Growth Hormone Secretion, Serum, and Cerebral Spinal Fluid Insulin and Insulin-Like Growth Factor-I Concentrations in Pigs with Streptozotocin-Induced Diabetes Mellitus

(43503)

C. R. BARB,[1,*] N. M. COX,[†] C. A. CARLTON,[†] W. J. CHANG,[‡] AND R. F. RANDLE[§]

Animal Physiology Research Unit, USDA, ARS, Russell Research Center, Athens, Georgia 30613; Department of Animal and Dairy Sciences[†] and College of Veterinary Medicine,[§] Mississippi State University, Starkville, Mississippi 39762; and Department of Animal and Dairy Science,[‡] University of Georgia, Athens, Georgia 30602*

Abstract. **Diabetes mellitus was induced using streptozotocin in five gilts between 8 and 12 weeks of age. Gilts were maintained with exogenous insulin (INS) except during experimental periods. Four litter-mate gilts served as controls. At 9 months of age, all gilts were ovariectomized, and 30 days after ovariectomy, Experiment (Exp) 1 was conducted. Jugular vein catheters were inserted and blood samples were collected every 10 min for 8 hr. Experiment 2 was conducted when gilts were 11 months of age. Venous blood and cerebrospinal fluid (CSF) samples were collected in the absence (Phase I) or presence (Phase II) of INS therapy. In Experiment 1, plasma glucose concentrations were greater ($P < 0.05$) in diabetic (465 ± 17 mg/100 ml) than in control (82 mg ± 17 mg/100 ml) gilts, whereas serum INS was lower ($P < 0.0001$) in diabetic gilts (0.3 ± 0.02 vs 0.9 ± 0.05 ng/ml) and insulin-like growth factor-I was similar in diabetic and control gilts (32 ± 3 vs 43 ± 4 ng/ml, respectively). Mean serum GH concentration was 2-fold greater ($P < 0.02$) in diabetics (2.8 ± 0.4 ng/ml) than in control gilts (1.2 ± 0.2 ng/ml). Diabetic gilts exhibited a greater ($P < 0.05$) number of GH pulses than control gilts (3.2 ± 0.4 vs 1.5 ± 0.3/8 hr, respectively). In addition, GH pulse magnitude was markedly elevated ($P < 0.02$) in diabetic (5.8 ± 0.4 ng/ml) compared with control gilts (3.3 ± 0.6 ng/ml). Mean basal serum GH concentrations were greater ($P < 0.07$) in diabetic (2.2 ± 0.5 ng/ml) compared with control gilts (1.0 ± .1 ng/ml). In Experiment 2, CSF concentrations of insulin-like growth factor-I, INS, GH, and protein were similar for diabetic and control gilts in both phases. Serum GH levels were similar for diabetics and controls in Phase I, but were greater ($P < 0.05$) in diabetics than in controls in Phase II. CSF glucose levels were greater in diabetic than in control gilts in both the presence ($P < 0.003$) and absence ($P < 0.0002$) of INS therapy, whereas plasma glucose was greater ($P < 0.003$) in diabetic than in control gilts in the absence of INS, but returned to control concentrations in the presence of INS. However, serum GH levels were unchanged after INS therapy in the diabetic gilts. In conclusion, altered GH secretion in the diabetic gilt may, in part, be due to elevated CSF glucose concentrations, which may alter GH-releasing hormone and/or somatostatin secretion from the hypothalamus.** [P.S.E.B.M. 1992, Vol 201]

[1] To whom requests for reprints should be addressed at Animal Physiology Research Unit, USDA, ARS, P.O. Box 5677, Athens, GA 30613.

Received November 25, 1991. [P.S.E.B.M. 1992, Vol 201]
Accepted June 5, 1992.

0037-9727/92/ 2012-0223$3.00/0

Abnormalities in the pattern of growth hormone (GH) secretion are commonly observed with insulin-dependent diabetes mellitus in humans (1–3). However, neither the mechanism responsible for nor the physiological consequences of altered GH secretion are fully understood. In order to modulate supraphysiological blood GH concentrations, it is necessary to define the relationships between excessive GH

secretion and intermediate metabolites and metabolic hormones. Both insulin-like growth factor-I (IGF-I; 4, 5) and insulin (INS; 6) suppressed GH secretion from pituitary cells *in vitro*. Moreover, intracerebroventricular administration of IGF-I in the rat (7) and INS in the pig (8) inhibited GH secretion. In addition, a recent study by Barb *et al.* (9) demonstrated that elevated blood glucose or free fatty acids suppressed or delayed the GH response to growth hormone-releasing hormone (GHRH) in the pig. However, little is known about the relationship between pattern of GH secretion and serum and cerebrospinal fluid (CSF) concentrations of IGF-I, INS, and glucose during diabetes. Therefore, this study was conducted to determine the influence of diabetes on pattern of GH secretion and serum and CSF concentrations of IGF-I, INS, and glucose.

Materials and Methods

General. Nine cross-bred gilts from three litters were used in two experiments. Littermates were assigned to either diabetic (n = 5) or controls (n = 4). Between 8 and 12 weeks of age (17–31 kg body wt), five pigs were treated with streptozotocin (150 mg/kg) to induce diabetes, as described by Meurer *et al.* (10). The diabetic gilts were maintained on exogenous pig INS (Lente insulin; Squibb Novo, Inc., Princeton, NJ) at an initial dose of 0.25 IU/kg given once every 24 hr to control hyperglycemia. Blood glucose concentrations were monitored at 3 hr after INS injection daily using Dextrostix (Miles, Inc., Elkhart, IN) and a portable meter. Adequate control was defined as blood glucose concentration between 30 and 80 mg/100 ml, and doses of INS were adjusted twice weekly to meet this criterion. For the duration of each study, gilts were housed in individual pens. Between Experiments 1 and 2, gilts were housed in groups with access to either concrete or gravel lots. Gilts were fed a corn-soybean meal ration (16% crude protein) supplemented with vitamins and minerals according to National Research Council (1988) guidelines. Pigs weighed 21 ± 0.3 kg at time of diabetes induction and were weighed weekly. Control pigs were limit fed so that growth rates were equivalent to those of diabetic gilts, which were fed *ad libitum*. Age and body weight at puberty averaged 185 ± 2.4 days and 103 ± 6 kg for controls and 192 ± 2.4 days and 111 ± 5 kg for diabetic pigs, respectively.

Experiment 1. At 9 months of age, all gilts were ovariectomized 30 days before the experiment to eliminate the influence of ovarian steroids on GH secretion. Gilts were fitted with an indwelling jugular vein catheter (11) and insulin therapy was removed on Day 0. Catheters were flushed with 3.5% sodium citrate twice daily for 4 days. On Day 4, beginning at 0800 hr, blood samples were taken at 10 min intervals for 8 hr. Blood samples were allowed to clot at 4°C for 24 hr and serum was harvested, except samples for glucose, which were drawn into heparinized tubes containing sodium fluoride. Samples were stored at −20°C until assayed for GH, IGF-I, INS, and glucose. All samples were quantified for GH by radioimmunoassay, as described by Barb *et al.* (12), and IGF-I by radioimmunoassay as described previously (10). The intra-assay and interassay coefficients of variation were 3.2% and 13.6% for GH and 13% and 15% for IGF-I, respectively. Assay sensitivities were 0.4 ng/ml for GH and 6 ng/ml for IGF-I. Plasma and CSF glucose concentrations were measured by the glucose oxidase-peroxidase method as described by Cox *et al.* (13). Serum and CSF insulin were measured by a procedure reported previously (13). Intra- and interassay coefficients of variation were 8% and 15%, respectively, and sensitivity was 0.04 ng/ml.

Experiment 2. The study was conducted in two phases approximately 40 days after Experiment 1. The diabetic gilts had been regulated and maintained on exogenous insulin during that time. Comparisons of serum and CSF concentrations of GH, INS, glucose, and IGF-I were conducted. Seven days before the study, insulin replacement therapy was discontinued. CSF was extracted according to a modified procedure by Boogerd and Boudewyn-Peters (14). Gilts were fasted 12 hr before induction of anesthesia with a 10% solution of Biotal (6 mg/kg iv, sodium thiamylal; Boehringer Ingelheim Animal Health, Inc., St. Joseph, MO). Anesthesia was then maintained on Metafane (methoxyflurane; Pitman-Moore, Washington Crossing, NJ) and nitrous oxide. An 18-g, 8-in spinal needle was inserted exactly midline in the lumbosacral area and CSF was collected as it flowed freely; if CSF was unattainable, then the cisterna magna was used as the CSF source. Specific gravity and protein content were determined at the time of extraction using a refractometer. Venous blood samples were obtained simultaneous to CSF removal. CSF (vol 3–10 ml) was centrifuged at 2700 rpm for 5 min and the supernatant was stored at −20°C for later hormonal analyses, as described in Experiment 1. Fourteen days after Phase I, Phase II of the study was conducted and diabetic gilts remained on insulin therapy. To prevent hypoglycemia due to their fasted state, the diabetic animals received onehalf their regular insulin dosage the day of CSF removal.

Statistical Analysis. Serum GH concentrations were subjected to general linear model split plot-in-time analysis of variance procedures of the Statistical Analysis System (15). The statistical model included treatment, pig, time, and treatment × time interaction. The effects of treatment were tested using animal within treatment as the error term. Time and treatment × time were tested using animal × time within treatment as the error term. For each pig, means for IGF-I, INS, and glucose were calculated on serum samples collected every 2 hr during the 8-hr sampling periods on Day 4

and were subjected to one-way analysis of variance procedure (15).

For each gilt, mean serum GH concentrations, basal GH concentrations, number of GH pulses, and GH pulse magnitude were determined by Pulsar analysis using a 5% criterion of variation (16). Data were then subjected to a one-way analysis of variance procedure (15). To determine differences between diabetic and control gilts, serum and CSF, GH, IGF-I, INS, and glucose concentrations and CSF protein values were subjected to a one-way analysis of variance procedure (15).

Results

Experiment 1. Plasma glucose concentrations were greater ($P < 0.05$) and serum INS was lower ($P < 0.0001$) in diabetic than in control gilts, whereas serum IGF-I concentrations were similar (Table I). Serum GH concentrations were greater ($P < 0.02$) for diabetic compared with control gilts (Fig. 1). Profiles for serum GH concentrations for two individual gilts each from the diabetic and control treatment groups are depicted in Figure 2. Indices of GH secretion are presented in Table II. Mean serum GH concentrations were 2-fold greater ($P < 0.02$) in diabetic than in control gilts. Diabetic gilts exhibited a greater ($P < 0.05$) number of GH pulses than control animals. In addition, GH pulse magnitude was markedly elevated ($P < 0.02$) in diabetic gilts compared with control gilts. Mean basal serum GH concentrations were greater ($P < 0.07$) in diabetic than in control gilts.

Experiment 2. Specific gravity of CSF averaged 1.006 in diabetic and control animals in both phases. Insulin, IGF-I, GH and glucose concentrations for diabetic pigs in the absence and presence of INS and in controls are presented in Table III. In Phase I (diabetic pigs off insulin), fasting plasma glucose levels were

Table I. Mean Serum GH Concentrations, GH Peak Frequency, and GH Peak Magnitude for Diabetic and Control Gilts[a]

Treatment	Gilts (*n*)	Serum GH (ng/ml)	Frequency of GH peaks/8 hr	GH peak magnitude (ng/ml)
Control	4	1.2 ± 0.2*	1.5 ± 0.3‡	3.3 ± 0.6*
Diabetic	5	2.8 ± 0.4†	3.2 ± 0.4§	5.8 ± 0.4†

[a] Data are expressed as mean ± SE. Means in a column with different symbols differ: *,†, $P < 0.02$; ‡,§, $P < 0.05$.

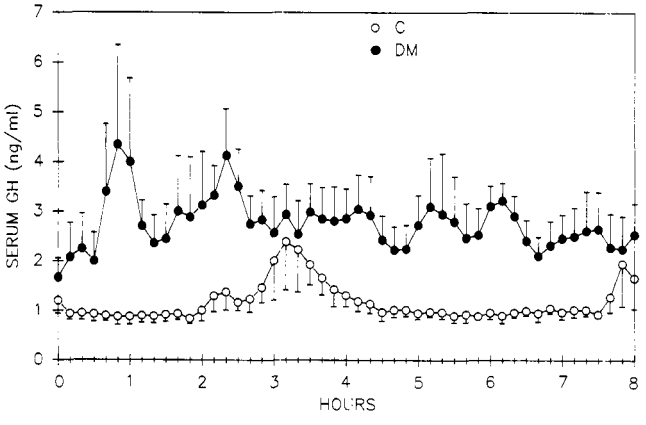

Figure 1. Serum GH concentrations (mean ± SE) in gilts with diabetes mellitus ([DM] $n = 5$) and controls ([C] $n = 4$). Serum GH concentrations were greater ($P < 0.02$) in diabetic compared with control gilts.

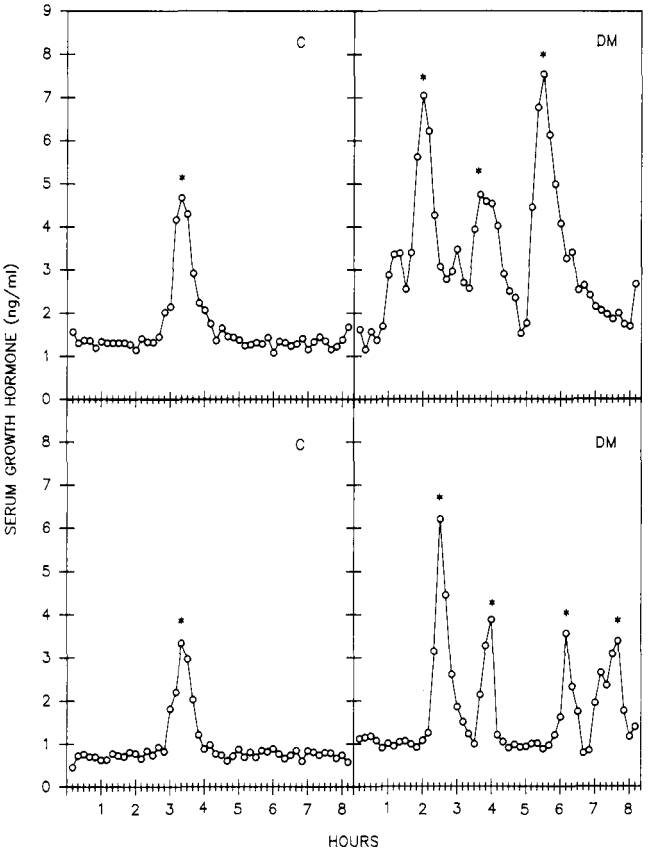

Figure 2. Serum GH concentrations in four individual gilts, two each from the diabetes mellitus (DM) and control (C) treatment groups. The asterisk denotes a GH pulse.

Table II. Mean Plasma Glucose, Serum INS, and IGF-I Concentrations for Diabetic and Control Gilts[a]

Treatment	Gilts (*n*)	Plasma glucose[b] (mg/100 ml)	Serum INS[c] (ng/ml)	Serum IGF-I[c] (ng/ml)
Control	4	82 ± 17‡	0.9 ± 0.05‖	43 ± 4
Diabetic	5	465 ± 17§	0.3 ± 0.02¶	32 ± 3

[a] Data are expressed as mean ± SE. Means in a column with different symbols differ: ‡,§, $P < 0.05$; ‖,¶, $P < 0.0001$.
[b] Mean of samples collected over a 114-hr period starting on Day 4.
[c] Mean of samples collected every 2 hr during the 8-hr sampling period on Day 4.

Table III. Serum and CSF, GH, INS, IGF-I, and Glucose Concentrations for Diabetic Gilts in the Absence (Phase I) or Presence (Phase II) of INS Therapy and Control[a]

Item	CSF		Serum or Plasma	
	Control	Diabetic	Control	Diabetic
Phase I: Diabetics without insulin				
GH (ng/ml)	2.1 ± 0.3	2.1 ± 0.2	4.8 ± 1.3	6.6 ± 1.1
INS (ng/ml)	0.15 ± 0.02	0.21 ± 0.07	0.34 ± 0.05	0.27 ± 0.02
IGF-I (ng/ml)	3.9 ± 1.8	2.4 ± 0.4	50 ± 13	31 ± 11
Glucose (mg/dl)	51 ± 3*	162 ± 14†,**	62 ± 3‡	310 ± 26§,**
Phase II: Diabetics with insulin				
GH (ng/ml)	1.9 ± 0.3	2.3 ± 0.3	3.5 ± 0.2‖	7.7 ± 1.6¶
INS (ng/ml)	0.11 ± 0.02	0.12 ± 0.01	0.58 ± 0.21	2.6 ± 1.0
IGF-I (ng/ml)	2.7 ± 4	2.4 ± 0.2	49 ± 12	49 ± 5
Glucose (mg/dl)	49 ± 3‡	76 ± 5§,††	62 ± 3	58 ± 2††

[a] Data are expressed as means ± SE. Means in a row with different symbols are different: *,† $P < 0.0002$; ‡,§ $P < 0.003$; ‖,¶ $P < 0.05$. Means in a column with different symbols are different: **,†† $P < 0.0001$.

higher ($P < 0.003$) in the untreated diabetic than in the control animals. CSF glucose levels were higher ($P < 0.0002$) for diabetic than for controls, but there were no differences in CSF INS and GH levels and serum GH concentrations in the diabetic and control gilts. Serum and CSF IGF-I levels were similar between groups in both phases. In Phase II (INS replacement), regulated plasma glucose levels in the diabetic pigs dropped from 310 ± 26 to 58 ± 2 mg/dl ($P < 0.0001$) and was not different from the controls (62 ± 3 mg/dl). Although CSF glucose levels in the diabetic animals decreased from 162 ± 14 to 76 ± 5 mg/dl ($P < 0.0001$), there was still a difference ($P < 0.003$) between diabetic and control pigs (76 ± 5 and 49 ± 3 mg/dl, respectively). Total protein was similar in the diabetic gilts from Phase I to II. Serum INS increased in the diabetic pigs with exogenous INS administration. CSF INS concentrations in diabetic animals decreased in Phase II, as did serum and CSF glucose concentrations. However, serum GH concentrations remained unchanged in diabetic pigs when compared with Phase I concentrations, but serum GH levels were greater ($P < 0.05$) in diabetics than controls for Phase II, whereas CSF GH levels were similar between the groups.

Discussion

Serum GH concentrations and GH pulse frequency were greater after induction of diabetes in the face of elevated plasma glucose concentrations compared with controls in the present study. These findings are similar to those reported by Asplin *et al.* (3) in which GH pulse frequency and amplitude were greater in patients with poorly controlled diabetes compared with nondiabetic patients. In contrast, serum GH concentrations were lower in the face of marked hyperglycemia in streptozotocin-induced diabetic rats compared with controls (17). These observations demonstrate species variation in the hypothalamic pituitary axis response to diabetes. There has been interest in recent years in defining the mechanisms that subserve the abnormalities in GH secretory pattern in diabetes. Alterations could be associated with differences in secretion and/or clearance rates of GH (18). However, it remains to be substantiated to what extent clearance rates of GH contribute to the aberrations of GH secretion observed in diabetes (19). Alternatively, nutrient and/or metabolic derangement as a result of diabetes may contribute to abnormal GH secretion. We demonstrated previously that hyperglycemia delayed the GH response to GHRH in pigs (9). In the present study, the GH pulse-generating mechanism appears to be desensitized to hyperglycemia, since both plasma and CSF concentrations of glucose were greater than controls. In support of this idea, Press *et al.* (20) reported failure of glucose to suppress pituitary GH response to GHRH in diabetic patients.

The pulsatile pattern of GH secretion reflects pituitary somatotrope response to hypothalamic GHRH and somatostatin secretion (21). Growth hormone pulses occur during periods of increased GHRH secretion coincident with or immediately preceding a reduction in somatostatin secretion into the hypophyseal-portal blood during periods of expected GH pulses (22). The chronic elevation of plasma and CSF glucose concentrations observed during the period of INS withdrawal in Phase I and the continued elevation of CSF glucose concentrations after INS therapy in Phase II suggest that elevated CSF glucose may act to alter the endogenous thythm of GHRH and somatostatin. In support of this idea, Lengyel *et al.* (23) reported that, in the rat, glucose inhibited and 2-deoxyglucose stimulated somatostatin secretion from hypothalamic fragments *in vitro*. Therefore, increased GH pulse frequency in diabetic pigs compared with controls, in the present study, would suggest an alteration in the rhythm of GHRH and somatostatin secretion. An alternative to a hypothalamic site of action for diabetes-induced alteration in GH secretion is a direct effect on the

pituitary somatotrope. The GHRH-stimulated GH release in diabetic subjects was greater than (24) or similar to controls (20). Moreover, anterior pituitary cells in culture from diabetic rats exhibited a marked increase in sensitivity to GHRH and a reduction in sensitivity to somatostatin with no change in basal secretion (25). The findings are in partial agreement with Olchovsky *et al.* (17), who reported a reduction in membrane somatostatin receptors in anterior pituitary cells of diabetic rats compared with controls. This decrease was accompanied by a reduction in GH content and basal secretion. These aberrations in pituitary function cited above may reflect diabetes-induced hypothalamic dysfunction, which, in turn, alters pituitary somatotrope activity.

Recently, IGF-I was shown to participate in feedback inhibition of GH secretion at the level of both the pituitary and hypothalamus in the rat. Brain intraventricular injection of IGF-I suppressed episodic release of GH (7, 26) and stimulated hypothalamic somatostatin (27). Insulin-like growth factor-I inhibited both acute release of GH and GH mRNA levels *in vitro* in rats (5, 28). In order to evaluate the potential role of IGF-I in GH secretion during diabetes, serum and CSF concentrations of IGF-I were measured. Both serum and CSF concentrations of IGF-I were similar in diabetic and control gilts. In contrast, systemic IGF-I concentrations were lowered by diabetes in the human (2, 3, 29) and rat (17). Rieu and Binoux (30) reported in humans that after removal of INS therapy for 2 or 3 days, serum IGF-I concentrations were depressed and serum GH levels elevated. In the present study, INS therapy had been removed for 4 days in Experiment 1 and 7 days in Experiment 2 prior to the respective studies. Therefore, we believe that duration of INS withdrawal should have been sufficient to alter serum and CSF IGF-I concentrations. Insulin, another potential factor in the modulation of GH synthesis and secretion (5, 6, 8), can also be eliminated as a possible regulator of GH secretion, since diabetic pigs were insulinopenic.

In conclusion, data from the present study support the idea that elevated GH concentrations observed during poorly controlled diabetes are associated with an alteration in pulsatile secretion of GH. These perturbations in GH secretion in diabetic gilts may be due, in part, to elevated serum and CSF glucose concentrations, which alter GHRH and/or somatostatin secretion from the hypothalamus, which, in turn, alters pituitary somatotrope activity.

The authors wish to thank Benny Barrett for his technical assistance; Ruel L. Wilson, biometrician, southern region, ARS, for his statistical advice; and Dr. D. J. Bolt, USDA, Beltsville, MD, for providing the growth hormone used in radioimmunoassay.

Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

1. Vigneri R, Squatrito S, Pezzino V, Filetti S, Branca S, Polosa P. Growth hormone levels in diabetes: Correlation with the clinical control of the disease. Diabetes **25**:167–172, 1976.
2. Press M, Tamborlane WV, Sherwin RS. Importance of raised growth hormone levels in mediating the metabolic derangements of diabetes. N Engl J Med **310**:810–815, 1984.
3. Asplin CM, Faria ACS, Carlsen EC, Vaccaro VA, Barr RE, Iranmanesh A, Lee MM, Veldhuis JD, Evans WS. Alterations in the pulsatile mode of growth hormone release in men and women with insulin-dependent diabetes mellitus. J Clin Endocrinol Metab **69**:239–245, 1989.
4. Berelowitz M, Szabo M, Frohman LA, Chu L, Hintz RL. Somatomedin-C mediates growth hormone negative feedback by effects on both the hypothalamus and the pituitary. Science **212**:1279–1281, 1981.
5. Yamashita S, Melmed S. Insulin-like growth factor I action on rat anterior pituitary cells: Suppression of growth hormone secretion and messenger ribonucleic acid levels. Endocrinology **118**:176–182, 1986.
6. Melmed S. Insulin suppresses growth hormone secretion by rat pituitary cells. J Clin Invest **73**:1425–1433, 1984.
7. Tannenbaum GS, Guyda HJ, Posner BI. Insulin-like growth factors: A role in growth hormone negative feedback and body weight regulation via brain. Science **220**:77–79, 1983.
8. Barb CR, Cox NM, Matamoros IA, Kraeling RR, Rampacek GB. Growth hormone (GH) secretion after intracerebroventricular (ICV) administrations of insulin (INS) in the gilt [Abstract]. J Anim Sci **68**(suppl 1):310, 1990.
9. Barb CR, Kraeling RR, Barrett JB, Rampacek GB, Campbell RM, Mowles TF. Serum glucose and free fatty acids modulate growth hormone and luteinizing hormone secretion in the pig. Proc Soc Exp Biol Med **198**:636–642, 1991.
10. Meurer KA, Cox NM, Matamoros IA, Tubbs RC. Decreased follicular steroids and insulin-like growth factor-I and increased atresia in diabetic gilts during follicular growth stimulated with PMSG. J Reprod Fertil **91**:187–196, 1991.
11. Barb CR, Kraeling RR, Rampacek GB, Fonda ES, Kiser TE. Inhibition of ovulation and LH secretion in the gilt after treatment with ACTH or hydrocortisone. J Reprod Fertil **64**:85–92, 1982.
12. Barb CR, Estienne MJ, Kraeling RR, Marple DN, Rampacek GB, Rahe CH, Sartin JL. Endocrine changes in sows exposed to elevated ambient temperature during lactation. Domest Anim Endocrinol **8**:117–127, 1991.
13. Cox NM, Stuart MJ, Althen TG, Bennett WA, Miller HW. Enhancement of ovulation rate in gilts by increasing dietary energy and administering insulin during follicular growth. J Anim Sci **64**:507–516, 1987.
14. Boogerd W, Boudewyn-Peters AC. A simple method for obtaining cerebrospinal fluid from a pig model of herpes encephalitis. Lab Anim Sci **36**:386–388, 1986.
15. SAS, Inc. SAS user's guide. Gary, NC: Statistical Analysis System Institute, 1982.
16. Merriam GR, Wachter KW. Algorithms for the study of episodic hormone secretion. Am J Physiol **6**:E310–E318, 1982.
17. Olchovsky D, Bruno JF, Wood TL, Gelato MC, Leidy JW Jr, Gilbert JM Jr, Berelowitz M. Altered pituitary growth hormone (GH) regulation in streptozotocin-diabetic rats: A combined defect of hypothalamic somatostatin and GH-releasing factor. Endocrinology **126**:53–61, 1990.
18. Boucher BJ, Butterfield WJH, Whichelow MJ. The disappear-

ance of labeled human growth hormone from the forearm circulation in normal and diabetic subjects. Clin Sci **37**:721–730, 1969.
19. Navalesi R, Pilo A, Vigneri R. Growth hormone kinetics in diabetic patients. Diabetes **24**:317–327, 1975.
20. Press M, Tamborland WV, Thorner MO, Vale W, Rivier J, Gertner JM. Pituitary response to growth hormone-releasing factor in diabetes. Diabetes **33**:804–806, 1984.
21. Tannenbaum GS, Ling N. The interrelationship of growth hormone (GH)-releasing factor and somatostatin in generation of the ultradian rhythm of GH secretion. Endocrinology **115**:1952–1957, 1984.
22. Plotsky PM, Vale W. Patterns of growth hormone-releasing factor and somatostatin secretion into the hypophysial-portal circulation of the rat. Science **230**:461–463, 1985.
23. Lengyl AMJ, Grossman A, Nieuwenhuyzen-Kruseman AC, Ackland J, Rees LH, Besser M. Glucose modulation of somatostatin and LHRH release from rat hypothalamic fragments in vitro. Neuroendocrinology **39**:31–38, 1984.
24. Krassowski J, Felber JP, Rogala H, Jeske W, Zgliczynski S. Exaggerated growth hormone response to growth hormone-releasing hormone in type I diabetes mellitus. Acta Endocrinol **117**:225–229, 1988.
25. Sheppard MS, Eatock BA, Bala RM. Altered release of growth hormone from dispersed adenohypophysial cells of streptozotocin diabetic rats. I. Effects of growth hormone releasing factor and somatostatin. Can J Physiol Pharmacol **67**:1315–1320, 1989.
26. Abe RL, Grumbach MM, Kaplan SL. Human growth hormone and somatomedin C suppress the release of growth hormone in unanesthetized rats. Endocrinology **112**:1319–1323, 1983.
27. Berelowitz M, Dudlak D, Frohman LA. Release of somatostatin-like immunoreactivity from incubated rat hypothalamus and cerebral cortex. J Clin Invest **69**:1293–1301, 1982.
28. Yamashita S, Weiss M, Melmed S. Insulin-like growth factor I regulates growth hormone secretion and messenger ribonucleic acid levels in human pituitary tumor cells. J Clin Endocrinol Metab **63**:703–735, 1986.
29. Tan K, Baxter R. Serum insulin-like growth factor I levels in adult diabetic patients. J Clin Endocrinol Metab **63**:651–655, 1986.
30. Rieu M, Binoux M. Serum levels of insulin-like growth factor (IGF) and IGF binding protein in insulin-dependent diabetics during an episode of severe metabolic decompensation and the recovery phase. J Clin Endocrinol Metab **60**:781–785, 1985.