GLUCOSE RESPONSE TO EXOGENOUS INSULIN AND KINETICS OF INSULIN METABOLISM IN OBESE AND LEAN HEIFERS

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ABSTRACT

Changes in serum concentrations of glucose and insulin after iv injection of a low (20 mU/kg) and high (200 mU/kg) dose of bovine insulin were used to quantify insulin resistance and calculate kinetic variables of injected insulin, respectively, in four obese and four lean heifers. Serum samples from jugular venous blood were collected 60, 45, 30, 15 and 1 min before and 2.5, 5, 10, 20, 30, 40, 60, 80, 100, 120, 150, 180, 210 and 240 min after each treatment. Mean (± SE) pretreatment concentration of insulin (µU/ml) was higher (P<.01) in obese (50 ± 6.6) than lean (20 ± 1.8) heifers, even though glucose concentrations were similar in both groups (71 ± 2.9 mg/100 ml). Concentrations of insulin after each treatment were similar in both groups and returned to pretreatment values by 60 and 120 min after injection of the low and high doses, respectively. Glucose concentrations during the first 40 min after treatment with the low dose were lower (P<.05) in lean than obese heifers, but were similar in both groups during the first 40 to 60 min after the high dose of insulin. The high insulin dose decreased (P<.05) glucose concentrations below those of the low dose in each group, but the difference was greater (P<.01) in obese than lean heifers. These results indicated that obese heifers were insensitive to the glucoregulatory effects of exogenous insulin, although the maximum responses to insulin were similar. Fractional removal rates (k, min⁻¹) and biological half-lives (t½, min) for injected insulin were affected (P<.01) only by insulin dose. For all heifers, mean (± SE) values of k decreased from .124 ± .77 to .057 ± .004 and values of t½ increased from 5.7 ± .4 to 12.4 ± .8 after the high compared with the low dose of insulin. Mean (± SE) secretion rates of insulin (µU·kg⁻¹·min⁻¹) were greater (P<.01) in obese (218 ± 33) than lean (96 ± 11) heifers.

(Key Words: Obesity, Insulin, Kinetics, Cattle.)

Introduction

Obesity in nonruminant animals is associated with basal hyperinsulinemia, the extent of which is positively correlated with the degree of obesity (Bagdade et al., 1967), and cellular resistance to the glucoregulatory effects of both endogenous and exogenous insulin (Rabinowitz and Zierler, 1962; Karam et al., 1963). Concentrations of insulin in serum also were correlated positively with degree of obesity in Holstein heifers (J. P. McCann and T. J. Reimers, unpublished data). Resistance to the glucoregulatory effects of insulin was present in these same heifers because serum concentrations of glucose were similar in lean and obese heifers in spite of the hyperinsulinemia associated with obesity.

Insulin resistance means that normal concentrations of insulin produce a less-than-expected biological response (Kahn, 1978). Insulin resistance is associated with decreased sensitivity to the effects of insulin, and this can occur with or without a decrease in the maximum response to insulin (Kahn, 1978). Experiments reported here were done to characterize insulin resistance in obese heifers by providing the same low and high doses of insulin to obese and lean heifers. Changes in glucose concentrations in serum after each insulin treatment were used to determine if obese heifers were insensitive to insulin, with or without a decreased maximum response to insulin. In addition, serum concentrations of insulin after each insulin treatment were used to estimate kinetic variables of insulin metabolism in obese and lean heifers.
Materials and Methods

Animals. Four obese and four lean Holstein heifers between 3 and 4 yr of age and of the same height (133 ± 1.3 cm) were used. Obese heifers had greater (P<.05) body weights (553 ± 16 kg vs 453 ± 8 kg), body condition scores (4.6 ± .1 vs 3.2 ± .2) and depths of ultrasonically determined subcutaneous rib fat (4.6 ± .7 mm vs 1.7 ± .3 mm) than lean heifers. We have found these indices of obesity to be accurate measurements of differences in degree of obesity in sheep, where they were as useful as the significantly greater weights of omental, cardiac and perirenal fat depots determined at necropsy (J. P. McCann, T. J. Reimers and E. N. Bergman, unpublished data). Obesity in the heifers was considered nutritional in origin, occurring before this experiment when heifers were fed corn silage ad libitum. Beginning at least 2 mo before the experiment, heifers were fed different amounts of corn silage according to their maintenance requirements. This diet maintained steady-state body condition, as indicated by no significant change in body weight during this experiment.

Insulin Resistance. Bovine insulin was injected via a cannula into the jugular vein of each heifer at a dose of 20 mU/kg body weight on d 16 (1830 to 1930 h) of the estrous cycle (d 0 = estrus) and at a dose of 200 mU/kg body weight at the same time on d 17. All injections were from the same vial of insulin. Cannulae were flushed with physiological saline (10 ml) immediately after each injection. A blood sample (10 ml) collected 1 min after each injection was discarded.

Blood samples (8 ml) were collected via the same cannula at 60, 45, 30, 15 and 1 min before injection of insulin and at 2.5, 5, 10, 20, 30, 40, 60, 80, 100, 120, 150, 180, 210 and 240 min after each injection. Blood was dispensed immediately after collection in equal amounts into duplicate glass tubes maintained in an ice-water bath. One of the duplicates contained no additive (insulin assay), while the other contained 10 mg of sodium fluoride to prevent glycolysis (glucose assay). Blood was stored at 4 C for 12 to 16 h before serum was recovered by centrifugation and stored at -22 C until assayed for insulin (Reimers et al., 1982) and glucose.

Insulin concentrations in samples after injection of insulin that were greater than the highest insulin standard (310 µU/ml) were reassayed after serially diluting each in freshly prepared 7% bovine serum albumin (BSA, fraction V) in 0.01 M phosphate-buffered saline (PBS). Serial dilutions (in 7% BSA-PBS) of serum collected from heifers before and after injection of insulin displaced [125I] insulin in a manner parallel with each other and with the standard insulin. The BSA-PBS solution did not contain any immunoreactive insulin.

Kinetics of Insulin Metabolism. Equations describing removal rates of injected insulin were calculated as follows. The mean plus one standard deviation of the five preinjection concentrations of insulin for each heifer was subtracted from the post-treatment concentrations of insulin for that heifer for each insulin dose before concentrations were converted to natural logs. The relationship between log-transformed insulin concentrations and time after insulin injection was determined by polynomial regression analysis. Initially, all log-transformed values for obese or lean heifers for each insulin dose were used to determine whether the best fit for each group of data was described by a single- (i.e., monoexponential removal rate) or multiple-degree regression (i.e., biexponential removal or greater). A single-degree polynomial was the best fit for each group of data, indicating that an open one-compartment model, or monoexponential removal rate, described sufficiently the kinetics of insulin metabolism in this experiment (Ritschel, 1980).

Monoexponential removal-rate equations for concentrations of injected insulin in serum were then calculated from the regression equations for each heifer by substituting the antilog value of the y-axis intercept for A₀ (concentration of insulin in serum at time zero), and by substituting slope of the regression line for k (fractional removal rate constant). Biological half-lives (t½, min) for injected insulin were calculated as .693 / k (Ritschel, 1980). Values of A₀ and k then were used to calculate the volume of distribution (Vd) and secretion rate of insulin for each heifer according to the

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1. Eli Lilly and Co., Indianapolis, IN (Regular Iletin II; 100 U/ml).
2. Abbott Hospitals, North Chicago, IL (Abhocath).
3. Becton-Dickinson, Rutherford, NJ.
4. Sigma Chemical Co., St. Louis, MO.
5. Gilford Diagnostics, Cleveland, OH (Trinder method).
6. Calbiochem-Behring, La Jolla, CA.
following equations: \( V_d = \text{insulin dose (\( \mu U/\text{heifer} \))} \div A_0; \) secretion rate = steady-state concentration of insulin (mean of pretreatment values) \( \times \) metabolic clearance rate (\( V_d \times k \)).

Statistical Analyses. Differences in insulin and glucose concentrations among groups were tested by the method of Gill (1979). Selection of polynomial degree for best fit of data in regression analysis was based on the F-value from regression analysis of variance and Student's t-value (\( P < .05 \)) for each degree of fit used. Differences in components of mean equations for insulin removal and in insulin secretion rates and volumes of distribution of insulin due to insulin dose and body condition were determined using 2 \( \times \) 2 factorial analysis of variance (Steel and Torrie, 1960).

Results

Insulin Resistance. Concentrations of glucose were similar in both groups before each treatment (figure 1). The lowest concentrations of glucose occurred 40 min after treatment with the low dose and 40 to 60 min after treatment with the high dose of insulin in both lean and obese heifers. Glucose concentrations during the first 40 min after treatment with the low dose of insulin were lower (\( P < .05 \)) in lean than obese heifers, but were similar in both groups at this time after the high dose of insulin. The high dose of insulin decreased (\( P < .05 \)) concentrations of glucose below those measured after the low dose in each group. However, the magnitude of this decrease was greater (\( P < .01 \)) in obese than in lean heifers. Glucose concentrations were similar in obese and lean heifers from 60 to 240 min after treatment with the low dose of insulin. In contrast, concentrations of glucose after the high insulin dose returned to base line 60 min sooner in obese than in lean heifers.

Concentrations of insulin in peripheral serum were similar in obese and lean heifers for the first 40 min after the low dose (figure 2) and for the first 120 min after the high dose (figure 3) of insulin. However, concentrations of insulin were greater (\( P < .01 \)) in obese than in lean heifers after these times and also before treatment (figures 2 and 3).

Kinetics of Insulin Metabolism. Neither body condition or interaction between dose of insulin and body condition affected any of the kinetic variables of insulin metabolism. Removal of injected insulin from blood in both groups after the low and high doses of insulin is shown in figure 4. The initial concentration, mean fractional removal rate and \( t/2 \) for exogenous insulin were similar in lean and obese heifers for each dose of insulin (table 1). However, the

Figure 1. Mean (± SE) concentrations of glucose in obese and lean heifers before and after treatment (arrows) with a low (lower panel) and high dose (upper panel) of bovine insulin.
Figure 2. Mean (± SE) concentrations of insulin in obese and lean heifers before and after injection (arrow) of a low dose of bovine insulin. Note log scale (base 10) on ordinate.

Figure 3. Mean (± SE) concentrations of insulin in obese and lean heifers before and after injection (arrow) of a high dose of bovine insulin. Note log scale (base 10) on ordinate.
Figure 4. Removal curves of insulin after an injection of a low (20 mU/kg) and high (200 mU/kg) dose of bovine insulin to obese and lean heifers. Points are mean ± SE. Lines show the mean fractional removal rate equation (see table 1) for each insulin dose in obese and lean heifers. Note log scale (natural) on ordinate.

Table 1. Kinetics of Insulin Metabolism in Obese and Lean Heifers after Injection of a Low (20 mU/kg) and High (200 mU/kg) Dose of Insulina,b

<table>
<thead>
<tr>
<th>Item</th>
<th>Obese Low</th>
<th>Obese High</th>
<th>Lean Low</th>
<th>Lean High</th>
</tr>
</thead>
<tbody>
<tr>
<td>A0, μU/ml</td>
<td>758 (192)</td>
<td>2,940 (251)</td>
<td>612 (118)</td>
<td>2,700 (321)</td>
</tr>
<tr>
<td>k, min⁻¹</td>
<td>.122 (.01)</td>
<td>.06 (.005)</td>
<td>.127 (.01)</td>
<td>.055 (.005)</td>
</tr>
<tr>
<td>t½, min</td>
<td>5.96 (.77)</td>
<td>11.84 (.31)</td>
<td>5.52 (.25)</td>
<td>12.88 (1.28)</td>
</tr>
</tbody>
</table>

aValues are mean ± SE (in parentheses).

bMeans in each row for the high dose were different (P<.01) from those for the low dose.
TABLE 2. VOLUMES OF DISTRIBUTION AND SECRETION RATES OF INSULIN IN OBESE AND LEAN HEIFERS AFTER INJECTION OF A LOW (20 mU/KG) AND HIGH (200 mU/KG) DOSE OF INSULIN

<table>
<thead>
<tr>
<th>Item</th>
<th>Obese</th>
<th>Lean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Volume of distribution, ml/100 g of body weight</td>
<td>3.1b</td>
<td>7.0c</td>
</tr>
<tr>
<td></td>
<td>(.6)</td>
<td>(.6)</td>
</tr>
<tr>
<td>Secretion rate, ( \mu U \cdot kg^{-1} \cdot min^{-1} )</td>
<td>210b</td>
<td>237b</td>
</tr>
<tr>
<td></td>
<td>(38)</td>
<td>(52)</td>
</tr>
</tbody>
</table>

a Values are mean ± SE (in parentheses).

b, c Means within a row with the same superscript do not differ (P > .05).

that primed-plus-continuous infusion rather than injection of insulin is considered a more useful technique for estimating kinetic variables of insulin. Nevertheless, Sherwin et al. (1974) and McGuire et al. (1979) showed that injection and a primed-plus-continuous infusion of insulin provided similar estimates of insulin kinetics in humans.

Values of \( t_{\frac{1}{2}} \) and \( k \) reflect body catabolism of insulin, which includes binding of insulin to receptors on plasma membranes and insulin degradation within the liver, kidney and possibly peripheral tissues (Katz and Rubenstein, 1973; Brockman and Bergman, 1975; Duckworth et al., 1981). Our values for \( t_{\frac{1}{2}} \) after the low dose of insulin were comparable with approximate values of 8 min (Stoll et al., 1971), 6 min (Stoll et al., 1971) and 4 to 9 min (Ørskov and Christensen, 1966; Williams et al., 1968; Turner et al., 1971; Sönksen et al., 1973) that have been reported for normal baboons, swine and humans, respectively. Trenkle (1971) injected high doses of bovine insulin (500 to 800 mU/kg) into fed sheep and obtained values of \( t_{\frac{1}{2}} \) of 11 to 13 min, similar to those in our heifers after the high dose of insulin. Obesity in humans had no effect on body catabolism of insulin in one study (McGuire et al., 1979), but increased catabolism slightly in another (Genuth, 1972). Data in table 1 show that insulin catabolism in obese and lean heifers was quantitatively similar, indicating that differences in blood concentrations of insulin were not due to slower catabolism of insulin in obese heifers. Indeed, data in table 2 show that the basal hyperinsulinemia in obese heifers was the result of a greater secretion rate of insulin.

Insulin secretion rates of 5 to 14 mU kg\(^{-1}\) h\(^{-1}\) in our study agree well with 7.8 mU kg\(^{-1}\) h\(^{-1}\) calculated by Brockman and Bergman (1975) using blood flows and arteriovenous differences in insulin concentrations across the pancreas of sheep. Insulin secretion rates in the obese heifers were similar to those of 12 to 13 mU kg\(^{-1}\) h\(^{-1}\) in nonobese humans (Sherwin et al., 1974; Eaton et al., 1980) and 15.4 mU kg\(^{-1}\) h\(^{-1}\) in sheep that may have been obese (Trenkle, 1971).

Volume of distribution of insulin after the low dose of insulin was similar to a plasma volume\(^*\) of approximately 4% of body weight in heifers (McCann, 1981) and cows (Reynolds, 1953). There was an approximate twofold increase in \( V_d \) and twofold decrease in \( k \) when the dose of insulin was increased tenfold, suggesting a slower exchange of insulin between other compartment(s) and serum, or that slowly exchanging compartment(s) dominated after the high but not after the low dose. The ability of body tissues to catabolize insulin was not saturated after either dose of insulin because both removal rates followed first-order kinetics rather than Michaelis-Menten (saturation or capacity-limited) kinetics (Ritschel, 1980). However, insulin kinetics in this study and in the study of insulin kinetics in nonruminants (Tiran et al., 1979) were nonlinear because they were not directly

\*Plasma volume here refers to the aqueous portion of blood in vivo and is considered equivalent to serum volume.
proportional to the dose of insulin (Ritschel, 1980).

**Literature Cited**


