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Blockade of the Growth Hormone (GH) Receptor Unmasks Rapid GH-Releasing Peptide-6-Mediated Tissue-Specific Insulin Resistance

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ABSTRACT

The roles of GH and its receptor (GHR) in metabolic control are not yet fully understood. We studied the roles of GH and the GHR using the GHR antagonist pegvisomant for metabolic control of healthy nonobese men in fasting and nonfasting conditions. Ten healthy subjects were enrolled in a double blind, placebo-controlled study on the effects of pegvisomant on GHRH and GH-releasing peptide-6 (GHRP-6)-induced GH secretion before and after 3 days of fasting and under nonfasting conditions (n = 5). Under the condition of GHR blockade by pegvisomant in the nonfasting state, GHRP-6 (1 μg/kg) caused a increase in serum insulin (10.3 ± 2.1 *vs.* 81.3 ± 25.4 mU/L; $P < 0.001$) and glucose (4.2 ± 0.3 *vs.* 6.0 ± 0.6 mmol/L; $P < 0.05$) concentrations. In this group, a rapid decrease in serum free fatty acids levels was also observed. These changes were not observed under GHR blockade during fasting or in the absence of pegvisomant. We conclude that although these results were obtained from an acute study, and long-term administration of pegvisomant could render different results, blockade of the GHR in the nonfasting state induces tissue-specific changes in insulin sensitivity, resulting in an increase in glucose and insulin levels (indicating insulin resistance of liver/muscle), but probably also in an increase in lipogenesis (indicating normal insulin sensitivity of adipose tissue). These GHRP-6-mediated changes indicate that low GH bioactivity on the tissue level can induce changes in metabolic control, which are characterized by an increase in fat mass and a decrease in lean body mass. As a mechanism of these GHRP-6-mediated metabolic changes in the nonfasting state, direct nonpituitary-mediated GHRP-6 effects on the gastroentero-hepatic axis seem probable. (*J Clin Endocrinol Metab* **86:** 590–593, 2001)

THE ROLES OF GH and its receptor (GHR) in the metabolic control of man are not fully understood yet. During fasting, serum GH levels increase significantly, whereas serum total and free insulin-like growth factor I (IGF-I) as well as insulin levels decline (1–3). Also, GH-deficient (GHD) patients show, in parallel with acromegalic patients with high GH concentrations, a reduction in insulin sensitivity (4–6). In contrast to acromegalics, GHD patients have an increase in fat mass (4, 6–8). Pegvisomant is a genetically manipulated GH molecule that disables functional dimerization of the two GH receptor (GHR) molecules involved in signal transduction due to a single mutation at the site II of the GH molecule. Pegvisomant is pegylated to increase the serum half-life. Currently, the compound is used in phase II and III trials for the treatment of acromegaly (9–12). Pegvisomant potentially can also be used in studies in normal individuals to obtain more insight into the role of GH, its receptor, and its secretagogues in metabolic control. We performed a double blind, placebo-controlled, cross-over study comparing the effects of placebo *vs.* a single sc injection of 80 mg pegvisomant on GHRH- and GH-releasing peptide-6 (GHRP-6)-induced GH secretion both before and after a 3-day period of fasting in 10 healthy young male subjects. We also repeated the same study in 5 of the 10 subjects, but under nonfasting conditions. During this open label study period, all subjects received pegvisomant.

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Subjects and Methods

Ten healthy male subjects, 20–30 yr of age (mean ± SD age, 23.4 ± 2.7 yr; range, 20–28), with a normal body weight (mean ± SD body mass index, 21.8 ± 1.8 kg/m^2; range, 19.7–25.8) were asked to participate. None of the subjects had a relevant medical history or used medication. All 10 subjects participated in the cross-study, and 5 of these 10 subsequently participated in the single arm study (see below). The study was approved by the local ethical committee and all subjects gave written informed consent before enrolment in the study.

The study consisted of two parts: a double blind, placebo-controlled, cross-over study comparing GHR blockade with placebo before and after 3 days of fasting (with free access to noncaloric fluids), and a single arm, open label study in which the effect of GHR blockade under nonfasted conditions (continuation of subject's normal daily diet) was investigated. All study periods in both protocols lasted 4 days, during which the subjects were admitted to the Clinical Research Unit (CRU). Except for fasting, study periods in both protocols were identical. In the fasting study all subjects had to fast from 2400 h on the day before admission until after the first test. They were admitted to the CRU at 0730 h, at which time two indwelling iv catheters were inserted in both forearms. On days 1 and 4, a GHRP-6 or GHRH test was performed between 0800–0900 h. A second test (GHRH or GHRP-6 test) was performed between 1600–1700 h (exactly 8 h after the first test). Between the two tests, a standard light meal was served to all subjects on day 1, but not to subjects in the fasting group on day 4. This light meal consisted of two slices of bread, with one unit of butter, one slice of cheese, one unit of fruit jelly, and one unit of milk (caloric content, 1764 kJ). Immediately after the second GH stimulatory test either 80 mg Pegviso-

mant or placebo were administered sc. All subjects fasted from midnight on day 1 until the end of the study.

In the nonfasting arms of the study, all subjects also had to fast from 2400 h on the day before admission until after the first test. Again, they were admitted to the CRU at 0730 h, at which time two indwelling iv catheters were inserted in both forearms. On days 1 and 4, a GHRP-6 test was performed between 0800–0900 h. A GHRH test was performed exactly 8 h after the GHRP-6 test. Between the two tests, a standard light meal, as described above, was served to all subjects on days 1 and 4. Immediately after the GHRH test on day 1, 80 mg Pegvisomant were administered sc. A wash-out period of at least 3 weeks was chosen in between each of the admission periods for each subject. To detect changes in concentrations over the day, blood samples for endocrine and metabolic parameters were taken daily at intervals of 4 h throughout every study period of 4 days. These samples were used to calculate mean GH concentrations over the day.

GH stimulation tests

Either GHRH (Ferring Pharmaceuticals Ltd., Hoofddorp, The Netherlands) or GHRP-6 (CLINALFA AG, Laufelingen, Switzerland) was administered as an iv bolus injection of 1 μg/kg BW. Blood samples were drawn every 15 min from −15 to 120 min after injection. In the cross-over study, tests were performed in random order.

Study medication

GHR antagonist (Pegvisomant; Somavert) and placebo were supplied by Sensus Drug Development Corp. (Austin, TX).

Assays

Samples were measured for endogenous GH in a two-site immunoassay that does not cross-react with pegvisomant. The interassay coefficients of variation (CVs) are 4.1% at 4.0 μg/L and 3.8% at 20 μg/L. The intraassay CVs are 3.4% at 0.25 μg/L, 1.9% at 2.5 μg/L, and 4.5% at 25 μg/L (Medical Klinik Innenstadt, Ludwig-Maximillians University, Munich, Germany) (9). Serum IGF-I was determined using a commercially available RIA (Biosource Technologies, Inc., Nivelles, Belgium; intra- and interassay CVs, 5.0% and 9.6%, respectively). This IGF-I assay measures IGF-I in acid-ethanol extracts. Free IGF-I concentrations were assessed using a commercially available immunoradiometric assay (Diagnostics Systems Laboratories, Inc., Webster, TX; intra- and interassay CVs, 10.3% and 10.7%, respectively). Glucose was determined with an automatic hexokinase method (Roche, Almere, The Netherlands). Insulin was assessed by RIA (Medgenix Diagnostics, Brussels, Belgium; intra- and interassay CVs, 13.7% and 8.0%, respectively). Free fatty acids were determined with an enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany; intra- and interassay CVs, 1.1% and 4.1%, respectively).

Statistical analysis

Means were compared with the Wilcoxon matched pairs test. All P values are two-sided; $P < 0.05$ was considered significant. Unless otherwise noted, all results are reported as the mean ± SEM. Analyses were performed using Prism version 3.00 for Windows (GraphPad Software, Inc., San Diego, CA). The area under the curve was calculated by the trapezoidal rule.

Results

GH

No significant differences were observed in serum GH concentrations on day 1, expressed as the area under the curve for all GH data obtained in 24 h, excluding GH concentrations that were obtained during the two stimulatory tests. On day 4, however, serum GH concentrations increased in all fasting subjects, whereas only in the fasting, pegvisomant-pretreated subjects was this increase in GH significant (0.4 ± 0.4 ng/mL on day 1 *vs.* 2.1 ± 0.7 ng/mL on day 4).

Insulin

In Fig. 1A, serum insulin concentrations are shown on days 1 and 4 of each of the study periods. No significant changes in serum insulin concentrations were observed after the administration of 1 μg/kg GHRP-6. However, only in the presence of pegvisomant and in the nonfasting state was a rapid and significant increase in insulin levels found (day 4; insulin, 0800 h *vs.* 2400 h, 10.3 ± 2.1 *vs.* 81.3 ± 25.4 mU/L; $P <$ 0.001).

Glucose

No significant changes were observed in glucose after GHRP-6 administration. Again, only in the presence of pegvisomant and in the nonfasting state was a significant increase in serum glucose levels observed (day 4; glucose, 0800 h *vs.* 2400 h, 4.2 ± 0.3 *vs.* 6.0 ± 0.6 mmol/L; $P < 0.05$; Fig. 1B).

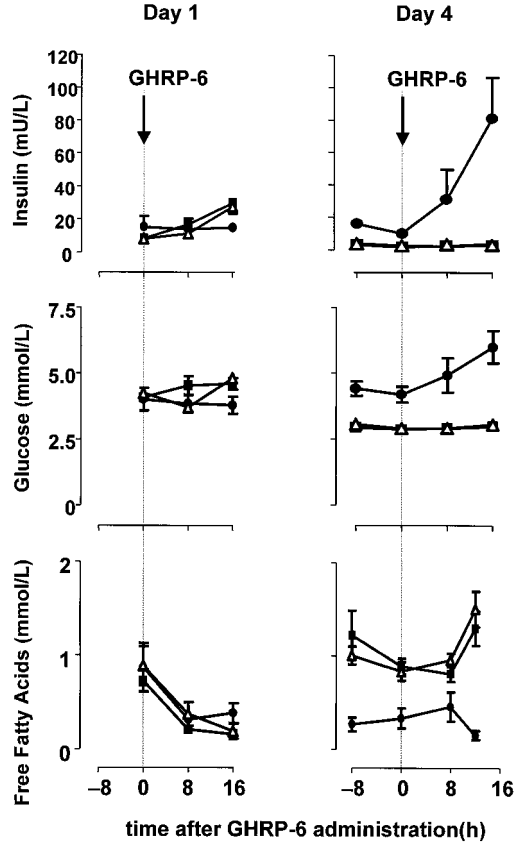

FIG. 1. Serum insulin (milliunits per L; A), serum glucose (millimoles per L; B), and serum FFA (moles per L; C) concentrations after sc administration of 1 μg GHRP-6 in normal healthy subjects with or without fasting and with or without the presence of a GH blocker. ■, Fasting, placebo (n = 10); △, fasting, pegvisomant (n = 10); ●, nonfasting, pegvisomant (n = 5). All GHRP-6 tests were performed in all subjects of all study groups (including the nonfasting group) after an overnight fasting period. All subjects in the nonfasting group continued their normal daily diet throughout the study. Although different symbols were used on day 1 for each study group, study conditions with regard to fasting were identical for all subjects on this day. Pegvisomant was administered only after the two stimulatory tests were finished.

Free fatty acids

Finally, only in the five nonfasting subjects and only in the presence of pegvisomant was an acute and significant decrease in serum free fatty acid (FFA) levels observed (day 4; FFA, 0800 h *vs.* 1600 h, 0.33 ± 0.1 *vs.* 0.15 ± 0.03 mmol/L; $P < 0.05$). Interestingly, on day 4, FFA levels did increase after GHRP-6 administration in the fasting state regardless of the presence of a GHR blockade (0800 h *vs.* 1600 h, 0.89 ± 0.01 *vs.* 1.30 ± 0.18 mmol/L; $P < 0.05$; Fig. 1C). A decrease in serum FFA concentrations in all groups was observed after GHRP-6 administration on day 1.

IGF-I

Serum total IGF-I levels did not change during the fasting period. However, we observed an increase in IGF-I levels on day 2, which was probably induced by the two stimulatory tests on day 1 (mean IGF-I at baseline, 26.6 ± 2.6 nmol/L; at 1600 h on day 2, 31.9 ± 3.3 nmol/L). After day 2, total IGF-I levels began to decrease, but this decrease did not reach the level of significance on day 4. There was a slight, but significant, decrease during fasting in the presence of pegvisomant compared with the IGF-I levels in controls on day 4 (controls 0800 h on day 4 *vs.* pegvisomant 0800 h on day 4, 27.9 ± 4.0 *vs.* 23.6 ± 4.2 nmol/L; $P < 0.05$). Furthermore, blockade of the GHR did significantly reduce serum free IGF-I levels. This decrease was not further influenced during fasting (day 1 0800 h *vs.* day 4 0800 h, 0.12 ± 0.02 *vs.* 0.04 ± 0.00 nmol/L).

None of these parameters changed after the administration of 1 μg/kg GHRH (data not shown).

Discussion

In this study pegvisomant was used to study the roles of GH and its receptor in the metabolic control of the fasting status. To our surprise, we found that the administration of a standard dose of 1 μg/kg GHRP-6 induced an acute and significant increase in serum insulin and glucose and a significant decrease in FFA levels in normal individuals pretreated with pegvisomant when not fasted. Moreover, GHRP-6 administration caused a significant increase in FFA in the fasting state regardless of the presence of a GHR blockade. Why these changes were not observed after the administration of GHRH is not clear. Possibly, the effects of GHRH on metabolic processes are either not important or are mainly mediated by GH action, which in this study was reduced by pegvisomant and/or fasting. All of these GHRP-6-mediated changes in insulin, glucose, and FFA concentrations in the pegvisomant-pretreated, nonfasting group returned to baseline values the next day (data not shown). The decrease in serum FFA levels to normal nonfasting levels on day 1 in all groups is probably caused by an exaggerated food intake the night before admission, as all subjects were aware of the coming fasting period, which started on day 1 at 2400 h.

As they were only found in the presence of a GHR blocker, one must conclude that these GHRP-6-induced changes in insulin, glucose, and FFA levels are not modulated by the GH system. These observations suggest that a certain degree of GH action in peripheral target tissues, such as the pancreas, adipose tissue, and liver, control these GHRP-6-dependent changes in serum insulin, glucose, and FFA levels. Apparently there is a delicate balance between the GH-GHR system and the GH secretagogue (GHS)-GHS receptor (GHS-R) system in the control of the insulin-glucose-FFA system in healthy men, whereas blockade of the GHR unmasks tissue-specific insulin sensitivity. This tissue-specific sensitivity results in increased lipogenesis (and therefore potentially an increase in fat mass), whereas insulin resistance of carbohydrate metabolism might eventually lead to insulin resistance-related changes, as observed in type 2 diabetes mellitus (13–17). Strikingly, the metabolic changes observed in this study parallel those that occur during physiological aging (18–22). Interestingly, such changes have also been observed to a certain degree in some studies with some other GHS (23–26). The fact that GHRP-6 administration induces these metabolic effects could hypothetically be explained by an up-regulation of the GHS-R in the presence of pegvisomant (27, 28). Our data also provide some insight in the mechanisms that might underlie the observed reduced insulin sensitivity in both acromegalics and GH-deficient patients, with respect to carbohydrate metabolism, and opposite changes in lipogenesis and lipolysis in GHD *vs.* acromegaly (29–31). Apparently, the high GH concentrations in acromegaly augment glycolysis and lipolysis, and as a consequence, hyperinsulinism develops. In conditions in which GH signaling is lacking in peripheral tissue (*e.g.* GHD); however, GHS-mediated glycolysis increases, and hyperinsulinism develops, which, in turn, induce lipogenesis. Unpublished observations (A. J. van der Lely) support this delicate balance between insulin and GH action, as a number of acromegalic patients who were treated weekly with high dosages of 80 mg pegvisomant, sc, developed hypertrophy of sc adipose tissue at the injection site. This was a reversible phenomenon, as during the follow-up period off medication these changes in adipose tissue quickly resolved.

The mechanisms responsible for the observed GHRP-6-mediated increase in serum glucose and insulin concentrations during GHR blockade in the nonfasting state are not known. Strikingly, on day 1, when all subjects were also in the nonfasting state but without the presence of GHR blockade, no changes in serum glucose and/or insulin concentrations were observed after GHRP-6 administration. Possible candidates for the observed metabolic changes, such as cortisol, glucagon, glucagon-like peptide-1, or somatostatin, all lack at least one of the characteristic metabolic reactions observed in this study (increases in glucose and insulin as well in lipogenesis). It is unlikely that glucocorticoids are responsible for the increase in serum glucose and insulin concentrations, because on day 4, serum cortisol and ACTH levels decreased rapidly during the first 8 h after GHRP-6 administration, as expected on the basis of a normal diurnal pattern (data not shown). Although the metabolic effects of glucagon on glucose and insulin levels are the same as those observed after GHRP-6 administration, it has strong lipolytic activities as well (32–35). Glucagon-like peptide-1 has both lipogenic and insulinogenic actions, but it decreases glucose levels, as it is a powerful glucogenic factor (36). Whether the recently discovered and first known endogenous ligand of the GHS receptor (37) shares the observed GHRP-6-mediated changes in metabolic parameters (and the possible subse-

quent changes in body composition) will be answered when data on the effects of ghrelin administration become available. Finally, changes in paracrine somatostatin activity within the pancreatic islets would either increase or decrease insulin secretion, with opposite changes in glucose levels. Possibly, with this study we have observed direct GHS-R-mediated effects on glycolysis and insulin secretion.

Finally, Fig. 1 clearly shows that in the fasting groups, the presence of pegvisomant was not significantly important for the changes observed in glucose, insulin, and FFA levels. Apparently in this situation, loss of GH action and/or a drop in IGF-I levels seem not to be the major players in fasting-related changes in these parameters. In the nonfasting state, however, and in the presence of pegvisomant, a drop in free, but not total, IGF-I was observed. However, we observed an increase in IGF-I levels on day 2 in all subjects, which most likely was induced by the two stimulatory tests on day 1. After day 2, total IGF-I levels began to decrease, but this decrease did not reach the level of significance on day 4. Other studies are necessary to rule out confounding effects of changes in IGF-I on all noted findings. The question of which item (loss of GH action or decrease in free IGF-I concentrations) is responsible for the observed changes in glucose, insulin, and FFA levels after injection of GHRP-6 remains to be answered.

In conclusion, we found that under the condition of GHR blockade, GHRP-6 has profound stimulatory effects on serum insulin and glucose levels, which reflect a state of insulin resistance. These GHRP-6-induced changes only occur in the nonfasting state in the presence of GHR blockade. In the same condition there is an increase in lipogenesis, which indicates tissue-specific differential changes in insulin sensitivity that actually could lead to undesired changes in body composition. We also conclude that these possible effects of GHRP-6 are GH independent, and that in man some degree of GH action is necessary to prevent these GHRP-6-induced metabolic changes. Moreover, we postulate that direct GHS-R-mediated effects are involved in the induction of the metabolic alterations as well as subsequent changes in body composition, which are characteristic of the insulin resistance syndrome.

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