Modulation of basal glucose metabolism and insulin sensitivity by growth hormone and free fatty acids during short-term fasting

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Abstract

Background and aims: The metabolic response to fasting involves an increase in circulating levels of growth hormone (GH) and free fatty acids, and resistance to insulin’s actions on glucose metabolism. Stimulation of lipolysis and insulin resistance are well-described effects of GH. The present study was designed to test the degree to which the insulin antagonistic effects of GH on glucose metabolism are mediated through stimulation of lipolysis during fasting.

Methods: Seven normal subjects were examined on three occasions during a 40-h fast with infusion of somatostatin, insulin and glucagon for the final 18 h: (expt. i) with GH replacement, (expt. ii) with GH replacement and antilipolysis with acipimox, and (expt. iii) without GH and with antilipolysis.

Results: Basal glucose turnover was significantly reduced by addition of acipimox (rate of disappearance (Rd) glucose (mg/kg/min): 1.91 ± 0.08 (expt. i), 1.69 ± 0.05 (expt. ii), 1.61 ± 0.08 (expt. iii); P < 0.01), whereas insulin-stimulated glucose uptake was significantly increased (glucose infusion rate (M-value) (mg/kg/min): 1.66 ± 0.22 (expt. i), 2.47 ± 0.10 (expt. ii), 2.00 ± 0.31 (expt. iii); P < 0.05). Addition of GH during inhibition of lipolysis failed to affect basal and insulin-stimulated glucose metabolism significantly.

Conclusion: Thus, the present data provide strong evidence that the insulin antagonistic effects of GH on fasting glucose metabolism are causally linked to concomitant stimulation of lipolysis.

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Introduction

It is well described that prolonged fasting induces insulin resistance (1), which may be related to high circulating levels of growth hormone (GH) and free fatty acids (FFA) (2). Several studies have shown that GH antagonises the effects of insulin on lipid and glucose metabolism, although the underlying mechanism remains uncertain. After an overnight fast physiological elevations in plasma GH concentrations in humans have been shown to induce insulin resistance in the liver as well as in skeletal muscle (3, 4). In fasted dogs, administration of GH was associated with an increase in plasma insulin levels, unchanged glucose turnover, but resistance to the effects of administered insulin (5). In GH-deficient adults continued GH replacement during fasting decreased the glucose infusion rate (M-value) during a glucose clamp, reflecting decreased sensitivity of glucose metabolism to insulin and increased palmitate turnover, reflecting increased lipid mobilization and utilization (6).

Because GH promotes lipolysis in adipose tissue and elevation of GH is associated with increased plasma FFA levels (7–13), increased lipid availability has been suggested to be responsible for the insulin resistance observed with elevated GH levels. After GH infusion both GH and FFA levels are positively correlated with the increase in peripheral and hepatic insulin resistance (14), whereas overnight lowering of FFAs with acipimox (an antilipolytic agent) has been shown to improve insulin sensitivity (15). Moreover, postabsorptive insulin resistance induced by GH administration in GH-deficient adults was grossly diminished by concomitant suppression of FFAs (16). Put together, these data may suggest that insulin resistance during fasting is causally linked to GH-induced stimulation of lipolysis. This assumption has not, however, been experimentally tested.

The present study was designed to define the roles of FFAs and GH in the regulation of glucose metabolism during fasting and, more specifically, to test whether the insulin antagonistic effects of GH on glucose metabolism are mediated through stimulation of lipolysis and whether inhibition of lipolysis leads to increased insulin sensitivity. For this purpose we examined seven healthy subjects on three occasions with and without GH and
pharmacological antilipolysis during 40 h of fasting together with infusion of somatostatin to clamp hormone levels.

Subjects and methods

Experimental protocol (Fig. 1)

Seven healthy male subjects (mean age 23 ± 1 years; body mass index 24 ± 1 kg/m²) were examined on three occasions during a 40-h fast with infusion of somatostatin, insulin and glucagon for the final 18 h to control hormone levels: (expt. i) with GH replacement (+ GH, − acipimox), (expt. ii) with GH replacement and inhibition of lipolysis (+ GH, + acipimox), and (expt. iii) without GH and with inhibition of lipolysis (− GH, + acipimox) (Fig. 1). Somatostatin (200 µg/h) was infused together with insulin (Actrapid, 100 IU/ml, Novo Nordisk, Copenhagen, Denmark; infusion rate adjusted to maintain plasma glucose levels between 3 and 5 mmol/l (0.1 mU/kg/min to 0.05 mU/kg/min)) and glucagon (GlucaGen, 1 ng/ml, Novo Nordisk; the infusion rate being adjusted to maintain stable glucose levels (1.0 to 1.5 mg/kg/min)). The somatostatin experiments were identical in terms of hormone replacement doses, except for GH (Norditropin, 12 IU/ml, Novo Nordisk; 4.5 IU partly as bolus injections, partly as continuous infusion) and acipimox (Olbetam; 250 mg p.o. every 4th h). To avoid hypoglycemia during fasting with GH suppression and antilipolysis, all participants temporarily received glucose intravenously (0.2 mg/kg/min for a period of 0.5 to 1.5 h); exactly the same amount of glucose was given in exactly the same manner in the experiment with GH replacement. Since potential hypoglycemia was anticipated during GH suppression, the − GH, + acipimox experiment was conducted first in all subjects, in order to allow for control of exogenous glucose. The subjects were blinded to this procedure. The remaining two experiments were performed in random order with at least 6 weeks elapsed between each experiment. At each investigation a 3-h ‘basal’ period was followed by a euglycemic 3-h glucose clamp. No participants received glucose during the basal period, as glucose infusion was tapered at least 2 h before investigations of substrate metabolism were started. Prior to the study, subjects were on a weight maintaining diet for at least 3 months. All subjects gave their written informed consent, and the study was approved by the regional ethics committee and conducted according to the Declaration of Helsinki.

Assays

The plasma glucose level was measured in duplicate immediately after sampling on a glucose analyzer (Beckman Instruments, Palo Alto, CA, USA). Whole blood glycerol, lactate and β-hydroxybutyrate (BOH) were analyzed by autoluminometric enzymatic methods (17). Levels of serum FFAs were determined by a commercial kit (Wako Chemicals, Neuss, Germany). A double monoclonal immunoluminometric assay (Delfia, Wallac, Finland) was used to measure serum GH, while plasma glucagon and insulin-like growth factor-I (IGF-I) levels were measured by in-house assays (18–20). Insulin was determined by a commercial immunological kit (DAKO, Glostrup, Denmark) (Immuno- clear, Stillwater, MN, USA). Catecholamines were measured by liquid chromatography (21). Cortisol was measured by an automated chemiluminescence system (Chiron Diagnostics, Fernwald, Germany).

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i. 40 h fast with suppression of GH and exogenous GH replacement (+GH, −acipimox)

ii. 40 h fast with suppression of GH, exogenous GH replacement, and inhibition of lipolysis (+GH, +acipimox)

iii. 40 h fast with suppression of GH and inhibition of lipolysis (−GH, +acipimox)

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<th>Hours of fasting</th>
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Figure 1 The experimental design (see text for details).
Euglycemic hyperinsulinemic glucose clamp and indirect calorimetry

All studies were performed with the participants in the supine position. At 0800 h a bolus of 20 μCi [3-3H]glucose (New England, Nuclear, Boston, MA, USA) was given, followed by a continuous infusion of 20 μCi/h. Three hours were allowed for the isotope to equilibrate. From 1100 to 1400 h a constant amount (0.6 mU/kg/min) of insulin (Actrapid, Novo Nordisk) was infused; based on measurements every 10 min plasma glucose was clamped at 5.0 mmol/l by infusion of variable rates of a 20% glucose solution. To minimize rapid dilution of the labeled glucose pool with unlabeled glucose, [3-3H]glucose was added to the glucose infused during the clamp (100 μCi/500 ml 20% glucose). During the clamp, hepatic glucose production (EGP) was calculated by subtracting the amount of exogenous glucose necessary to maintain euglycemia (M-value) from the isotopically determined overall appearance rate for glucose. To prevent hypoaminoacidemia during insulin stimulation, we infused a commercially available amino acid solution Vamin 14 g nitrogen/l, 0.0176 ml/kg/min. One liter contained the following amino acids: 12 g l-alanine, 8.4 g l-arginine, 2.5 g aspartate, 420 mg l-cysteine, 4.2 g l-glutamate, 5.9 g glycine, 5.1 g l-histidine, 4.2 g l-isoleucine, 5.9 g l-leucine, 6.8 g l-lysine, 4.2 g l-methionine, 5.9 g l-phenylalanine, 5.1 g l-proline, 3.4 g l-serine, 4.2 g l-threonine, 1.4 g l-tryptophane, 170 mg l-tyrosine, 5.5 g l-valine in sterile water. Indirect calorimetry (Deltatrac monitor, Datex Instrumentarium, Helsinki, Finland) allowed measurements of respiratory exchange ratios (RERs). Net lipid and glucose oxidation rates (Rd(ox)) were calculated from the above measurements. Net nonoxidative glucose disposal (Rd(non-ox)) was calculated by subtracting oxidative glucose disposal (Rd(ox)) from total glucose disposal (Rd) measured isotopically.

Microdialysis

The microdialysis technique allows repeated sampling of interstitial fluid from adipose tissue and was used for measurement of interstitial levels of glucose, lactate and glycerol. A microdialysis catheter (CMA 60, CMA, Stockholm, Sweden) was placed in the abdominal s.c. adipose tissue after anesthetization of the skin with 0.05 ml lidocaine at the site of perforation of the skin. The microdialysis catheter used has a molecular cutoff of 20 kDa. Immediately after placement, perfusion of the catheters with physiological perfusion fluid (perfusion fluid T1, CMA: Na⁺, 147 mmol/l; K⁺, 4 mmol/l; Ca²⁺, 2.3 mmol/l; Cl⁻, 156 mmol/l; pH, 6; osmolality, 290 mosmol/kg), at a flow rate of 0.3 ml/min, with the use of a portable pump (CMA 106, CMA), was accomplished. At this flow rate, the rate of recovery with the microdialysis catheter is almost 100% (22).

The microdialysis catheter was placed at t = 0 min. After 1 h of calibration with perfusion of the microdialysis catheter, allowing local edema and hemorrhage to subside, sampling started at t = 60 min and continued, every 60 min, until t = 360 min. The first sample was thus withdrawn at t = 120 min. It reflects the integrated level of interstitial glucose from t = 60 min to t = 120 min. In the figure the sample is assigned 90 min (Fig. 2). This principle was used for all samples. The observed changes in interstitial glycerol concentration can be seen as an index of lipolysis (23–25).

Forearm technique

Muscle substrate balance can be assessed by the forearm method, which quantifies muscle uptake and
release of metabolites. Arterialized and deep-venous blood samples were drawn simultaneously from the heated dorsal hand vein and the deep antecubital vein respectively. Before the blood samples were drawn the hand blood flow was interrupted for at least 1 min by inflation of a wrist cuff to 250 mmHg. Immediately after the blood samples were drawn, the forearm blood flow was measured by venous occlusion plethysmography.

Statistics
Results are expressed as means±S.E.M. The Kolmogorov-Smirnov test was used to test for normal distribution. Statistical comparisons between the study periods (+GH, −acipimox; +GH, +acipimox; −GH, +acipimox) were assessed by a two-way analysis of variance (ANOVA). If this test was positive, post hoc comparison was carried out by means of a paired t-test (to test the effect of lipolysis (expt. i vs expt. ii) and the effect of GH (expt. ii vs expt. iii)). For time series (i.e. circulating hormones) the area under the curve (AUC) was calculated by the trapezoidal method and comparisons were made by analysis of variance. A P-value below 0.05 was considered significant. Unless specified otherwise data referred to below were obtained with arterialized blood based on triplicate measurements during the last 30 min of the basal and clamp periods.

Results
Circulating hormones and protein metabolites
Inhibition of lipolysis led to an 80% increase in GH concentration despite somatostatin infusion (Table 1). Total IGF-I (tIGF-I) and free IGF-I (fIGF-I) decreased and the counterregulatory hormone, epinephrine, increased. The addition of GH during inhibition of lipolysis was associated with an increase in glucagon and a decrease in norepinephrine.

The total plasma concentration of amino acids was not affected by administration of GH or acipimox (total amino acids (μmol/l): 2470±87 (expt. i), 2552±53 (expt. ii), 2378±181 (expt. iii); P > 0.05). Overall, the gluconeogenic amino acids did not differ although alanine decreased when GH was added during antilipolysis (alanine (μmol/l): 235±12 (expt. i), 255±9 (expt. ii), 325±17 (expt. iii); P < 0.01).

Lipid metabolism
Acipimox administration lowered circulating concentrations of lipid intermediates (FFA and glycerol) (Table 2) and halved the concentration of BOH. FFA and glycerol tended to escape the action of the drug after 12 h since an increase in circulating concentrations of FFA, glycerol, and interstitial glycerol (Fig. 2) was seen in the presence of GH.

Table 1 Serum concentration (means±S.E.) of hormones.

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<td>19.7±1.0</td>
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<td>tIGF-I (μg/l)</td>
<td>6.1±0.4</td>
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<td>fIGF-I (μg/l)</td>
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<td>Norepinephrine (pg/ml)</td>
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Table 2 Serum concentration (means±S.E.) of hormones.

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<tr>
<td>GH (μg/l)</td>
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<td>tIGF-I (μg/l)</td>
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<td>fIGF-I (μg/l)</td>
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NS, not significant.
Circulating levels of metabolites (means ± S.E.).

1 GH, acipimox, 2 GH, acipimox vs acipimox (expt. ii), 3 GH vs acipimox (expt. iii) (ANOVA).

Table 2  Circulating levels of metabolites (means ± S.E.).

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<th>FFA (μmol/l)</th>
<th>Fructose (μmol/l)</th>
<th>BOH (μmol/l)</th>
<th>Glucose (mmol/l)</th>
<th>Lactate (μmol/l)</th>
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<td>151 ± 67</td>
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<td>457 ± 25</td>
<td>17 ± 4</td>
<td>646 ± 115</td>
<td>4.9 ± 0.1</td>
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Glucose metabolism and insulin sensitivity

Plasma glucose was decreased 20% by inhibition of lipolysis whereas lactate was increased (Table 2). Addition of GH during antilipolysis was associated with a significant increase in glucose, whereas lactate decreased. Steady-state interstitial glucose values were approximately 15% lower than in plasma (Fig. 2) but the three study periods showed proportional concentrations compared with plasma. After 34 h of fasting a significant increase in glucose oxidation was observed during fasting with suppression of lipolysis (Rd(ox) (mg/kg/min): 1.16 ± 0.16 (expt. i), 1.86 ± 0.21 (expt. ii), 1.52 ± 0.21 (expt. iii); P < 0.05), whereas energy expenditure (EE) decreased (EE (kcal/24 h): 1860 ± 61 (expt. i), 1620 ± 58 (expt. ii), 1594 ± 118 (expt. iii); P < 0.05). During the clamp lipid oxidation tended to be decreased during fasting with antilipolysis (lipid ox (mg/kg/min) (clamp): 0.74 ± 0.08 (expt. i), 0.48 ± 0.14 (expt. ii), 0.40 ± 0.08 (expt. iii); P = 0.09).

Forearm blood flow and exchange of metabolites

Forearm blood flow as assessed by plethysmography was not affected by administration of either GH or acipimox. Consequently, forearm exchange of metabolites are given as arterio-venous differences in plasma
concentrations of metabolites. Insulin-stimulated uptake of FFAs was significantly decreased during antilipolysis (a–v difference fatty acids (μmol/l): 68 ± 12 (expt. i), 19 ± 8 (expt. ii), −4 ± 12 (expt. iii); P < 0.01), whereas no significant change was found in glucose uptake. Lactate was released during fasting with antilipolysis and GH replacement (a–v difference lactate (μmol/l): 4 ± 29 (expt. i), 259 ± 18 (expt. ii), 54 ± 44 (expt. iii); P = 0.05).

**Discussion**

The present study was designed to assess the acute effect of GH and FFAs on glucose metabolism and insulin sensitivity during short-term fasting. The main findings are first that inhibition of lipolysis induces a clearcut decrease in basal glucose turnover and EGP and increases insulin sensitivity, and secondly that administration of GH during inhibition of lipolysis fails to affect basal and insulin-stimulated glucose metabolism significantly.

Several studies have shown that GH postabsorptively antagonises the effects of insulin on glucose and lipid metabolism (4, 9, 26). During fasting and stress, GH secretion is augmented and studies in GH-deficient adults have demonstrated that GH replacement during fasting in GH-deficient subjects increases lipid oxidation and decreases insulin sensitivity (6, 27). The mechanisms by which GH induces insulin resistance during fasting remain to be precisely defined. Randle and colleagues in 1963 proposed the existence of a glucose fatty acid (28), and although the underlying mechanisms are still the subject of dispute, the concept of substrate competition has been supported by numerous studies. Suppression of circulating levels of FFA, induced by pharmacological antilipolysis, increased insulin sensitivity (15, 29, 30), and lipid infusion has been observed to inhibit glucose uptake and carbohydrate oxidation (31, 32). Postabsorptively, the insulin resistance induced by GH administration in

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**Table 3**

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<tr>
<th>GH</th>
<th>Acipimox</th>
<th>GH</th>
<th>Acipimox</th>
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<th>Acipimox</th>
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<td>1.61 ± 0.08</td>
<td>1.68 ± 0.05</td>
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<tr>
<td>Clamp Glucose Rd (mg/kg/min)</td>
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<td>2.00 ± 0.31</td>
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<tr>
<td>M-value (mg/kg/min)</td>
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<td>2.67 ± 0.13</td>
<td>2.44 ± 0.11</td>
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</table>

Figure 3 Insulin sensitivity as assessed by M-value (means ± s.e.) during the hyperinsulinemic clamp. Shaded bar, +GH; –acipimox; open bar, +GH, +acipimox; solid bar, –GH, +acipimox. P < 0.05. The shaded bar is significantly different from the open bar.
GH-deficient adults was clearly reduced by concomitant suppression of FFA (16). In as much as insulin sensitivity, judged by the M-value, tended, if anything, to be increased (2.5 vs 2.0 mg/kg/min) during GH administration when lipolysis was blocked, our results clearly suggest that the ability of GH to induce insulin resistance during fasting is entirely secondary to stimulated lipolysis. The specific mechanism by which FFAs influence glucose metabolism in muscle tissue has not been resolved yet. Recent data challenge the original mechanisms proposed to be operative according to the Randle hypothesis, as the FFA-induced decrement in glycogen synthesis has been shown to be preceded by a lowering of intracellular glucose-6-phosphate (33), and FFA have been demonstrated to lower intracellular glucose concentrations (34). Taken together, these observations suggest that FFA lower glucose disposal primarily by inhibition of transmembrane glucose transport (35).

Traditionally, insulin was believed to inhibit endogenous glucose production exclusively through direct actions. However, studies in dogs (36–39) have suggested that at least some of insulin’s effects are indirect and may be mediated by lowering of plasma FFA. The role of FFAs in the regulation of glucose production in vivo seems relatively complex, involving both direct effects and indirect effects secondary to alterations in circulating concentrations of glucoregulatory hormones. In normal overnight-fasted human volunteers, experimental increments in FFA levels induced by the combined infusion of triglycerides and heparin are usually not associated with any significant change in glycemia or endogenous glucose production (40–42). The potential increase in hepatic glucose output induced by FFA is antagonized by their stimulatory effect on insulin secretion, and several reports indicate that the suppression of this hormonal effect by somatostatin is necessary to unveil the hyperglycemic action of FFA (41, 43). Similarly, the experimental lowering of FFA levels postabsorptively does not inhibit (29, 30, 44–48) and may even stimulate hepatic glucose output (49, 50), possibly through a reduction in insulin secretion and an increase in gluconeogenic pathways. In the present study we intended to control FFA, insulin, glucagon, and GH. During the basal period, as would be expected, inhibition of lipolysis was associated with a decrease in glucose production. This is in line with results from Mittelman et al. (51) showing that suppression of lipolysis under constant basal insulin causes suppression of EGP, despite a significant rise in catecholamines.

Despite the fact that glucagon was administered in exactly the same amount in all three situations, the plasma level was significantly decreased during fasting with suppression of GH and inhibition of lipolysis (expt. iii). The low circulating levels during antilipolysis and suppression of GH are presumably due to an increase in glucagon clearance. The regulation of glucagon clearance remains controversial. The circulating serine protease dipeptidyl peptidase IV seems to be a primary enzyme involved in the degradation and inactivation of glucagon (52); regulation of this peptide, however, remains to be clarified. In the present study the low glucagon levels found during suppression of GH and antilipolysis (expt. iii) could add to the decrease in glucose production in this situation, but since glucagon levels were comparable during GH replacement (expt. i) and GH replacement and antilipolysis (expt. ii), the decrease in EGP caused by antilipolysis cannot be explained by glucagon. It should, however, be noted that rates of glucose disposal during the clamp also tended to be elevated during GH administration and that glucagon is not thought to have any effects on the disposal of glucose.

The levels of FFAs were suppressed by acipimox, which is known to exert its antilipolytic effect by lowering the intracellular level of cyclic adenosine monophosphate (cAMP) and thereby inhibiting the activity of the hormone-sensitive lipase (53). That the effects of antilipolysis on glucose metabolism may have been due to an intrinsic effect of acipimox, cannot entirely be excluded. When FFA levels are clamped to separate any direct or pharmacological effects of acipimox on glucose uptake from secondary effects of inhibiting lipolysis, glucose oxidation during a euglycemic, hyperinsulinemic clamp (29, 54) in healthy subjects and suppression of EGP by insulin in noninsulin-dependent diabetic (NIDDM) subjects (46) are not affected by acipimox. On the other hand, during hyperinsulinemia Fulcher et al. (54) demonstrated a minute increase in glucose disposal due to increased non-oxidative glucose disposal after acipimox administration. In the presence of basal insulin concentrations peripheral glucose uptake is not altered acutely in acipimox-treated NIDDM subjects (30, 55). Thus, the bulk of evidence suggests that acipimox does not have any direct effects on glucose metabolism.

In conclusion, our results show that experimental lowering of circulating FFA levels with acipimox during fasting suppresses endogenous glucose production, increases insulin sensitivity and abolishes any anti-insulin effects of GH on glucose metabolism. Thus stimulation of lipolysis must be a pivotal — and perhaps the only - mechanism for the insulin antagonistic actions of GH on fasting glucose metabolism.

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