Potentiation of Response to Insulin and Anti-Insulin Action by Two Human Pituitary Peptides in Lean Agouti *A/a*, Obese Yellow A^{vy}/A, and C57BL/6J-*ob/ob* Mice[1] (42896)

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Abstract. **Insulin-like and anti-insulin effects of human growth hormone (hGH) were examined by determining the effects of two peptides representing portions of the hGH molecule in lean agouti *A/a* and obese yellow A^{vy}/A and *ob/ob* mice. The peptides were the amino terminal segment, residue 1–43 (hGH_{1-43}), which has been shown to potentiate the response to insulin and another peptide, hyperglycemic peptide (HP), with unknown structure, which has anti-insulin activity. The anti-insulin component is an acidic low molecular weight peptide which co-purifies with hGH but was not recognized by antibodies to intact hGH and did not cross-react with anti-hGH_{1-43} antiserum. The purpose of these studies was to further understand the multiple actions of hGH and its acute and chronic effects on response to insulin. Injections of hGH_{1-43} dramatically enhanced the effect of insulin on glucose clearance of obese yellow A^{vy}/A and *ob/ob* mice and increased the insulin-stimulated glucose oxidation in adipose tissue of yellow mice, but had no direct effect on blood glucose or insulin levels of either genotype. Administration of HP to obese yellow mice produced hyperglycemia and suppressed serum insulin concentrations. Tissues from lean agouti and obese yellow mice treated with HP *in vitro* showed decreased basal and insulin-stimulated glucose oxidation as well as decreased ^{14}C incorporation into lipids. Chronic treatment of obese yellow and *ob/ob* mice with HP increased fasting blood glucose and impaired glucose tolerance. The effect of HP was more pronounced in obese yellow mice and the *ob/ob* mice were more sensitive to the diabetogenic actions of intact hGH. These data provide further evidence for the existence of two opposing biologic activities derived from disparate amino acid sequences in hGH. Additionally, the data indicate that assays using obese yellow A^{vy}/A mice can distinguish the effects of hGH from those of the individual peptides to a greater degree than assays using obese *ob/ob* mice.** [P.S.E.B.M. 1989, Vol 191]

Human growth hormone (hGH) produces contradictory actions (insulin-like and anti-insulin) on carbohydrate metabolism both *in vivo* and *in vitro* (1). These multiple and complex actions of hGH have been very poorly understood. Purified pituitary GH preparations are known to be heterogeneous, containing both posttranscriptional and posttranslational modifications which include low molecular weight peptides, high molecular weight aggregates, interchain disulfide bonding and proteolytically altered forms (2–4). Our working hypothesis is that the opposing actions of hGH are produced by two separate regions of the hormone. The amino terminal portion of hGH (hGH_{1-43}) potentiates the tissue response to insulin, and another, still unidentified portion of the molecule, the hyperglycemic or diabetogenic peptide (HP), antagonizes insulin action and causes insulin resistance. Whereas *in vivo* and *in vitro* studies indicate that hGH_{1-43} has none of the direct effects of insulin, it greatly enhances sensitivity to insulin (5–7). On the other hand, HP directly antagonizes the response and

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sensitivity of tissue to insulin and inhibits insulin secretion in obese mice (7). Furthermore, GH administration to GH-deficient animals has a transient insulin-like action on blood glucose (hypoglycemia) followed by a more permanent state of hyperglycemia (8) and insulin resistance (9, 10). Acute treatment with hGH_{1-43} does not affect blood glucose concentrations in rats (6).

The fact that the 20-kDa variant of hGH (hGH_{20K}), which lacks 15 amino acids (residues 32–46) (11), lost most of the insulin-like activity of hGH (12–15) but retained growth-promoting (2, 16) lactogenic (2) and diabetogenic (13) activities suggested that hGH_{32-46} might be involved in the induction of insulin-like effects (17). Several recent reports have now confirmed that hGH_{32-46}, when given to rodents (5, 18, 19), dogs (20), and primates (21), has enhanced responses to insulin. Other investigators have also found that the amino terminal fragment of hGH (hGH_{1-15}) and portions of that sequence have insulin-potentiating activity both *in vivo* and *in vitro* (22–25).

Administration of large doses of hGH produced a reversible glucose intolerance in man and animals (1). When the hormone was subjected to limited proteolytic digestion, diabetogenic activity was enhanced (26–28). Furthermore, a low molecular weight human pituitary peptide was shown to be about 50 times more active than intact hGH in producing hyperglycemia in the dog (29). In the yellow A^{vy}/A mouse, the low molecular weight diabetogenic factor at a dosage of 10 μg/day for 3 days impaired glucose tolerance, whereas a preparation of highly purified hGH that had been stripped of the low molecular weight peptide, showed no significant diabetogenic activity at a 20-fold higher concentration (7).

Therefore, the purpose of this study was to determine how these two naturally occurring peptides, hGH_{1-43} and HP, modulate the tissue responses to insulin *in vivo* and *in vitro*. Previous work had indicated that these pituitary peptides were effective in nanogram quantities, suggesting their physiologic relevance in carbohydrate metabolism. The effects of hGH_{1-43}, hGH, and HP on lean agouti and obese yellow as well as *ob/ob* mice were examined using three approaches: (i) we assessed the actions of the peptides on concentrations of glucose and insulin in blood; (ii) we studied the effects of the peptides on glucose clearance; and (iii) we determined the effects of the peptides on glucose metabolism in adipose tissue *in vitro*. By being able to attribute the two opposing effects of hGH on response to insulin to two separate pituitary peptides, we may obtain insight into the mechanism of action of the intact hormone.

Materials and Methods

Experimental Design. Mice (5–9 months old) were injected with hGH_{1-43} either daily for 3 days (sc) or 60 min (ip) before the glucose tolerance test (GTT). They were fasted for 9 hr before the initiation of GTT. The hGH peptides were administered alone or with insulin. Blood was collected by retro-orbital sinus puncture to measure glucose and insulin concentrations. Nonfasted mice were used to assess tissue sensitivity to insulin in the presence of hGH_{1-43} or HP. Glucose metabolism was assayed by measuring [^{14}C]glucose oxidation to $^{14}CO_2$ of adipose tissue *in vitro* in the presence or absence of insulin and the hGH peptides.

Animals. Female "viable yellow" A^{vy}/A (BALB/c × VY)F_1 hybrid mice (45–65 g body wt) and their lean agouti A/a littermates (20–30 g) were bred in-house by mating BALB/c/AnNCr1BR females (Charles River Laboratories Inc., Wilmington, MA) with VY/WffC3H/NCTR-A^{vy}/a males (National Center for Toxicological Research, Jefferson, AR). To compare the effects of the peptides in two different obese animal models, C57BL/6J-*ob/ob* mice (50–70 g) purchased from The Jackson Memorial Laboratory (Bar Harbor, ME) were also used. All animals were housed 5–7 per cage, were fed *ad libitum* with Wayne Lab Blox diet (Allied Mills Inc., Chicago, IL), and had free access to water. The facility was maintained with an ambient room temperature of 22 ± 1°C and kept on a 12-hr light/dark cycle. The mice were weighed and handled at least twice a week throughout the experimental period.

Hormones and Pituitary Peptides. Highly purified hGH (406-38-2; 3 IU/mg) and four preparations of hyperglycemic peptides: HP-406-16-4, HP-406-30-6, HP-406-38-4, and HP-406-59-5 were used in this study. They were all prepared as described earlier (3) and generously supplied by Dr. U. J. Lewis of The Whittier Institute, La Jolla, CA. Upon further purification of HP over a Sephadex G-50 column with 10% acetic acid, the active fraction which produced hyperglycemia and glucose intolerance contained at least three peptides: a low molecular weight acidic peptide (17-kDa hGH) accompanied by what appears to be a dimer of the 17-kDa hGH (35kDa) and a 22-kDa hGH component which is probably some deamidated 22-kDa hGH (Fig. 1). Because purified 22-kDa hGH has only weak hyperglycemic activity in our assays, the amount of 22-kDa hGH in the HP preparation will be without effect. We have not conclusively identified the 17-kDa hGH which is suspected to be the diabetogenic peptide, but it does not cross-react with polyclonal antisera to either intact hGH or hGH_{1-43}. The hGH_{1-43} fragment was prepared by the solid phase peptide synthesis method (30) and was a generous gift from Dr. N. Ling, Salk Institute, La Jolla, CA. The purity of the synthetic hGH_{1-43} was confirmed by high pressure liquid chromatography and amino acid analyses. Human insulin used for injections in the GTT was Humalin R, of recombinant DNA origin, whereas that used for metabolic assays *in vitro* was crystalline porcine insulin; both were purchased from Eli Lilly and Co., Indianapolis, IN.

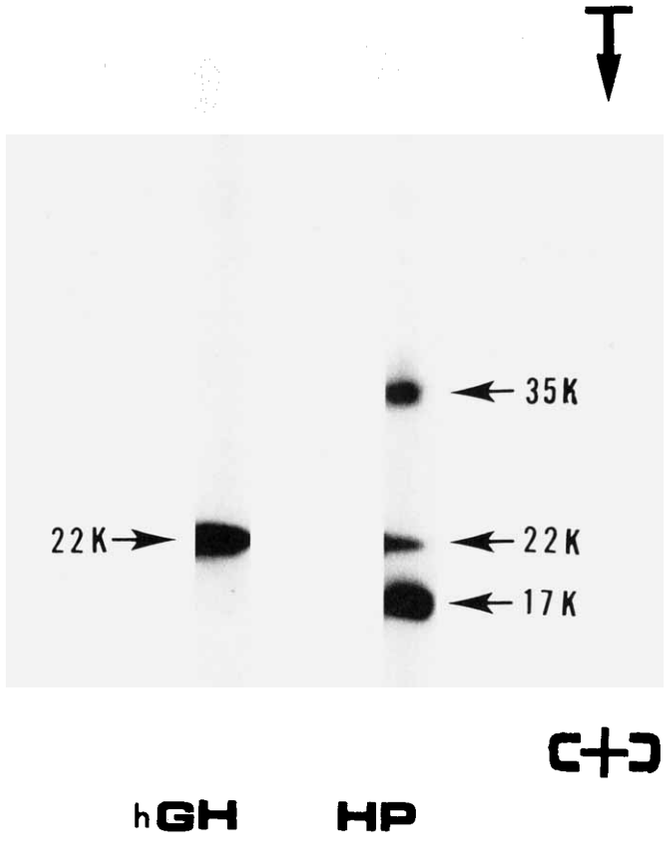

Figure 1. Polyacrylamide-gel electrophoretic mobility patterns of purified hGH and a highly active HP preparation. Electrophoresis was carried out in sodium dodecyl sulfate slab gel with 12% acrylamide and run under reducing conditions (with 2-mercaptoethanol). The gel was stained with Coomassie blue. M_r values were obtained by using molecular weight standards.

Animal Treatment. *Acute treatment.* To measure the acute effects of the peptides, two groups of mice of each genotype, one fasted for 9 hr and one nonfasted, were each divided into four sets. Each set of mice received injections (ip) of either saline, hGH_{1-43} (100 μg/mouse), HP (100 μg/mouse), or insulin (20 milliunits/mouse). Sixty minutes after injections, blood was obtained for glucose and insulin measurements by either retro-orbital sinus puncture or by collection of trunk blood after decapitation.

Chronic treatment. Mice were injected sc once daily for 3 days with saline, hGH_{1-43} (10–100 μg/mouse), HP (10–100 μg/mouse), or hGH (100 μg/mouse). On the fourth day, the mice were fasted for 9 hr and blood samples were obtained by retro-orbital sinus puncture for glucose analyses.

Serum Glucose and Insulin Measurements. Blood was allowed to clot at room temperature for 20 min, placed on ice at 4°C for 6 hr, and then centrifuged at 1500*g* for 30 min. Serum was collected and kept frozen at −70°C for glucose and insulin analyses. Glucose was measured by the glucose oxidase method using a Glucose Analyzer-2 (Beckman Instruments Inc., Fullerton, CA), and insulin was measured by radioimmunoassay using a Coat-a-Count insulin kit (Diagnostic Products Corp., Los Angeles, CA).

Glucose Tolerance Test. To measure the acute effects of hGH_{1-43} on glucose clearance, mice were fasted for 9 hr. An initial blood sample (25 μl) to determine the fasting concentration of glucose was obtained by retro-orbital sinus puncture using very narrow graduated capillary tubes. The mice received injections (ip) of 200 μl of a glucose solution (1 mg/g body wt for *ob/ob* mice and 2 mg/g body wt for lean agouti and obese yellow mice). The doses reflect the fact that *ob/ob* mice are known to have severely impaired glucose clearance rates compared with the other mice (7, 28). To assess the effect of the peptide and insulin, the mice received simultaneous injections of either hGH_{1-43} (100 μg/mouse), insulin (20 milliunits/mouse), or a combination of hGH_{1-43} and insulin. Blood samples were collected at intervals of 30 and 60 min over a period of 2 to 3 hr after injections. To each of the blood samples collected, 40 μl of 0.3 *N* $ZnSO_4 \cdot 7H_2O$ and 40 μl of 0.3 *N* $Ba(OH)_2$ were added to precipitate blood protein and cells. The samples were centrifuged and the clear supernatants were collected for glucose determinations.

The diabetogenic effect of HP was assessed as described by Reagan (31). Briefly, each genotype was separated into five animals per group. In order that all mice serve as their own controls, they first received injections (sc) of saline once a day for 3 days. On the fourth day, the mice were given injections (sc) of 2 μg of dexamethasone sodium phosphate (Merck, Sharp and Dohme, West Point, PA) and were fasted for a period of 9 hr. Fasting blood samples (25 μl) were collected and the GTT was repeated as described above. The mice were allowed to rest for 4 days. Then for 3 days, each group of mice received daily injections of either hGH (100 μg/mouse) or one of various doses of HP (10–100 μg/mouse); on the fourth day the GTT was repeated.

Glucose Metabolism. Male mice were given injections of saline, hGH_{1-43} (10–100 μg/mouse), or HP (10–100 μg/mouse). For chronic treatments, the animals were sacrificed 2 hr after the last injection (ip). Epididymal adipose tissue was quickly removed and placed in Krebs-Ringer-Hepes (KRH) buffer (pH 7.4) containing 1% bovine serum albumin and 1 m*M* glucose. Adipose tissue from at least five mice was pooled by group and randomized in each assay. Tissue from different mice in the same treatment group was pooled in order to obtain enough tissue for various metabolic assays and also to provide the mean for the group relative to the other treatment groups. Preliminary studies indicated that results were not improved by assaying tissue from each individual animal separately. Tissue segments (30–40 mg) were transferred into six separate 20-ml vials containing fresh KRH buffer, 1% bovine serum albumin, and 350,000 dpm per vial of 1 m*M* [U-^{14}C]glucose (specific activity, 250–360 mCi/mmol; Research Products International Corp., Mount Pros-

pect, IL) either without insulin (basal) or with insulin (250 or 500 microunits/ml) to make a total volume of 1 ml. The tissue samples were then thoroughly gassed with 95% O_2–5% CO_2 and $^{14}CO_2$ was collected after a 2-hr incubation as described previously (5, 6). Radioactivity was counted using a liquid scintillation LS-200 spectrophotometer (Beckman Instruments Inc., Fullerton, CA) with counting efficiency measured by external standard.

To study the effects of the peptide *in vitro*, mice were sacrificed and adipose tissue was removed. The tissue segments were transferred into vials of KRH buffer containing 1% bovine serum albumin and 1 m*M* [U-^{14}C]glucose in the absence or presence of hGH_{1-43} (1–10 μg/ml), HP (0.001–0.1 μg/ml), hGH (1–10 μg/ml), or insulin (500 microunits/ml) and were incubated for 4 hr in a shaking water bath at 37°C. A longer incubation period (4 hr) with HP provided a more consistent result. The amount of $^{14}CO_2$ released was trapped and counted. To measure ^{14}C incorporated into total lipids, the tissue segments were washed thoroughly with KRH buffer, minced, transferred into vials, and extracted with an acidic isopropanol/heptane mixture at 37°C for 3 hr and then counted.

Statistical Analysis. For each study at least three separate experiments were conducted, and no less than five observations were made for each treatment group. In each experimental determination, five to seven mice per group were used and all data are expressed as mean ± SEM. Statistical comparison between treatment groups was done using Student's *t* test, and probability values less than 0.05 were considered to be statistically significant. When more than two comparisons were made, the data were analyzed by two-way analysis of variance, followed by Duncan's test for multiple range.

Results

Effect of hGH_{1-43} and HP on Blood Glucose and Insulin. Injections of hGH_{1-43} in fasted and nonfasted yellow mice did not significantly affect blood glucose or insulin concentrations (Table I). As expected, blood glucose levels of fasted yellow mice were slightly lower than those in the nonfasted group. Also, serum insulin levels of fasted yellow mice were correspondingly lower. The treatment of nonfasted yellow mice with either saline or hGH_{1-43} resulted in a significant increase in blood glucose concentrations. This was probably the response to being handled, resulting in glucose output by the liver because of glycogen breakdown, since these changes were not present in the fasted mice. When the increase in blood glucose (Δ) from yellow mice that received saline injections was compared with those treated with hGH_{1-43}, no significant difference was observed (Table I). Furthermore, injections of hGH_{1-43} had no effect on serum glucose or insulin concentrations in fasted yellow mice. Because of the wide variation in blood glucose concentration following injection in the nonfasted state, the effects of HP were tested only in fasted yellow mice. Treatment of the fasted yellow mice with HP dramatically increased ($P < 0.0001$) blood glucose concentrations (173 ± 7 vs 487 ± 29 mg/dl) and significantly decreased ($P < 0.01$) insulin concentrations (128 ± 24 vs 58 ± 10 microunits/ml) when compared with the saline-treated controls (Table I). Intact hGH at a dose of 100 μg/mouse had no effect on the concentrations of glucose and insulin in yellow mice (Table I). Therefore, acute treatment with HP has direct diabetogenic activity in these mice.

Effect of hGH_{1-43} and HP on Glucose Tolerance Test. The glucose clearance rate in yellow mice that had received hGH_{1-43} alone was the same as that in saline-treated controls, and only insulin showed a slight but not significant increase (Fig. 2). However, a combination of hGH_{1-43} and insulin given to yellow mice resulted in a significant ($P < 0.05$) increase in their glucose clearance rates compared with the results for the other treatment groups. When areas under the curve for GTT experiments were calculated, similar statistical

Table I. Acute Effects of Injections of hGH_{1-43}, HP, and intact hGH on Serum Glucose and Insulin Levels of Obese Yellow A^{vy}/A (BALB/c × VY)F_1 Hybrid Mice[a]

	Serum glucose (mg/dl)			Serum insulin (microunits/ml)		
	0 min	60 min	Δ	0 min	60 min	Δ
Nonfasted						
Control (saline)	182 ± 7 (5)	261 ± 12 (5)	79	182 ± 36 (5)	133 ± 26 (5)	−49
hGH_{1-43} (100 μg)	192 ± 12 (5)	270 ± 25 (5)	78	144 ± 20 (5)	124 ± 20 (5)	−20
Fasted						
Control (saline)	155 ± 7 (5)	160 ± 4 (5)	5	166 ± 14 (5)	154 ± 25 (5)	−12
hGH_{1-43} (100 μg)	173 ± 8 (10)	163 ± 5 (10)	−10	162 ± 24 (9)	164 ± 12 (9)	2
HP (100 μg)	173 ± 7 (8)	487 ± 29 (8)	314*	128 ± 24 (7)	58 ± 10 (7)	−70**
hGH (100 μg)	159 ± 11 (6)	180 ± 9 (6)	21	145 ± 17 (6)	156 ± 10 (6)	11

[a] Data are expressed as mean ± SEM. The number of animals is in parentheses. Obese yellow A^{vy}/A mice were either fed *ad libitum* or fasted for 9 hr and blood samples were obtained. The mice were injected with saline (control), hGH_{1-43} (100 μg/mouse), HP (100 μg/mouse), or intact hGH (100 μg/mouse) and blood samples from each group were collected 60 min later for glucose and insulin analyses. * $P < 0.0001$ compared with fasted control, hGH_{1-43}-, and hGH-treated groups. ** $P < 0.01$ compared with fasted control, hGH_{1-43}-, and hGH-treated groups.

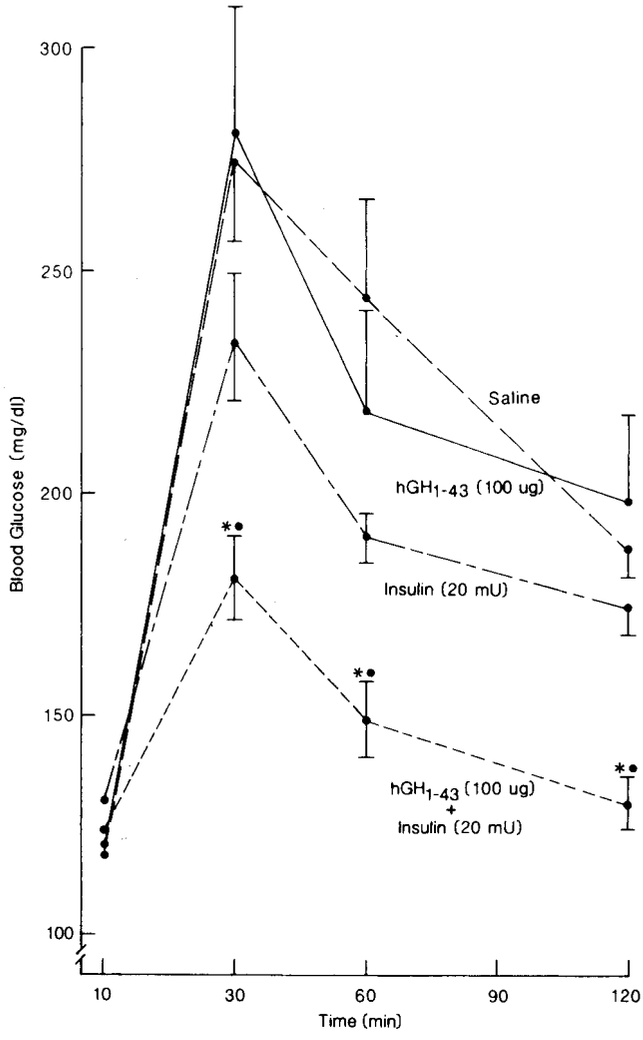

Figure 2. Effect of hGH_{1-43} and insulin on glucose tolerance in obese yellow A^{vy}/A mice. Mice were fasted for 9 hr, blood samples were obtained, and glucose solution (2 mg/g body wt) was injected (ip) without or with hGH_{1-43} (100 μg/mouse) or insulin (20 milliunits/mouse), or a combination of hGH_{1-43} and insulin. Blood sampling was done for 120 min at 30- and 60-min intervals. Each point represents the mean ± SEM of at least eight mice from two separate experiments. *$P < 0.01$ compared with saline (control) and hGH_{1-43}; •$P < 0.05$ compared with insulin.

significance was obtained. No change in glucose clearance rate was observed when lean agouti mice were treated with hGH_{1-43}, and the insulin-stimulated clearance rate was not increased further by hGH_{1-43}.

For comparison, the effect of the insulin-potentiating peptide (hGH_{1-43}) on glucose tolerance was examined in fasted *ob/ob* mice (Table II). Although hGH_{1-43} alone had no effect when compared with saline-treated control, injections with insulin alone in *ob/ob* mice slightly but significantly improved glucose clearance ($P < 0.025$), and when hGH_{1-43} and insulin were given the rate of glucose clearance was significantly increased further ($P < 0.05$). This indicates that hGH_{1-43} is also an effective *in vivo* potentiator of insulin response in *ob/ob* mice.

In order to determine the effective dose of HP, the obese yellow mice were treated with various doses of HP or hGH and the results were compared with those for yellow mice treated with saline. Injections of hGH (100 μg/day) did not result in a significant fasting hyperglycemia and only slightly but significantly ($P < 0.05$) impaired glucose tolerance at the 60- and 120-min points (Fig. 3); blood glucose concentrations, however, were not different from those of the controls at 180 min. Injections of HP in yellow obese mice at a dose of 10 μg/day increased, although not significantly, the fasting blood glucose concentrations, but they significantly impaired glucose tolerance at all times tested ($P < 0.05$) (Fig. 4). This indicates that HP at a dose of 10 μg/day had a more pronounced effect on glucose tolerance than 100 μg/day of hGH in obese yellow mice. At a higher dose of 50 μg/day, HP significantly ($P < 0.05$) increased fasting blood glucose concentrations and impaired glucose tolerance (Fig. 4), which was further increased at an HP dose of 100 μg/day ($P < 0.01$).

In order to compare the response of the obese yellow mouse to that of the *ob/ob* mouse to HP, two doses of the HP fraction (10 and 100 μg/day) and a single dose of hGH (100 μg/day) as a control were used. Treatment of *ob/ob* mice with hGH resulted in fasting hyperglycemia and significantly ($P < 0.05$) impaired glucose tolerance (Fig. 5). Although previous studies (13) indicated that hGH is diabetogenic at concentrations as low as 10 μg/day, HP at such a low dose had no effect in the *ob/ob* mouse (Fig. 6). But at a higher dose of HP (100 μg/day) the *ob/ob* mouse showed a significant ($P < 0.05$) increase in fasting blood glucose concentrations, and the glucose tolerance curve was significantly ($P < 0.05$) impaired (Fig. 6). At such a high dose of HP, the extent of glucose impairment was the same as that obtained with hGH treatment at all time points examined (Fig. 5). Therefore, it appears that the *ob/ob* mouse is somewhat less sensitive than the yellow mouse to the diabetogenic actions of HP. However, although HP is equipotent to hGH in the *ob/ob* mouse (Figs. 5 and 6), it is at least 10 times more active than hGH in the yellow mouse.

***In Vitro* Glucose Metabolism in Adipose Tissue.** In lean agouti and obese yellow mice, hGH_{1-43} added *in vitro* did not stimulate the basal or increase further the insulin-stimulated [U-^{14}C]glucose oxidation to $^{14}CO_2$ of adipose tissue. In contrast, when the peptide was administered *in vivo*, it significantly enhanced insulin action on glucose oxidation *in vitro* in mice (5) and rats (6). The *in vivo* effect of HP on blood glucose concentration and glucose clearance in the obese yellow mouse at a dose as low as 10 μg prompted us to study the action of HP on glucose metabolism in excised adipose tissue *in vitro*.

Incubation with HP at a dose of 10 ng/ml significantly inhibited the basal ($P < 0.05$) as well as the

Table II. Potentiation by hGH_{1-43} of Effect of Exogenous Insulin on Glucose Clearance in Obese C57BL/6J-*ob/ob* Mice[a]

Treatment		Serum glucose (mg/dl)		
		0 min	30 min	180 min
Control (saline)	(10)	194 ± 15	608 ± 49	568 ± 58
hGH_{1-43} (100 μg)	(5)	202 ± 22	578 ± 17	542 ± 62
Insulin (20 milliunits)	(5)	169 ± 7	509 ± 11*	407 ± 46*
hGH_{1-43} (100 μg) + insulin (20 milliunits)	(5)	170 ± 11	444 ± 24**	339 ± 22**

[a] Data are expressed as mean ± SEM. The number of animals is in parentheses. Obese *ob/ob* mice were fasted for 6 hr, blood samples were obtained, and glucose solution (1 mg/g body wt) was injected without or with hGH_{1-43} (100 μg/mouse), insulin (20 milliunits/mouse), or a combination of hGH_{1-43} and insulin. Blood sampling was done at 30 and 180 min after the aministration of glucose and concentration of serum glucose was assessed. * $P < 0.025$ compared with control and hGH_{1-43}-treated groups. ** $P < 0.05$ compared with insulin-treated group.

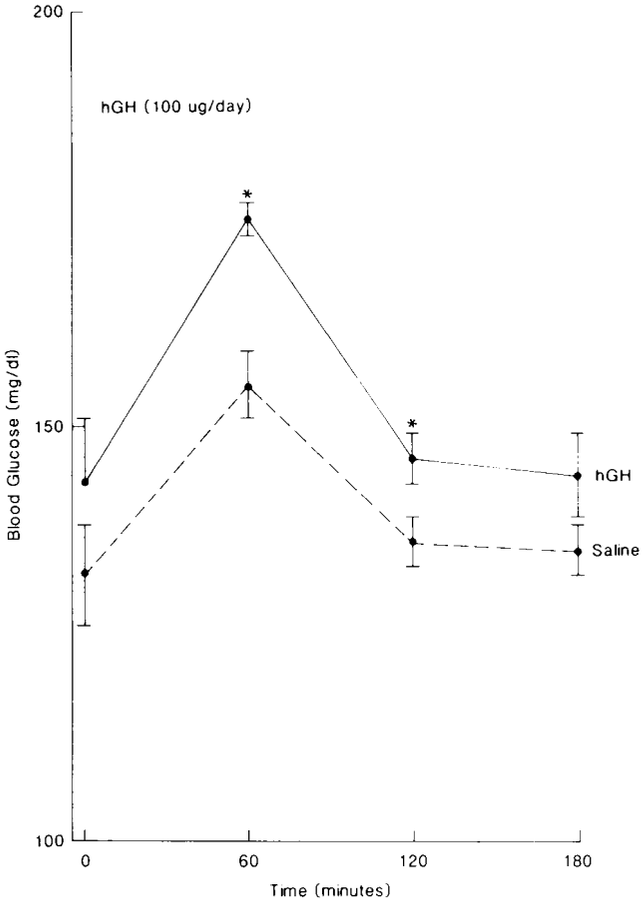

Figure 3. Effect of hGH on glucose tolerance in obese yellow A^{vy}/A mice. Mice were treated for 3 days with saline or hGH (100 μg/day). On the fourth day the mice were fasted for 9 hr, blood samples were obtained, and glucose solution (2 mg/g body wt) was injected (ip). Blood sampling was done for 180 min at 60-min intervals. Each point represents the mean ± SEM of at least 15 mice from three separate experiments. *$P < 0.05$ compared with saline-treated controls.

insulin-stimulated ($P < 0.01$) glucose oxidation rates of adipose tissue from yellow mice (Fig. 7). Furthermore, in tissue from lean agouti mice, HP at a dose of 100 ng/ml also significantly inhibited basal ($P < 0.05$) and insulin-stimulated ($P < 0.01$) glucose oxidation (Fig. 8), whereas the basal ^{14}C incorporation into total lipids was significantly ($P < 0.05$) inhibited at an HP dose of only 10 ng/ml (Fig. 9). No such effect was observed from hGH at a dose of 100 ng/ml in tissue from both agouti and yellow mice. It is interesting to note that while tissue from agouti mice showed a 5- to 6-fold stimulation with insulin, yellow mice had less than a 2-fold increase, indicating again that the tissue from yellow mice is less sensitive to insulin stimulation (7). In support of these results is our recent observation that membranes prepared from the liver of yellow mice have lower apparent insulin binding affinities (K_a) than those of corresponding agouti littermates (0.51 ± 0.14 nM^{-1} vs 1.67 ± 0.45 nM^{-1}, respectively).

Discussion

This was the first detailed study designed to compare two human pituitary-derived low molecular weight peptides to hGH in mice of various genotypes, and to determine how these peptides are involved in the expression of insulin-potentiating activity on one hand and insulin-resistance and anti-insulin activities both *in vivo* and *in vitro* on the other hand. In agreement with previous studies (3, 5, 6, 20, 22–25, 29) our current observations support the hypothesis that there are at least two peptides responsible for the various biologic effects of hGH. Also, our data support the notion that the actions of hGH are associated with amino acid sequences at distinct regions of the molecule. The amino terminal fragment (hGH_{1-43}) potentiates the response to insulin, whereas another portion (HP) is involved in antagonizing insulin action. Other workers have suggested that disparate "active centers" exist in the hGH molecule and that the growth-promoting effect may be independent of both insulin-like and diabetogenic activities (2, 3, 6, 12–14, 17, 32, 33). These findings suggest that the insulin-like action is independent of the sequences responsible for growth-promoting effects (6). There is still no convincing evidence to establish whether the peptide sequences involved in anti-insulin activity are growth promoting. Separation of the insulin-like (or insulin-potentiating) and anti-insulin actions through the use of these pituitary pep-

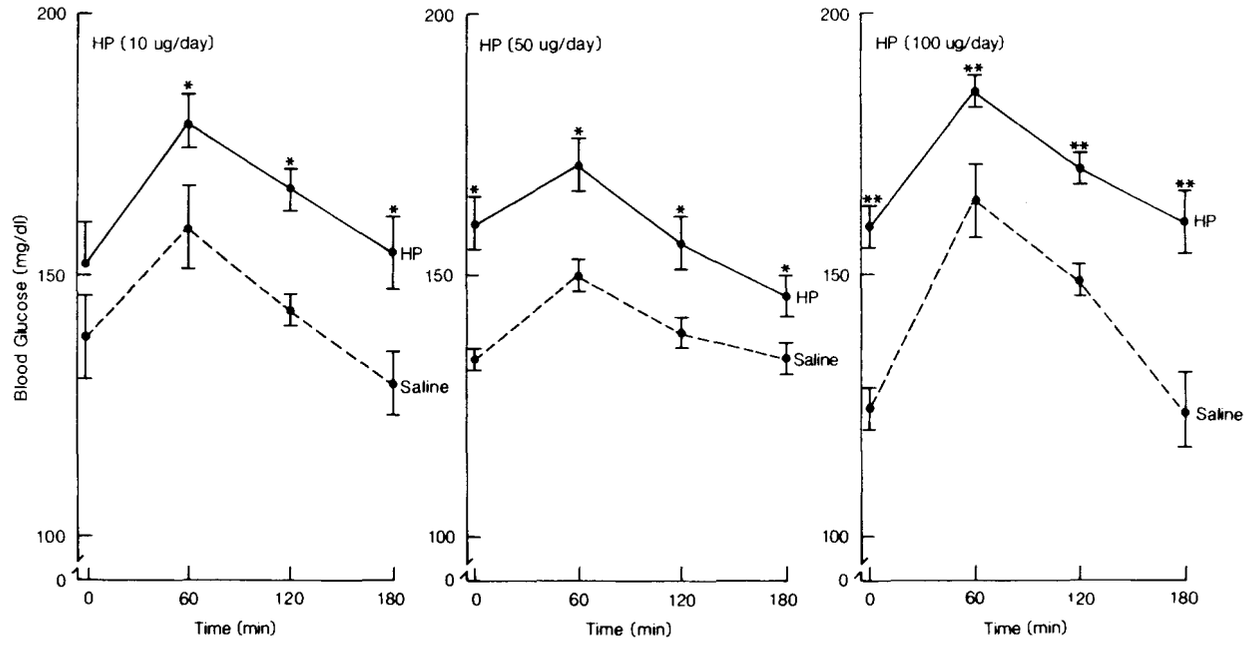

Figure 4. Effect of HP on glucose tolerance in obese A^{vy}/A yellow mice. Mice were treated for 3 days with saline or various doses of HP (10, 50, and 100 μg/day). On the fourth day the mice were fasted for 9 hr, blood samples were obtained, and glucose solution (2 mg/g body wt) was injected (ip). Blood sampling was done for 180 min at 60-min intervals. Each point represents the mean ± SEM of at least 10 mice from three separate experiments. $^{*}P < 0.05$; $^{**}P < 0.01$ compared with saline-treated controls.

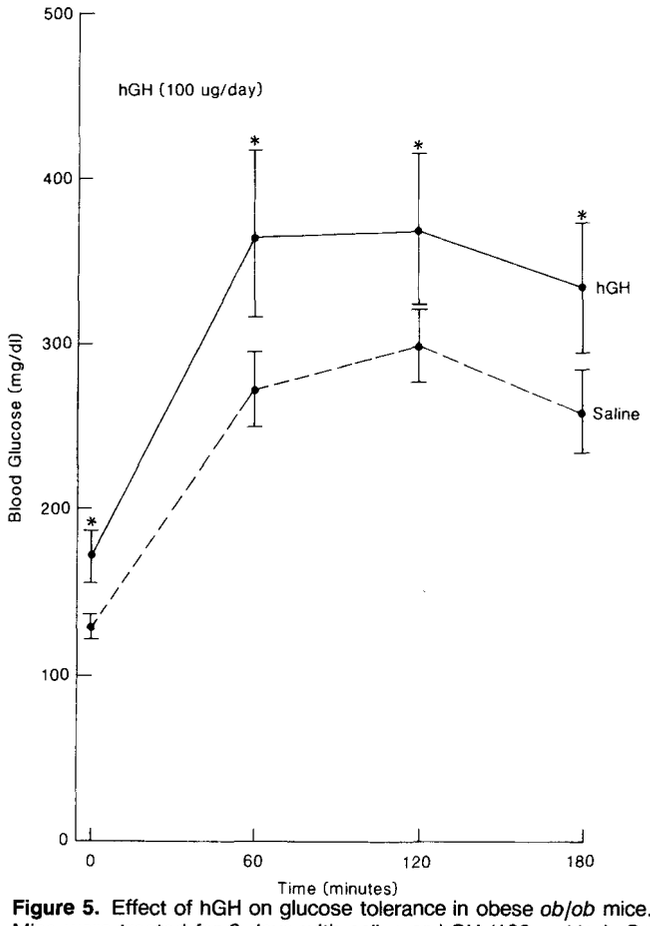

Figure 5. Effect of hGH on glucose tolerance in obese *ob/ob* mice. Mice were treated for 3 days with saline or hGH (100 μg/day). On the fourth day the mice were fasted for 9 hr, blood samples were obtained, and glucose solution (1 mg/g body wt) was injected (ip). Blood sampling was done for 180 min at 60-min intervals. Each point represents the mean ± SEM of at least 11 mice from two separate experiments. $^{*}P < 0.05$ compared with saline-treated control.

tides should contribute to the understanding of the actions of hGH.

Frigeri *et al.* (34) reported that the insulin-like action of hGH on adipose tissue from hypophysectomized rats and adipocytes from normal rats was neutralized by the presence of polyclonal anti-insulin antibodies *in vitro*. In support of this observation, Gause *et al.* (35) and Guller *et al.* (36) indicated that hGH needs insulin for its action on target cells. Stevenson *et al.* (20) found that hGH_{32-46} enhanced glucose clearance in the dog by stimulating insulin secretion and thus increasing glucose utilization, and Mondon *et al.* (18, 19) observed that hGH_{32-46} enhanced glucose utilization by peripheral tissues of normal rats without suppressing hepatic glucose outflow. In our study, treatment with hGH_{1-43} did not affect insulin concentrations but enhanced insulin action in lowering blood glucose concentrations and increasing glucose oxidation in adipose tissue of yellow mice. Therefore, we concluded that insulin is required for demonstrating the direct insulin-like activity of hGH, which suggests an insulin-potentiating function for hGH. The question of when or how the insulin-like activity of hGH is expressed is fundamental to understanding its actions. As hGH_{1-43} does not appear to compete with hGH or insulin at the receptor level, it is possible that it causes changes on these receptors or even other hormone receptors such as those of glucagon, glucocorticoid or catecholamines, all of which are involved in carbohydrate metabolism. Although it seemed reasonable to assume that hGH_{1-43} affects tissue membranes so as to result in changes in glucose metabolism, this did not prove to be the case. For example, exposing the tissue directly to hGH_{1-43} *in*

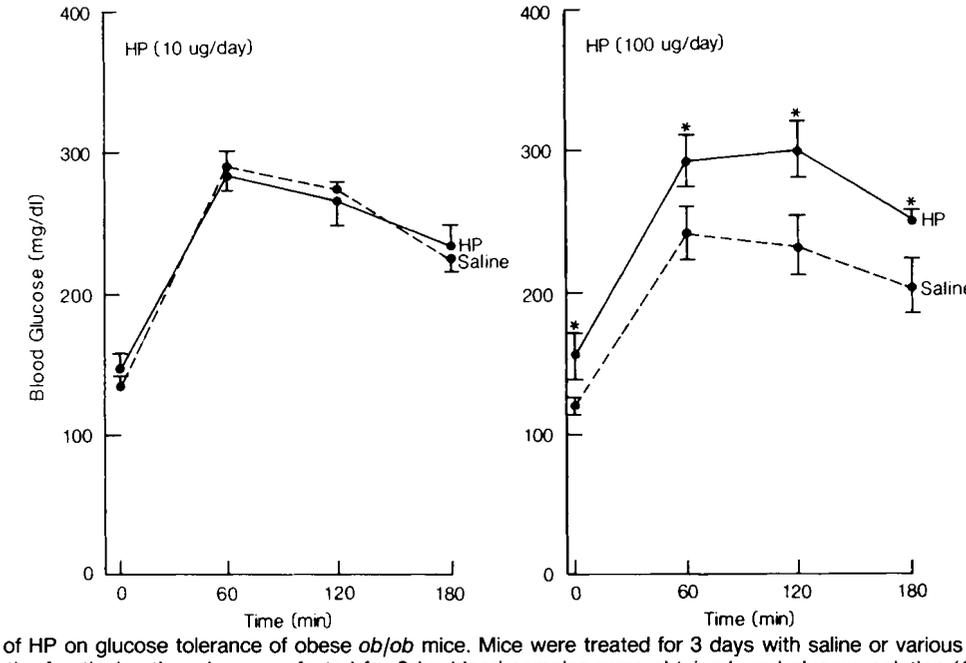

Figure 6. Effect of HP on glucose tolerance of obese *ob/ob* mice. Mice were treated for 3 days with saline or various doses of HP (10 and 100 μg/day). On the fourth day the mice were fasted for 9 hr, blood samples were obtained, and glucose solution (1 mg/g body wt) was injected (ip). Blood sampling was done for 180 min at 60-min intervals. Each point represents the mean ± SEM of at least 10 mice from two separate experiments. $^{*}P < 0.05$ compared with saline-treated controls.

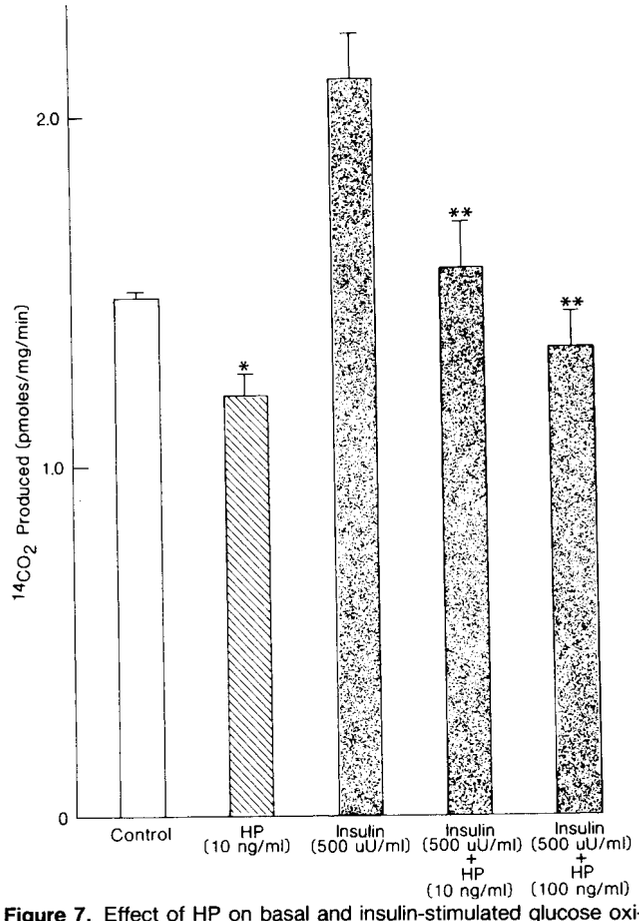

Figure 7. Effect of HP on basal and insulin-stimulated glucose oxidation in adipose tissue from obese yellow A^{vy}/A mice measured *in vitro*. Adipose tissue was incubated in the presence or absence of HP (10 or 100 ng/ml) and insulin (500 microunits/ml) for 4 hr and the rate of [U-^{14}C]glucose oxidation to $^{14}CO_2$ was measured. Each bar represents the mean ± SEM of at least 12 replicates from two separate experiments. $^{*}P < 0.05$ compared with controls; $^{**}P < 0.05$ compared with insulin alone.

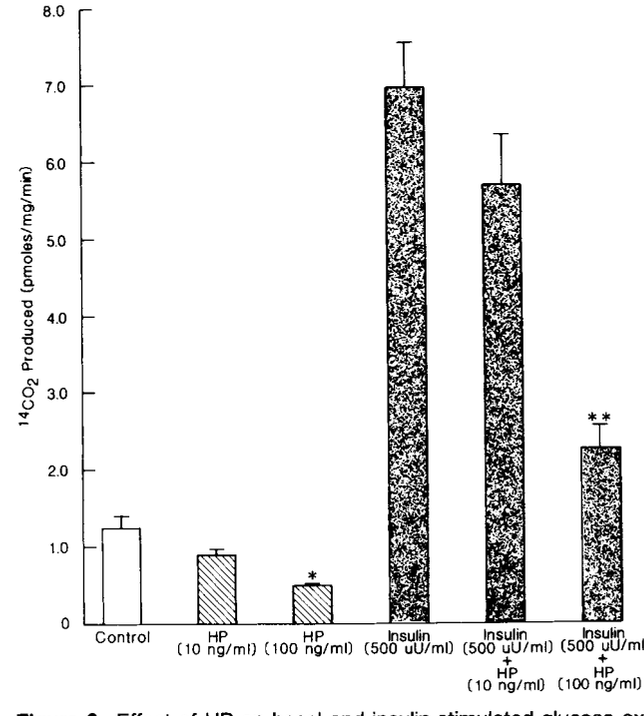

Figure 8. Effect of HP on basal and insulin-stimulated glucose oxidation in adipose tissue from lean agouti *A/a* mice measured *in vitro*. Adipose tissue was incubated in the presence or absence of HP (10 and 100 ng/ml) and insulin (500 microunits/ml) for 4 hr and the rate of [U-^{14}C]glucose oxidation to $^{14}CO_2$ was measured. Each bar represents the mean ± SEM of at least 10 replicates from two separate experiments. $^{*}P < 0.01$ compared with controls; $^{**}P < 0.001$ compared with insulin alone.

vitro did not stimulate glucose oxidation, and hGH_{1-43} only enhanced insulin action following *in vivo* administration (5). *In vivo*, both acute and chronic treatment with hGH_{1-43} enhanced insulin action on glucose clear-

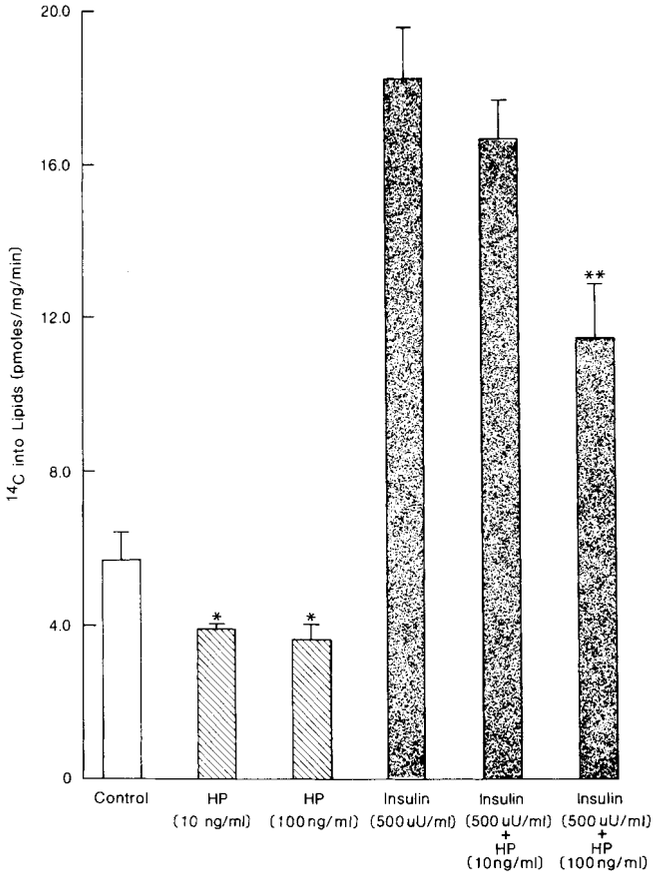

Figure 9. Effect of HP on basal and insulin-stimulated lipogenesis in adipose tissue from lean agouti *A/a* mice measured *in vitro*. Adipose tissue was incubated in the presence or absence of HP (10 and 100 ng/ml) and insulin (500 microunits/ml) for 4 hr and the rate of ^{14}C incorporated into total lipids was measured. Each bar represents the mean ± SEM of at least 10 replicates from two separate experiments. $^{*}P < 0.05$ compared with controls; $^{**}P < 0.01$ compared with insulin alone.

ance and metabolism in yellow mice and diabetic rats (5, 6). Our evidence using hGH_{1-43} supports the concept of an insulin-potentiating action for hGH rather than a direct insulin-like action. The enhancement of insulin action by hGH_{1-43} could be due to increased insulin binding, decreased insulin resistance, or other postreceptor mechanisms. Other possibilities include the production of a second messenger following treatment of the peptide *in vivo* which then acts to potentiate insulin action.

The contradictory actions of hGH on carbohydrate metabolism have often led to misunderstanding and misinterpretation of its metabolic effects (1). Other workers have disagreed on the multiple actions of hGH and suggested that the observed actions were mainly due to contaminating pituitary peptides of low molecular weight (1, 3, 7, 29). That a low molecular weight fragment of hGH (5,000–10,000 kDa) is responsible for the diabetogenic activity of the pituitary was previously proposed by two other laboratories (28, 37). Lostroh and Krahl (28) reported that hGH_{44-77} contains the anti-insulin activity whereas Bornstein *et al.* (37) considered the carboxyl terminal portion ($hGH_{171-191}$) to be the region of anti-insulin activity. The amino acid sequence of the lower molecular weight hGH (17-kDa hGH) that was in our HP preparation will be needed before the relation of our material to that used by other investigators is known. Arguments that the most active diabetogenic substance in the pituitary gland is a form of GH have been presented (38). Cameron *et al.* (39) showed that when adipose tissue was exposed *in vitro* to *S*-carboxymethylated hGH (a diabetogenic derivative of hGH), the basal and insulin-stimulated glucose oxidation was unaffected. They concluded that the diabetogenic action of hGH in the *ob/ob* mice is characterized by sustained hyperinsulinemia which eventually triggers hyperglycemia and peripheral insulin resistance. On the other hand, we found chronic treatment of yellow mice with large doses of hGH (100 μg/day) resulted in only a mild hyperglycemia, a slight impairment of glucose tolerance, and no change in circulating insulin concentrations (7). Although hGH and HP are equipotent in producing diabetogenic activity in *ob/ob* mice, HP impairs glucose tolerance at a very low dose in yellow mice but not in *ob/ob* mice. The difference between these two genotypes of obese mice could be due to the degree of obesity, since in obese yellow mice fat constitutes a smaller proportion of body weight than in *ob/ob* mice when compared with their respective littermate (40). Furthermore, hGH at low doses (10 and 100 ng/ml) did not decrease the *in vitro* basal or insulin-stimulated glucose oxidation in adipose tissue from yellow mice, and only at a very high dose of hGH (1000 ng/ml) did we find a significant inhibitory response (data not shown). In contrast, treatment of yellow mice with small doses of HP not only decreased plasma insulin concentrations but also caused hyperglycemia, impaired glucose tolerance, and increased insulin resistance. When adipose tissue from yellow and agouti mice was directly exposed to nanogram concentrations of HP *in vitro*, HP severely inhibited basal and insulin-stimulated glucose metabolism. Even on the basis of molar concentrations, the effects of HP were at least 10 times greater than those of intact hGH in producing glucose intolerance and inhibiting glucose oxidation. We conclude that yellow mice not only provide a sensitive assay for assessing the insulin-potentiating and anti-insulin actions, but can also distinguish the effects of hGH from those of hGH_{1-43} and HP.

There are several possible explanations for the effect of HP in mice. First, it could be due to inhibition of insulin secretion in response to exogenously administered glucose, to stimulation of catecholamines and glucagon release, or to increased insulinase activity. Second, treatment with HP could have inhibited the response to insulin on glucose uptake and oxidation. Third, HP could have induced insulin resistance by decreasing insulin binding or by some other postreceptor effect. The fourth possibility could be due to the

direct effect of HP on carbohydrate metabolism in inhibiting glucose transport in peripheral tissues, in increasing glucose output or in decreasing glucose metabolism in the liver.

These studies indicate that the amino terminal portion of hGH (hGH_{1-43}) enhances the action of insulin whereas HP, a still unidentified peptide, has anti-insulin activity. The existence of opposing biologic activities in two distinct regions of the hGH molecule not only suggests the presence of active peptide sequences but also that these peptides can be separated by proteolytic cleavage of the intact hormone or produced by a specific processing mechanism. Whether these two regions of hGH are directly involved in modulating insulin responses or whether an imbalance of the peptides results in this effect is not known. As these peptides are active under physiologic conditions and are found in significant amounts in the pituitary, their metabolic relevance is implied. However, until specific radioimmunoassays for measurement of these two peptides in both the blood and pituitary gland are available, assigning specific functions to them will have to wait.

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