Insulin resistance and abomasal motility disorders in cows detected by use of abomasoduodenal electromyography after surgical correction of left displaced abomasum

Davide Pravettoni, DVM, PhD; Klaus Doll, DVM; Markus Hummel, DVM; Elena Cavallone, BSc; Michela Re, DVM; Angelo G. Belloli, DVM

Objective—To determine the correlation between insulin concentrations and myoelectrical activity of the abomasum in cows with a left displaced abomasum (LDA).

Animals—14 dairy cows with an LDA at the onset of lactation.

Procedure—During surgical correction of an LDA, 3 pairs of electrodes were placed in the smooth muscle of the gastrointestinal tract (abomasal body, pars pylorica, and duodenum) of each cow. Electromyographic recordings were obtained once per day for 7 days. Samples were collected and tested to determine concentrations of insulin, glucagon, cortisol, glucose, β-hydroxybutyrate, and nonesterified fatty acids.

Results—All 14 cattle had high glucose and insulin concentrations at the time of admission, independent of ketosis. Concentrations of glucose and insulin decreased slowly after surgical treatment and were associated with a progressive increase in abomasoduodenal myoelectric activity. The 14 cows were allocated into 2 groups (suspected insulin-resistant cattle, n = 7; suspected non–insulin-resistant cattle, 7) on the basis of persistent hyperinsulinemia during the postoperative period. Seven days after surgery, the abomasoduodenal myoelectric patterns were still significantly lower for the insulin-resistant cows, compared with patterns for the non–insulin-resistant cows.

Conclusions and Clinical Relevance—Insulin resistance appears to be common in cows with an LDA. Analysis of results of this study reveals that abomasal atony in cows with an LDA depends on persistence of high serum concentrations of insulin. Results of this study could provide an explanation for a pathogenetic factor of LDAs and the frequent relapses of cattle affected by this condition. (Am J Vet Res 2004;65:1319–1324)
suggests that LDAs are associated with disorders of the intrinsic nervous system combined with reduced sensitivity of the muscle tissue to cholinergic stimuli. Analysis of results of other studies suggests that abomasal atony is the determinant pathogenetic factor for an LDA. The existence of this atony, which precedes distention and displacement of the abomasum, has been proven in vivo by use of abomasal electromyography. In 1 of those studies, investigators recorded electromyographic data from the body of the abomasum, pylorus, and duodenum in 6 cows with an LDA to determine the influence of visceral atony on the pathogenesis of the disease. During that study, a decrease in electric activity was recorded during relapses when the abomasum moved to the left side of the abdomen and an increase in activity was recorded when the abomasum spontaneously repositioned itself. The authors of that study did not observe the few hours of inactivity documented in another study, which are considered to be important in the development of the pathologic changes.

High basal insulin concentrations could play a role in the pathogenesis of an LDA, causing abomasal atony. In fact, the abomasal emptying rate in young heifers can be experimentally delayed by administration of insulin, independent of glycemia. The insensitivity of body tissues to the action of insulin on glucose clearance does not contradict a possible effect of insulin on gastric muscle because there are differing insulin receptors for each biological action of insulin. A comparable inhibitory effect of insulin on gastric motility in dogs attributable to transmembrane metabolic changes or to a direct effect of insulin on gastric muscle has been reported. A similar result has been confirmed in cattle and sheep. The purposes of the study reported here were to verify high concentrations of glucose in association with high concentrations of insulin in cows with an LDA and to correlate the concentration of insulin with abomasal atony.

Materials and Methods

Animals—Fourteen Holstein-Friesian dairy cows (1 from each of 14 herds in the district of Hesse, Germany) were used in the study. Each cow was at the onset of lactation (interval from parturition until admission ranged from 3 to 40 days; mean ± SD, 14.8 ± 12.3 days), and each cow had an LDA. Cows ranged from 2.5 to 7 years of age (mean, 4.28 ± 1.62 years). Milk production of each cow was 20 to 40 L/d. Cows had been fed a diet consisting of a mixture of hay, corn, and grass silage supplemented with concentrates in accordance with each cow’s milk production. During hospitalization, cows were provided a diet of mixed hay ad libitum that was supplemented by concentrate in accordance with each cow’s milk production; the ration was provided twice daily (7:30 AM and 7:30 PM).

Cows were selected from sick cattle examined at the Klinik für Wiederkauer und Schweine, Justus-Liebig-Universität, Gießen, Germany. For this study, we considered a group of cows that was not affected by any other complicating diseases, such as metritis, mastitis, moderate or severe peritonitis, or lameness, and that had not received any type of drug (especially glucose solutions or corticosteroids) that could affect concentrations of glucose and glycemia-related hormones. The study protocol was approved by the Animal Welfare Commission of the district of Middle Hesse, Germany.

Clinical examination—Each cow was admitted to the clinic and then subjected to a clinical examination. On the basis of medical history, each cow had inappetence for several days and milk production had decreased. Physical examination was used to diagnose an LDA (percussion and ballottement with simultaneous auscultation of the left abdominal wall were used to identify a high-pitched resonant echo and fluid-splashing sounds associated with an LDA).

Collection of blood samples—Blood samples were collected from a jugular vein of each cow within 30 minutes after admission (day 0). An aliquot of blood was placed into sodium fluoride–containing tubes, another aliquot of blood was placed into EDTA-containing glass tubes, and another aliquot of blood was placed into glass tubes for serum. Tubes were centrifuged, and the plasma and serum were harvested and immediately frozen at −20°C until analyzed. Subsequent blood samples were collected at 8 AM (ie, immediately after morning feeding) on days 1 to 7 (ie, each of the 7 days of hospitalization).

Surgery—Surgical correction of an LDA was performed within 1 hour after admission. Omentotomies was performed in accordance with the surgical procedure described by Hummel et al. Following neuroleptic analgesia achieved by IV administration of a combination of xylazine hydrochloride (0.05 mg/kg) and ketamine hydrochloride (5 mg/kg), each cow was positioned in left lateral recumbency. The right abdominal wall was prepared for aseptic surgery, and procaine was used for local infiltration of sites for a lateral incision in the ventral paracostal region (laparotomy site) and right paralumbar fossa (exit site of the electrodes). Three pairs of electrodes were implanted (1 in the smooth muscle of the body of the abomasum, 1 in the pars pylorica [at the greater curvature], and 1 in the proximal portion of the duodenum). A titanium device exited the skin and contained wires from the retrievable stainless-steel electrodes. The electrodes were left in place for 7 days; then they were removed by applying gentle retraction to the titanium device.

Electromyography—Electromyographic measurement frequency was 214 Hz. For wireless monitoring of myoelectric activity, an extracorporeal amplifier-encoder-transmitter was connected to the titanium device of each cow. The receiver of the telemetry system was connected to a personal computer that recorded the signals. Signals were then processed by use of a software program. Mean spike frequency and maximal and minimal potentials were calculated every 2 seconds.

During days 1 to 7 of the postoperative period, 1-hour telemetric measurements were performed daily between 8 and 9 AM. These measurements were obtained immediately following collection of venous blood samples.

Analytic procedures—Plasma harvested from blood samples collected into tubes that contained sodium fluoride was used for measurement of concentrations of glucose. Serum samples were used for measurement of concentrations of nonesterified fatty acids (NEFA) and β-hydroxybutyrate. Glucose concentrations were measured by use of the hexokinase method. The NEFA concentrations were determined enzymatically by use of acyl-coenzyme A synthetase, acyl-coenzyme A oxidase, and peroxidase. Concentrations of β-hydroxybutyrate were determined enzymatically by use of a colorometric method that involved β-hydroxybutyrate dehydrogenase.
Plasma-EDTA samples were used to determine concentrations of insulin, glucagon, and cortisol. Hemolyzed samples were not used. Insulin, glucagon, and cortisol concentrations were determined by use of radioimmunoassay in accordance with analytic procedures described in the material supplied with each test reagent kit.

Assignment of cattle to insulin-resistant and non-insulin-resistant groups—Concentrations of hormones, glucose, β-hydroxybutyrate, and NEFA were used to assign cows to 2 groups (suspected insulin-resistant group and suspected non-insulin-resistant group). Cows were considered insulin resistant when they had at least 2 insulin concentrations during the postoperative period (days 1 to 7) that were higher than the highest limit of a reference range (3.65 to 11.34 μU/mL; mean ± SD, 7.49 ± 1.92 μU/mL); the reference range was calculated from samples obtained from 50 healthy lactating dairy cows between 1 to 7 after surgery. Insulin, glucagon, and cortisol concentrations decreased after surgery.

During the hospitalization period after surgical correction of the LDA, hormone and metabolites between groups were performed (Table 1). Cows with an LDA concentration higher than the reference range were assigned to the suspected insulin-resistant group. Insulin concentrations during the postoperative period of hospitalization was expressed as the total integral calculated by the equation:

\[ \Sigma_t (\text{potential}_{\text{max}}[t] - \text{potential}_{\text{min}}[t]) \times 2 \]

where potential_{\text{max}} is the maximal potential, potential_{\text{min}} is the minimal potential, t represents the time points at which samples were obtained, and 2 represents the 2-second interval between calculations.

**Results**

Cows with an LDA—Concentrations of insulin, glucagon, cortisol, glucose, β-hydroxybutyrate, and NEFA in the cows at time of admission (day 0) and during the hospitalization period after surgical correction (days 1 to 7) were calculated (Table 1). All concentrations decreased after surgery.

Mean ± SD value of total electromyographic activity (integrations of the recorded myoelectric activities) of the body of the abomasum, pars pylorica, and duodenum was calculated by integrating the potential differences registered every 2 seconds for the daily 1-hour recording period during the 7 days after surgery (Figure 1). A progressive increase in myoelectric activity was observed for the body of the abomasum and duodenum, which reached a maximum on day 6. The body of the abomasum had a slight increase in myoelectric activity until day 3, which then stabilized with mild fluctuations until day 7. The pars pylorica had a fluctuating pattern and peaked at day 6, whereas the duodenum had a continuous increase in myoelectric activity from days 1 to 6. In every anatomical structure, a slight decrease in myoelectric activity was recorded on day 7. However, within each anatomical structure, there was not a significant difference in myoelectric activity among days. High SD values documented the heterogeneity of the samples when considered together.

Comparison between insulin-resistant and non-insulin-resistant cows—On the basis of the aforementioned criteria, 7 cows were assigned to the suspected insulin-resistant group and 7 cows were assigned to the suspected non-insulin-resistant group. Comparisons of concentrations of the various hormones and metabolites between groups were performed (Table 2). Similarly, myoelectric activity was compared between groups (Figure 2).

<table>
<thead>
<tr>
<th>Day*</th>
<th>Insulin (μU/mL)</th>
<th>Glucagon (pg/mL)</th>
<th>Cortisol (nmol/L)</th>
<th>Glucose (mmol/L)</th>
<th>β-HB (mmol/L)</th>
<th>NEFA (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>17.2 ± 14.5</td>
<td>130.7 ± 98.7</td>
<td>79.9 ± 69.5</td>
<td>4.5 ± 1.4</td>
<td>1.8 ± 1.6</td>
<td>1.3 ± 0.5</td>
</tr>
<tr>
<td>1</td>
<td>12.0 ± 5.5</td>
<td>87.2 ± 29.3</td>
<td>31.5 ± 38.5</td>
<td>4.3 ± 0.6</td>
<td>1.3 ± 0.9</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>10.3 ± 5.9</td>
<td>72.8 ± 25.8</td>
<td>18.3 ± 17.1</td>
<td>3.5 ± 0.7</td>
<td>0.9 ± 0.5</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>11.9 ± 6.0</td>
<td>65.9 ± 32.7</td>
<td>20.7 ± 23.5</td>
<td>3.7 ± 0.7</td>
<td>0.5 ± 0.2</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>14.4 ± 5.6</td>
<td>66.8 ± 32.0</td>
<td>15.7 ± 15.3</td>
<td>3.6 ± 0.7</td>
<td>0.5 ± 0.2</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>5</td>
<td>12.3 ± 6.5</td>
<td>84.1 ± 27.0</td>
<td>16.2 ± 12.8</td>
<td>3.8 ± 0.5</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>6</td>
<td>10.4 ± 4.2</td>
<td>65.6 ± 28.8</td>
<td>16.1 ± 12.9</td>
<td>3.5 ± 0.4</td>
<td>0.5 ± 0.2</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td>7</td>
<td>10.1 ± 4.6</td>
<td>68.4 ± 19.6</td>
<td>11.7 ± 9.9</td>
<td>3.4 ± 0.3</td>
<td>0.8 ± 0.3</td>
<td>0.4 ± 0.2</td>
</tr>
</tbody>
</table>

Reference range†: 3.65–11.34 (pg/mL), 26.9–102.50 (μU/mL), 12.0–110.0 (mmol/L), 87.2–293.0 (pg/mL), 31.5–38.5 (nmol/L), 3.65–11.34 (mmol/L), 0.2–0.3 (mmol/L), 0.01–0.79 (mmol/L).

Data are given as mean ± SD. *Day 0 was the day of admission and surgical correction of the LDA, and days 1 to 7 were the postoperative period of hospitalization. Reference ranges were established for our laboratory by use of samples obtained from 50 clinically normal lactating dairy cows during the 3 weeks after parturition. β-HB = β-Hydroxybutyrate. NEFA = Nonesterified fatty acids.
In insulin-resistant cows, evaluations of hormone patterns revealed a persistence of high concentrations of insulin on days 3 to 5 after surgery with a peak on day 4, which was significantly (P = 0.01) higher than the peak concentration for the non–insulin-resistant group (Figure 2). Changes in glucagon concentrations were similar for the 2 groups. Cortisol concentrations had a pattern similar to that for insulin concentrations, with a peak on day 4. Glycemia values for the 2 groups of cows differed significantly on day 4. For both groups, β-hydroxybutyrate concentrations differed significantly on day 3. The NEFA concentrations were similar for both groups, and the mean values were within the reference range by day 2.

Patterns of myoelectric activity were evaluated for the body of the abomasum, pars pylorica, and duodenum, respectively, for the insulin-resistant and non–insulin-resistant groups (Figures 3–5). A progressive increase in the myoelectric activity of the body of the abomasum, pars pylorica, and duodenum was observed for the non–insulin-resistant group. Mean values for the body of the abomasum were > 6,000 µV·s from days 3 to 7. For the pars pylorica

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (µmol/L)</td>
<td>IR</td>
<td>18.7 ± 16.6</td>
<td>12.0 ± 6.6</td>
<td>11.0 ± 5.6</td>
<td>12.2 ± 6.4</td>
<td>16.6 ± 4.7</td>
<td>12.1 ± 3.8</td>
<td>10.9 ± 5.3</td>
<td>11.4 ± 5.5</td>
</tr>
<tr>
<td></td>
<td>Non-IR</td>
<td>15.7 ± 13.2</td>
<td>10.8 ± 3.2</td>
<td>8.3 ± 6.3</td>
<td>8.6 ± 4.3</td>
<td>9.1 ± 1.5</td>
<td>9.0 ± 3.3</td>
<td>9.0 ± 1.7</td>
<td>8.1 ± 2.7</td>
</tr>
<tr>
<td>Glucagon (pg/mL)</td>
<td>IR</td>
<td>136.8 ± 104.0</td>
<td>96.2 ± 29.4</td>
<td>82.2 ± 25.6</td>
<td>72.5 ± 27.9</td>
<td>76.2 ± 23.7</td>
<td>74.4 ± 25.5</td>
<td>82.2 ± 22.2</td>
<td>78.4 ± 13.6</td>
</tr>
<tr>
<td></td>
<td>Non-IR</td>
<td>132.5 ± 106.0</td>
<td>84.9 ± 23.6</td>
<td>64.0 ± 25.5</td>
<td>63.6 ± 38.9</td>
<td>61.4 ± 39.5</td>
<td>58.7 ± 27.1</td>
<td>51.4 ± 28.9</td>
<td>60.5 ± 22.2</td>
</tr>
<tr>
<td>Cortisol (nmol/L)</td>
<td>IR</td>
<td>79.0 ± 81.5</td>
<td>22.1 ± 21.0</td>
<td>17.0 ± 12.9</td>
<td>21.6 ± 16.3</td>
<td>20.0 ± 19.2</td>
<td>17.5 ± 16.4</td>
<td>15.0 ± 16.7</td>
<td>10.5 ± 13.7</td>
</tr>
<tr>
<td></td>
<td>Non-IR</td>
<td>74.8 ± 63.1</td>
<td>34.7 ± 51.8</td>
<td>12.6 ± 11.4</td>
<td>19.9 ± 33.0</td>
<td>11.7 ± 10.1</td>
<td>14.2 ± 9.2</td>
<td>15.1 ± 9.0</td>
<td>10.9 ± 4.1</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>IR</td>
<td>4.2 ± 1.1</td>
<td>4.1 ± 0.4</td>
<td>3.7 ± 0.8</td>
<td>3.9 ± 0.9</td>
<td>4.0 ± 0.8</td>
<td>3.8 ± 0.5</td>
<td>3.8 ± 0.3</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Non-IR</td>
<td>4.8 ± 1.7</td>
<td>4.4 ± 1.0</td>
<td>3.1 ± 0.5</td>
<td>3.4 ± 0.3</td>
<td>3.2 ± 0.4</td>
<td>3.3 ± 0.5</td>
<td>3.3 ± 0.4</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>β-HB (mmol/L)</td>
<td>IR</td>
<td>2.2 ± 2.0</td>
<td>1.7 ± 1.1</td>
<td>1.0 ± 0.5</td>
<td>0.7 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>0.7 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Non-IR</td>
<td>1.4 ± 1.2</td>
<td>0.9 ± 0.6</td>
<td>0.8 ± 0.5</td>
<td>0.4 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.3</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>NEFA (mmol/L)</td>
<td>IR</td>
<td>1.2 ± 0.6</td>
<td>1.0 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>0.5 ± 0.3</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Non-IR</td>
<td>1.5 ± 0.3</td>
<td>1.0 ± 0.4</td>
<td>0.7 ± 0.3</td>
<td>0.5 ± 0.2</td>
<td>0.5 ± 0.3</td>
<td>0.4 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>0.4 ± 0.2</td>
</tr>
</tbody>
</table>

*Values with different superscript letters differ significantly (**P = 0.005 and ***P < 0.05).
*See Table 1 for remainder of key.
had differing degrees of insulin resistance. In 1 study, investigators observed differing degrees of insulin resistance in cows with an LDA that was considered comparable to forms of non–insulin-dependent diabetes mellitus observed in humans and other domestic mammals. Forms of insulin resistance or reduced sensitivity of peripheral tissues to the action of insulin have been described in humans in association with obesity. Overfeeding cows during the nonlactating period causes postpartum lipomobilization and disorders of glucose homeostasis. Obesity causes reversible insulin resistance in humans that results in downregulation of the cellular receptors for insulin attributable to reduced affinity of the receptors or through a postreceptor fault. In cattle, insulin resistance can be induced by high concentrations of fatty acids, growth hormones, stress, infections, and endotoxins. Causes of insulin resistance in other animal species include persistent hyperprogesteronism, hyperadrenocorticism, hypothyroidism and hyperthyroidism, and bacterial infections. Bacterial infection and subsequent endotoxins seem unlikely in the cattle of our study because of the exclusion criteria for the selection of cows at the time of admission.

It was important to establish relationships that existed between an LDA and insulin resistance, which are often associated. Analysis of results of the study reported here indicated that in cows with an LDA, high insulin concentrations are associated with reduced abomasal activity. In another study, investigators described a correlation between hyperinsulinemia and a decrease in the abomasal emptying rate (measured by use of a duodenal fistula) and concluded that hormones of glycemic metabolism may cause inhibition of abomasal motility. Investigators in another study also observed an inhibitory effect of insulin on gastric motility in dogs attributable to transmembrane metabolic changes or a direct effect of insulin on gastric smooth muscle. Insulin plays a role in the control of jejunal motor activity in sheep. However, abomasal electromyography is not an efficient method for evaluating the abomasal emptying rate, compared with the use of a duodenal fistula. In our study, the abomasal emptying rate was not measured; however, we observed that hyperinsulinemia was associated with reduced myoelectric activity of the abomasum during the postoperative period.

Electromyography of the abomasum reveals circadian variations associated with feeding. However, myoelectric activity is reduced in cows affected with an LDA, even after surgical correction, compared with data obtained from healthy control cows. During the study reported here, serum cortisol concentrations were high only at admission, probably because of transport stress. However, the stress had abated by day 1, and cortisol concentrations had returned to within the reference range. Consequently, cortisol did not affect the other hormonal and metabolic variables during the convalescent period.

Analysis of the results of our study suggested that insulin resistance that results in abomasal atony would appear to be an important pathogenic factor for an LDA. Despite clinical recovery, disorders of glucose...
metabolism and abomasal motility persisted until at least 7 days after surgical correction. These unresolved dysfunctions could be 1 explanation for the frequent recurrence of LDA in dairy cows.

References