ABSTRACT

Changes in insulin concentrations of blood plasma were studied in lactating cows during and after a 1-h intravenous glucose infusion in 5 spontaneously ketonemic, 10 nonketonemic (normal), and 4 starved cows.

A biphasic increase in insulin with a maximum 10 to 20 min after the start of the glucose infusion was followed by a sustained (1 to 2 h) elevation in the normal and ketonemic animals. In starved cows only the later phase was detectable. Insulin was higher in normal than in starved and ketonemic cows during both phases of the insulin secretion in spite of approximately identical glucose. Differences between starved and ketonemic cows were not major.

Average rates of decline in sugar concentrations after the end of the infusion were higher in normal and ketonemic [half times $143 \pm 23$ (standard deviation) and $166 \pm 22$ min] than in starved cows ($281 \pm 47$ min). The low basal insulin of plasma and poor responses of insulin secretion following glucose infusions indicate sluggish $\beta$-cell function in ketonemic cows.

INTRODUCTION

The content of ketone bodies in blood varies widely in apparently healthy cows under normal feeding conditions. In accordance with common clinical experience on the occurrence of bovine ketosis, the greatest incidence of high plasma acetoacetate (Acac) is during the first mo postpartum (12). The high ketone bodies are accompanied by lowered glucose in plasma. Theories for development of ketosis have centered about the concept of glucose imbalance since the early observations by Sjølemma (31) of hypoglycemia in clinical cases. Krebs (20) drew attention to the close association between ketogenesis and the requirements for a high rate of gluconeogenesis during lactation. Excessive transfer of carbohydrates to the milk leaves insufficient glucose for extramammary tissues. This stimulates mobilization of body fat which leads to ketogenesis. The fact that mammary extraction of glucose is high even at glucose contents in the hypoglycemic range substantiates this hypothesis (23, 24).

The role of insulin in the development of hypoglycemia and ketonemia during milk production is understood incompletely. Kronfeld (22) postulated that a hypersecretion of insulin could be a precipitating factor for hypoglycemia during bovine ketosis. Except for the observations by Kronfeld (21) of high insulin in three ketotic cows, most evidence seems to indicate low insulin in cows with subclinical ketosis (17, 19, 26, 30). Since basal insulin in plasma and responses of insulin to a stimulus as a glucose tolerance test seem to be correlated in man (1, 5, 10), low basal insulin could indicate low pancreatic reserves for insulin release in ketonemic cows. The present investigation was to study the secretory capacity of the pancreatic $\beta$-cells in normal and in spontaneously ketonemic cows with an intravenous glucose infusion test. For comparison starved cows, similar to ketonemic cows in that they have restricted supplies of glucose, also were studied.

METHODS

Animals

Three groups of Norwegian Red Cows (Table 1) included 10 apparently normal, 5 spontaneously ketonemic, and 4 cows which had been starved previous to the experiment. Healthy cows, 3 to 9 yr old, without previous
TABLE 1. Milk production (average and range), stage of lactation, and pre-infusion concentrations in plasma of the measured metabolites (average and standard deviation).

<table>
<thead>
<tr>
<th>Group</th>
<th>Days post-partum</th>
<th>Milk production (kg/day)</th>
<th>Sugar (mg/100 ml)</th>
<th>Plasma Acac (ng/ml)</th>
<th>Insulin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>13–240</td>
<td>16–13–23</td>
<td>73.7</td>
<td>.4</td>
<td>.75 .30</td>
</tr>
<tr>
<td>Ketonic</td>
<td>15–54</td>
<td>23–18–27</td>
<td>50.8</td>
<td>14.0</td>
<td>.40 .10</td>
</tr>
<tr>
<td>Starved</td>
<td>57–148</td>
<td>19–14–24 (4–11)</td>
<td>70.0</td>
<td>2.5</td>
<td>.33 .06</td>
</tr>
</tbody>
</table>

*Range of milk yields during the last 24 h of the starvation period.

histories of ketosis since last calving were used both for starvation experiments and as normals. The clinical status of the ketonic cows differed since two showed signs of impaired appetite on the day of the infusion while three apparently had normal appetites (Table 2). The contents of Acac in plasma were all within the range of ketone body concentrations in clinical cases of ketosis (27).

**Feeding**

The cows were fed, according to body weight and milk production, diets of hay, grass silage, and concentrates. The cows were not fed in the morning on the day of the experiment, but milking was as usual.

**Starvation**

Food was withheld for 48 h before glucose infusions. During this period milk yield decreased from an average of 19 kg before starvation to 7 kg during the last 24 h of starvation.

**Glucose Infusions**

A priming dose of glucose – 300 mg/kg body weight – was injected into the jugular vein as a 35 to 50% solution in 3 to 7 min. Immediately afterwards a continuous infusion of 8 to 10 mg per kg per min was started. The infusion was given at 12 to 15 ml/min for 60 min after the start of the priming injection.

**Blood Sampling**

Polyethylene catheters were inserted into the jugular veins under local anesthesia 1 h before the experiments were started. Glucose was infused through one of the catheters while the other one was used for blood sampling. Between samplings the catheter was filled with heparinized saline (5 IE heparin/ml).

Blood samples were withdrawn 60 and 30 min before the infusion and subsequently 10, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160, 180, and 210 min after the start of infusion. Starved animals also were sampled at 240, 270, and 300 min. Heparinized plasma was separated immediately, cooled on ice, and frozen within a maximum of 5 h. Plasma aliquots for sugar and Acac determinations were kept at -70 C until analyzed to minimize losses of Acac in storage.

**Analytical Procedures**

Insulin in plasma was measured by radioimmunoassay with dextran-coated charcoal to separate bound and free insulin (29). Bovine insulin was used as a standard and for the production of antibodies. Hove (17) reported some impairment in the recovery of insulin added to plasmas of ketonic cows. Corrections for this recovery were omitted in the present study since it would not interfere with interpretation of the data.

Sugar and Acac of plasma were determined by automated spectrophotometric methods.
### TABLE 2. Case histories and insulin responses of the ketonemic cows.

<table>
<thead>
<tr>
<th>Clinical findings</th>
<th>Plasma analyses on the day of experiment</th>
<th>Plasma insulin 30 to 120 min after start of infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acac</td>
<td>Sugar</td>
</tr>
<tr>
<td>Cow 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical ketosis: Impaired appetite one day before the infusion.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Given 200 g sodium propionate. New anorexic episode 3 days after the infusion.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18.7</td>
<td>72.0</td>
</tr>
<tr>
<td>Cow 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical ketosis: Impaired appetite on the day before the experiment.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18.3</td>
<td>37.0</td>
</tr>
<tr>
<td>Cow 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subclinical ketosis: Normal appetite. Anorexic 6 days before the infusion.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment: prednisolone initially and 200 g sodium-propionate daily for 3 days.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.5</td>
<td>42.0</td>
</tr>
<tr>
<td>Cow 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subclinical ketosis: Normal appetite.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.3</td>
<td>52.5</td>
</tr>
<tr>
<td>Cow 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subclinical ketosis: Normal appetite.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.4</td>
<td>43.2</td>
</tr>
</tbody>
</table>
sugar by reduction of ferricyanide, and Acac by the nitroprusside reaction in dialysates of plasma (3).

**Statistical Evaluation and Calculations**

Wilcoxon tests for unpaired data (two tailed) were used to calculate probabilities. A P<.05 was statistically significant. The rate of decrease in sugar concentration in the post infusion period was calculated as the slope of the sugar curve in plasma starting at the end of the infusion. The decline of sugar was taken to be linear with time on a semilogarithmic scale, thus permitting the use of linear regression analysis.

**RESULTS**

Figures 1 and 2 show the effect of glucose infusion on the three parameters as average curves for the three groups of animals. Evidently the nonketonemic animals responded with higher increments in plasma insulin than the starved and the ketonemic (P<.01). In most of the cows a biphasic pattern of insulin secretion could be seen, as is also apparent from the average curves in Fig. 1. A maximum in insulin occurred within 10 to 20 min, followed by a lower and sustained elevation for 1 to 2 h after the end of the infusion (Fig. 1). Systematic differences between subclinically and clinically ketotic cows could not be demonstrated during the period of maximal insulin responses (Table 2).

It further appeared that in the starved animals plasma sugar concentrations rose continuously throughout the period of glucose infusion and reached higher than in the other animals. In the ketonemic animals a marked decrease in plasma Acac from one-third to one-fourth of the initial level was observed after the glucose infusion. This antiketogenic effect of glucose could be detected in the starved and normal cows as well.

The difference in insulin between normal and ketonemic cows was not reflected in the rate of fall in blood sugar concentrations during

**Fig. 1.** Plasma insulin, sugar, and acetoacetate concentrations in 10 normal (●) and 5 ketonemic (○) cows. Means and standard errors of the means.

**Fig. 2.** Plasma insulin, sugar, and acetoacetate concentrations in 4 starved cows. Individual plasma Acac, otherwise means and standard errors of the means.
DISCUSSION

In accordance with (6, 17, 26, 30), the ketonemic cows were characterized by lower insulin and sugar in plasma than the normal (P<.01) before the infusion. No difference in insulin of plasma could be detected between starved and ketonemic cows in spite of the much higher sugar concentrations in plasma of the former (Table 1). The positive correlation between pre-feeding or basal sugar and insulin reported in (17), therefore, seems restricted to fed animals.

Hyperglycemia, the stimulus used in the present study, is without importance in adult ruminants under physiological conditions (2). In spite of this, elevated glucose in plasma is a potent stimulus to secretion of insulin. In other species it is accepted generally that glucose stimulates the release of stored insulin as well as the pancreatic synthesis of insulin. An initial peak in insulin concentration was observed within 20 min after start of glucose infusion in the present study. This was followed by a sustained elevation of insulin concentrations 80 to 140 min after start of infusion (Fig. 1). In similar glucose infusion tests in man these two phases of insulin secretion have been taken to represent an initial release from pancreatic insulin stores followed by increased insulin production and release (7). This interpretation is in accord with the postulated mechanism of insulin secretion involving different “pools” in the \( \beta \)-cells (9, 28). In the starved cows the initial peak was barely perceptible, whereas the insulin concentrations increased steadily during the hours following the infusion. This second phase was more protracted than in the two other groups of cows.

The low secretory responses of insulin after glucose infusions should be compared to the findings of reduced insulin in plasma in ketotic (6) and ketonemic (17) cows. Schwalm and Schultz (30) observed a similar, although statistically not significant, trend towards low insulin in a group of subclinically ketogenic cows. Further, Hove and Halse (19) reported a gradual decrease in insulin release to a feeding stimulus with decreasing prefeeding glucose in plasma. In highly ketonemic cows the increments in insulin of plasma were absent. None of the animals in the studies referred to has been sampled during the hours immediately before onset of clinical symptoms. The low basal insulin and sluggish responses to both physiological (feeding) and artificial stimuli (glucose infusion) are difficult to reconcile with the postulate of Kronfeld (22) that a period of transient hyperinsulinism might precipitate the final hypoglycemia and hypophagia at the onset of clinical symptoms.

Experiments in man (25) and cattle (19) demonstrate that blood glucose in the period preceding an experiment is of great importance for the amount of insulin released in response to a secretory signal. The low insulin responses to glucose infusions in the ketonemic animals, therefore, probably result from a pancreas with a low secretory capacity for insulin developed during the days or weeks of hypoglycemia which regularly accompanies high ketones. Low insulin in subclinical ketosis does not preclude an active uptake of glucose by mammary cells since observations by Kronfeld (23) and Hove (18) indicate that great variations in insulin in blood may occur without observable changes in mammary consumption of glucose.

Hypocalcemia and increased sympathetic activity can abolish the release of insulin to various stimuli (4, 14, 15, 25), and changes in these factors might have interfered with release of insulin. Calcium in plasma was not measured in my study, but reduced calcium cannot explain the small increments in insulin in the ketonemic cows since calcium in plasma is affected only moderately by even severe hyperketonemia (13). Starvation invariably decreases calcium in plasma. Halse (11) observed average reductions of about 20% after 48 h of starvation of dairy cows. Although the findings of Littledeike et al. (25) indicate that greater reductions in calcium of plasma are required to abolish insulin secretion, an effect of lowered calcium on insulin release during starvation cannot be excluded. Norepinephrine in plasma increases during a 43 h fast in man (8), and such an increase could contribute to a reduction in insulin secretion in starved cows. Hypoglycemia is also a potent stimulus to
catecholamine secretion in man (8), but the possible significance of increased catecholamines in mediating the lowered insulin response in ketonemic cows must await further studies.

In accordance with (16), rates of decline in sugar concentrations were almost identical in ketonemic and normal cows. This probably can be explained by the high rate of milk secretion in the ketonemic cows which, due to high demands for glucose, could permit a rapid repair of the hyperglycemia in absence of great increases in insulin. In agreement with this a lower rate of glucose utilization due to low milk production in the starved cows might, at least partly, explain the much slower decline in this group.

In summary the low basal insulin and low insulin increments in ketonemic cows after stimulation seem to be consistent with the concept of glucose imbalance in ketosis since these adaptions in insulin secretion will contribute to carbohydrate conservation by reducing carbohydrate utilization in insulin sensitive tissues, and to fat utilization.

ACKNOWLEDGMENTS

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