INSULIN RESPONSIVENESS TO GLUCOSE AND TISSUE RESPONSIVENESS TO INSULIN IN LACTATING, PREGNANT, AND NONPREGNANT, NONLACTATING BEEF COWS

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ABSTRACT

Insulin responsiveness to glucose and tissue responsiveness to insulin, using the hyperglycemic clamp and the hyperinsulinemic euglycemic clamp techniques, were measured in lactating, late pregnant, and nonpregnant, nonlactating (NPNL) beef cows. The glucose infusion rate (GIR) in the hyperglycemic clamp technique was higher ($P < .05$) in lactating cows than in NPNL cows. The plateau in plasma insulin concentration (insulin responsiveness) was higher ($P < .05$) in lactating cows than in late pregnant and NPNL cows. Pregnant cows tended to have higher GIR and lower plateau in plasma insulin concentration than NPNL cows. In the hyperinsulinemic euglycemic clamp technique, GIR (tissue responsiveness to insulin) was higher ($P < .05$) in lactating cows than in late pregnant cows; values for NPNL cows were intermediate. We conclude that insulin responsiveness to glucose and tissue responsiveness to insulin were enhanced during lactation but tended to be decreased during late pregnancy in beef cows.

Key Words: Glucose, Insulin, Beef Cows, Lactation, Pregnancy


Introduction

Utilization and partitioning of nutrients change drastically during pregnancy and lactation in cows; both are controlled by endocrine status (Bines and Hart, 1982; Collier et al., 1984). Blood concentrations of glucagon, growth hormone, and insulin are related to milk production in dairy cows (Herbein et al., 1985; Sartin et al., 1988). Insulin is one of the important hormones controlling nutrient metabolism. Plasma insulin is lower during lactation than during the dry period in dairy cows (Vasilatos and Wangsness, 1981; Sartin et al., 1985b). Responsiveness and sensitivity of insulin using the glucose clamp technique have been reported in pregnant sheep (Hay et al., 1984) and rabbits (Hauguel et al., 1987) and in lactating sheep (Metcalf and Weekes, 1988; Faulkner and Pollock, 1990) and goats (Debras et al., 1989), but data concerned with pregnant and lactating beef cows have not been available.

The objective of this experiment was to measure insulin responsiveness to glucose and tissue responsiveness to insulin using the hyperglycemic clamp technique and the hyperinsulinemic euglycemic clamp technique in lactating, late pregnant, and nonpregnant, nonlactating (NPNL) beef cows.

Materials and Methods

Animals. Sixteen Japanese Shorthorn cows were used in the experiment. The Japanese Shorthorn breed was established by crossing native Japanese cattle with English Shorthorn (Egaisu, 1988). They yield more milk (11.2 kg/d) than other Japanese beef cows (approximately 5 kg/d for Japanese Black; Tominaga et al., 1959). These cows were housed in a free-stall barn and were allotted into three groups: lactating ($n = 5$, 2 to 5 wk postpartum, $613 \pm 47$ kg), nonlactating late pregnant ($n = 3$, 3 to 8 wk prepartum, $614 \pm 44$ kg), and NPNL cows ($n = 8$, $666 \pm 21$ kg). They were fed 4 kg/d of a mixed concentrate (75.2% DM; 25%
jugular veins; the one for sampling was concentration by from the other catheter every 5 cm above the preinfusion blood glucose designed to achieve a circulating concentration three times immediately before the initiation of glucose infusion. Blood samples were taken glycemic clamp technique blood glucose concentration was determined blood samples were obtained from the catheters without noticeable stress to the cows. Initially about 2 mg.kg⁻¹.min⁻¹ and glucose infusion rate was infused by a multichannel peristaltic pump⁴ inserted catheters² were inserted into both external catheterization the experiment started. Both were filled with a sterile solution of trisodium citrate³. After recovery from catheterization the experiment started. All blood samples were obtained from the catheters without noticeable stress to the cows.

Hyperglycemic Clamp. In the hyperglycemic clamp technique (DeFronzo et al., 1979), glucose (34.8% sterile solution) was infused by a multichannel peristaltic pump through the jugular catheter for 2 h. Basal blood glucose concentration was determined three times immediately before the initiation of glucose infusion. Glucose infusion rate was initially about 2 mg.kg⁻¹.min⁻¹ and was designed to achieve a circulating concentration 50 mg/dl above the preinfusion blood glucose concentration by 30 min after the initiation of glucose infusion. Blood samples were taken from the other catheter every 5 min and glucose concentrations were determined within 1 min after taking the blood samples. Immediately after determination of glucose concentration, the glucose infusion rate was adjusted to maintain the desired glucose concentration by changing the pump dial. Residual blood samples taken at 10-min intervals were stored in ice-water until centrifugation. The amount of glucose infused was recorded every 10 min throughout the 2-h period. Cows did not have access to feed during the infusion period.

Hyperinsulinemic Euglycemic Clamp. This procedure was conducted approximately 28 h after the end of the hyperglycemic clamp procedure. In the hyperinsulinemic euglycemic clamp technique (Weekes et al., 1983), insulin (2 U/ml) dissolved in sterile solution of .9% sodium chloride and 2.5% potassium chloride was infused continuously through the jugular catheter by the peristaltic pump at the constant rate of 6.0 mU.kg⁻¹.min⁻¹ for 2 h after determination of the preinfusion blood glucose concentration. Glucose solution (34.8%) was infused through the jugular catheter by the other peristaltic pump at a variable rate to maintain the preinfusion blood glucose levels. Blood sampling and monitoring of glucose infusion rate were performed at 5-min and 10-min intervals during the 2-h period of the experiment, respectively.

Analyses. Blood glucose concentration was determined using an automated glucose analyzer⁶. After centrifugation (2,000 × g, 15 min), the supernatant fluids were stored at −20°C for insulin assay. Plasma insulin was assayed by a RIA kit⁷. Intra- and interassay CV were 4% and 12%, respectively.

Calculations. The glucose infusion rate and plasma insulin concentration during the last half of the 2-h period were relatively stable; their mean values were termed the glucose infusion rate (GIR) and the plasma insulin plateau concentration, respectively (Sano et al., 1990). Insulin responsiveness to glucose and tissue responsiveness to insulin were represented by the plasma insulin plateau concentration in the hyperglycemic clamp technique and the GIR in the hyperinsulinemic euglycemic clamp technique, respectively.

Differences between the preinfusion mean and values after the initiation of glucose or insulin infusion were evaluated by Student’s paired t-test. Differences among lactating, late pregnant, and NPNL cows on parameters of insulin responsiveness and tissue responsive-

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TABLE 1. CHEMICAL COMPOSITION OF DIETS⁸

<table>
<thead>
<tr>
<th>Item</th>
<th>Mixed concentrate</th>
<th>Orchardgrass hay</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME, Mcal/kg⁹</td>
<td>2.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Ash, %</td>
<td>5.1</td>
<td>8.2</td>
</tr>
<tr>
<td>CP, %</td>
<td>27.8</td>
<td>8.4</td>
</tr>
<tr>
<td>Ether extract, %</td>
<td>2.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Crude fiber, %</td>
<td>3.5</td>
<td>41.7</td>
</tr>
<tr>
<td>Nitrogen-free extract, %</td>
<td>60.7</td>
<td>40.2</td>
</tr>
</tbody>
</table>

⁸Dry matter basis.
⁹Calculated.

distillers feeds, 25% brewer’s dried yeast, 19% wheat bran, 18% corn, 4% wheat germ meal, 4% soybean meal, and 5% mineral and vitamin mixture) and given ad libitum access to orchardgrass hay (87% DM). Hay consumption was approximately 12 kg·cow⁻¹·d⁻¹. Lactating cows maintained BW, late pregnant and NPNL cows were in positive energy balance. Chemical composition of diets is shown in Table 1. They were kept in a stanchion stall during the catheterization and blood sampling. Polyethylene catheters² were inserted into both external jugular veins; the one for sampling was inserted 30 cm and the one for infusion 40 cm. Both were filled with a sterile solution of 3.8% trisodium citrate³. After recovery from catheterization the experiment started. All blood samples were obtained from the catheters without noticeable stress to the cows.

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2Imamuraya, Co. Ltd., Japan.
3Wako Pure Chemicals, Japan.
4Model AC-2120, Atto Co. Ltd., Japan.
5Actrapid monocomponent porcine insulin, Novo, Denmark.
6Model GLU-1, Toa Electronics Ltd., Japan.
7IRI ‘Eiken’, Eiken Chemical Co. Ltd., Japan.
Plateau concentrations were higher in pregnant and lactating cows (Figure 1). The plasma insulin concentration of plasma insulin was not different among the groups. Plasma insulin increased (P < .01) during glucose infusion in all groups; concentrations during the last half of glucose infusion were constant in late pregnant and NPNL cows but increased in lactating cows (Figure 1). The plasma insulin plateau concentrations were higher (P < .05) in lactating cows than in late pregnant and NPNL cows and tended to be lower in late pregnant cows than in NPNL cows (Table 2, Figure 1).

Hyperinsulinemic Euglycemic Clamp. Blood glucose concentrations were almost clamped at the basal levels during the 2-h period of insulin infusion accompanied by simultaneous glucose infusion (Figure 2). Concentrations were 98 ± 1, 99 ± 2, and 99 ± 1% of the desired goal during the last half of the 2-h period in lactating, late pregnant, and NPNL cows, respectively. The glucose infusion rate increased progressively during the initial hour of the 2-h period, but it was almost constant thereafter. The GIR in lactating cows was greater (P < .05) than that in late pregnant and NPNL cows (Table 3). No difference (P > .05) in the GIR was observed between NPNL cows and the other two groups. The plasma insulin plateau concentrations were lower (P < .05) in lactating and late pregnant cows than in NPNL cows.

**Discussion**

This experiment was designed to study insulin secretory response to exogenous glucose and tissue responsiveness to exogenous insulin in lactating, late pregnant, and NPNL cows. The GIR estimates differences between whole-body glucose utilization and endogenous glucose production during the glucose clamp period. Tissue responsiveness to exogenous insulin represents responsiveness of glu-
Figure 1. The concentrations of blood glucose and plasma insulin and glucose infusion rate before and during the hyperglycemic clamp technique in lactating, late pregnant, and nonpregnant, nonlactating (NPNL) cows. Vertical bars represent SE of means.

cose uptake by tissues, although insulin influences lipid and nitrogen metabolism as well as glucose metabolism (Faulkner and Pollock, 1990). Blood glucose turnover rate is enhanced during lactation and pregnancy in cows (Bickerstaffe et al., 1974), goats (Buckley et al., 1982; Chaiyabutr et al., 1982; Debras et al., 1989), and sheep (Bergman, 1963; Bergman and Hogue, 1967; Wilson et al., 1983). The GIR in the hyperglycemic clamp technique may be related to this enhanced glucose turnover rate during lactation and pregnancy, although significant differences were not observed between late pregnant and NPNL cows (Table 2). In the hyperinsulinemic euglycemic clamp technique, the plasma insulin plateau

Figure 2. The concentrations of blood glucose and plasma insulin and glucose infusion rate before and during the hyperinsulinemic euglycemic clamp technique in lactating, late pregnant, and nonpregnant, nonlactating (NPNL) cows. Vertical bars represent SE of means.
concentrations in lactating and late pregnant cows were lower ($P < .05$) than those in NPNL cows, even though insulin was infused identically on a BW basis for each cow. This suggests that there might be differences in plasma insulin clearance among cows of different physiological states (Debras et al., 1989). This might be related to uptake of insulin by the lactating mammary gland (Faulkner and Pollock, 1990) and perhaps to uptake by the pregnant uterus. Another possibility is that insulin uptake by the liver changes during lactation and pregnancy.

Although pregnant dairy cows had essentially the same basal insulin levels as the lactating and NPNL dairy cows, the amount of insulin secreted during infusion of glucose or propionate was higher in pregnant cows than in the lactating and NPNL cows in a previous study (Sartin et al., 1985a,b). In contrast, basal insulin concentrations and insulin responses to injected glucose differed little among lactating, pregnant, and dry ewes (Bassett, 1989). Our results indicated that insulin responsiveness to glucose and tissue responsiveness to insulin in beef cows tended to be lower in late pregnant cows than in NPNL cows. Hauguel et al. (1987) showed that, in rabbits, insulin sensitivity of insulin-sensitive tissues decreased during late pregnancy. This may be associated with a decrease in the number of insulin receptors during late pregnancy (Vernon et al., 1981). Although insulin presumably increases glucose uptake by peripheral tissues (Trenkle, 1981), uterine glucose uptake of sheep was not influenced during the hyperinsulinemic euglycemic clamp technique (Hay et al., 1984). Therefore, insulin seems to act unfavorably on fetal glucose uptake. This decrease in tissue responsiveness to insulin in late pregnant cows might protect fetuses from hypoglycemia.

Both basal concentration of insulin and insulin response to secretagogues were reported to be lower in lactating than in dry dairy cows (Lomax et al., 1979; Sartin et al., 1985b) and to be lower in high-yielding lactating cows than in low yielders (Sartin et al., 1988). In contrast, basal insulin concentrations did not change over lactation in young beef cows (Chang et al., 1984). Insulin responsiveness and tissue responsiveness to insulin in the present experiment were markedly higher ($P < .05$) in lactating beef cows. Energy balance during lactation generally is maintained in beef cows, whereas in dairy cows negative energy balance generally is observed (Sartin et al., 1985a,b). This may be related to an inherent difference between beef and dairy cows because feeding level influenced insulin sensitivity (Metcalf and Weekes, 1988). Increased insulin responsiveness during lactation may be attributed to enhanced utilization of glucose, because the ratio of plasma insulin plateau concentrations to infused glucose was almost the same between lactating and NPNL cows (Table 2). Faulkner and Pollock (1990) also suggested that the sensitivity of glucose utilization was increased in lactating sheep compared with nonlactating sheep. However, Debras et al. (1989) reported that the insulin-stimulated glucose utilization above basal levels was greatly impaired during early lactation compared with the dry period in goats; they suggested that a decrease in insulin sensitivity in some insulin-sensitive tissues might occur. Type of diet (Janes et al., 1985) and feeding level (Metcalf and Weekes, 1988) also may be related to this inconsistency.

In summary, lactating beef cows had enhanced insulin responsiveness and tissue responsiveness to insulin. This characteristic of beef cows may be involved in depositing nutrients to peripheral tissues, because they lose less of their body weight during lactation than dairy cattle do (Hart et al., 1978).

**Implications**

Insulin responsiveness and insulin action on peripheral tissues changed during the production cycle. Beef cows, such as the Japanese Shorthorn, partition nutrients to deposition of body tissues rather than to milk even during early lactation.

**Literature Cited**


