±0.2</td>
<td>0.9±0.2</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>3.8±1.0</td>
<td>2.8±0.3</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.9±1.2</td>
<td>2.1±1.0</td>
</tr>
</tbody>
</table>

HDL and LDL, high- and low-density lipoproteins respectively.

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Table 2: Medical background of the pituitary disease in patients.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age (Years)</th>
<th>Aetiology</th>
<th>Treatment</th>
<th>Pituitary deficiencies</th>
<th>Hormonal substitution</th>
<th>Estimated duration of GHD (years)</th>
<th>GH peak (mIU/l) after stimulation with:</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>50</td>
<td>NS</td>
<td>op + rtr</td>
<td>ATG</td>
<td>cty</td>
<td>17</td>
<td>IH 0.1 mIU/l</td>
</tr>
<tr>
<td>M</td>
<td>44</td>
<td>NS</td>
<td>op + rtr</td>
<td>AT</td>
<td>ct</td>
<td>12</td>
<td>Clonidine 2.4 mIU/l</td>
</tr>
<tr>
<td>M</td>
<td>43</td>
<td>NS</td>
<td>op + rtr</td>
<td>ATG</td>
<td>cty</td>
<td>8</td>
<td>Clonidine 0.9 mIU/l</td>
</tr>
<tr>
<td>M</td>
<td>41</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>24</td>
<td>Clonidine 0.1 mIU/l</td>
</tr>
<tr>
<td>M</td>
<td>36</td>
<td>C</td>
<td>rtr</td>
<td>ATG</td>
<td>cty</td>
<td>18</td>
<td>IH 0.3 mIU/l</td>
</tr>
<tr>
<td>F</td>
<td>49</td>
<td>NS</td>
<td>—</td>
<td>ATGD</td>
<td>cted</td>
<td>3</td>
<td>IH 1.8 mIU/l</td>
</tr>
<tr>
<td>F</td>
<td>49</td>
<td>P</td>
<td>op + rtr</td>
<td>AG</td>
<td>ce</td>
<td>22</td>
<td>Clonidine 0.5 mIU/l</td>
</tr>
<tr>
<td>F</td>
<td>44</td>
<td>C</td>
<td>op</td>
<td>ATG</td>
<td>cte</td>
<td>6</td>
<td>IH 0.2 mIU/l</td>
</tr>
<tr>
<td>F</td>
<td>44</td>
<td>I</td>
<td>—</td>
<td>A</td>
<td>c</td>
<td>8</td>
<td>IH 4.8 mIU/l</td>
</tr>
<tr>
<td>F</td>
<td>31</td>
<td>NS</td>
<td>op</td>
<td>ATGD</td>
<td>cted</td>
<td>3</td>
<td>IH 1.2 mIU/l</td>
</tr>
</tbody>
</table>

(5 GH-deficient–ACTH-sufficient patients)

| M     | 46          | I         | —         | —                     | —                    | OM                              | 23 IH; clonidine 0.1 mIU/l; 2.4 mIU/l |
| M     | 29          | NS        | rtr       | —                     | —                    | 10                              | IH 5.3 mIU/l                        |
| M     | 27          | I         | —         | —                     | —                    | 5                               | IH 2.4 mIU/l                        |
| M     | 22          | JA        | op        | —                     | —                    | 4                               | IH; clonidine 7.2 mIU/l; 6.2 mIU/l   |
| M     | 22          | I         | —         | —                     | —                    | 5                               | IH 4.6 mIU/l                        |

Abbreviations are as shown below.

Aetiology: C, treated Cushing's disease; I, idiopathic; JA, juvenile astrocytom corpus pineal; NS, non-secreting adenoma; P, prolactinoma.

Treatment: op, surgical operation; rtr, radiation treatment.

Pituitary insufficiencies: A, ACTH; T, TSH; G, FSH/LH; D, ADH.

Hormonal substitution: c, cortisone; t, thyroxine; e, oestrogen; y, testosterone; d, desmopressin.

Medication: CA, calcium carbonate; CH, cholecalciferol; CP, calcipotriol; FC, fludrocortisone acetate; FU, furosemide; OM, omeprazole; SI, simvastatin; SO, sotalolhydrochloride.

Stimulation test: IH, Insulin hypoglycaemia.

Figure 1: Experimental design.
EGP was measured by the isotope dilution technique using [3-3H]glucose (Amersham), administered as a priming dose of 8.3 µCi/m² and a constant infusion of 0.083 µCi/m²·min for 270 min. The basal plasma tracer specific activity is linearly related to the rate of tracer infusion per m² of body surface area. In order to achieve a similar basal plasma specific activity in all subjects, the tracer amount was adjusted for body surface area (10). To achieve tracer equilibrium in the plasma glucose pool we allowed 150 min to pass before the insulin infusion was started. Plasma glucose was clamped by a variable labeled glucose infusion. The glucose infusion was labeled with [3-3H]glucose to maintain plasma glucose specific activity at baseline levels. Labeling of the glucose infusion was based upon previous estimates of the expected glucose infusion and hepatic glucose production rate during the clamp (11). The radiochemical purity of the tracer was 99% as reported by the manufacturer.

Indirect calorimetry was performed in the fasting state and during the last 45 min of the insulin clamp to estimate net rates of carbohydrate and lipid oxidation. A computerized open-circuit system was used to measure gas exchange through a transparent plastic canopy (Deltatrac, Datex, Helsinki, Finland).

Lean body mass was measured by bioelectrical impedance analysis (BIA). The bioelectrical impedance technique estimates total body water using a two-terminal portable impedance analyzer (BIA 101, RJJ, Akern, Copenhagen, Denmark). Electrodes were placed on the dorsal surface of the left hand and foot. Measurements were made in the supine position in the morning after voiding (12).

Samples for measurements of plasma glucose, serum insulin, FFA and [3-3H]glucose specific activity were drawn in accordance with the flowchart in Fig. 1. To prevent in vitro lipolysis, the samples for FFA measurements were collected in pre-chilled tubes (13). The FFA samples from the entire group were analyzed at the same time.

The plasma glucose concentration was measured in duplicate on a Beckman glucose analyzer (Beckman, Fullerton, CA, USA). Serum insulin was measured by an ELISA (DAKO, Cambridgeshire, UK) with an intra-assay coefficient of variance (CV) of 7.5% and an interassay CV of 7%. FFAs were measured using an enzymatic calorimetric ACS-ACOD-MEHA method (Wako Chemicals GmbH, Neuss, Germany), with an intra-assay CV of 3.2% and an inter-assay CV of 5.7%. [3-3H]glucose specific activity was measured in duplicate from the supernatant of 0.5 M perchloric acid extract of serum samples after evaporation of radiolabelled water. Serum and urine urea were measured through the oxidation of NADH to NAD by enzymatic calorimetric ACS-ACOD-MEHA method (Synchron LX Systems Chemistry Information, Synchron LX 20; Beckman Coulter, Inc., Palo Alto, CA, USA). Blood eosinophil leucocytes were measured by the VCS principle (volume, conductivity, scatter) using the Coulter LH 750 system (Beckman Coulter). Serum cortisol was analyzed with the Beckman Access Immunoassay System (Beckman Coulter). The CV for cortisol measurements at a concentration of 65 nmol/l was 11% and at a concentration of 470 nmol/l, 5%. The detection limit was 11 nmol/l.

**Calculations**

Fasting EGP was calculated by dividing the [3-3H]glucose infusion rate by the steady-state plateau of [3-3H]glucose specific activity in plasma during the last 30 min of the fasting tracer infusion period. During administration of insulin and glucose a non-steady-state condition in plasma [3-3H]glucose specific activity exists. At high rates of glucose uptake the classical model of Steele is known to produce negative estimates of EGP. When adding [3-3H]glucose to the variable exogenous glucose infusion, the plasma [3-3H]glucose specific activity was maintained constant, and only a few negative numbers of EGP were adapted as zero in calculations. The infusion rate of exogenous glucose was integrated over 20 min intervals and subtracted from the total rate of glucose appearance to obtain the rate of residual EGP during the clamp. Total body glucose metabolism was calculated by adding the mean rate of EGP during the last 60 min of the insulin clamp to the mean glucose infusion rate during the same period. Non-oxidative glucose metabolism, mainly storage of glucose as glycogen, was calculated as the difference between total body glucose metabolism and glucose oxidation, as determined by indirect calorimetry.

Net rates of glucose and lipid oxidation were calculated from indirect calorimetric measurements in the fasting state and during the last 45 min of the insulin clamp. Protein oxidation was calculated from the overnight urinary urea nitrogen excretion collected by the patient and urinary urea nitrogen excretion obtained during the insulin clamp. Corrections were made for urea clearance (14).

**Statistical analysis**

Values are presented as the means±S.D. or the median (interquartile range), if the variable is not normally distributed. The significance of differences between changes during the placebo and cortisol periods was tested by Wilcoxon ranked sign test for paired data and between groups by the Mann–Whitney U test for unpaired data. All statistical analyses were performed with NCSS 2000 (Number Cruncher Statistical System) software (PASS 6.0 for Windows; Kaysville, UT, USA). $P < 0.05$ was considered statistically significant.
Results

Validation of study design (Fig. 2)

There were no differences in the basal serum cortisol concentrations before the clamp between the A and B protocols (175 ± 161 vs 187 ± 196 nmol/l; P = not significant (NS)). As expected, the basal cortisol concentrations in the patients with isolated GHD (group C) were significantly higher than in groups A and B (539 ± 62 nmol/l; P < 0.01). At 2.5 h after intake of cortisol (at the start of the clamp), serum cortisol concentrations were significantly higher in group A than in group B (324 ± 156 vs 132 ± 136 nmol/l; P = 0.006) and not significantly different from group C (177 ± 104 nmol/l). During the clamp, the cortisol concentrations declined in both groups A and B, with no difference between the two experiments during the last hour of the clamp (4.5 h after intake of cortisol) (161 ± 91 vs 127 ± 143 nmol/l) but significantly lower than in group C (356 ± 87 nmol/l; P = 0.01).

In addition, the number of blood eosinophilic leukocytes decreased significantly in group A compared with group B (164 ± 91 £ 10^9/l vs 216 ± 94 £ 10^9/l; P = 0.04), which was maintained during the last hour of the clamp.

Glucose and insulin concentrations (Fig. 3)

There were no differences in the plasma glucose concentrations between protocols A, B and C, either in the basal state (5.1 ± 0.4, 5.1 ± 0.4 and 5.7 ± 1.3 mmol/l respectively) or during the clamp (5.5 ± 0.2, 5.5 ± 0.2 and 5.5 ± 0.2 mmol/l respectively). Likewise, the basal serum insulin concentration did not differ between groups A, B and C (68.4 ± 20, 60.7 ± 28 and 108 ± 89 pmol/l respectively). In addition, during the clamps the steady-state insulin concentrations were maintained at a similar level in all three protocols (222 ± 61, 238 ± 65 and 279 ± 160 pmol/l respectively).

FFA concentration and lipid oxidation (Fig. 3 and Table 3)

There were no differences in basal serum FFA levels between groups A, B and C (467 ± 230, 453 ± 190 and 621 ± 360 µmol/l respectively). During the insulin clamp, the FFA levels were suppressed to the same extent in all three experiments (106 ± 53, 106 ± 48 and 132 ± 140 µmol/l respectively).

The rates of lipid oxidation did not differ between groups A, B and C, either in the fasting (1.11 ± 0.30, 1.13 ± 0.31 and 1.47 ± 0.65 µmol/kg/min) or during the clamp (0.927 ± 0.15, 1.02 ± 0.24 and 1.12 ± 0.61 µmol/kg/min respectively).

Glucose metabolism (Table 3)

The rate of insulin-stimulated glucose uptake did not differ between the experiments A, B and C (11.8 ± 1.8, 13.2 ± 3.9 and 15.6 ± 2.9 µmol/kg/min). Neither the fasting (4.62 ± 2.9 vs 4.18 ± 2.8 µmol/kg/min) nor the insulin-stimulated rates (6.62 ± 2.8 vs 6.56 ± 2.6 µmol/kg/min) of glucose oxidation were affected by cortisol replacement and they were not different from group C (3.38 ± 4.9 and 7.21 ± 5.9 µmol/kg/min). Consequently, cortisol replacement had no significant effect on non-oxidative glucose metabolism (5.18 ± 2.5 vs 6.65 ± 4.5 µmol/kg/min); these values were not

Figure 2 Blood eosinophil leucocytes and serum cortisol concentrations in the fasting state (-150 to 0 min) and during the clamp (0 to +120 min). □, cortisol (A); ⊗, placebo (B); ●, control (C). n = 10 per study group for cortisol/placebo and n = 5 for control group.

Figure 3 Plasma glucose, serum insulin and serum FFA concentrations in the fasting state (-150 to 0 min) and during the clamp (0 to +120 min). ○, cortisol; ◊, placebo; ●, control. n = 10 per study group for cortisol/placebo and n = 5 for control group.
different from those in group C (12.6 ± 2.5 μmol/kg/min). The fasting rates of EGP were unaffected by cortisol (11.7 ± 0.96 vs 12.0 ± 1.2 μmol/kg/min) and were not significantly different from those in group C (12.6 ± 2.5 μmol/kg/min). There were also no significant differences in residual EGP during the clamp between the three protocols (2.21 ± 1.9, 1.51 ± 1.6 and 1.93 ± 3.7 μmol/kg/min respectively).

Energy expenditure (Table 3)

The fasting rates of energy expenditure were similar for groups A and B (6.8 ± 1.3 vs 6.8 ± 1.3 MJ/24 h) and were not significantly different from group C (7.8 ± 1.1 MJ/24 h).

Protein oxidation (Table 3)

The fasting (4.28 ± 1.1, 4.80 ± 2.1 and 4.43 ± 3.1 μmol/kg/min) and insulin-stimulated (3.89 ± 2.2 vs 3.32 ± 1.6 and 4.01 ± 2.9 μmol/kg/min respectively) rates of protein oxidation were similar for groups A, B and C.

Comparison between GH-deficient subjects with multiple hormonal deficiencies and isolated GHD (Table 3)

No significant differences were observed between GH-deficient individuals with multiple hormonal deficiencies and individuals with isolated GHD regarding glucose and lipid metabolism.

Discussion

The results challenge the view that other pituitary deficiencies such as ACTH/cortisol and their replacement would significantly modify the effect of GH on insulin sensitivity in GH-deficient patients. This argument is based upon the findings of similar rates of total body glucose uptake and EGP after cortisol administration, prior to the clamp, as placebo. Estimates of insulin-stimulated glucose uptake were recorded during the last hour of the clamp. At this time point, the cortisol concentrations after oral cortisol administration had already declined to levels similar to those seen during the placebo and were significantly lower than those encountered in individuals with isolated GH deficiency but maintained ACTH production. This finding clearly emphasizes the short serum half-life of administered oral cortisol. In plasma, only approximately 4% of cortisol circulates free while the majority is bound to corticosteroid-binding globulin in plasma. The enzyme 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) converts inactive cortisone to active cortisol in a number of tissues including liver, muscle and adipose tissue. In contrast to cortisol, cortisone circulates in much larger doses unbound in plasma and thereby represents an inactive glucocorticoid storage pool (15). GH inhibits 11β-HSD1 activity through an IGF-I-mediated effect (16). Therefore, it is quite possible that if the diurnal GH concentrations show large variations in GH-deficient patients with GH replacement therapy this will also influence the HPA axis.

However, the biological half-life of cortisol does not necessarily reflect the plasma half-life. As an estimate of the biological effect of cortisol we measured the number of eosinophil leucocytes in
peripheral blood. Judged from this, blood eosinophils were still suppressed during the last hour of the clamp suggesting that a biological effect of cortisol could be expected during this time period. Of course, we do not know whether the response of different tissues (e.g. muscle) to glucocorticoids follows the same time course as for blood leukocytes.

Regardless of these considerations, the estimates of fasting EGP were performed at a time point when the cortisol effect was maximal. Although cortisol is known to stimulate gluconeogenesis no effect of cortisol replacement was seen on fasting EGP. The effect of cortisol on gluconeogenesis could partially be mediated by its stimulatory effect on lipolysis, which, in turn, is known to drive gluconeogenesis (17). In keeping with this, no effect was seen on FFAs or rates of lipid oxidation after cortisol.

In addition to studying the effect of cortisol replacement therapy on glucose metabolism the design also allowed us to compare the metabolic effects of GH between GH-deficient patients with multiple pituitary deficiencies and patients with isolated GHD. Although there was a tendency for a higher rate of glucose uptake, particularly non-oxidative glucose metabolism, in patients with isolated GHD compared with those with GHD plus multiple deficiencies, this could partially be attributed to the younger age of the subjects with isolated GHD. Some caution is, however, warranted in the interpretation of these data given the small number of individuals with isolated GHD (Table 3).

In conclusion this study shows that it is unlikely that morning cortisol administration prior to a euglycaemic clamp would significantly influence the estimates of hepatic and total body insulin sensitivity. Some caution must be given regarding the interpretation of the data due to the short plasma half-life of administered oral cortisol.

**Acknowledgements**

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