EFFECT OF FASTING ON HORMONES AND METABOLITES IN PLASMA OF FAST-GROWING, LEAN AND SLOW-GROWING OBESE PIGS \(^1,2,3\)

P. J. Wangsness, W. A. Acker, J. H. Burdette, L. F. Krabill \(^4\) and R. Vasilatos

The Pennsylvania State University, University Park 16802

Summary

Plasma concentrations of glucose, free fatty acids (FFA), insulin and growth hormone (GH) were determined immediately after food removal and then hourly for 24 hours. Blood was sampled from six lean and six obese pigs at 10 weeks of age via indwelling catheters. Plasma glucose decreased but was similar in both pig strains shortly after feed removal; at the end of the 24-hr fast, plasma glucose was higher (P<.01) in lean pigs. Plasma FFA concentrations were similar in lean and obese pigs and increased five-fold within 24 hr of fasting. Plasma insulin was higher (P<.05) in obese pigs than in lean pigs immediately after food removal only (21.4 ± 3.0 vs 9.8 ± 2.4 µU/ml). Pattern of GH secretion over 24 hr was episodic; average plasma GH was lower in obese pigs than in lean pigs (2.8 ± .7 vs 9.4 ± 1.9 ng/ml). In summary, FFA mobilization was similar in lean and obese pigs and increased five-fold within 24 hr of fasting. Plasma insulin was higher (P<.05) in obese pigs than in lean pigs immediately after food removal only (21.4 ± 3.0 vs 9.8 ± 2.4 µU/ml). Pattern of GH secretion over 24 hr was episodic; average plasma GH was lower in obese pigs than in lean pigs (2.8 ± .7 vs 9.4 ± 1.9 ng/ml). In summary, FFA mobilization was similar in lean and obese pigs, GH concentrations were lower in plasma of obese pigs and relative differences in plasma glucose and insulin between pig strains were influenced by time after feed removal.

(Key Words: Plasma Hormones, Metabolites, Pigs, Growth, Fasting, Obesity.)

Introduction

To aid in the identification of key metabolic and(or) endocrine factors that could help explain extremes in composition (fat versus protein) and rate of growth, we have compared the lean fast growing Yorkshire domestic pig with a genetically obese, slow growing strain of feral pig (Ossabaw). Previous work has revealed several metabolic and endocrine differences between these two strains of pigs, which differ markedly in body composition when compared at equal age or equal weight (Buhlinger et al., 1978). The initial report on the obese syndrome in the Ossabaw pig (Martin et al., 1973) and subsequent studies (Ezekwe and Martin, 1975; Martin and Herbein, 1976; Wangsness et al., 1977) have indicated that the feral Ossabaw strain has fewer muscle nuclei, greater lipogenic capacity of adipose tissue, mild insulin insensitivity and less growth hormone than the Yorkshire. Most of the previous work, especially that on plasma hormones and metabolites (Wangsness et al., 1977), was with pigs already fasted from 14 to 16 hours. Because time relative to feeding might influence metabolite and hormone status, we were interested in comparing pigs at various stages of fasting. The objectives of the present study were to determine concentrations of glucose, free fatty acids (FFA), insulin and growth hormone (GH) in plasma at several times after feed removal in Ossabaw and Yorkshire pigs and to relate these measurements to certain physiological and metabolic characteristics of the pigs.

Materials and Methods

Animals and Blood Sampling. Six lean female Yorkshire and six female feral-obese Ossabaw pigs, approximately 10 weeks of age, were used. The Yorkshire pigs weighed an average of 16.5 kg while the Ossabaws averaged...

---

2 This research supported in part by NIH Biomedical Sciences Support Grant and NIH Grant HD 11121 to P. J. Wangsness. The authors acknowledge J. F. Kavanaugh and L. C. Griel, Jr. for assistance in surgical preparations and S. Hoy for typing the manuscript.
3 From thesis submitted by W. Acker in partial fulfillment of the requirements for the M.S. degree. Results were presented in part at the 62nd Annu. Meet. of the FASEB, Atlantic City, NJ, April 1978 (Abstr. No. 2001).
4 Upjohn and Co., Kalamazoo, MI 49001.
10.5 kilograms. All pigs were surgically fitted with indwelling aortic catheters established via a femoral artery (Wangsness et al., 1977). After surgery and for the duration of the experiment, the pigs were housed in individual plywood pens at The Pennsylvania State University Animal Maintenance Center. A standard corn and soybean starter-growing diet (Cote and Wangsness, 1978) was fed ad libitum.

Blood was sampled hourly for 24 hr beginning with the removal of feed at 0800 hours. Lighting was continuous for 5 days prior to (for adjustment) and during the sampling period. The 5-ml blood samples were placed on ice after collection and separated by centrifugation; plasma was stored in 1-ml vials at −60°C. Catheters were flushed with physiological saline after each blood sampling, and thus fluid lost by blood sampling was replaced with saline.

**Plasma Analyses.** Packed cell volume was monitored to ensure that blood withdrawals were not excessive. Glucose was measured in plasma by the glucose oxidase procedure. Free fatty acid concentration was determined in .5 ml plasma by the microtitration technique of Kelley (1965). Palmitic acid (64 mg/250 ml heptane) was used as a standard instead of oleic acid. Plasma immunoreactive insulin and GH concentrations were determined by double antibody radioimmunoassay as described previously (Wangsness et al., 1977).

**Calculations.** To evaluate differences between lean and obese pigs in GH secretion over a 24-hr period, we measured areas under the secretion curves. The GH secretion curve for each animal was plotted in triplicate by a computer program. A known rectangular area (usually 24 hr x 50 ng/ml) containing the secretion curve was cut out and weighed. We then weighed the curve itself and expressed it as a percentage of the known area weight to find the unknown area under the curve. Triplicates were then averaged, with negligible error.

Analysis of variance of plasma constituents was by a nested factorial design. Main effects were strain, pig nested within strain and time (table 1). Comparisons between pig strains for plasma constituents at selected individual blood sampling times were performed by Bonferroni's t-test (Neter and Wasserman, 1974) for plasma insulin and glucose because these were significant interactions in the analysis of variance. Four t-test comparisons were made, the first two and last two blood sampling times after feed removal. In subsequent discussion, the pigs will be referred to as lean and obese.

**Results and Discussion**

Mean GH concentrations in plasma collected over the 24-hr period were lower (P<.01) in obese than in lean pigs (2.4 ± .4 vs 9.4 ± .8 ng/ml). Mean area under the plotted GH curves for the same period was also lower (P<.01) for the obese pigs than the lean pigs (68.5 ± 14.2 vs 246.4 ± 52.1 hr × ng/ml). These observations are consistent with earlier work (Wangsness et al., 1977), which showed a decreased GH

### Table 1. F Values from Analysis of Variance for Pig Strain, Pig within Strain and Time Effects on Plasma Glucose, Insulin, Growth Hormone (GH) and Free Fatty Acids (FFA)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>F for plasma measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose</td>
</tr>
<tr>
<td>Pig straina</td>
<td>6.2c</td>
</tr>
<tr>
<td>Pig (strain)</td>
<td>20</td>
</tr>
<tr>
<td>Timeb</td>
<td>20.8d</td>
</tr>
<tr>
<td>Strain X timeb</td>
<td>3.6d</td>
</tr>
<tr>
<td>Pig (strain) X time</td>
<td>207</td>
</tr>
</tbody>
</table>

aTested against pig (strain).
bTested against pig (strain) X time.
cP<.05.
dP<.01.

1 Sigma Chemical Co., St. Louis, MO 63178.
response of obese pigs than of lean pigs with insulin-induced hypoglycemia and a lower plasma GH concentration at 14 hr of fasting. Lower serum and pituitary GH have been reported in a strain of swine selected over 14 generations for high backfat (Althen and Gerrits, 1976). Suppressed baseline GH in the obese pig, along with possible decreased GH response to feeding, may be in part responsible for its slower growth rate and impaired muscle development (Buhlinger et al., 1978).

The 24-hr pattern of GH concentrations of all pigs was episodic (figure 1A), and there was considerable variation among pigs within each strain. Examples of individual data for both lean (figure 1B) and obese (figure 1C) pigs demonstrate this variability. Secretion was pulsatile, with troughs of undetectable concentrations in both lean and obese pigs and increases as high as 40 ng/ml in the lean pigs and 17 ng/ml in the obese pigs. Differences between lean and obese animals in diurnal patterns, which have been reported for rats (Tannenbaum and Martin, 1976), were not discernable. Tannenbaum and Martin sampled blood at 15-min intervals and reported that the light-dark cycle acted as an external cue or Zietgeber which set the biological "clock" for periodicity of GH secretion. When rats were exposed to constant light, basic GH rhythm of a secretory surge of GH release approximately every 3 hr was the same; however, the cycle was not entrained to time of day (i.e., it became free running). In the present study there was constant light. Therefore, GH secretory pattern of pigs may have been free running; this would have caused overlap of secretory peaks among pigs, and distinct peaks and troughs would not have been observed when averages were calculated (figure 1). Further studies, incorporating more frequent blood sampling and a light-dark cycle, are in progress to discern possible strain differences in diurnal GH secretory patterns.

Insulin (P<.01) in plasma decreased with time in both pig strains (figure 2), as expected (Cahill, 1971), but insulin concentrations were not different (P>.13) by analysis of variance over the entire 24-hr period (table 1). The

Figure 1. Average plasma growth hormone concentrations in lean (n = 6) and obese (n = 6) pigs with time after feed removal (A). Examples of individual growth hormone profile over 24 hr for a lean (B) and an obese (C) pig.
strain x time interaction (P<.01) for insulin can be explained by a difference (P<.05) between lean and obese pigs at the first sampling time and an absence of differences (P>.05) between the strains at the two final sampling times. However, at the first two sampling times, plasma glucose concentrations (figure 3) were similar (P>.05) in the two pig strains. Hyperinsulinemia, measured as increased fasting insulin and (or) increased insulin response to stimulation, together with normal or increased blood glucose, is generally interpreted as insulin resistance. Thus, the observations in the present study are consistent with previous reports of decreased insulin sensitivity and impaired glucose tolerance (Wangsness et al., 1977). Two additional factors, however, might also account for the increased insulin concentrations in obese pigs. First, the Ossabaw pigs were reported to consume approximately 15 to 25% more dry matter per unit body weight than the Yorkshire pigs (Wangsness et al., 1980). Thus, a somewhat greater food intake may have stimulated greater insulin release. However, feed intake was not measured in the present study. Second, the response of pancreatic beta cells to stimuli (e.g., absorbed amino acids) associated with feeding may be greater in obese pigs. This hypothesis is supported by work which showed that circulating insulin concentrations, after provocative stimulation by arginine, were greater in Ossabaw pigs than in Yorkshire pigs (Wangsness et al., 1977). Hyperresponsiveness of beta cells of the pancreas also has been reported in obese rodents (Bray and York, 1971).

After 14 hr, when pigs were presumed to be in the postabsorptive state, plasma insulin concentrations (figure 2) appeared similar in the two strains of pigs, whereas plasma glucose appeared higher in lean pigs. At the last two sampling times, glucose was higher (P<.05) in lean pigs. This observation, along with the absence of differences (P>.05) between pig strains at the first two sampling times after feed removal, resulted in the strain x time interaction (P<.01) for glucose (table 1). The reason for the difference in glucose concentrations at the end of the sampling period is not apparent. Measurements of plasma concentrations of glucocorticoids and glucagon, two hormones related to glucose metabolism, might have explained these differences in glucose between the lean and obese pigs. It is clear from the present study, however, that length of fast influences in plasma glucose and insulin between pig strains.

Concentrations of FFA in plasma were similar in lean and obese pigs throughout the 24-hr period after feed removal (figure 4). Both pig strains exhibited a fivefold increase in FFA concentrations within 24 hours. These results suggest a similar potential for lipolysis during
HORMONES AND METABOLITES WITH FASTING IN PIGS

Figure 4. Plasma free fatty acid concentrations in lean (n = 6) and obese (n = 6) pigs with time after feed removal. No difference (P<.05) between lean and obese.

fasting, but other reports do not agree on the extent to which reduced lipolytic capacity in vivo may contribute to excessive lipid deposition in obese pig strains. Weisenberg and Allen (1973) assessed in vitro lipid mobilization from adipose tissue by measuring hormone-sensitive lipase activity and by measuring FFA release in vitro from adipose tissue upon epinephrine stimulation; they found that lean and obese pigs seemed able to mobilize lipid to a similar degree. These results are in agreement with data from the present study and with Buhlinger et al. (1978), who reported similar basal lipolysis per total adipose tissue mass in Yorkshire and Ossabaw pigs. However, in contrast to the results of the present study, there are several reports of suppressed lipolysis in vitro (Trygstad et al., 1972) and in vivo (Bakke, 1975; Wood et al., 1977) in obese pig strains. More detailed studies, including measurements of various serum lipid fractions and turnover, as well as intracellular rates of reesterification, are needed to assess accurately the possible contribution of altered lipid metabolism in producing increased adiposity and reduced muscle development in Ossabaw pigs. Also, the extent to which insulin or other antilipolytic factors may influence the lipolytic action of GH is unknown.

In summary, plasma glucose, insulin, GH and FFA concentrations in lean and obese pigs were influenced by time after food removal. FFA concentrations increased with time after removal but were the same in both strains of pigs. Immediately after feed removal, insulin was increased in obese pigs but glucose concentration was similar to that in lean pigs; at the end of the 24-hr fasting period, insulin concentrations were similar, but glucose was lower in obese pigs than in lean pigs. GH concentrations were lower in obese pigs than in lean pigs.

Literature Cited


