EFFECTS OF PORCINE GROWTH HORMONE ON GLUCOSE METABOLISM OF PIGS: II. GLUCOSE TOLERANCE, PERIPHERAL TISSUE INSULIN SENSITIVITY AND GLUCOSE KINETICS

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ABSTRACT

This study was conducted to determine whether the increase in serum glucose observed in pigs treated chronically with pGH is due to an increase in hepatic glucose output or to an impairment in glucose clearance. Barrows (n = 4 per treatment) were treated with pituitary derived pGH (ppGH), recombinant pGH analog (rpGH) or vehicle. Pigs were treated for 28 d by daily i.m. injections. Insulin tolerance and glucose tolerance tests (GTT) were performed on d 19 and 21, respectively, following treatment with pGH. Glucose turnover was quantified on d 28 using [6-³H]glucose. Chronically treating pigs with pGH resulted in a significant decrease (26%; P < .05) in glucose clearance, as determined by the GTT. Glucose clearance was affected similarly by ppGH and rpGH. Intra-arterial glucose infusion markedly increased plasma insulin concentration in pGH-treated pigs. Peak plasma insulin response was 87% and 58%, respectively, higher (P < .05) in ppGH- and rpGH-treated than in control pigs. Insulin infusion elicited a marked hypoglycemia in pigs; however, the extent and duration of hypoglycemia were significantly less in pGH-treated pigs (ppGH or rpGH). Glucose production rates were 23% higher (P = .085) in ppGH-treated than in control pigs. These results establish that the hyperglycemia induced by pGH is the result of an increase in hepatic glucose output and a concurrent impairment in glucose clearance.

(Key Words: Pigs, Growth Hormone, Glucose Turnover, Insulin, Glucose Tolerance.)


Introduction

The previous paper established the temporal nature of the porcine growth hormone (pGH)-induced increase in plasma glucose and insulin concentrations in pigs (Gopinath and Etherton, 1989). It is evident that pGH perturbs the glucose-insulin axis in pigs. It is not clear, however, whether the pGH-dependent increase in serum glucose is the result of an increase in hepatic glucose output or, alternatively, whether it reflects an impairment in glucose clearance. To resolve this, we conducted the present study to determine whether pGH affects glucose turnover and/or glucose clearance in pigs chronically treated with pGH. In addition, we conducted in vivo insulin tolerance tests to establish the extent to which pGH alters insulin insensitivity in peripheral tissues. The findings indicate that pGH increases hepatic glucose output and impairs glucose clearance. This is associated with a significant loss of insulin sensitivity.
Materials and Methods

Animals and Treatments. Yorkshire barrows (average BW = 60 kg) were used in all the experiments. Barrows were fitted with femoral artery catheters and maintained as described in the previous paper (Gopinath and Etherton, 1989). Barrows were assigned randomly to the following three treatment groups (n = 4/treatment): pituitary pGH (ppGH 70 µg·kg⁻¹·d⁻¹), recombinant pGH analog (rpGH 70 µg·kg⁻¹·d⁻¹) and control. Pigs in the control group were injected with vehicle. The methods used for pGH solubilization and injection were described in the previous paper (Gopinath and Etherton, 1989). Pigs were treated daily for 28 d. All experiments were performed with this group of pigs. Pigs were housed in individual pens and were fed a corn-soybean meal ration formulated to contain 18% crude protein (Etherton et al., 1986). Lysine-HCl was added to the diet (.5%) to ensure adequate amino acid availability. Treatment of pigs with pGH for 28 d increased average daily gain 23% (836 vs 1,032 g, P < .05).

Experiment 1: Glucose Tolerance Test. The effects of intra-arterial glucose loading on fasting plasma glucose and insulin response were studied after 21 d of pGH treatment. After a 12- to 14-h fast, pigs were given an intra-arterial infusion of dextrose (50% dextrose, sterile) calculated to provide 1 g of glucose/kg BW. The catheters were immediately flushed with 5 ml of sterile saline. Blood samples (5 to 10 ml) were collected at 10-min intervals for 30 min before and at 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 120, 150, 180 and 240 min following glucose infusion. Blood samples were collected in tubes containing heparin as an anticoagulant and were immediately centrifuged to separate the plasma, which was then stored at −20°C until analyzed for metabolites.

Experiment 2: Insulin Tolerance Test. The hypoglycemic effect of an intra-arterial infusion of insulin was determined in order to assess insulin sensitivity of peripheral tissues in pigs treated with pGH. Pigs were treated with ppGH or rpGH for 18 d. On d 19, after an overnight fast (12 to 14 h), pigs were given an intra-arterial injection of porcine insulin (125 U/kg³·BW). This concentration of insulin was found in preliminary studies to cause hypoglycemia in pigs. The catheters were flushed immediately with 5 ml of sterile saline and blood samples (5 to 10 ml) were collected at 10-min intervals for 30 min before and at 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 120, 150, 180 and 240 min following insulin infusion. Blood samples were collected in tubes containing heparin as an anticoagulant and were immediately centrifuged to separate the plasma.

Experiment 3: Glucose Kinetics. Glucose turnover in control and ppGH treated pigs was quantified using [6-³H]glucose (Cote et al., 1982). The rationale for using this particular tracer has been discussed by Cote et al. (1982). Because ppGH and rpGH affected glucose clearance and insulin sensitivity of peripheral tissues similarly, glucose kinetics were studied only in pigs treated with ppGH and control animals. Pigs were fasted overnight (12 to 14 h) on d 27. The following morning they were given 250 µCi of [³H]glucose (D-[6-³H]glucose) in saline (5 ml) via a femoral artery catheter. The catheter was flushed immediately with 5 ml of saline and blood samples (5 to 10 ml) were collected at 10-min intervals for 30 min before and at 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 150 and 180 min following glucose infusion. The catheters were filled with .04 M sodium citrate between sampling periods. Blood samples were collected in tubes containing heparin as an anticoagulant and were centrifuged immediately to separate the plasma in order to minimize glucose breakdown. Plasma samples were stored at −20°C until analysis.

Analyses of Hormones and Metabolites

Plasma glucose and insulin were determined as described previously (Chung et al., 1985; Etherton et al., 1986, 1987). Plasma samples collected during the glucose turnover experiment were deproteinized (Somogyi, 1945) and

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6Lot 9164-C, donated by Pitman-Moore Inc., Terre Haute, IN.
7Lot 117-064, donated by Pitman-Moore Inc., Terre Haute, IN. This analog lacks the first seven amino acids at the NH₂ terminus of the molecule (Evock et al., 1988).
8TechAmerica Group, Inc., Elwood, KS.
9Lot 615-2H2-300; 26.8 U/mg; donated by R. E. Chance, Eli Lilly Co., Indianapolis, IN.
8Amersham Corp., Arlington Heights, IL.
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the radioactivity associated with glucose was determined using an ion-exchange procedure (Mills et al., 1981). Specific radioactivity (SRA) of glucose was determined by dividing total radioactivity by the glucose concentration of the isolated glucose fraction and was expressed as dpm/mg glucose.

Data Analysis. Area under the plasma glucose and insulin response curves was determined by Simpson’s rule of integration using an IBM personal computer. Treatment differences were tested by Student’s t-test. The plasma glucose SRA curves from 5 to 240 min post [3H]glucose infusion (Figure 1) were analyzed using a decision-making Fortran computer program, AUTOAN (Sedman and Wagner, 1976), and were found to be best described by the biexponential equation:

\[ C = Ae^{-at} + Be^{-bt} \]

where \( C \) is the glucose SRA at time, \( t \); \( A \) is the Y intercept of the fast component of the disappearance curve; \( a \) is the disposition rate constant in the fast component of the disappearance curve; \( B \) is the Y intercept of the slow component of the disappearance curve; and \( b \) is the disposition rate constant in the slow component of the disappearance curve. Fasting plasma glucose concentration in control or pGH-treated pigs remained relatively constant (Figure 1), indicating that glucose steady state prevailed during the turnover experiment. At steady state, glucose replacement rate has been shown to equal glucose utilization rate (Katz et al., 1974).

The kinetic parameters of glucose metabolism were calculated as follows: Replacement or production rate of glucose (\( R_O \)), glucose minimal mass (\( M_{\text{min}} \)), and the outflow of glucose from the sampling pool (\( R_{11} \)) were calculated using the exponential equations described by Katz et al. (1974). Fractional clearance rate, metabolic clearance rate and half-life of glucose in the fast and slow phases of the disappearance curves were determined according to Shipley and Clark (1972). Glucose space was calculated according to Judson and Leng (1972) and expressed as liters of glucose space or as a percentage of body weight. Minimal transit time of glucose was calculated as \( M_{\text{min}}/R_O \) (Katz et al., 1974). Treatment differences were tested by Student’s \( t \)-test. (Ryan et al., 1976). Values are expressed as mean ± standard error.

Results

Chronically treating pigs with pGH increased fasting plasma glucose concentration (Figure 1) and resulted in a significant decrease in glucose clearance rate after intraarterial glucose challenge (Figure 2). When glucose clearance rates were calculated from the natural log glucose concentration vs time curves (Figure 2) there was a 24 to 27% decrease (\( P < .05 \)) in clearance rate in pGH-treated pigs compared with control pigs. Glucose clearance was affected similarly by ppGH and rpGH (\( P > .05 \)). As expected, glucose infusion markedly increased plasma insulin concentration (Figure 3). In pigs treated with either ppGH or rpGH the secretory (peak insulin values) response was greater (\( P < .05 \)) than in control pigs (60 to 90% higher; Table 1; Figure 3). The insulin secretory response was not statistically different between ppGH and rpGH (Table 1).

The increase in plasma insulin response to glucose in the face of an impaired glucose clearance suggested that pGH decreased insulin sensitivity. This clearly was the case when the insulin tolerance test data was examined (Figure 4). As expected, infusion of insulin elicited a marked hypoglycemia in control pigs. In pGH-treated pigs, however, the extent and duration of hypoglycemia were less (\( P < .05 \)). Glucose response to insulin injection, however, was similar in pigs treated with either source of pGH.

Glucose turnover rate or production rate (\( R_O \)) was 23% higher (\( P = .085 \)) in pigs treated with ppGH than in the controls (Table 2). Glucose production rate relative to BW or metabolic BW was not increased (\( P > .1 \)) even though the response was 15 to 17% higher in ppGH-treated than in control pigs (Table 2). There were no differences in metabolic clearance rate or fractional clearance rate of glucose between control and ppGH-treated pigs. Similarly, there were no differences in glucose space and mean transit time between treatments. Minimal mass of glucose was higher (34%; \( P = .1 \)) in pGH-treated than in the control pigs. When minimal mass of glucose was expressed per kg BW, however, there was no statistical difference, even though there was a 26% increase in pGH-treated pigs (data not shown).

A comparison of glucose clearance observed under conditions of steady state (Exp.
Figure 1. Semilogarithmic plot of plasma glucose specific radioactivity in control pigs and pigs treated with pituitary porcine growth hormone (ppGH) (top panel), and plasma glucose concentration during [1H]glucose turnover experiment (bottom panel). Each point represents the mean ± SE of values obtained from three control or four ppGH-treated pigs. The area under the plasma glucose response curve for pGH-treated pigs was greater (P < .05) than that for control pigs.

3) and glucose loading (Exp. 1) is presented in Table 3. Although there were no differences in glucose clearance rates during the steady state, upon glucose loading, clearance rates of glucose were about 24 to 27% lower in pGH-treated than in control pigs. Compared with the steady state situation (Exp. 3), there was a 233% increase in the rate at which glucose was cleared during glucose loading in control pigs. This increase in clearance rate of glucose in pGH-treated pigs under the above conditions was 158% (Table 3).

Discussion

Findings of the present study indicate that pGH-dependent hyperglycemia and hyperinsulinemia are the result of alterations in hepatic glucose output and a concurrent impairment in glucose removal. Even though the diabetogenic effects of GH in man and rodents have been well documented (Davidson, 1987), the mechanisms behind its diabetogenic actions are not clear. Cameron et al. (1987) suggested that the hyperinsulinemia and glucose intolerance of mice chronically treated with GH were due primarily to increased peripheral tissue resistance to insulin. Although the diabetogenic effects of pGH have been observed (Chung et al., 1985; Etherton et al., 1986, 1987; Wray-Cohen et al., 1987; Evock et al., 1988; Gopinath and Etherton, 1989), the findings of
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TABLE 1. PEAK PLASMA INSULIN RESPONSE TO GLUCOSE INFUSION IN CONTROL AND pGH-TREATED PIGS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Peak insulin responseb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.6 ± 2.7d</td>
</tr>
<tr>
<td>ppGHc</td>
<td>19.8 ± 2.3e</td>
</tr>
<tr>
<td>rpGHd</td>
<td>16.8 ± 1.7e</td>
</tr>
</tbody>
</table>

Pigs were treated with pGH (70 µg/kg) by daily i.m. injections for 21 d. Following a 12- to 14-h fast, each pig was infused intra-arterially with 1 g glucose/kg BW (glucose loading).

bPeak insulin response represents the mean of insulin concentrations obtained at 5, 10, 15 and 20 min following glucose infusion. Values are ng/ml.

cppGH = pituitary pGH; rpGH = recombinantly derived pGH.
d,eMeans with different superscripts differ (P < .05).

The present report are the first to establish the changes in glucose turnover in pigs following chronic treatment with pGH.

The impaired glucose clearance in pigs chronically treated with pGH is in accord with the glucose intolerance observed following oral or i.v. glucose loading in man and rodents treated with GH (Rosenfeld et al., 1982; Sherwin et al., 1983; Kostyo et al., 1984; Stred et al., 1987). Similarly, glucose intolerance was observed in acromegalic patients (Beck et al., 1965; Trimble et al., 1980). Porcine GH-treated pigs exhibited a 24 to 27% reduction in their ability to clear glucose following an intra-arterial glucose load. Based on this, it appears that pGH blunts glucose uptake and utilization in peripheral tissue. It is difficult to discern from the present study the tissues in which glucose uptake is impaired. We have found that in vivo and in vitro pGH decreases fatty acid synthesis and glucose transport in pig adipocytes (Walton et al., 1986, 1987; K. Magri and T. Etherton, unpublished data). Based on this, it is evident that adipose tissue is a site at which pGH decreases glucose uptake. We have found recently that treating pigs for 7 d with the same dose of pGH (70 µg/kg BW) reduced glucose transport into adipocytes by about 60% (K. Magri and T. D. Etherton, unpublished data). This decrease in glucose uptake parallels quite well the decrease observed in rates of fatty acid synthesis (a 60% reduction) from glucose in the same pigs. What remains to be resolved is the quantitative effect of an impairment in glucose uptake by pGH in adipose tissue to the observed impairment in glucose clearance on a whole-body basis. The quantitative importance of glucose uptake by adipose tissue as a proportion of total body glucose uptake has not been established for the pig. It can be speculated, however, that a sizeable proportion of glucose-

TABLE 2. KINETIC PARAMETERS OF GLUCOSE METABOLISM IN CONTROL AND pGH-TREATED PIGS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 3)</th>
<th>pGH (n = 4)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose space, litersd</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% BW</td>
<td>27.2 ± 1.4d</td>
<td>31.2 ± 4.7</td>
<td>NSc</td>
</tr>
<tr>
<td>Mean transit time, min</td>
<td>32.5 ± 1.5</td>
<td>35.0 ± 5.5</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose minimal mass (Mmin), g</td>
<td>60.7 ± 2.0</td>
<td>66.3 ± 6.4</td>
<td>NS</td>
</tr>
<tr>
<td>Turnover rate (Roe), mg/min</td>
<td>21.9 ± 1.7</td>
<td>29.5 ± 3.7</td>
<td>.1</td>
</tr>
<tr>
<td>mg/(min·kg)</td>
<td>361 ± 26</td>
<td>443.8 ± 25</td>
<td>.085</td>
</tr>
<tr>
<td>mg/(min·kg)</td>
<td>4.3 ± .3</td>
<td>5.0 ± .3</td>
<td>.2</td>
</tr>
<tr>
<td>mg/(min·kg)</td>
<td>13.1 ± .9</td>
<td>15.3 ± .9</td>
<td>.1</td>
</tr>
<tr>
<td>Metabolic clearance rate, ml/min</td>
<td>449.3 ± 36</td>
<td>466.6 ± 38</td>
<td>NS</td>
</tr>
<tr>
<td>Fractional clearance rate, liters/min</td>
<td>-.0165 ± .001</td>
<td>-.0155 ± .002</td>
<td>NS</td>
</tr>
</tbody>
</table>

Pigs were chronically treated with pituitary pGH for 27 d. On d 28, pigs were fasted for 12 to 14 h and glucose turnover was determined using [3H]-glucose (D- [6-3H] glucose). The plasma glucose SRA vs time curves were analyzed using a biexponential equation (C = Ae -αt+Be -βt) and the kinetic parameters of glucose metabolism were calculated as described in Materials and Methods.

dGlucose space as liters of glucose.

cNS = not significant.
Figure 2. Plasma glucose concentrations before and following intra-arterial glucose infusion (1 g/kg BW; time indicated by the arrow) in control pigs or pigs treated with pituitary porcine growth hormone (ppGH) or recombinantly derived pGH (rpGH) (bottom panel), and glucose clearance curves obtained by plotting natural logarithm glucose concentration vs time (top panel). Each point represents the mean ± SE of values obtained from four pigs. The slopes of the glucose clearance curves were: control = −0.055 ± 0.002; pGH (ppGH and rpGH combined) = −0.041 ± 0.002 min⁻¹. Glucose clearance rates in pGH-treated pigs (ppGH and rpGH combined) were different from those in controls (P < .05).

Carbon (perhaps 20 to 40% of glucose cleared/d) is used for lipid synthesis and oxidation in adipocytes from pigs of 70 kg BW. This speculation is based, in part, on the rate of lipid deposition in normal pigs (180 to 200 g/d), the rate glucose oxidation in pig adipocytes, the estimated rate of glucose production (R₀), the fact that most lipid deposited (80%) in pig fat is the result of de novo synthesis (Hood and Allen, 1973), the observation that glucose is the principal endogenous carbon source for fatty acid synthesis (O’Hea and Leveille, 1969) and that adipose tissue is the preeminent tissue site of fatty synthesis in the pig. The effects of pGH in adipose tissue result in a redirection of an appreciable quantity of glucose to other tissues. What remains to be resolved is the effect that pGH has on glucose uptake by muscle, the insulin sensitivity of this tissue and whether the response to pGH in muscle differs from that in adipose tissue. It is unknown whether the insulin resistance is due to a receptor or postreceptor defect in insulin action. Insulin binding to porcine or murine adipocytes (Cameron et al., 1987; K. Magri and T. Etherton, unpublished data), and human monocytes (Brautsch-Marain et al., 1982; Rosenfeld et al., 1982) was unaltered after GH therapy. Based on this we speculate that the insulin resistance observed in pGH-
TABLE 3. COMPARISON OF GLUCOSE CLEARANCE IN CONTROL AND pGH-TREATED PIGS DURING CONDITIONS OF STEADY STATE GLUCOSE (Exp. 3) AND GLUCOSE LOADING (Exp. 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glucose clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Steady state</td>
</tr>
<tr>
<td>Control</td>
<td>-.0165 ± .001a</td>
</tr>
<tr>
<td>pGH</td>
<td>-.0155 ± .002</td>
</tr>
<tr>
<td>pGH-pd</td>
<td>NSa</td>
</tr>
</tbody>
</table>

aFractional clearance rate (min⁻¹) for [³H]-glucose disappearance calculated as the reciprocal of minimal transit time. Each value is the mean ± SE of values obtained from three (control) or four (pGH) pigs.

bFractional rate constant (min⁻¹) for disappearance of intra-arterial glucose load of 1 g/kg BW obtained from Exp. 1. Each value is the mean ± SE of values obtained from four pigs.

cPercentage increase in fractional rate constant for glucose removal during conditions of steady state glucose vs glucose loading.

daProbability of difference.

eNS = not significant.

treated pigs is due to a postreceptor defect in insulin action. It should be emphasized, however, that no studies have been reported that have examined the effects of pGH treatment on insulin binding to membrane preparations from pig muscle.

Treatment of pigs with either ppGH or rpGH caused similar impairments in glucose tolerance and insulin action in peripheral tissues. This is in accord with our earlier observations that both ppGH and rpGH elicit similar growth-promoting and metabolic effects (Walton et al., 1987; Evock et al., 1988).

Collectively, our studies demonstrate that the growth-promoting and metabolic effects are intrinsic properties of the pGH molecule.

The increase in glucose production in pGH-treated pigs in the face of existing hyperinsulinemia (Table 1; Figure 3) suggests an impairment in hepatic sensitivity to insulin. Hepatic insulin resistance had been reported in acromegalic patients and in nondiabetic humans following short-term GH infusion (Rizza et al., 1982; Hansen et al., 1986). Moreover, the ability of insulin to suppress glucose production and to stimulate glucose utilization is impaired in acromegalic patients (Hansen et al., 1986). Increased hepatic glucose production has been reported in GH-treated dogs (Bishop et al., 1967; Rathgeb et al., 1970) and dairy cows in peak lactation (McDowell et al., 1987). Based on the estimates obtained from the tracer study, however, it appears that pGH did not impair glucose clearance in pigs that were in glucose steady state (Exp. 3). However, upon glucose loading (e.g., following a meal), the mechanisms necessary to effect an increase in glucose clearance were blunted by pGH.

In conclusion, we have demonstrated that treatment with pGH causes glucose intolerance and peripheral tissue insulin resistance in pigs. Treatment with pGH also increased glucose production in pigs in spite of the existing hyperinsulinemia, suggesting an impairment in hepatic sensitivity to insulin. Further studies are necessary to understand the mechanisms by which pGH blunts glucose uptake and causes insulin resistance and to establish what postre-
ceceptor defects exist in insulin action. In addition, it will be important to resolve whether any differential effects of pGH on tissue sensitivity to insulin occur among the different insulin-sensitive target tissues. Finally, it will be useful to develop a working model of pGH action that integrates these effects during a time in which the rate of muscle protein deposition is dramatically increased by pGH.

Literature Cited

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