Hypophysectomy or Adrenalectomy of Rats with Insulin-Dependent Diabetes Mellitus Partially Restores Their Responsiveness to Growth Hormone (43810)

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Abstract. **The studies reported herein were conducted to confirm that the pituitary gland is involved in maintaining growth hormone (GH) resistance in rats with insulin-dependent diabetes mellitus (IDDM) and to determine whether the adrenocorticotropic hormone (ACTH)-adrenal cortical axis is responsible. The rats were made diabetic by injecting streptozotocin (85 mg/kg body wt) IP once daily on two consecutive days. They were then injected with 15 IU insulin SC twice daily on two consecutive days to enable them to survive hypophysectomy or adrenalectomy. Intact nondiabetic (NonDb), diabetic (Db), hypophysectomized diabetic (HxDb), and adrenalectomized diabetic (AxDb) rats were injected twice daily with 50 μg porcine (p) GH or with 0.9% saline for 2 weeks following the surgeries.**

Serum glucose levels of the saline-injected Db, HxDb, and AxDb rats were significantly greater than those of the NonDb rats by 106%, 65% and 49%, respectively. However, the levels in the HxDb and AxDb animals were significantly lower than those of the Db group by 20% and 28%, respectively. Injections of pGH into NonDb rats increased serum glucose concentrations by 38%, over their saline-treated controls, and by 29% in AxDb rats. This diabetogenic effect of GH was not seen in any other group.

Administration of pGH to Db rats failed to increase body weight gain, tail growth, tibial epiphysial plate width, or serum IGF-I concentration over saline-injected controls. By contrast, HxDb and AxDb rats injected with pGH showed significant increases in all four growth parameters. Total serum IGF-I concentrations in AxDb rats injected with pGH equaled those in NonDb controls. To determine whether the lack of corticosterone (B) in the AxDb rats was responsible for the reduced hyperglycemia and restored responsiveness to pGH, AxDb rats were given B in their drinking water at 5 or 25 μg/ml. Administration of B reduced the beneficial effects of adrenalectomy by restoring hyperglycemia and growth impairment, and partially restored resistance to the pGH injections.

These studies confirm that the pituitary contributes to diabetic growth impairment and show that the ACTH-adrenal cortical axis is primarily responsible for the GH-resistant state that develops in rats with IDDM. [P.S.E.B.M. 1994, Vol 207]

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Reduced growth is a common problem associated with insulin-dependent diabetes mellitus (IDDM) and the basis of this impairment is not well understood. In rats with IDDM, growth retardation is accompanied by a decrease in serum insulin-like growth factor-I (IGF-I) and growth hormone (GH) levels (1), and refractoriness to GH treatment *in vivo* (2, 3) and to IGF-I *in vitro* (4). GH-stimulated production

of IGF-I by the liver is not maintained in IDDM or in fasted animals and is associated with a reduced number of hepatic GH receptors (5–8) and a postreceptor defect (9). Growth can be partially restored in diabetic rats by administering a high dose of IGF-I (10) or a low dose of insulin into the hepatic portal vein (11), without restoring euglycemia.

Previous research in animals showed that hypophysectomy alleviates the metabolic symptoms of IDDM (12, 13) and recent studies demonstrated that hypophysectomy of rats with IDDM partially restored their responsiveness to GH *in vivo* (14) and restored the responsiveness of their cartilage to IGF-I *in vitro* (4). Therefore, the pituitary gland contributes to the metabolic imbalance and growth impairment associated with IDDM.

Early studies also showed that GH exacerbates the metabolic symptoms in IDDM (1). However, in contrast with humans, rats with IDDM have reduced GH levels and defective or reduced GH receptors. Therefore, it is unlikely that GH is responsible for the resistance to anabolic hormones in these rodents. However, the reduction in GH receptor concentration and function during IDDM may significantly contribute to GH resistance in both models. Other data indicate that the ACTH-adrenal cortical axis may be a major counter regulatory mechanism to insulin in rats with IDDM. Streptozotocin (STZ)-induced diabetes increases plasma and urinary corticosterone (B) concentrations, decreases thymus weight, and increases adrenal weight. These responses are accompanied by increased metabolic activity in pituitary corticotropes and decreased sensitivity to glucocorticoid negative feedback (15–19). The studies reported herein were conducted to confirm the effects of hypophysectomy on rats with IDDM and to determine whether pituitary stimulation of adrenal B production is responsible for maintaining GH resistance.

Material and Methods

Animals. Male Long-Evans rats weighing 120–140 g were purchased from Simonsen's Laboratories, Inc. (Gilroy, CA). They were maintained in an environmentally controlled room at 23 ± 1°C, with a 12-hr light:dark cycle, and were fed *ad libitum*. All experiments were described in detail in a protocol that was approved by our Institutional Animal Care and Use Committee. The care and use of the rats were in strict compliance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals.

Treatments, Surgeries, and Experimental Design. Rats were made diabetic by two intraperitoneal (ip) injections of streptozotocin (STZ) (Sigma Chemical Co., St. Louis, MO) at 85 mg/kg body wt. The STZ was dissolved in sterile 0.9% NaCl immediately before use and the injections were given on two consecutive days. Urine glucose and ketone bodies were checked in the morning of the third day with Chemstrip urine test strips (Boehringer Mannheim, Indianapolis, IN) and rats with <5% urine glucose and those that were ketotic were excluded in order to reduce the range of the severity of diabetes among the animals. The remaining rats were injected subcutaneously twice daily for 2 days with 15 IU recombinant human insulin (Novolin; Novo Nordisk Pharmaceuticals Inc., Princeton, NJ) to normalize their metabolic status before surgery. This therapy enabled the animals to survive the procedures of hypophysectomy or adrenalectomy. On the following day, they were anesthetized by an ip injection of 100 mg/ml ketamine-HCl (Aveco, Fort Dodge, IA) and 10 mg/ml acepromazine maleate (Fermenta, Kansas City, MO) solution at a dose of 1 ml/kg body wt. They were then either hypophysectomized (Hx) by a transauricular method (20) or were bilaterally adrenalectomized (Ax). The latter animals were given 0.9% NaCl to drink. Rats were injected ip with 100 μl of either sterile 0.9% NaCl or porcine (p) GH (Monsanto Co., St. Louis, MO) twice daily for 14 days. The pGH was dissolved in a 0.03 *M* $NaHCO_3$ and 0.15 *M* NaCl solution at pH 9.5 and brought up to an injection volume of 50 μg/100 μl with sterile 0.9% NaCl. Corticosterone (B) was dissolved in 100% ethanol and then added to the 0.9% saline drinking water of two of the four groups of AxDb rats at a concentration of 5 μg B/ml 0.9% saline and 0.2% ethanol in an initial experiment and at 25 μg/ml in a later experiment.

Measurements. Body weights and tail lengths were recorded at the beginning and end of the 14-day treatment period for each rat. Terminal serum glucose and total serum IGF-I concentrations and tibial epiphysial plate widths (TEPW) were measured after termination on the morning of Day 15. Non-fasted serum glucose concentrations were determined with a Sigma glucose colorimetric assay with o-toluidine reagent (Sigma). Total serum IGF-I concentrations were determined by a standard double antibody disequilibrium radioimmunoassay for human IGF-I (21) after an acid-acetone cryoextraction to remove IGF binding proteins (22, 23). A polyclonal anti-human IGF-I antiserum (UB3-189) was used in conjunction with [^{125}I]-hIGF-I (Monsanto) tracer. Iodinations were performed with iodogen (Pierce, Rockford, IL).

Data Analysis. Data were analyzed by a one- or two-way analysis of variance coupled to Fisher's least significance difference test of multiple mean comparisons using the Systat 5 computer program for the Macintosh (SYSTAT, Inc., Evanston, IL). In each figure, significant differences are indicated by different letters ($P \leq 0.001$–0.05).

Results

The nonfasted serum glucose levels in the rats with IDDM were increased by 105% ($P < 0.001$) over those in the nondiabetic (NonDb) controls (Fig. 1a). The hyperglycemia in the Db controls was reduced in the HxDb controls by 20% ($P < 0.05$) and in the AxDb controls by 28% ($P < 0.01$). However, the glycemic status was not normalized in the HxDb and AxDb groups. Treatment with pGH increased the serum glucose levels by 38% in the NonDb ($P < 0.05$) rats and by 30% in the AxDb rats ($P < 0.01$). This diabetogenic effect was not seen in the Db or HxDb animals.

The body weight gain (BWG), tail growth (TG), and TEPW of the NonDb controls were greater ($P \leq 0.01$) than those of all other control groups (Fig. 1b–d). These growth indices were reduced in the Db controls by 55%, 44%, and 21%, respectively ($P < 0.001$ for each), and in the AxDb controls by 62% ($P < 0.001$), 57% ($P < 0.001$), and 31% ($P < 0.01$), respectively, compared with the NonDb controls. There were no differences in any of the growth parameters between the saline-injected Db and AxDb rats. The HxDb controls lost an average of 12.3 ± 2.7 g ($P < 0.001$) and their TG and TEPW were reduced by 96% ($P < 0.001$) and 58% ($P < 0.001$), respectively. Treatment with pGH had no affect on BWG or TG in NonDb or Db rats. It did increase the BWG in the HxDb rats by 41.9 g ($P < 0.001$), and the BWG and TG in the AxDb rats by 32.1 g ($P < 0.001$) and 8.6 mm ($P < 0.001$), respectively, compared with their controls. The TEPW of the NonDb, HxDb, and AxDb rats injected with pGH increased by 42% ($P < 0.001$), 117% ($P < 0.01$), and 62% ($P < 0.01$), respectively. However, the TEPW of the Db rats was unaffected.

Differences in total serum IGF-I concentrations among the experimental and control groups (Fig. 2) were similar to the differences in their TEPW measurements (Fig. 1d). In the Db controls, IGF-I concentrations were reduced by 32% ($P < 0.05$), relative to those in NonDb controls, and they were reduced by 91% ($P < 0.001$) in HxDb controls. The IGF-I concentrations in the AxDb controls were not significantly different from those in the NonDb or Db controls. Compared with their saline-injected controls, injections of pGH significantly increased serum IGF-I levels in NonDb, HxDb, and AxDb rats by 391 ($P < 0.001$), 217 ($P < 0.01$), and 231 ng/ml ($P < 0.01$), respectively, but had no effect in the Db rats.

In the B replacement experiments, all of the body growth indices showed that growth of both saline-injected control groups in the low-dose B replacement (5B) experiment was considerably greater than those of the controls of the high-dose B (25B) experiment. Increased use of the animal room by other investigators while the 25B experiment was conducted, over that occurring during the 5B experiment, may be responsible for this difference. Nevertheless, each experiment is internally consistent with appropriate controls.

The serum glucose levels of the saline-injected AxDb rats that were given the 5B drinking water were increased by 32% ($P < 0.05$) compared with controls drinking saline (Fig. 3a). This effect was accompanied by inhibition of TG by 29% ($P < 0.05$) and TEPW by 32% ($P < 0.05$) (Fig. 3c and d), but there was no effect on their BWG (Fig. 3b). This lack of an effect on BWG

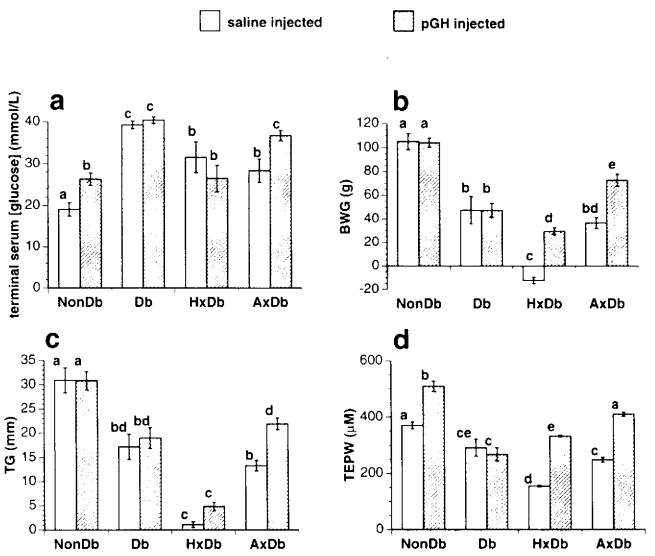

Figure 1. Terminal serum glucose concentration, body weight gain (BWG), tail growth (TG), and final tibial epiphysial plate width (TEPW) in intact nondiabetic (NonDb), diabetic (Db), hypophysectomized diabetic (HxDb), and adrenalectomized diabetic (AxDb) rats (pGH = 50 μg 2×/day). Values are mean ± SEM and n = 6–8. Significant differences are indicated by different letters ($P \leq 0.05$).

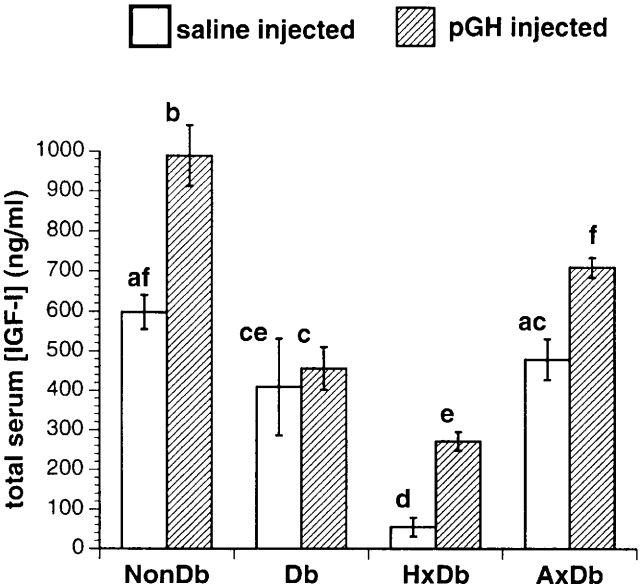

Figure 2. Total serum IGF-I concentration in NonDb, Db, HxDb, and AxDb rats. Otherwise as in the legend for Figure 1.

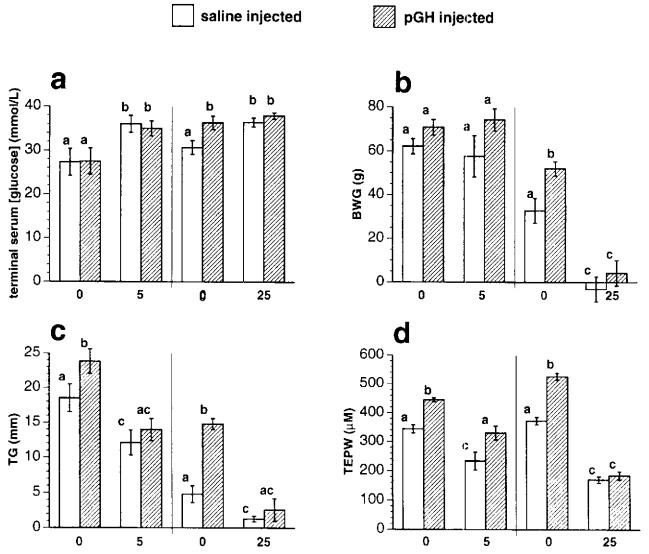

Figure 3. Terminal serum glucose concentration, BWG, TG, and final TEPW in AxDb rats with replacement of 0, 5, and 25 μg B/ml 0.9% saline drinking water. The 5B and 25B experiments were run independently, therefore statistical comparisons were made within, but not between, experiments. Otherwise as in the legend for Figure 1.

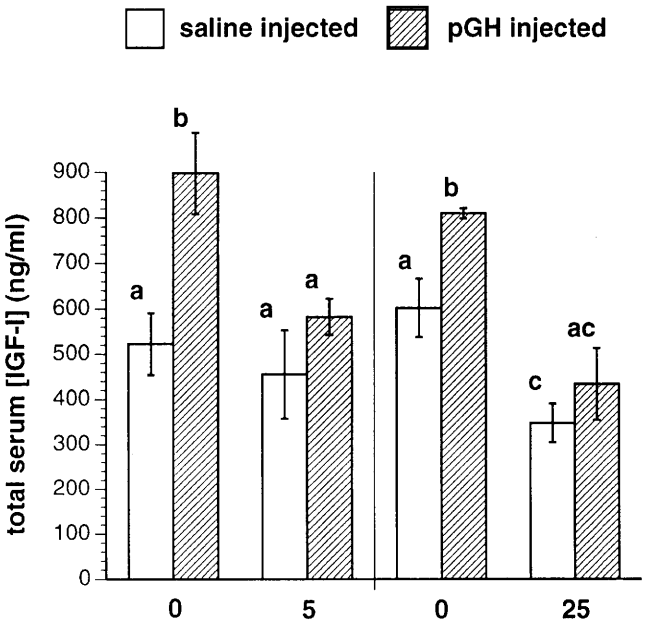

Figure 4. Total serum IGF-I concentration in AxDb rats with replacement of 0, 5, and 25 μg B/ml 0.9% saline drinking water. The 5B and 25B experiments were run independently, therefore statistical comparisons were made within, but not between, experiments. Otherwise as in the legend for Figure 1.

may be due to intestinal engorgement of rats drinking 5B, whereas the controls were not engorged. Injection of pGH caused significant increases in TG by 40% ($P < 0.05$) and TEPW by 29% ($P < 0.01$) of the saline-injected controls, but they failed to significantly increase BWG. The low dose of B inhibited the TG response to pGH, but did not reduce the increase in TEPW.

The high dose of B clearly restored GH resistance in the AxDb animals. The saline-injected controls that were given the 25B drinking water had a 19% ($P < 0.01$) elevation in serum glucose levels and showed a striking reduction in all three growth indices. The hyperglycemic effect of pGH injection was evident in the controls given no B treatment and these animals showed significant increases in BW, TG, and TEPW (Fig. 3a–d) by 59% ($P < 0.05$), 208% ($P < 0.001$), and 41% ($P < 0.001$), respectively. The 25B treatment completely nullified all four effects of the pGH.

The serum IGF-I concentrations in the saline-injected controls of the 5B and 25B experiments, and in the saline-injected 5B rats, were not significantly different (Fig. 4). However, the IGF-I concentration in the controls of the 25B experiment were reduced ($P < 0.01$) by 254 ng/ml compared to those in the 25B controls. Injection of pGH elevated the IGF-I levels in the controls of the 5B and 25B experiments by 375 ng/ml ($P < 0.01$) and 201 ng/ml ($P < 0.05$), respectively, and both doses of B completely suppressed this effect.

Discussion

Our present results are consistent with previous studies which demonstrated that serum IGF-I levels and growth were significantly reduced in rats with IDDM (1). The animals used in our experiments were not severely growth retarded, probably because their IDDM state was only moderately severe and nonketotic. Nevertheless, their responsiveness to pGH injection was completely abolished. Our data confirm the results of English *et al.* (14) that hypophysectomy reduces the hyperglycemia in rats with IDDM and partially restores their responsiveness to GH, although there are some quantitative differences between their results and ours. Serum glucose concentrations of the HxDb rats reported by English *et al.* (14) were completely normalized, whereas in our experiment, the levels were significantly reduced, but not normalized. The equivalent dose of pGH restored serum IGF-I concentrations to equal those of NonDb controls in the study of English *et al.* (14), but to only 50% of control levels in our study. Nevertheless, the diabetogenic action of GH was not seen in the HxDb rats in either study. Furthermore, the growth responses of HxDb rats to pGH were similar in both studies and appear to be unrelated to the degree of normalization of serum IGF-I concentrations.

Robinson *et al.* (24) and Carlsson *et al.* (25) showed that the growth impairment that accompanies IDDM was primarily a result of intracellular starvation due to the metabolic imbalance. The finding that hypophysectomy normalized glycemic status of rats with IDDM and restored their responsiveness to GH (14) and IGF-I (4) is consistent with this hypothesis. However, in our study, the GH-treated AxDb rats had serum glucose levels equal to those of the Db controls

(with or without pGH injection), yet the AxDb rats showed significant responses to the GH (Fig. 1a–d). Accordingly, our results with the AxDb animals suggest that the beneficial effects of hypophysectomy in Db rats are due to removal of ACTH, with consequent B deficiency.

Although serum B levels were not measured in our study, hypophysectomy reduces circulating ACTH and B levels and causes adrenal atrophy (26–28). The terminal serum glucose levels of the saline-injected AxDb rats were significantly lower than those of the Db rats, but they were also significantly higher than those of the NonDb rats. Both replacement doses of B elevated serum glucose concentrations in the AxDb rats, which confirms that elevated B levels contribute to diabetic hyperglycemia. The 5B dose was only partially effective at reducing GH responsiveness in the AxDb rats; only the TG and serum IGF-I responses to pGH were significantly reduced. By contrast, the 25B dose completely blocked all measured responses to pGH.

Although the HxDb and AxDb rats showed significant growth responses to the pGH, the diabetogenic action of GH was seen only in the NonDb and AxDb rats (Fig. 1a). These data are consistent with a recent study which showed that an insulin-sensitizing drug could also disassociate the somatotropic and diabetogenic actions of GH (29), of which the latter is due mainly to a postreceptor defect in insulin action (30). Both of these effects were abolished by B replacement in the AxDb animals. Thus, hypophysectomy, but not adrenalectomy, reduces this effect of GH. It has been shown that the number of glucose transporters in adipocytes increases when rats are Hx (31). Furthermore, GH positively regulates membrane incorporation of Type 4 glucose transporters in these cells (32, 33). Therefore, it is possible that the inability of pGH to increase serum glucose levels in the HxDb rats may also be due to differential distribution of the types of glucose transporters in these animals compared with the NonDb and AxDb rats.

None of the indices of growth in the saline-injected AxDb rats was greater than those in the Db animals. Thus, it would appear that although the activated ACTH-adrenal cortical axis is responsible for impaired GH responsiveness in the Db rats, it does not cause their impaired growth. However, secretion of GH in NonDb rats is influenced by several factors, including blood glucose and B concentrations. Although glucocorticoid excess reduces circulating GH levels, glucocorticoids normally help to maintain blood GH levels (34–36). IDDM and adrenalectomy both lower serum levels of GH, therefore, serum GH concentrations may have been lower in the AxDb rats than in animals with only one of these endocrine deficits. This may account for the lack of improved growth in the AxDb rats even though their glycemic status and responsiveness to exogenous GH were partially normalized.

Unterman *et al.* (37) reported that when Db ats were Ax, basal IGF-I levels were increased, but they remained significantly lower than those of controls. When B was replaced, IGF-I levels were reduced to equal those in Db rats. In our experiments, adrenalectomy did not significantly increase serum IGF-I levels compared with the Db rats (Fig. 2), but it did result in IGF-I levels that were not significantly lower than the NonDb controls. Furthermore, the high dose of B replacement reduced the IGF-I levels in the AxDb rats (Fig. 4). The Db controls used by Unterman *et al.* (37) had lower IGF-I levels than did those of our study, possibly due to a more severe state of insulin deficiency. The rats used by Unterman *et al.* (37) received a higher dose of STZ than did those used in our study (140 vs 85 mg/kg body wt). The more severely diabetic animals used in their study may have been more responsive to adrenalectomy than were our rats.

The results of several studies indicate that IDDM does not affect the affinity of hepatic somatogenic receptors for GH, but the number and functional status of these receptors may vary with the severity and duration of the disorder (5, 6, 38). A postreceptor defect is probably responsible for GH resistance in rats with moderately severe IDDM rather than a loss of somatogenic binding sites (7). Interestingly, Bornfeldt *et al.* (8) found that GH receptor mRNA levels were reduced in various tissues of diabetic rats, but not in the liver. Bryson and Baxter (39) reported that adrenalectomy partially reversed the reduction in hepatic GH receptors that occurred in rats with severe IDDM. This effect probably did not contribute to the improved responsiveness to GH in our AxDb rats because they were not severely Db. However, adrenalectomy may have corrected the postreceptor defect that occurs in rats with IDDM.

In conclusion, our results suggest that pituitary stimulation of adrenal B production is responsible for exacerbating the hyperglycemia of IDDM, and causes the GH resistance that occurs in that state. This conclusion serves to reemphasize that complications that arise during IDDM may be due to the actions of counterregulatory hormones rather than to insulin deficiency *per se*. Glucocorticoids inhibit basal and GH stimulated IGF-I production in NonDb and Hx rats and may be responsible for growth impairment associated with Cushing's syndrome, despite normal IGF-I levels (40–44). Furthermore, chronic administration of cortisone acetate to rats reduced serum IGF-II levels and impaired the ability of GH to raise them (45). Interestingly, GH and IGF-I resistance also exists in fasted or malnourished animals (46–50). Although the blood metabolite profile in animals with these nutritionally deprived states are opposite to those in ani-

mals with IDDM, circulating levels of counterregulatory hormones, including glucocorticoids, are elevated in all three states (51–53) and those of insulin are depressed. Therefore, it is unlikely that blood glucose levels are directly responsible for GH resistance. However, glucocorticoids may contribute to the maintenance of GH resistance in fasted and protein-deprived animals as well as in animals with IDDM.

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