Effects of Hypophysectomy and the Insulin-Like and Anti-Insulin Pituitary Peptides on Carbohydrate Metabolism in Yellow A^{vy}/A (BALB/c × VY)F_1 Hybrid Mice (42942)

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Abstract. **The amino-terminal portion of human growth hormone, residues 1- 43 (hGH_{1-43}), has insulin-potentiating action, while a hyperglycemic pituitary peptide (HP), which co-purifies with human growth hormone (hGH), is antagonistic to the action of insulin. The effects of hGH, hGH_{1-43}, and HP on glucose metabolism were assessed in young (4–5 weeks) and adult (6–8 months) hypophysectomized yellow A^{vy}/A mice which lacked any interfering endogenous pituitary hormones, and compared with age-matched intact obese yellow A^{vy}/A and lean agouti A/a mice. Treatment with hGH_{1-43} or HP did not promote body growth in hypophysectomized yellow mice; but after 2 weeks of treatment with hGH, there was a significant increase in body weight ($P < 0.05$). Treatment with HP raised blood glucose and lowered insulin concentrations in obese yellow mice, but not in agouti or hypophysectomized yellow mice. The severely impaired glucose tolerance of the hypophysectomized yellow mice was improved by acute (60 min) and chronic (3 days) treatment with hGH_{1-43} as well as by 2 weeks of treatment with hGH; in contrast, HP had no effect. Glucose oxidation in adipose tissue from obese yellow mice was low and showed essentially no response to stimulation by insulin at doses lower than 1000 microunits/ml. Basal glucose oxidation rates in adipose tissue taken from agouti and hypophysectomized yellow mice were significantly higher ($P < 0.001$) than those in tissue from obese yellow mice, and the rates responded significantly ($P < 0.05$) to 100 microunits/ml insulin. The insulin binding affinities in liver membranes from agouti mice were higher than those from either obese or hypophysectomized yellow mice. The insulin receptor densities were similar in both agouti and obese yellow mice, but higher in hypophysectomized yellow mice ($P < 0.05$). Treatment with hGH_{1-43} slightly increased, although not significantly, the insulin receptor density in yellow obese mice while hGH showed essentially no change. Therefore, hypophysectomy appeared to increase tissue response and decrease insulin resistance by increasing receptor numbers and lowering the circulating insulin levels. Furthermore, the insulin-like action of hGH was elicited directly *in vivo* by hGH_{1-43} in hypophysectomized yellow mice.** [P.S.E.B.M. 1989, Vol 191]

Human growth hormone (hGH or hGH_{22k}) is known to promote body growth and to show insulin-like and anti-insulin actions in rodents, both *in vivo* and *in vitro* (1). Somatogenic, insulin-like, and anti-insulin actions are characteristics of hGH and growth hormone (GH) from many species (1, 2). Multiple actions of hGH may arise from different regions of the macromolecule, suggesting that distinct biologic activities of hGH can be ascertained using peptides which are partial sequences of hGH. Both the amino-terminal and carboxyl-terminal regions of growth hor-

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mones (GHs) consist of α-helices and in addition two more helical structures are found in the mid-region of the native molecule (3). The three-dimensional structure of hGH_{22K} changes dramatically when the peptide is cleaved. The conformation of various peptides derived from the native molecule (hGH_{22K}) may also be altered. This could result in a peptide with enhanced or diminished activity or completely devoid of biologic activity. That the structural changes due to cleavage of hGH_{22K} result in the modification of the biologic activity of the native molecule is very possible. Moreover, low molecular weight peptides that may be hGH fragments with metabolically opposing properties (insulin-like and anti-insulin) are found in the pituitary (2, 4, 5). The two opposing properties of hGH are enigmatic and their physiologic significance is still not clear. Some workers have suggested the possibility of more than one GH receptor, each capable of binding a specific form of GH such as hGH_{20K} (a 20-kDa variant of hGH) or hGH_{22K} but with different affinities in various tissues (6, 7). The fact that several low molecular weight hGH fragments are found in significant amounts circulating in blood (8) indicates that these pituitary peptides may be important modulators of carbohydrate metabolism.

The pituitary-derived peptides can be separated into those which both enhance insulin action and decrease insulin resistance, and those which inhibit insulin action and induce insulin resistance in tissues. Some recent reports have indicated that the amino-terminal fragments of hGH showed an insulin-potentiating action (9–16), while the carboxyl terminus was antagonistic to insulin action (17). Recent studies with an amino-terminal portion of hGH (hGH_{1-43}) have shown that it enhances insulin action on adipose tissue from obese mice and diabetic rats and potentiates the action of insulin on glucose clearance (11, 13–16). Earlier studies have indicated that the presence of ambient insulin is necessary for eliciting insulin-like action of GH (18, 19). An apparent contradiction to this is that GH also induces insulin resistance in various tissues (20, 21). Human GH is known to be diabetogenic when given at higher than physiologic doses in certain animals such as dogs (22, 23), cats (24), genetically obese mice (16, 25, 26), and even humans (27). This diabetogenic action of hGH has been demonstrated by GH from all species so far studies (28). But the fact that highly purified hGH shows diminished insulin antagonistic actions (16, 26, 29) indicates that other more potent anti-insulin peptides are present in the pituitary (30). Lewis *et al.* (29) have isolated several low molecular weight peptides which co-purified with hGH, and they showed them to be 50–100 times more potent than hGH in anti-insulin activities. These hyperglycemic (diabetogenic) peptides (HP) do not cross-react with anti-hGH or anti-hGH_{1-43} antisera (16).

During the search for a suitable animal model for the study of the insulin-like and anti-insulin actions of pituitary peptides, a "viable yellow" A^{vy}/A (BALB/c × VY)F_1 hybrid mouse was shown to be very responsive to these peptides (11, 16, 26). To eliminate the effects of endogenous GH and other pituitary peptides which might influence the metabolic assays, we have initiated studies in hypophysectomized yellow mice. Since GH deficiency is associated with increased insulin sensitivity and since prolonged treatment with GH induces insulin resistance (31), the acute and chronic effects of hGH and those of hGH_{1-43} and HP on blood glucose and insulin concentrations, glucose clearance, glucose metabolism, and insulin binding in tissues of hypophysectomized yellow mice were compared with those seen in intact obese yellow and lean agouti mice.

Materials and Methods

Animals. Female yellow A^{vy}/A (BALB/c × VY)F_1 hybrid mice and their agouti A/a siblings of two age groups (young, 4–5 weeks and adult, 6–8 months) were studied. The mice (five to six per cage) were kept at room temperature (22 ± 2°C) and a 12-hr light/12-hr dark cycle was maintained. They were fed *ad libitum* with Wayne Lab-Blox diet (Allied Mills, Inc., Chicago, IL) and had free access to water. The yellow mice develop symptoms of diabetes and obesity (hyperglycemia and hyperinsulinemia) at an age of 6–7 weeks (26).

Hypophysectomized Mice. The surgery was performed at our animal care facility as previously described by Lostroh and Jordan (32) and modified by Plotcher and Powley (33). Briefly, the mouse was anesthetized by injection (ip) of a nembutal solution at a dose of 0.1 mg/g body wt. The surgical procedure involved the gentle use of a micro-drill through a suture at the parapharyngeal side of the mouse head; the pituitary gland was slowly and carefully sucked out with a fine pointed glass pipette tip that was connected to a vacuum pump. Previous studies (32, 33) have indicated that sham operation causes only acute undue stress to the mice but does not alter the physiologic response when compared with unoperated mice. Thereafter, the mice were fed *ad libitum* with a special chow (hypophysectomy diet no. 902029; ICN Biochemicals, Cleveland, OH) and kept under conditions identical to those described above. In a preliminary study, we observed that alternating the diet for intact and hypophysectomized mice had no effect on the assay data. Because these hormone-deficient hypophysectomized yellow mice were not supplemented with hormonal therapy postoperatively, they were kept on special diet throughout the study. The hypophysectomized mice were rested for 10–12 days before blood samples were collected for glucose and insulin measurements.

Peptides and Hormones. The hyperglycemic peptides (HP-406-30-6 and HP-407-9-5) and the highly purified hGH (406-38-2; 3 IU/mg) were prepared from frozen human pituitaries in-house by Lewis et al. (2)

using the procedure described previously. The synthetic amino-terminal portion of hGH (hGH_{1-43}) was a generous gift from Dr. N. Ling, Salk Institute, La Jolla, CA. The purity of the peptide was tested and confirmed by high-performance liquid chromatography and amino acid analyses. Human insulin used for metabolic assays was Humulin R, of recombinant DNA origin (Eli Lilly & Co., Indianapolis, IN), and bovine insulin used for binding studies was purchased from Sigma Chemical Co. (St. Louis, MO). Dosages for effective response of hGH_{1-43}, hGH, and HP in mice were determined in a preliminary study and were also reported in our earlier studies (11, 14–16).

Serum Glucose and Insulin Levels. Blood (200 μl) from the mice was obtained by retro-orbital sinus puncture, kept 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 the glucose concentration was determined by the glucose oxidase method using a Glucose Analyzer-2 (Beckman Instruments Inc., Fullerton, CA). Insulin was measured by radioimmunoassay using a Coat-a Count insulin kit (Diagnostic Products Corp., Los Angeles, CA).

Glucose Tolerance Test. Glucose tolerance tests (GTT) were performed on mice that had been fasted for 9 hr. Fasting blood samples (25 μl) were obtained by retro-orbital sinus puncture, and the mice were then given injections (ip) of a glucose solution (2 mg/g body wt), alone or simultaneously with 100 μg of either hGH_{1-43}, HP, or hGH. Blood samples were collected at intervals of 30 min over a period of 2–4 hr following the injections and analyzed for glucose. In some cases, the animals were injected with peptides once daily for at least 3 days before initiation of GTT as described previously (25, 26, 28).

Glucose Oxidation. Four to six weeks after GTT, the mice were killed by cervical dislocation, and parametrial adipose tissue was quickly removed and placed in Krebs-Ringer Hepes buffer (pH 7.4) containing 1% bovine serum albumin (BSA) and 1 m*M* glucose. Tissue from at least four mice was pooled by group and randomly distributed in each assay. The tissue segments (40–50 mg/assay) were transferred into six separate reaction vials containing fresh Krebs-Ringer-Hepes buffer, 1% BSA, and 1 m*M* [U-^{14}C]glucose (specific activity, 250–360 μCi/μmol); Research Products International Corp., Mount Prospect, IL, with or without insulin (0–2500 microunits/ml) in a total volume of 1 ml; they were then gassed thoroughly with 95% O_2-5% CO_2. The $^{14}CO_2$ collection and counting was accomplished as described previously (11, 13). Livers were also dissected from the mice and used for the insulin binding studies.

Insulin Binding Studies. Liver plasma membranes were prepared according to Maeda *et al.* (34), with a pH 9.0 homogenization buffer that contained 200 m*M* glycine, 150 m*M* NaCl, 10 m*M* EDTA, 20 m*M* EGTA, 1 m*M* phenylmethylsulfonyl fluoride, and 100 KIU Trasylol/ml. The amount of membrane protein used in each assay was determined by solubilizing the membranes in 50 m*M* $NaHCO_3$ containing 0.5% sodium dodecyl sulfate, following the procedure of Smith *et al.* (35), using BSA as a standard and the bicinchoninic protein assay reagents (Pierce Chemical Co., Rockford, IL). Bovine insulin was radiolabeled to a specific activity of 160 μCi/μg with Na^{125}I (ICN-Biochemicals) by a modification of the method of Salacinski *et al.* (36). Briefly, 5 μg of bovine insulin in 10 μl of 40 m*M* HCl and 40 μl of 0.5 *M* sodium phosphate buffer (pH 7.4) were added to a polypropylene tube previously coated with 10 μg of Iodogen (Pierce Chemical Co.). Then Na^{125}I (5 μCi in 10 μl of phosphate buffer) was added to the mixture. The reaction was terminated after 90 min by dilution in 450 μl of phosphate buffer. Free ^{125}I was separated from ^{125}I-insulin by chromatography on a 0.9- × 25-cm G-25 column equilibrated in 50 m*M* phosphate buffer containing 0.1% BSA. The binding of ^{125}I-insulin (182 pg) to receptors was performed as described by Sjordin and Viitanen (37), for 90 min at room temperature in a total volume of 1 ml in the presence of increasing amounts of unlabeled bovine insulin (0–2.5 μg). The precipitation of insulin in 10% trichloroacetic acid after incubation with membranes was 70% for tissue from each source.

Statistical Analysis. Statistical comparison was done using Student's *t* test, and probability values less than 0.05 were considered to be statistically significant. For multiple comparisons, the data were analyzed by analysis of variance followed by Duncan's multiple range test. Equilibrium binding constants and receptor densities were obtained by use of the nonlinear curve fitting program "LIGAND" (38). All data are expressed as mean ± SE. For each analysis, data from at least three separate experiments were pooled with the exception of the glucose oxidation in hypophysectomized mice, where data from only two experiments were pooled and no less than five observations were made. For the insulin binding study, data from a single pair of a representative experiment are shown. In all experiments, 5–20 mice per group were used.

Results

Weight Gain and Body Composition. Hypophysectomy reduced the rate of weight gain in young yellow mice (Fig. 1A) and caused a progressive loss of weight in adult yellow mice (Fig. 1B). All hypophysectomized mice were carefully inspected for pituitary remnants and none was found, nor was there any overt evidence of brain damage. Chronic treatment of young hypophysectomized yellow mice with hGH (100 μg/day) produced a significant increase ($P < 0.05$) in weight (2.13 ± 0.20 g) when compared with saline-treated controls (1.06 ± 0.32 g), but not until the second and third weeks (Fig. 1C). By the end of the second week of

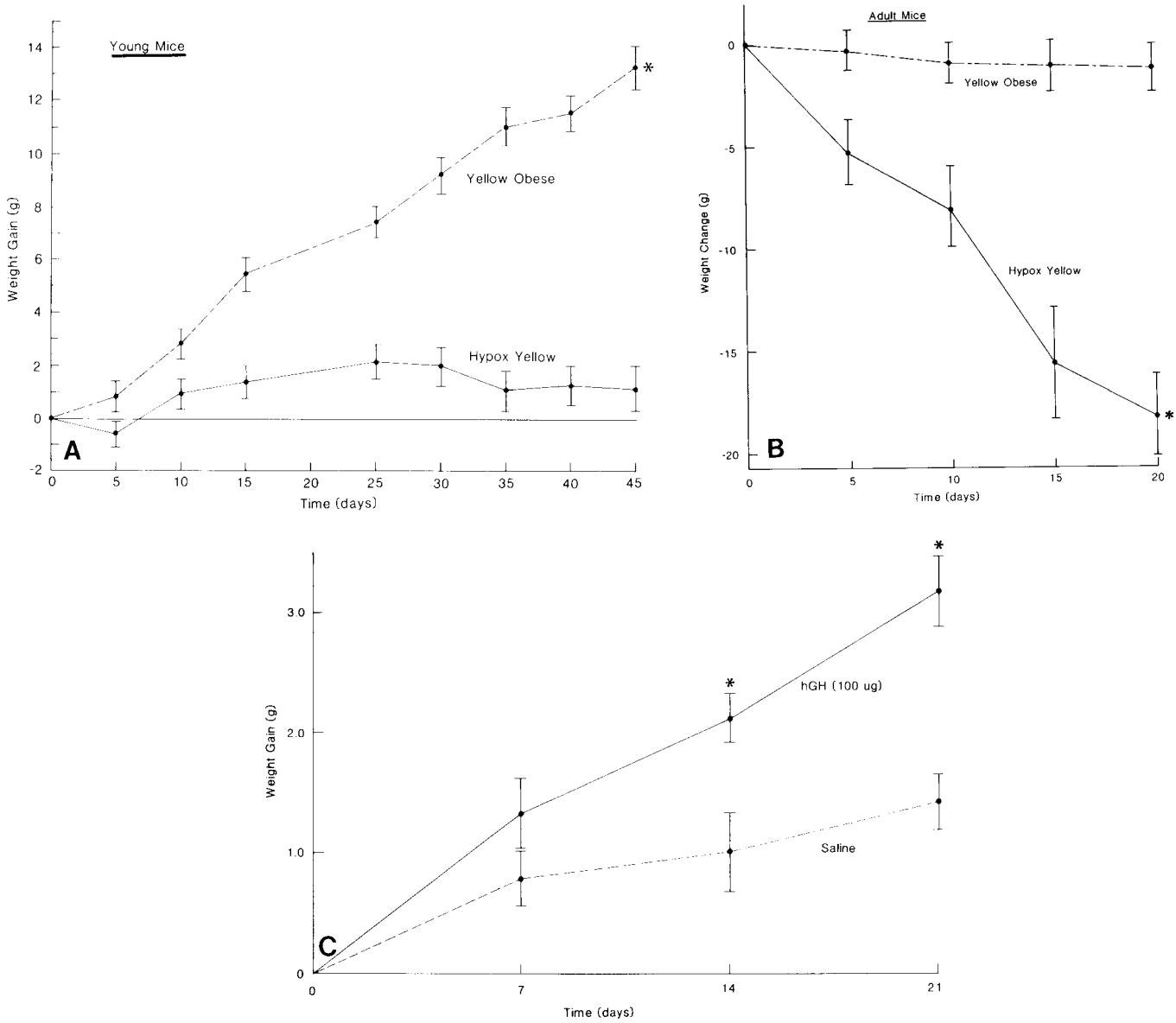

Figure 1. (A) Body weight gain for young intact and hypophysectomized yellow A^{vy}/A mice. Mice were weighed throughout the experimental period. Each point represents the mean weight gain of 20 intact yellow mice or 19 hypophysectomized yellow mice. The data are expressed as the mean ± SE. *$P < 0.001$ compared with hypophysectomized yellow mice. (B) Change in body weight for adult intact and hypophysectomized yellow A^{vy}/A mice. Mice were weighed throughout the experimental period. Each point represents either the mean weight change of 20 intact or 8 hypophysectomized yellow mice. The data are expressed as the mean ± SE. *$P < 0.001$ compared with intact yellow mice. (C) Effect of hGH on body weight gain of hypophysectomized yellow A^{vy}/A mice. Mice were injected (sc) once daily for 21 days with either saline or hGH (100 μg/day/mouse). Each point represents the mean weight gain of at least nine mice. The data are expressed as the mean ± SE. *$P < 0.05$ compared with saline-treated group.

treatment, no significant increase in weight was produced in hypophysectomized yellow mice treated with 100 μg of hGH_{1-43} (1.18 ± 0.14 g) or HP (1.28 ± 0.21 g).

At the end of the experiments, the weight of the obese yellow mice was twice that of either the agouti or the hypophysectomized yellow mice. Although the food intake of agouti and obese yellow mice was similar, the hypophysectomized yellow mice ate only half as much (Table I). The percentage of parametrial adipose tissue to total body weight was dramatically larger in obese yellow mice than in agouti mice (21% vs 1%, respectively). Hypophysectomized yellow mice had smaller body weight when compared with obese yellow mice, but had a significantly larger percentage of parametrial fat (7%) than agouti mice. The weights of livers and kidneys from obese yellow mice were slightly heavier than in agouti mice, but lungs, hearts, and spleens were similar, and they all were significantly ($P < 0.01$) larger than those of hypophysectomized yellow mice. On the other hand, the weights of muscular diaphragms from all groups of mice were identical.

Serum Glucose and Insulin. Basal serum glucose (Table II) and insulin (Table III) concentrations in

agouti and hypophysectomized yellow mice were similar, but obese yellow mice had slightly higher serum glucose and significantly higher ($P < 0.01$) insulin concentrations than either. Treatment with hGH_{1-43} did not significantly alter serum glucose or insulin concentrations for any group of mice (Tables II and III), neither did hGH (data not shown). Tables II and III also show that treatment with HP did not significantly alter the serum glucose or insulin concentrations of agouti or hypophysectomized yellow mice; but on the other hand, they show that, in obese yellow mice, HP significantly increased serum glucose ($P < 0.01$) and decreased insulin concentrations ($P < 0.01$).

Glucose Clearance. In young hypophysectomized yellow mice, the fasting blood glucose levels were lower but not significantly different from those of intact young yellow mice. Although glucose tolerance was significantly impaired ($P < 0.01$), the blood glucose concentrations reached the basal levels within 2 hr after GTT (Fig. 2A). On the other hand, in adult hypophysectomized yellow mice, fasting blood glucose concentrations were significantly lower ($P < 0.01$) than those in intact adult yellow mice, and glucose tolerance was impaired dramatically (Fig. 2B). Expressing the data as blood glucose increment from 0 time (Δ), the basal levels in the intact adult yellow mice were reached within 2 hr, whereas in the adult hypophysectomized yellow mice, they were not attained at 4 hr, indicating an impaired glucose clearance rate.

Because of a high mortality rate in the adult hypophysectomized yellow mice (>80% died within 2 weeks after surgery), only young hypophysectomized yellow mice were used to assess the direct actions of hGH, hGH_{1-43}, and HP. Acute injections of hGH and HP had no effect on glucose clearance in young hypophysectomized yellow mice, but hGH_{1-43} (100 μg/mouse) significantly ($P < 0.05$) improved glucose clearance in 1 hr (Fig. 3) and also following a 3-day treat-

Table I. Body Weight, Food Intake, and Weight of Various Organs in Young Lean Agouti *A/a*, Obese Yellow A^{vy}/A, and Hypophysectomized Yellow A^{vy}/A Mice[a]

	Weight/mouse (g)[b]		
	Agouti	Obese yellow	Hypophysectomized yellow
Body weight	24.70 ± 0.8**[,†] (15)[c]	56.60 ± 1.9 (15)	22.30 ± 2.0** (15)
Parametrial adipose tissue	0.36 ± 0.02**[,†] (5)	11.69 ± 2.11 (10)	1.48 ± 0.23** (6)
Liver	1.23 ± 0.03*** (5)	1.90 ± 0.24 (10)	0.83 ± 0.13* (6)
Kidney (pair)	0.34 ± 0.01*** (5)	0.44 ± 0.01 (10)	0.19 ± 0.02* (6)
Diaphragm	0.09 ± 0.01 (5)	0.08 ± 0.01 (10)	0.08 ± 0.01 (6)
Lung (pair)	0.22 ± 0.01 (5)	0.24 ± 0.01 (10)	0.16 ± 0.01* (6)
Heart	0.13 ± 0.01 (5)	0.16 ± 0.04 (10)	0.07 ± 0.01* (6)
Spleen	0.14 ± 0.01 (5)	0.15 ± 0.01 (10)	0.05 ± 0.01* (6)
Food intake (g/day)	3.39 ± 0.5 (15)	3.45 ± 0.5 (15)	1.81 ± 0.3* (15)

Note. All data are expressed as mean ± SE.
[a] Mice were fed *ad libitum*, weighed daily, and their food intake was recorded twice a week for 3 weeks. Fresh wet weight of organs was measured within 10 min after sacrifice.
[b] Wet weight of tissues.
[c] Numbers in parentheses, number of animals.
* $P < 0.01$ compared with lean agouti and obese yellow mice. ** $P < 0.001$ compared with obese yellow mice. *** $P < 0.05$ compared with obese yellow mice. [†] $P < 0.001$ compared with hypophysectomized yellow mice.

Table II. Effects of hGH_{1-43} and HP on Concentration of Glucose in Serum of Young Nonfasted Lean Agouti *A/a*, Obese Yellow A^{vy}/A, and Hypophysectomized Yellow A^{vy}/A Mice[a]

	Serum glucose (mg/dl)								
	Saline-treated (9)[b]			hGH_{1-43}-treated (5)			HP-treated (5)		
	0 min	60 min	Δ	0 min	60 min	Δ	0 min	60 min	Δ
Agouti	83 ± 3	113 ± 3	30	80 ± 3	113 ± 3	33	91 ± 2	112 ± 4	21
Obese yellow	114 ± 6	143 ± 6	29	111 ± 2	143 ± 7	32	115 ± 6	198 ± 9	83*
Hypophysectomized yellow	90 ± 8	110 ± 7	20	84 ± 10	106 ± 5	22	91 ± 15	118 ± 11	27

Note. All data are expressed as mean ± SE.
[a] Mice were injected (ip) with saline, hGH_{1-43} (100 μg/mouse), or HP (100 μg/mouse) and blood samples (200 μl) from each group were obtained by retro-orbital sinus puncture before (0 time) and 60 min after treatment for glucose measurements.
[b] Numbers in parentheses, number of animals.
* $P < 0.01$ compared with all other groups.

Table III. Effects of hGH_{1-43} and HP on Concentration of Insulin in Serum of Young Nonfasted Lean Agouti *A/a*, Obese Yellow A^{vy}/A, and Hypophysectomized Yellow A^{vy}/A Mice[a]

	Serum insulin (microunits/ml)								
	Saline-treated (5)[b]			hGH_{1-43}-treated (5)			HP-treated (8)		
	0 min	60 min	Δ	0 min	60 min	Δ	0 min	60 min	Δ
Agouti	36 ± 3	30 ± 10	−6	42 ± 5	47 ± 3	5	42 ± 3	31 ± 4	−11
Obese yellow	146 ± 10	166 ± 11	20	144 ± 20	124 ± 20	−20	154 ± 10	94 ± 6	−60*
Hypophysectomized yellow	38 ± 4	36 ± 4	−2	44 ± 4	41 ± 5	−3	42 ± 4	30 ± 5	−12

Note. All data are expressed as mean ± SE.

[a] Mice were injected (ip) with saline, hGH_{1-43} (100 μg/mouse), or HP (100 μg/mouse) and blood samples (200 μl) from each group were obtained by retro-orbital sinus puncture before (0 time) and 60 min after treatment for insulin analyses by radioimmunoassay.

[b] Numbers in parentheses, number of animals.

* $P < 0.01$ compared with all other groups.

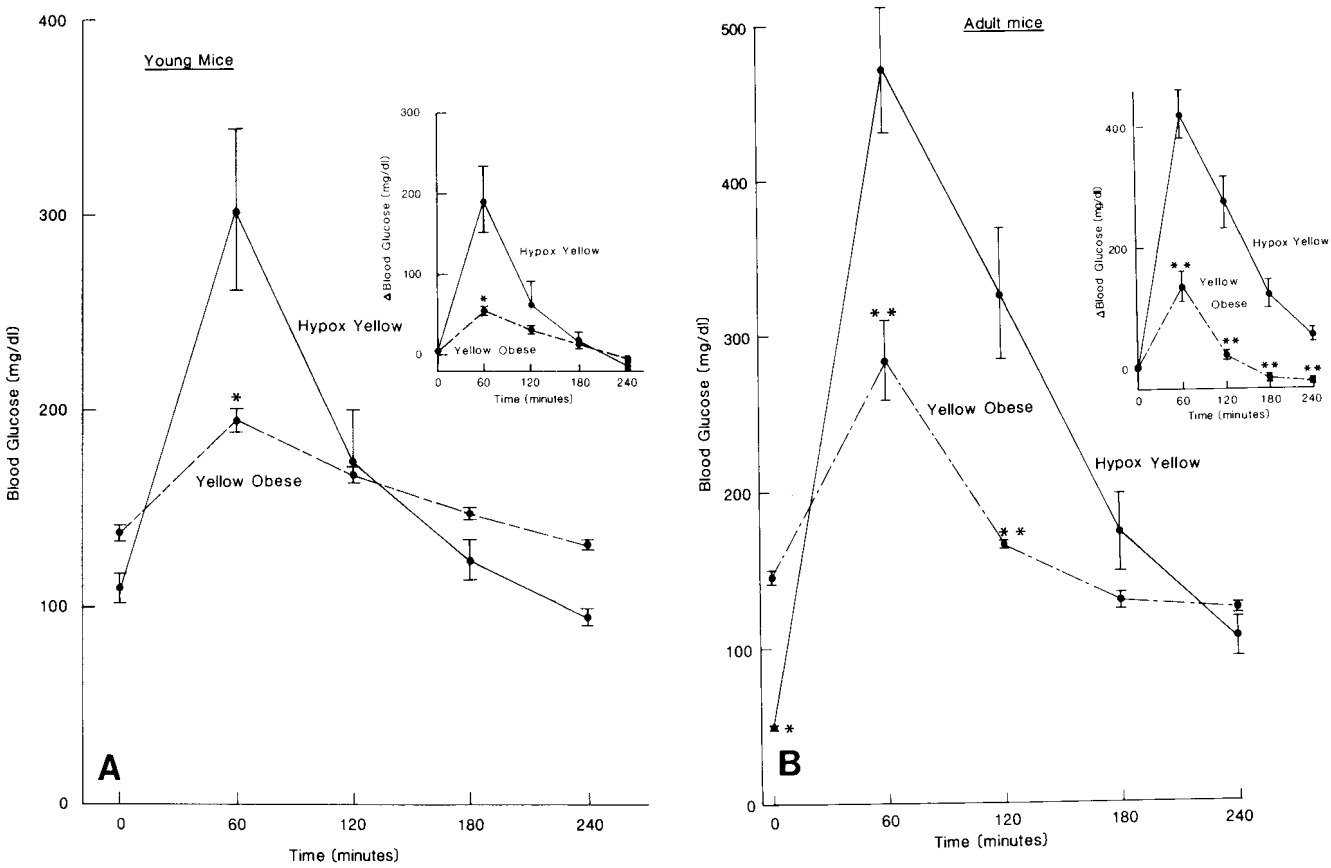

Figure 2. (A) GTT in young intact and hypophysectomized yellow A^{vy}/A mice. Mice were fasted for 9 hr and blood samples were obtained by retro-orbital sinus puncture. A glucose solution (2 mg/g body wt) was injected (ip) and blood sampling was done for 240 min at 60-min intervals. The inset represents the increment (Δ) from the initial fasted blood glucose level (see Table II). Each point represents the mean of at least 19 mice from three separate experiments. The data are expressed as the mean ± SE. *$P < 0.01$ compared with hypophysectomized yellow mice. (B) GTT in adult intact and hypophysectomized yellow A^{vy}/A mice. Mice were fasted for 9 hr and blood samples were obtained by retro-orbital sinus puncture. A glucose solution (2 mg/g body wt) was injected (ip) and blood sampling was done for 240 min at 60-min intervals. The inset represents the increment (Δ) from the initial fasted blood glucose level. Each point represents the mean of at least eight mice from two separate experiments. The data are expressed as the mean ± SE. *$P < 0.01$ compared with obese yellow mice. **$P < 0.01$ compared with hypophysectomized yellow mice.

ment ($P < 0.01$) (Fig. 4). When hGH was injected for at least 2 weeks into hypophysectomized yellow mice, it significantly ($P < 0.01$) improved glucose tolerance to a level indistinguishable from that of intact yellow mice (Fig. 5). Thus, the severe impairment of glucose clearance rate in hypophysectomized yellow mice was significantly improved by acute or chronic treatment with hGH_{1-43}, while intact hGH was only effective following a prolonged treatment.

Glucose Oxidation in Adipose Tissue. Obese yel-

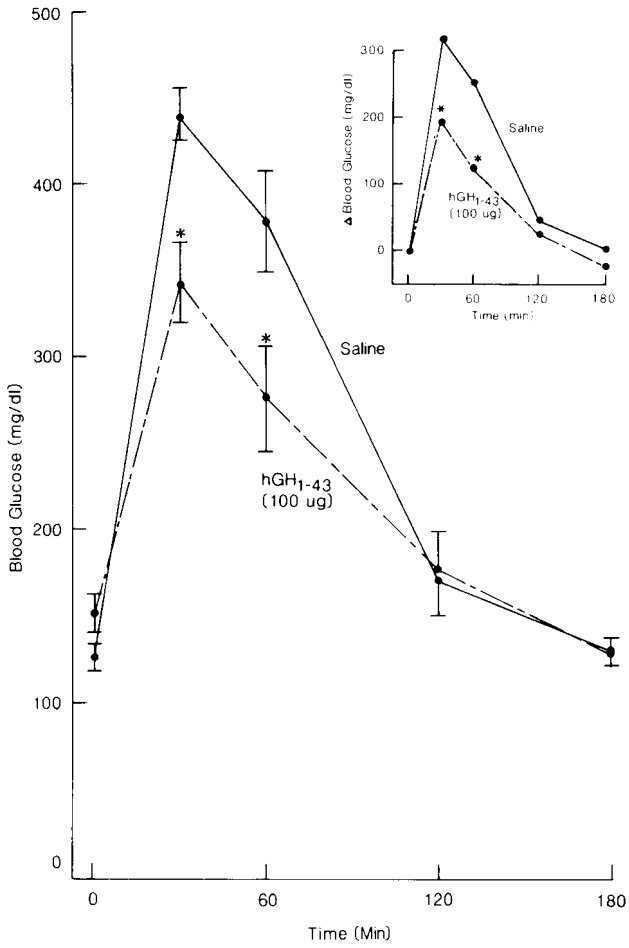

Figure 3. Acute effects of hGH_{1-43} on GTT in hypophysectomized yellow A^{vy}/A mice. Mice were fasted for 9 hr and blood samples were obtained by retro-orbital sinus puncture. A glucose solution (2 mg/g body wt) was injected (ip) without or with hGH_{1-43} (100 μg/mouse) and blood sampling was done for 180 min at 30- and 60-min intervals. The inset represents the increment (Δ) from the initial fasted blood glucose level (see Table II). Each point represents the mean of at least 10 mice from two separate experiments. The data are expressed as mean ± SE. *$P < 0.05$ compared with saline-treated group.

low mice had the lowest basal glucose oxidation rates of any of the groups studied, and only with high doses of insulin (1000–2500 microunits/ml) did their tissue show a significant increase ($P < 0.05$) in glucose oxidation (Fig. 6). In contrast, tissue from lean agouti mice had a higher basal glucose oxidation rate ($P < 0.05$) and, at a dose of only 100 microunits/ml, was more sensitive to insulin ($P < 0.001$) than tissue from obese yellow mice or hypophysectomized yellow mice ($P < 0.001$). This confirms our earlier finding (11, 15, 16, 26) that tissue from obese yellow mice is insulin resistant when compared with tissue from lean agouti mice. Furthermore, the maximum stimulation by insulin of glucose oxidation in tissue from lean agouti mice was at least 10-fold, whereas maximum stimulation in intact obese and hypophysectomized yellow mice was 1.5-fold and 2-fold, respectively (Fig. 6). In the present study, we have shown that hypophysectomized yellow mice have higher basal and insulin-stimulated rates of glucose oxidation in adipose tissue *in vitro* than do intact obese yellow mice ($P < 0.001$).

Insulin Binding. Our preliminary data indicated that hGH_{1-43} did not displace ^{125}I-insulin or ^{125}I-hGH from binding to mouse liver membranes and that ^{125}I-hGH_{1-43} did not bind to these membranes (data not shown). Due to the heterogeneity of HP relative to hGH and hGH_{1-43} and the obvious difficulty in radiolabeling a single entity of HP, no data on the effect of HP on ^{125}I-insulin and ^{125}I-hGH binding was collected. Analysis of ^{125}I-insulin binding to liver plasma membranes from lean agouti, obese yellow, and hypophysectomized yellow mice revealed a single noninteracting class of homogeneous insulin-binding sites. More complex models could not be fitted to the data. This analysis was performed by fitting the data to either a one-site or a two-site model where the goodness of fit was analyzed with the "LIGAND" computer program using the "Runs test" of Bennett and Franklin (39) and the F test on the residual variances of the two models.

The equilibrium binding constants derived from Scatchard plots are shown in Figure 7A. The binding constant obtained from the interaction of insulin with liver plasma membranes from lean agouti mice was three to four times greater than the binding constant obtained from intact obese yellow and hypophysectomized yellow mice ($P < 0.05$). Thus, hypophysectomy did not change the insulin receptor binding constant in liver plasma membranes. The receptor densities derived from Scatchard plots are shown in Figure 7B. Densities of insulin receptors in liver plasma membranes of lean agouti and obese yellow mice were not statistically different. When yellow mice were hypophysectomized, the density of liver plasma membrane insulin receptors increased by 3.2-fold ($P < 0.05$). Treatment of obese yellow mice with hGH_{1-43} for 3 days slightly increased ^{125}I-insulin binding to isolated adipocytes (by 20%) and to liver plasma membranes (334 ± 57 fmol/mg vs 250 ± 45 fmol/mg) when compared with that of saline-treated controls; but the increase was not statistically significant. Tissues from lean agouti mice showed no effect with hGH_{1-43}. Treating obese yellow mice with hGH, even at a dose 10 times the molar concentration used with hGH_{1-43}, showed essentially no significant effect on ^{125}I-insulin binding to adipocytes and liver plasma membranes (92 to 105% of that of saline-treated controls).

Discussion

In this study we have assessed the contribution of GH to insulin resistance in obese yellow A^{vy}/A mice and have determined what changes occur in response to insulin and hGH following hypophysectomy. Young nonovertly obese yellow mice tolerated the surgical procedure remarkably well and had a higher survival rate (95%) compared with adult obese yellow mice (<20%). For this reason, young yellow mice were used

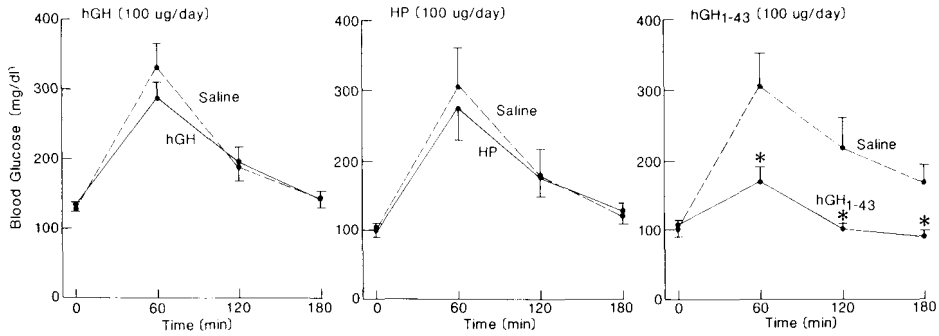

Figure 4. Chronic effects of injections of hGH, HP, and hGH_{1-43} on GTT in hypophysectomized yellow A^{vy}/A mice. Mice were injected (sc) daily for 3 days with saline and either hGH (100 μg/mouse), HP (100 μg/mouse), or hGH_{1-43} (100 μg/mouse). On the fourth day, the mice were fasted for 9 hr and blood samples were obtained by retro-orbital sinus puncture. A glucose solution (2 mg/g body wt) was injected (ip) and blood sampling was done for 180 min at 60-min intervals. Each point represents the means of at least eight mice from two separate experiments. The data are expressed as mean ± SE. $^*P < 0.01$ compared with saline-treated group.

in most of our studies. Earlier studies (33, 40) indicated that obesity progressed even after hypophysectomy in yellow mice. In agreement with this observation, our results showed that the amount of parametrial adipose tissue of hypophysectomized yellow mice was more than 4-fold greater than that of lean agouti mice. However, it appeared that hypophysectomy drastically diminished the progression of obesity, suggesting that the pituitary gland plays a role in the maintenance of obesity in yellow mice. Studies are now underway to determine the development of obesity and insulin resistance at an earlier age in the yellow mouse.

We observed that young hypophysectomized yellow mice maintained the same body weight which they had attained prior to surgery but hypophysectomy in adult obese yellow mice resulted in diminished food intake and a rapid weight loss. In our study, the weight of obese yellow mice increased by 0.30 g/day while Plotcher and Powley found that it increased by 0.28 g/day (33). Linear growth (25–30 days postoperation) in our hypophysectomized yellow mice was 0.09 g/day while theirs was 0.08 g/day, indicating that similar growth rates were obtained in both studies.

Although the hepatic insulin receptor densities of lean agouti and obese yellow mice were similar, the equilibrium binding constant was higher in agouti mice. Furthermore, the low insulin binding affinity of hepatic membranes from obese yellow mice was not altered by hypophysectomy (Fig. 7A). Hypophysectomized yellow mice showed a decrease in serum insulin concentration and a dramatic increase in hepatic insulin receptor density when compared with intact obese yellow mice. Elimination of GH and other pituitary hormones (by hypophysectomy) in yellow mice decreased circulating insulin levels and increased insulin binding; this may have alleviated insulin resistance, resulting in an increased tissue response to insulin.

Our findings that treatment of hypophysectomized yellow mice with hGH promoted body growth and that both hGH_{1-43} and hGH improved their impaired glucose clearance rate stress the importance of pituitary hormones in normalizing these parameters. Unlike hGH_{1-43}, hGH corrected an impaired glucose clearance in hypophysectomized yellow mice only by long-term treatment (2 weeks) but not by acute (60 min) or short-term (3 days and 7 days) treatment. Whether hGH is directly involved in the progression of obesity in yellow mice or plays an indirect role is still to be determined. What is clear from these results is that the pituitary hormones play an important regulatory role in carbohydrate metabolism.

Acute injections of hGH_{1-43} in lean agouti, obese yellow, and hypophysectomized yellow mice did not affect blood glucose nor insulin concentrations. In contrast, HP raised blood glucose and suppressed insulin in obese yellow mice, which suggests a direct effect for HP. The suppression of insulin concentration in the blood by HP could have contributed to the impairment of glucose clearance rate in obese yellow mice as suggested previously by Frigeri *et al.* (26) and Salem and Wolff (16). Unfortunately, we still have not conclusively identified the pituitary peptide fragment which elicits the hyperglycemic action of HP. What is known is that the HP fraction contains a 17-kDa fragment in addition to a 35-kDa (probably a dimer form of 17 kDa) and deamidated hGH_{22K} (16). The fact that the insulin-potentiating peptide (5 kDa, hGH_{1-43}) is found in relatively large quantities in the pituitary (at least 12 μg/gland) (41) suggests that the 17-kDa peptide comprises the remainder of the hGH_{22K} molecule (hGH_{44-191}) which is diabetogenic. Although this has not been confirmed, it is supported by the work of Lostroh and Krahl (42) which showed that hGH_{44-77} was hyperglycemic and Wade *et al.* (43) who considered the carboxyl-terminal portion ($hGH_{172-191}$) to contain anti-insulin activity. Such findings support the hypothesis that insulin-potentiating and anti-insulin actions reside in different regions of the hGH molecule. The hGH_{1-43} demonstrated both acute and chronic insulin-like effects on glucose clearance in hypophysectomized yellow mice. This observation does not rule out an insulin-potentiating action for hGH_{1-43} as these mice

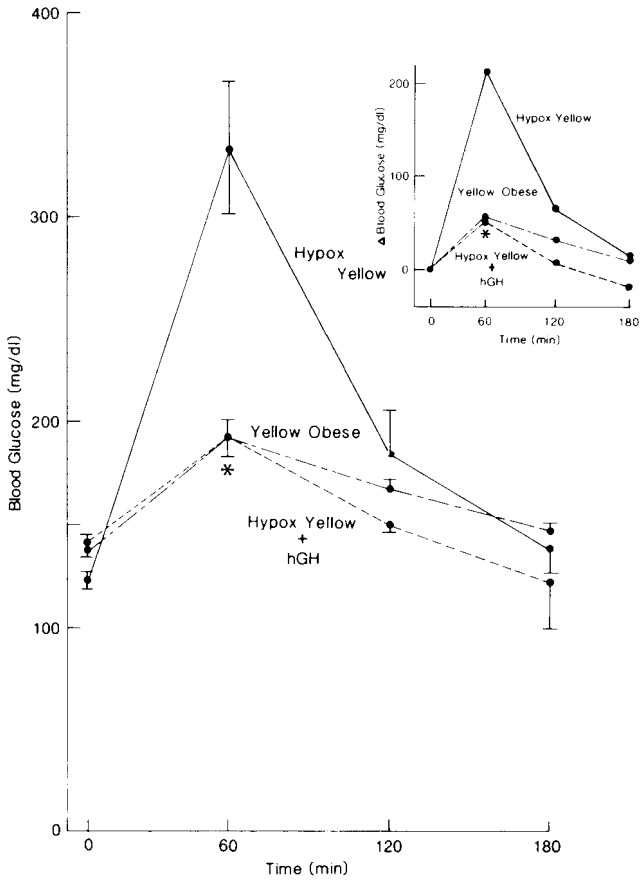

Figure 5. Chronic effects of injections of hGH on GTT in hypophysectomized yellow A^{vy}/A mice. Mice were injected (sc) once daily for 14 days with either saline or hGH (100 μg/mouse). On the last day, the mice were fasted for 9 hr and blood samples were obtained by retro-orbital puncture. A glucose solution (2 mg/g body wt) was injected (ip) and blood sampling was done for 180 min at 60-min intervals. The inset represents the increment (Δ) from the initial fasted blood glucose level (see Table II). Each point represents the mean of at least eight mice. The data are expressed as mean ± SE. $^*P < 0.01$ compared with saline-treated group.

maintained circulating insulin concentrations of 39 ± 4 microunits/ml. The failure of treatment with hGH for 7 days to promote body growth or to improve glucose clearance in hypophysectomized yellow mice while hGH_{1-43} directly enhanced glucose clearance is intriguing. It could be that hGH was unable to relieve the insulin resistance of the tissue or had first to be processed, presumable proteolytically modified prior to becoming active. To our surprise, treatment with HP neither affected glucose clearance nor body weight gain in hypophysectomized yellow mice.

Earlier studies using the extreme amino-terminal portions of hGH (hGH_{1-15}, hGH_{4-15}, hGH_{7-13}) provided evidence for the existence of insulin-potentiating peptides derived from hGH_{22K} (44–46). More recent studies indicated that administration of hGH_{1-43} or hGH_{32-46} to obese yellow mice enhanced responses to insulin action (11, 15, 16). In addition, similar effects were shown by others with hGH_{31-44} in rats (47), with hGH_{32-46} in rats (12) and in dogs (9), and with hGH_{32-38} in dogs (10). Upon further experimentation, we not only confirmed their observations in mice but also found no statistically significant change in insulin receptor number (334 ± 57 fmol/mg vs 250 ± 45 fmol/mg) nor binding affinity (.03 nM^{-1} vs 0.5 nM^{-1} by treatment with hGH_{1-43} when compared with that of saline-treated controls. Our studies also indicated that exposing the tissue directly to hGH_{1-43} did not enhance the rate of glucose oxidation *in vitro*, and that the insulin potentiating action was seen only after *in vivo* administration (11, 15, 16, 26). It is possible that the small increase in insulin binding seen in adipocytes from obese yellow mice treated with hGH_{1-43} contributed to the enhancement of insulin action on glucose oxidation (11, 15, 16). On the other hand, treatment with HP not only suppressed insulin levels but also inhibited the basal and the insulin-stimulated glucose oxidation of adipose tissue from lean agouti and obese yellow mice (15, 16, 26). The fact that hGH_{1-43} elicited only acute effects in intact obese yellow and hypophysectomized yellow mice *in vivo* and not *in vitro* further

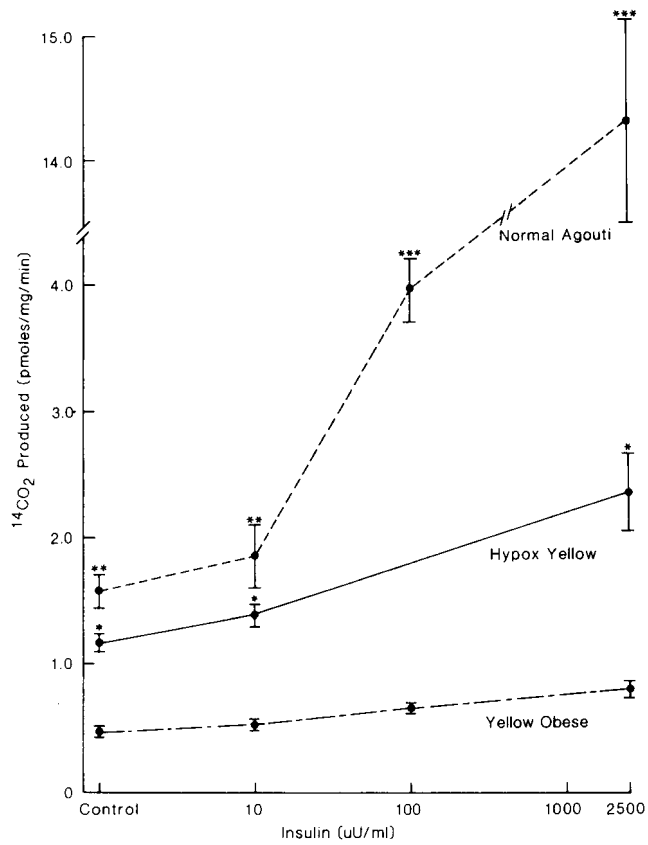

Figure 6. *In vitro* insulin sensitivity of adipose from lean agouti A/a, intact obese, and hypophysectomized yellow A^{vy}/A mice. Parametrial adipose tissue from each group was removed and the rates of [U-^{14}C]glucose oxidation to $^{14}CO_2$ as well as tissue sensitivity to various doses of insulin (10–2500 microunits/ml) added *in vitro* were measured. Each point represents the mean of 12 replicates from at least two separate experiments. The data are expressed as mean ± SE. $^*P < 0.001$ compared with intact obese yellow mice. $^{**}P < 0.05$ compared with hypophysectomized yellow mice; $^{***}P < 0.001$ compared with all other groups.

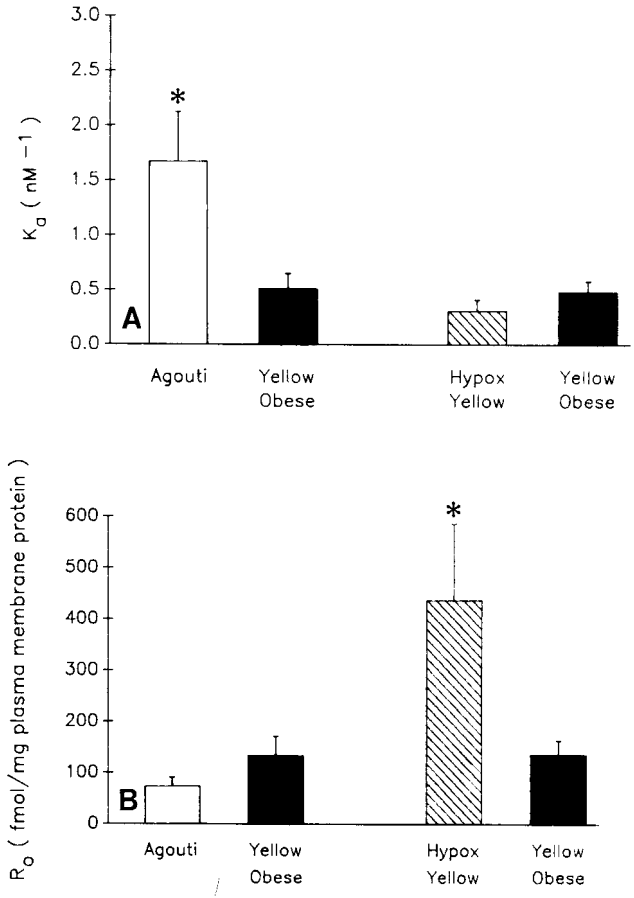

Figure 7. (A) Comparison of equilibrium binding constants for insulin in liver plasma membranes from lean agouti *A/a*, intact obese, and hypophysectomized yellow A^{vy}/A mice. Liver plasma membranes were incubated in the presence of [^{125}I]iodoinsulin and increasing amounts of unlabeled insulin. Each pair of vertical bars represents the mean of the binding constant derived from Scatchard plot analyses from a single representative experiment. The data are expressed as the mean ± SE. *$P < 0.05$ compared with all other groups. (B) Comparison of insulin receptor densities of liver plasma membranes from lean agouti *A/a*, intact obese, and hypophysectomized yellow A^{vy}/A mice. Liver plasma membranes were incubated in the presence of [^{125}I]iodoinsulin and increasing amounts of unlabeled insulin. Each pair of vertical bars represents the mean of the receptor density derived from Scatchard plot analyses from a single representative experiment. The data are expressed as the mean ± SE. *$P < 0.05$ compared with all other groups.

supports the notion that the peptide has no direct effect on tissue (11, 15, 16). It is reasonable to suggest that hGH_{1-43} stimulates another active substance which acts *in vivo* to produce the insulin-potentiating actions. Therefore, it appears that a wide range of amino-terminal fragments of hGH is involved in the enhancement of sensitivity to insulin action. In light of these findings, it is not surprising that hGH_{1-43}, which incorporates all of these peptide fragments, is more active than the other smaller fragments (11).

Glucose oxidation of the adipose tissue from lean agouti mice was extremely sensitive to stimulation by insulin when compared with that of tissue from obese yellow and even hypophysectomized yellow mice. It is interesting to note that the severity of insulin resistance in tissue from obese yellow mice was significantly alleviated following hypophysectomy. The increased sensitivity to insulin could also have been due to the decrease in the high circulating levels of insulin in hypophysectomized yellow mice. Stevenson *et al.* (9) indicated that insulin-like action (increased glucose uptake) of hGH_{32-46} in dogs could be explained by enhanced secretion of insulin, while hGH_{32-38} appeared to potentiate insulin action without changing insulin levels (10). In the present study, we did not observe any change in insulin levels by treatment with hGH_{1-43} or hGH to lean agouti, obese yellow, and hypophysectomized yellow mice; only hGH_{1-43} enhanced insulin actions in yellow mice. The suggestion by Stevenson *et al.* (9, 10) that these closely related peptides act through different mechanisms is doubtful. Our recent observation supports the work of Frigeri *et al.* (11) which showed that the peptides alter tissue sensitivity to insulin either by affecting insulin binding or by another postreceptor mechanism.

The insulin-potentiating and anti-insulin actions of hGH can be accounted for at least in part by low molecular weight pituitary peptides derived from hGH. The elimination of hGH by hypophysectomy in yellow mice presents a suitable animal model to study the direct and indirect actions of hGH_{1-43}, while the effect of HP is more distinctive in intact obese yellow mice. The peptide hGH_{1-43} elicits its insulin-potentiating actions without binding to receptors for hGH or insulin, and it appears to influence the actions of insulin by increasing receptor numbers or by other postreceptor mechanisms.

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