Serum Glucose and Free Fatty Acids Modulate Growth Hormone and Luteinizing Hormone Secretion in the Pig (43301)

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Abstract. **Two experiments were conducted to determine the effect of free fatty acids (FFA) and glucose treatment on growth hormone (GH) and luteinizing hormone secretion in the pig. In Experiment (Exp) 1, 15 prepuberal gilts received an intravenous infusion of FFA ($n = 5$; 3 ml of 10% Liposyn II/kg), glucose ($n = 5$; 1 g/kg), or saline ($n = 5$; 3 ml of 0.9%/kg). Jugular blood samples were collected every 15 min for 2 hr before and 3 hr after intravenous infusion of saline, FFA, and glucose. Synthetic [Ala^{15}]-h growth hormone-releasing factor-(1-29)NH_2 (1 μg/kg) and gonadotropin-releasing hormone (0.2 μg/kg) were administered 30 min after infusion (Time 0 = infusion). In Exp 2, eight prepuberal gilts received either FFA ($n = 4$) or saline ($n = 4$) as described in Exp 1, except that treatments were given every hour over a 10-hr period. Blood samples were collected every 15 min from 1 hr before to 10 hr after the start of FFA or saline infusion. In Exp 1, the peak GH response to growth hormone-releasing factor was delayed by 45 min ($P < 0.01$) by glucose treatment and suppressed ($P < 0.01$) by FFA treatment. The luteinizing hormone response to gonadotroph-releasing hormone was suppressed ($P < 0.03$) by glucose and enhanced ($P < 0.03$) by FFA. In Exp 2, the number of GH pulses was increased ($P < 0.05$) by FFA infusion and GH concentrations were positively correlated ($r = 0.58$, $P < 0.0003$) with FFA concentrations, while luteinizing hormone pulse amplitude was greater ($P < 0.01$) in FFA gilts than in saline gilts. These results indicate that FFA are more effective modulators of GH secretion than acute hyperglycemia, while metabolic status can alter pituitary responsiveness to gonadotropin-releasing hormone.** [P.S.E.B.M. 1991, Vol 198]

Nutritional status modifies serum growth hormone (GH) concentrations in steers (1), heifers (2), and pigs (3). Intermediate metabolites such as glucose, lipids, and amino acids have been postulated to be secondary controlling factors that modulate GH secretion (4). In the ewe, decreased peripheral or central nervous system glucose availability and exogenous administration of free fatty acids (FFA) antagonized growth hormone-releasing factor (GRF)-induced release of GH (5, 6), while pharmacologic depletion of circulating FFA enhanced GRF-induced release of GH (5). In the rat (7) and the human (8, 9), GRF-induced GH secretion was inhibited by FFA and glucose administration. The suppressive effects of FFA and glucose on GRF-induced GH release are probably due to increased secretion of somatostatin from the central nervous system (7, 10). In contrast, concomitant elevation of serum GH and glucose concentrations has been observed with insulin-dependent diabetes (11, 12). However, the mechanism responsible for this abnormal relationship is not fully understood.

Cameron *et al.* (13) suggested that the dynamic fluctuations of plasma hormones and metabolic substrates that occurred during the postprandial and postabsorptive periods provide signals to the brain that link metabolic status to the activation of the reproductive system. Nutritional and metabolic perturbations influence endocrine function in the pig. Armstrong and Britt (14) reported that chronic feed restriction in the gilt resulted in cessation of estrous cycles, lower plasma insulin concentrations, higher plasma FFA concentrations, and reduced luteinizing hormone (LH) pulse

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frequency, compared with controls. Booth (15) reported similar results in the feed-restricted prepuberal gilt. Moreover, Armstrong *et al.* (16) reported that preprandial concentrations of glucose were greater and FFA were lower in anestrous than in estrous sows during lactation. The authors speculated that altered energy metabolism during lactation could contribute to postweaning reproductive performance, although the mechanism by which this was effected was not determined.

Collectively, these data demonstrate that under certain physiological conditions (e.g., fasting, feed restriction, lactation, or diabetes), alterations in energy metabolism may influence hypothalamic-pituitary function. Therefore, we investigated whether alterations of blood glucose or FFA would modify pituitary response to the actions of GRF and gonadotropin-releasing hormone (GnRH) to release GH and LH, and, second, whether chronic elevation of FFA would alter pulsatile GH and LH secretion in the growing pig.

Materials and Methods

Experiment 1. Fifteen domestic cross-bred prepuberal gilts averaging 140 days of age and 67.9 ± 2.2 kg body wt were individually penned in an environmentally controlled building and exposed to a constant ambient temperature of 22°C and an artificial 12:12-hr light:dark photoperiod. Pigs were fed between 0700 and 0800 hr a corn-soybean meal ration (14% crude protein) supplemented with vitamins and minerals, according to Nutrition Research Council (17) guidelines. The experiment was conducted in two replicates with the following treatment groups: lipid emulsion (FFA; 3 ml of 10% Liposyn II [Abbott Laboratories, North Chicago, IL] emulsion/kg body wt), glucose (1 g/kg body wt), or saline (3 ml 0.9% solution/kg body wt) infused intravenously over a 5-min period starting at Time 0. The lipid emulsion (10% Liposyn II) consisted of the following fatty acids: linoleic (65.8%), oleic (17.7%), palmitic (8.8%), linolenic (4.2%), and stearic (3.4%) acids. The fatty acid content of Liposyn is generally comparable to that present in the circulation (18) of pigs, with the exception of linoleic acid, which comprised approximately 16% of total FFA in serum. Synthetic [Ala^{15}]-hGRF(1–29)-NH_2 (1 μg/kg body wt) and GnRH (Cystorlein; CEVA Laboratories, Inc., Overland Park, KS) (0.2 μg/kg body wt) in 2 ml of 0.9% saline were administered as an intravenous bolus. Before initiation of the experiment, a dose-response study (0.3, 1, and 3 μg/kg body wt) was performed with the GRF analog. The 1-μg/kg dose produced consistent GH responses. Based on previous observations (unpublished), the dose of GnRH used was optimal for assessing differences in pituitary sensitivity. In replicate 1, two gilts were assigned to the saline-treatment group, two to the FFA-treatment group, and three to the glucose-treatment group. In replicate 2, the remaining eight gilts were assigned to the above treatment groups such that a total of five gilts per treatment group was achieved across replicates.

On the day prior to treatment, an indwelling catheter was placed into the jugular vein of each pig (19). Blood samples were collected every 15 min for 2 hr before and 3 hr after intravenous infusion of saline, FFA, and glucose. GRF and GnRH were administered 30 min after infusion (Time 0 = infusion).

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 floride. Samples were stored at −20°C until they were assayed for GH, LH, glucose, and FFA. All samples were assayed for glucose using glucose oxidase kits (Sigma Chemical Co., St. Louis, MO) and for FFA using a colorimetric procedure (20). All samples were quantified for GH by radioimmunoassay, as described by Kraeling *et al.* (21) and modified by Barb *et al.* (22), and for LH by radioimmunoassay, as described previously (23, 24). The intraassay and interassay coefficients of variation were 3.2% and 13.6% for GH and 4.8% and 9.0% for LH, respectively. Assay sensitivities were 0.4 ng/ml for GH and 0.15 ng/ml for LH. Elevated serum FFA and glucose concentrations resulting from exogenous lipid and glucose infusion did not interfere with either the LH or GH radioimmunoassay. Using blood samples collected during the glucose and FFA infusion periods, parallelism was demonstrated by showing that estimates of GH and LH concentrations were not influenced by the volume of serum assayed (50–300 μl).

Experiment 2. Eight cross-bred prepuberal gilts averaging 150 days of age and 75.0 ± 2.0 kg body wt were housed as described in Experiment 1. All gilts were ovariectomized at 120 days of age. Four gilts each were randomly assigned to FFA or saline treatments utilizing dose and route of administration as described in Experiment 1, except that treatments were administered every hour over a 10-hr period in an effort to maintain chronic elevation of serum FFA concentrations. This treatment interval was based on the results of Experiment 1, in which FFA concentration remained elevated for 90 min above control levels. Blood was collected every 15 min from 1 hr before to 10 hr after the start of FFA or saline infusion. All samples were processed and quantified for GH, LH, and FFA, as described in Experiment 1.

Statistical Analysis. To determine differences between treatments for the GH and LH responses to the GRF/GnRH challenge, pretreatment mean serum GH and LH concentrations were subtracted from each value after the challenge. The data were then subjected to the general linear model split plot-in-time analysis of variance procedure of the Statistical Analysis System (25). The statistical model included treatment, time, repli-

cate, treatment × replicate, and treatment × time interactions. The effects of treatment and replicate were tested using animal within treatment × replicate as the error term. Time, treatment × replicate, and treatment × time were tested using treatment × time × replicate as the error term. Total area under the curve was estimated using the trapezoidal summation method for serum GH and LH responses. The data were then subjected to a one-way analysis of variance procedure. Differences between treatment means were determined by least-squares contrasts (25). Serum FFA and plasma glucose data from the two experiments were subjected to the general linear model split plot-in-time analysis of variance procedure of the Statistical Analysis System (25). If a significant treatment × time interaction was detected, then differences between treatments within a specific time were determined by least-squares contrasts (25).

For each gilt, mean serum hormone concentrations, basal hormone concentrations, the number of hormone pulses, and hormone pulse amplitude were determined by Pulsar analysis, using a 1% criterion of variation (26), during the 10-hr FFA-treatment period for Experiment 2. Data were then subjected to a one-way analysis of variance procedure. Pearson's correlations (25) were calculated among FFA and GH concentrations for control and FFA-treated animals in Experiment 2.

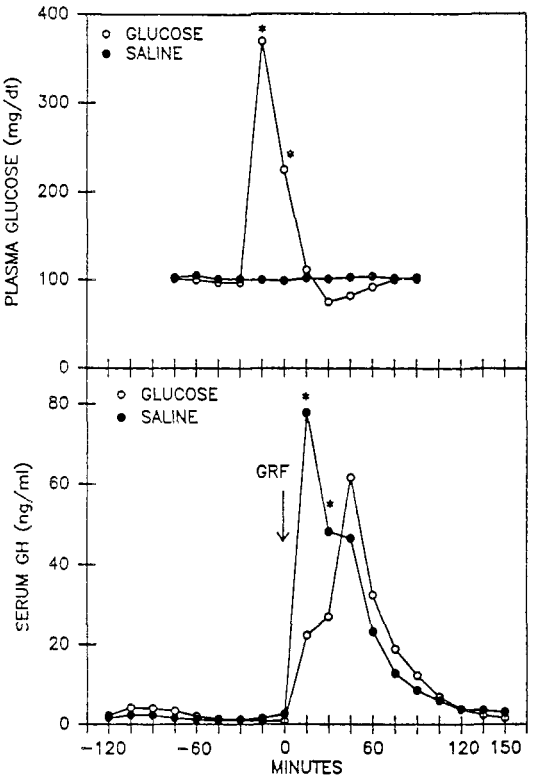

Figure 1. Mean plasma glucose (top panel) and serum GH (bottom panel) concentrations for gilts infused with glucose ($n = 5$) or saline ($n = 5$) 30 min before intravenous injection of GRF. Pooled SE were 11.5 mg/dl for glucose and 6.3 ng/ml for GH. Sample times at which significant effects of treatment were detected are indicated (*$P < 0.01$).

Results

Serum FFA and glucose concentrations in Experiment 1 were similar between saline-, (0.39 ± 0.01 mEq/liter, 102 ± 1 mg/dl), FFA- (0.36 ± 0.01 mEq/liter, 97 ± 1 mg/dl), and glucose- (0.34 ± 0.01 mEq/liter, 99 ± 1 mg/dl) treated pigs prior to infusion. Serum FFA and glucose increased ($P < 0.05$) after infusion of the respective compounds and reached peak concentrations of 1.82 ± 0.35 mEq/liter and 396 ± 30 mg/dl, respectively, by 15 min after infusion. Serum FFA concentrations were unaffected by glucose infusion, as plasma glucose concentrations were unaffected by FFA infusion. Saline infusion had no effect on either FFA or glucose concentrations (Figs. 1 and 2).

Concentrations of GH increased in all pigs after GRF challenge ($P < 0.05$). A treatment × time interaction ($P < 0.02$) was detected for the GH response to GRF. Peak concentrations of serum GH occurred within 15 min after injection of GRF in the saline pigs (Fig. 1). The peak GH response to GRF was delayed ($P < 0.01$) in the glucose- and FFA-treated gilts, with peak concentrations occurring at 45 and 30 min after injection of GRF, respectively (Figs. 1 and 2). Growth hormone concentrations at 15 min after GRF were greater ($P < 0.0002$) in saline-treated gilts (77.8 ± 19 ng/ml) than in FFA- (2.9 ± 0.5 ng/ml) and glucose- (22.3 ± 19.6 ng/ml) treated gilts (Figs. 1 and 2). Following GRF treatment, the area under the curve was greater ($P < 0.05$) in saline- (126 ± 3 ng·hr/ml) than FFA- (23 ± 3 ng·hr/ml) treated gilts, but was similar to that in glucose- (102 ± 23 ng·hr/ml) treated animals.

Mean serum LH concentrations were similar among treatments before injection of GnRH and averaged 0.20 ± 0.05 ng/ml, 0.20 ± 0.02 ng/ml, and 0.15 ± 0.01 ng/ml for saline-, FFA-, and glucose-treated animals, respectively. There was a treatment × time interaction ($P < 0.03$) for the LH response to GnRH. Peak serum LH concentrations achieved after GnRH were increased ($P < 0.005$) by FFA (2.8 ± 0.2 ng/ml) and suppressed ($P < 0.03$) by glucose (1.6 ± 0.2 ng/ml), compared with saline- (2.1 ± 0.2 ng/ml) treated gilts (Fig. 3). The LH response to GnRH as measured by the area under the curve was similar for all treatments and averaged 3.5 ± 0.9, 3.5 ± 0.7, and 2.2 ± 0.4 ng·hr/ml for saline-, FFA- and glucose-treated gilts, respectively.

In Experiment 2, serum FFA concentrations were episodic and followed the FFA infusion schedule, which resulted in a treatment × time interaction ($P < 0.05$, Fig. 4). Mean and basal serum GH concentrations, GH pulse frequency, and GH pulse amplitude are presented in Table I. Depicted in Figure 4 are profiles for mean serum GH concentrations for FFA- and saline-treated

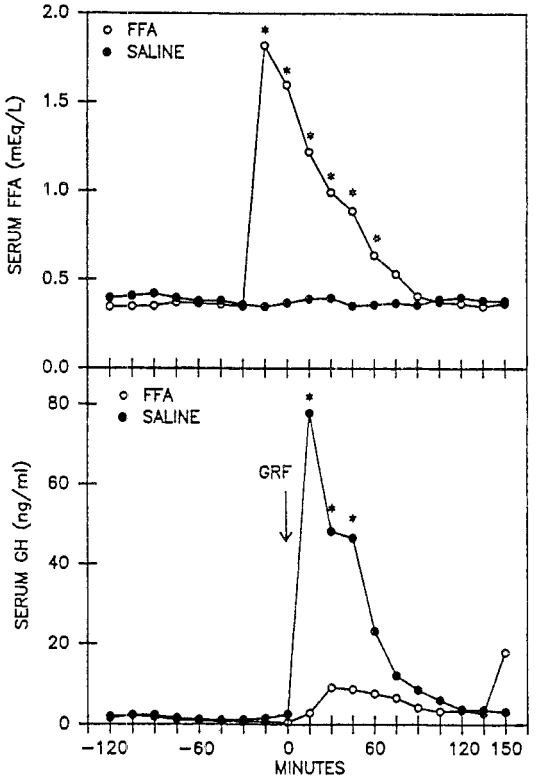

Figure 2. Mean serum FFA (top panel) and GH (bottom panel) concentrations for gilts infused with FFA (n = 5) or saline (n = 5) 30 min before intravenous injection of GRF. Pooled SE were 0.20 mEq/liter for FFA and 6.26 ng/ml for GH. Sample times at which significant effects of treatment were detected are indicated (*P < 0.01).

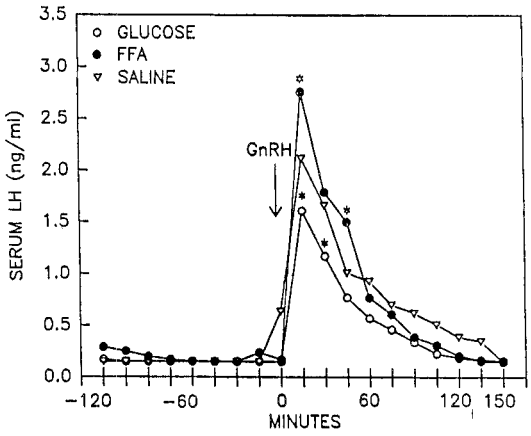

Figure 3. Mean serum LH concentrations in gilts infused with glucose (n = 5), FFA (n = 5), or saline (n = 5) 30 min before intravenous injection of GnRH. Pooled SE was 0.16 ng/ml. Sample times at which significant effects of treatment were detected are indicated (*P < 0.03).

gilts. The serum GH pulse frequency was increased (P < 0.05) by FFA infusion. In addition, GH concentrations were positively (r = 0.58, P < 0.0003) correlated with FFA concentrations in the FFA-treated gilts, while this relationship was not apparent in saline-treated animals. However, GH pulse amplitude and mean and basal GH concentrations were not altered by FFA infusion.

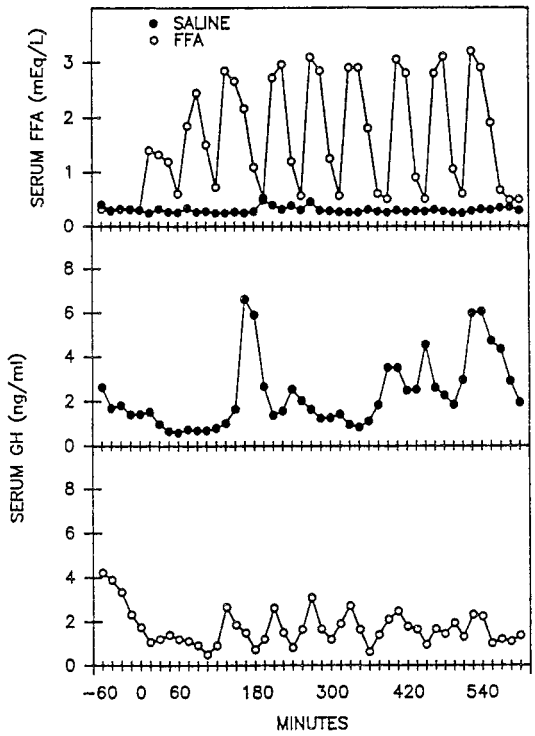

Figure 4. Mean serum FFA and GH concentrations in gilts infused every hour with either FFA (n = 4) or saline (n = 4). Time 0 = start of infusion. Pooled SE were 0.61 mEq/liter for FFA and 1 ng/ml for GH. A significant (P < 0.05) treatment × time interaction was revealed for FFA and GH concentrations. GH concentrations were positively correlated (r = 0.58, P < 0.0003) with FFA concentrations in the FFA-treated gilts.

Luteinizing hormone pulse amplitude was greater (P < 0.1) in FFA-treated gilts than in saline-treated gilts, while other indices of LH secretion were similar between the two treatments (Table II).

Discussion

The peak concentrations of FFA and glucose achieved in the present study approximated preprandial FFA concentrations observed in lactating sows (16) and glucose concentrations in diabetic gestating sow (27) and diabetic pigs (11, 27). Therefore, the changes in serum concentrations of GH and LH occurred in the face of physiological levels of FFA and glucose.

In the present study, acute hyperglycemia delayed, but did not suppress, the GRF-induced release of GH. A lack of GH suppression by hyperglycemia was also observed in the rat and cat (7, 28, 29). By contrast, in humans, hyperglycemia resulted in a marked inhibition of GH secretion after GRF treatment (9). Sartin *et al.* (5) reported recently that, in the ewe, decreased peripheral or central nervous system glucose availability antagonized the GRF-induced secretion of GH, while elevated plasma glucose concentrations had no effect. Similarly, in the monkey, hypoglycemia enhanced GH secretion and hyperglycemia had no effect (30). The apparent species differences by which hyperglycemia or

Table I. Mean and Basal Serum GH Concentrations, GH Pulse Frequency, and GH Pulse Amplitude for Saline- and Lipid-Treated Gilts in Experiment 2[a]

Treatment	No. of gilts	Mean GH concentrations (ng/ml)	Basal GH concentrations (ng/ml)	GH pulse frequency (pulses/10 hr)	GH pulse amplitude (ng/ml)
Saline	4	2.3 ± 0.6	1.2 ± 0.4	3.5 ± 0.6[b]	5.3 ± 1.7
FFA	4	1.5 ± 0.1	0.8 ± 0.1	6.0 ± 0.4[c]	2.6 ± 0.3

[a] Values expressed as mean ± SE.
[b,c] Means in a column with different superscripts are different ($P < 0.02$).

Table II. Mean and Basal Serum LH Concentrations, LH Pulse Frequency, and LH Pulse Amplitude for Saline- and Lipid-Treated Gilts in Experiment 2[a]

Treatment	No. of Gilts	Mean LH concentrations (ng/ml)	Basal LH concentrations (ng/ml)	LH pulse frequency (pulses/10 hr)	LH pulse amplitude (ng/ml)
Saline	4	0.5 ± 0.1	0.3 ± 0.1	9.0 ± 0.7	0.4 ± 0.1[b]
FFA	4	0.5 ± 0.1	0.3 ± 0.1	8.8 ± 0.5	0.7 ± 0.1[c]

[a] Values are expressed as mean ± SE.
[b,c] Mean in a column with different superscripts are different ($P < 0.1$).

glucopenia influences GH response to GRF are not clear. However, both the pituitary gland and hypothalamus are proposed as possible sites at which acute changes in glucose concentrations act to suppress GH release. Glucose-sensitive neurons are present in the hypothalamus (31) and are putative mediators of hypothalamic function. In support of this idea, Lengyel *et al.* (10) reported that, in the rat, glucose inhibited and 2-deoxyglucose stimulated somatostatin secretion from hypothalamic fragments *in vitro*. The ruminant brain can utilize alternative energy sources (32) and may not require a rapid increase in glucose production after brain glucopenia, as observed for monogastric species. Therefore, the ability of different species to utilize alternative energy sources may, in part, explain species variation in the GH response to alterations in blood glucose concentrations.

As in rats (7) and humans (8, 9), GRF-induced GH secretion was inhibited by FFA in the gilt. Recent studies by Sartin *et al.* (5) and Estienne *et al.* (6) demonstrated that exogenous FFA compromise the GH secretory response to exogenous GRF in sheep. Imaki *et al.* (7) reported that the suppressive effect of FFA on GRF-induced GH release in rats was abolished by pretreatment with antisomatostatin serum and, therefore, proposed that FFA inhibit GH secretion by stimulating somatostatin release. The GH response *in vitro* of porcine anterior pituitary cells to GRF was inhibited by somatostatin (33). Therefore, in the present study, the attenuated response to GRF displayed in FFA-treated gilts could theoretically be attributed to enhanced somatostatin release.

It has been suggested that FFA are taken up by hypothalamic tissue (34) and act by altering the bilayer structure of biological membranes, thereby interfering with movement and internalization of surface receptors (35) and altering the firing pattern of hypothalamic neurons *in vitro* (31, 36). Therefore, suppressed GH response to GRF may reflect a stimulation of somatostatin secretion from the hypothalamus. However, an alternative to a hypothalamic site of action for FFA-induced suppression of GRF-stimulated GH release is a direct effect of lipid on the adenohypophysis. Caprylic and oleic acids inhibited basal and GRF-induced GH release from rat pituitary slices *in vitro* (37), demonstrating that, at least in this species, the suppressive effects of lipid on somatotrope secretory activity may not depend entirely on suprapituitary input. However, palmitate was ineffective in altering GH release from bovine pituitary slices *in vitro* (38).

In Experiment 2, FFA treatment increased pulsatile GH secretion. This is in contrast to reports in the rat (7, 28), ewe (6), monkey (30), and human (8, 9), in which exogenous FFA administration decreased circulating concentrations of GH. The FFA treatment regime employed in Experiment 2 did not maintain serum FFA concentrations above control levels for the duration of the treatment period. We theorized, based on the results of Experiment 1, that FFA levels would remain elevated above control concentrations if Liposyn was infused every hour. Thus, the hourly fluctuations in serum FFA concentrations observed in Experiment 2 may have, in fact, resulted in a synchrony in the endogenous rhythm of GRF and somatostatin secretion from the hypothalamus and, subsequently, influenced somatotrope secretory activity. As cited above, FFA are taken up by hypothalamic tissue (34) and alter the firing pattern of hypothalamic neurons *in vitro* (31,

36). Therefore, GH pulses that occurred concomitant with FFA treatment may reflect an alteration of GRF and/or somatostatin secretion from the hypothalamus. Future studies are needed to address the influence of FFA on hypothalamic activity and neurohormone secretion in the pig.

Steiner *et al.* (39) and Cameron *et al.* (13) suggested that blood-borne signals reflecting metabolic status may influence the hypothalamic-pituitary axis with regard to gonadotropin secretion. Foster and Olster (40) reported that undernutrition inhibited LH secretion in lambs. Armstrong and Britt (14) reported that chronic feed restriction in the gilt resulted in lower plasma insulin concentrations, higher plasma FFA concentrations, higher basal glucose concentrations, and reduced LH pulse frequency when compared with controls. Booth (15) observed similar results following feed restriction in the prepuberal gilt. Moreover, Booth (15) found that glucose administration to feed-restricted gilts induced a rapid increase in episodic LH secretion similar to that observed in response to realimentation. Collectively, these data demonstrate that metabolic perturbation altered LH secretion. Therefore, it was hypothesized that FFA and glucose may act to alter pituitary responsiveness to GnRH and that chronic lipid administration would alter basal LH secretion. Our findings, that acute elevation of serum FFA levels enhanced and glucose concentrations suppressed the LH response to GnRH, while hourly administration of lipid increased LH pulse amplitude, support this idea. However, the glucose-induced suppression of the LH response to GnRH in the present study is in contrast to results reported by Booth (15). It should be noted that Booth (15) utilized feed-restricted animals in his studies. Therefore, age, body composition, or nutritional status may influence the responsiveness of the hypothalamic-pituitary axis to glucose. In addition, hyperglycemia may have altered GnRH-receptor affinity and/or number. In support of this hypothesis are the findings that glucose concentrations of 100–400 mg/dl resulted in a decrease in brain opioid-receptor affinity for [^{3}H] naloxone in the mouse (41). It is difficult to explain the FFA-enhanced LH response to GnRH. However, one possibility is that after FFA administration, they were immediately incorporated into cell plasma membranes, thereby altering the fluidity of the lipid bilayer (35). This could have resulted in amplification of the transmembrane signal following GnRH binding. Also, it could be argued that continuous intravenous infusion of lipid, in contrast to repeated injections as employed in Experiment 2, may be required to alter LH pulse frequency, but this seems unlikely since Estienne *et al.* (6, 42) failed to alter LH secretion in sheep after continuous lipid infusion.

Our results indicate that elevated serum FFA concentration is a more efficacious modulator of GH secretion than is acute hyperglycemia, while hyperglycemia suppressed and FFA enhanced the LH response to GnRH. Therefore, the results of the present study may explain in part altered endocrine function observed during periods of fasting, feed restriction, lactation, and obesity.

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