Evaluation of ornithine carbamoyl transferase and other serum and liver-derived analytes in diagnosis of fatty liver and postsurgical outcome of left-displaced abomasum in dairy cows

Emmanouil Kalaitzakis, DVM; Nikolaos Roubies, PhD; Nikolaos Panousis, DVM, PhD; Konstantinos Pourliotis, DVM; Eleni Kaldrymidou, DVM, PhD; Harilaos Karatzias, DVM, PhD

Objective—To evaluate postsurgical outcome in dairy cows with left-displaced abomasum (LDA) with regard to severity of fatty liver and assess the usefulness of preoperative determination of serum ornithine carbamoyl transferase (OCT) activity, bile acids concentration, and other variables for evaluating liver function during the postsurgical convalescence period.

Design—Prospective study.

Animals—68 Holstein cows.

Procedures—Blood and liver biopsy specimens were obtained during standing LDA surgery. Liver tissue was examined histologically and classified by severity of fatty change. Serum activities of liver-derived enzymes and concentrations of total lipids, triglycerides, bile acids, glucose, β-hydroxybutyric acid, bilirubin, and nonesterified fatty acids were determined.

Results—Most cows with LDA and cows with severe fatty liver were detected within the first month after calving. Postsurgical outcome was related to severity of fatty liver. All cows that died had severe fatty liver. Serum activities of OCT, aspartate aminotransferase, and glutamate dehydrogenase and serum total bilirubin concentration were sensitive indicators of fatty liver. Serum bile acids concentration was not an accurate indicator of fatty liver.

Conclusions and Clinical Relevance—Postsurgical outcome of cows undergoing surgery to correct LDA was related to fatty liver severity. Assessment of serum activities of OCT, aspartate aminotransferase, and glutamate dehydrogenase and serum total bilirubin concentration is recommended for diagnosis of fatty liver in dairy cows with LDA, whereas determination of bile acids concentration is not. The strong correlation between OCT activity and degree of hepatocellular damage supports use of this enzyme for assessing severity of fatty liver and predicting postsurgical outcome in cows with LDA. (J Am Vet Med Assoc 2006;229:1463–1471)

High-producing dairy cows are in an energy-deficient state during the early part of lactation because energy requirements for milk production exceed the capacity for feed consumption. This situation results in mobilization of body fat stores. Excessive lipomobilization increases fat accumulation in liver cells and leads to development of fatty liver (also known as hepatic lipidosis or fatty liver change). Although cattle with mild and moderate fatty liver do not necessarily have clinical signs, the condition has been associated with health and production problems. It has been proposed that disease risk increases in conjunction with liver fat concentration.

Among the diseases with which fatty liver has been associated is LDA, a common problem in high-producing dairy cows in early lactation. A typical clinical sign of LDA is gradual loss of appetite, which further aggravates energy deficit and results in enhanced lipomobilization and severe fatty liver. Despite successful surgical treatment, the general condition of cows with LDA may remain poor during the postoperative period because postsurgical convalescence of cows with LDA is related to the severity of fatty liver.

Early diagnosis of fatty liver and the degree of severity in cows with LDA are of special interest in estimating postsurgical prognosis. Although a definitive diagnosis of fatty liver can be made by means of histologic tissue examination, determination of certain serum biochemical variables helps in the assessment of fatty-liver severity and aids in determining postsurgical prognosis. Among the variables usually assessed are serum activities of AST, GDH, SDH, GGT, ALP, and ALT, and serum concentrations of glucose, NEFAs, bilirubin, β-HB, albumin, and urea nitrogen.

High serum concentrations of bile constituents (bilirubin and bile acids) have been reported in cows with fatty liver. Total serum bile acids concentration can be assessed to test the excretory capacity of the liver in dairy cows. However, the value of determining this analyte in diagnosis of fatty liver is controver-

**Abbreviations**

- LDA: Left-displaced abomasum
- AST: Aspartate aminotransferase
- GDH: Glutamate dehydrogenase
- SDH: Sorbitol dehydrogenase
- GGT: γ-glutamyltransferase
- ALP: Alkaline phosphatase
- ALT: Alanine aminotransferase
- NEFA: Nonesterified fatty acid
- β-HB: β-hydroxybutyrate
- OCT: Ornithine carbamoyl transferase
- GdL: Grades der Leberverfettung (degrees of fatty liver change)
sial because some authors consider serum bile acids determination to be a reliable indicator of liver dysfunction, whereas others do not. Apart from that debate, there is little information about the use of serum bile acids determination as a prognostic tool for the postsurgical convalescence of cows with LDA.

The usefulness of determination of serum OCT activity has not been widely studied. However, OCT is considered to be specific as an indicator of hepatocellular necrosis in ruminants and useful for diagnosis of severe fatty liver. A study on OCT made use of an assay developed for human, rather than bovine, serum. To the authors' knowledge, there are no reports concerning the usefulness of serum OCT activity for determination of postoperative prognosis in cattle undergoing surgery for LDA or for assessment of the severity of fatty liver.

The purpose of the present study was to investigate the postsurgical outcome of dairy cows with LDA with regard to the severity of fatty liver and to determine the prognostic value of preoperative measurement of OCT (quantified by use of a modified assay adapted for bovine serum), serum bile acids concentration, and other serum analytes in evaluating liver function in cows with LDA after corrective surgery. Occurrence and severity of fatty liver in cows with LDA were also evaluated.

Materials and Methods

Animal selection—Sixty-eight Holstein cows (13 of which were first-calf heifers) from 28 dairy farms in northern Greece were used. All owners gave informed consent for cattle to undergo the testing procedures. Samples were collected from September 2003 through December 2004. On-farm visits were conducted after LDA was diagnosed by a herd veterinarian. Information was collected, including history (age, parity, date of calving, recent health and production problems, and number of days from the onset of clinical signs of LDA) and body condition score (scale, 1 to 5), and a physical examination was performed. Surgical correction of the LDA was performed via laparotomy and a right flank approach in standing cows in each instance. Repositioning of the abomasum and omentoplasty were performed according to a described procedure.

Cows with evidence of infectious disease or other conditions unrelated to LDA or liver disease (eg, fever, vaginal discharge, metritis, and mastitis) were excluded from the study.

Sample collection—A blood sample was collected from a jugular vein with an 18-gauge needle and vacuum glass tubes from each cow immediately before the surgical procedure. After clotting, serum was separated by low-speed centrifugation (1,000 × g for 15 minutes), transferred to plastic vials, and stored in a refrigerator. Serum enzyme activity and β-HB, glucose, BUN, albumin, and total bilirubin concentrations were measured within 6 hours of collection, whereas serum total bile acids and NEFA concentrations were measured after 7 to 10 days of storage at −20°C.

Liver biopsy was performed by use of a liver biopsy needle through the open abdominal wall during surgery, after repositioning of the abomasum. Biopsy specimens (150 to 350 mg of tissue) were divided into 2 parts, one of which was fixed in neutral-buffered 10% formalin for histologic examination. The other part was stored at −20°C until determination of total lipid and triglyceride concentration.

Serum biochemical analysis—Spectrophotometric kinetic methods were used for determination of serum activities of AST, ALT, ALP, GGT, SDH, and GDH and concentration of β-HB. All measurements were obtained at a steady temperature (30°C). Colorimetric spectrophotometric methods were used for determination of OCT activity and total bilirubin, albumin, BUN, and glucose concentrations. Commercially available kits were used for determination of serum bile acids and NEFA concentrations.

Liver biochemical analysis—Total lipid concentration in liver tissue was measured via chloroform-methanol-water extraction. For triglyceride concentration, lipids extracted were saponified (by use of 1 mL of 0.5N potassium hydroxide solution and 1 mL of absolute ethanol) for 60 minutes at 70°C, and the resultant triacylglycerol was measured by the method of Eggstein and Kuhlmann. A spectrophotometer was used in all measurements.

Histologic examination—Biopsy specimens were fixed in neutral-buffered 10% formalin solution, cut to 3 to 4 µm in thickness, and stained with H&E. Specimens were examined via light microscopy for lipid content. Liver fat content was classified according to a 6-point scale of severity of fatty infiltration (ie, GdL). A score of 0 indicated that no fat droplets were visible and that the hepatocytes had a normal appearance, whereas a score of 5 was indicative of panlobular fatty infiltration. This classification was performed with the use of a point score scale. With this scale, scores were assigned on the basis of findings in the area from the central vein to the portal triad of the hepatic lobule, which was divided into 3 concentric regions. In each of those regions, scores were assigned according to the following guidelines: no lesion = 0 points; cloudy swelling = 0.5 points; cloudy swelling with small vacuoles (representing lipids washed out by alcohol during staining) = 1.0 point; many small vacuoles = 2.0 points; medium-sized vacuoles = 3.0 points; large vacuoles = 4.0 points; and appearance of stamp cells (hepatocellular cells that contain a large volume of lipid to the extent that cell contour is altered and nuclei are displaced) = 5.0 points. The most substantial lesion was used to assign the score for each region of the hepatic lobule. For example, if in 1 region large vacuoles were observed in some cells but small vacuoles and cloudy swelling were observed in other cells, the score would be 4.0 points. Points were summed, and a score for the lobule was obtained. For every specimen, 5 lobules were scored and the mean score was determined. From mean scores, each cow was classified as having 1 of 6 degrees of fatty liver (GdL 0 to 5) according to the following index: GdL 0 = 0 points; GdL 1 = 0.5 to 1.0 points; GdL 2 = 1.5 to 4.5 points; GdL 3 = 5.0 to 7.0 points; GdL 4 = 7.5 to 9.5 points; and GdL 5 = 10 points.

In 3 of the 68 cows, biopsy specimens did not contain 5 intact lobules for evaluation; in those instances, the evaluation was performed in 10 lobule parts. In each such tissue specimen, there was a mean ± SD of 6 ± 2.9 centrilobular regions and 12.2 ± 4.2 portal triad regions that could be evaluated histologically.

Groups—Cows were allocated among groups on the basis of the histologically determined GdL degree of fatty liver. No cows were classified in the GdL 0 or GdL 1 groups (ie, none had histologically visible lipids). Classification of GdL were used to describe the severity of fatty liver as follows: GdL 2 = mild fatty liver; GdL 3 = moderate fatty liver; GdL 4 = moderate-to-severe fatty liver; and GdL 5 = severe fatty liver. All cows that had no clinical improvement after surgical correction of the LDA and died within 4 weeks after surgery were classified in the GdL 5 group. Therefore, study cows were divided into the 5 groups according to GdL and the course of postsurgical convalescence as follows: GdL 2, GdL 3, GdL 4, GdL 5 that eventually recovered (GdL 5-recovered), and GdL 5 that eventually died (GdL 5-died).
Reference intervals—To obtain reference intervals for the biochemical variables tested, blood and liver samples were obtained from 110 healthy Holstein cows older than 2.5 years. The source was the same 28 dairy farms, which had an overall population of 2,950 dairy cattle. Cows were selected if they had no history of disease during the current milking period and were healthy at the time of sampling (liver biopsy specimens were obtained transectually) and there was an absence of histologically visible fat in liver tissue (ie, GdL < 2) as revealed later in histologic analysis of the biopsy specimen. All variables were measured via the same methods as the study cows. Samples were collected from 8:00 to 10:00 AM, after food had been withheld for 8 hours. For defining the reference interval, the 2.5 and 97.5 percentiles of those values were used as the lower and upper limit, respectively.

Statistical analysis—Analyses were performed by use of a software program. The homogeneity of variances was tested with the Levene test, and normality of distribution was evaluated by use of the Kolmogorov-Smirnov test. Differences in results among groups of cows, as defined by degree of GdL, were determined with the Kruskall-Wallis 1-way ANOVA. When significant differences among groups were observed, the Mann-Whitney means comparison test was used to identify which groups were different. The Spearman rank bivariate correlation was used to investigate the relationship between variables. For all comparisons, values of P < 0.05 were considered significant.

Results
Histologic evaluation—Liver tissues from all 68 cows contained at least some lipid (visible as vacuoles under light microscopy), indicating a classification of GdL > 1. The 68 cows were grouped as follows: GdL 2, n = 4 (including 4 first-calf heifers); GdL 3, 10 (including 4 first-calf heifers); GdL 4, 21 (including 9 first-calf heifers). Of cows in the GdL 5 group, 20 recovered (5 were first-calf heifers) and 21 died (5 were first-calf heifers) within 4 weeks after surgery (Table 1).

Table 1—Mean ± SE (range) values for serum, liver, and clinical variables by GdL group in 68 lactating Holstein cows with LDA and various degrees of fatty liver.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference interval*</th>
<th>GdL 2 (n = 4)</th>
<th>GdL 3 (10)</th>
<th>GdL 4 (13)</th>
<th>GdL 5-recovered (20)</th>
<th>GdL 5-died (21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGT (U/L)</td>
<td>11–26</td>
<td>21.50 ± 3.90 (17.5–32.0)</td>
<td>15.33 ± 1.73 (7.5–28.6)</td>
<td>23.23 ± 2.78 (13.0–45.5)</td>
<td>21.00 ± 2.69 (9.4–43.6)</td>
<td>38.26 ± 3.90 (16.0–91.0)</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>23.3–78.9</td>
<td>60.3 ± 4.02 (49–67)</td>
<td>67.85 ± 4.2 (41–92)</td>
<td>79.72 ± 7.34 (44–147)</td>
<td>109.52 ± 5.01 (40–342)</td>
<td>191.87 ± 71.17 (92–386)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>13–54</td>
<td>17.50 ± 6.34 (8–32)</td>
<td>32.80 ± 4.86 (15–61)</td>
<td>37.26 ± 4.13 (10–61)</td>
<td>45.69 ± 4.28 (16–90)</td>
<td>66.88 ± 8.09 (10–165)</td>
</tr>
<tr>
<td>SDH (U/L)</td>
<td>10–23.6</td>
<td>18.28 ± 5.16 (8–32)</td>
<td>12.03 ± 1.70 (8–24)</td>
<td>30.95 ± 5.20 (13–218)</td>
<td>18.64 ± 1.52 (8–32)</td>
<td>26.76 ± 3.50 (12–59)</td>
</tr>
<tr>
<td>GDH (U/L)</td>
<td>0.3–6.9</td>
<td>3.03 ± 0.92 (2.2–3.8)</td>
<td>4.92 ± 0.55 (2.5–7.5)</td>
<td>7.46 ± 0.63 (4.7–13.4)</td>
<td>9.83 ± 0.84 (3.4–18.4)</td>
<td>12.18 ± 1.22 (2.2–24.5)</td>
</tr>
<tr>
<td>OCT (U/L)</td>
<td>2.2–23.6</td>
<td>17.03 ± 2.88 (9.2–23.2)</td>
<td>21.31 ± 2.76 (9.7–38.6)</td>
<td>34.20 ± 2.56 (20–53.7)</td>
<td>47.01 ± 3.54 (24.3–79)</td>
<td>68.06 ± 4.85 (27.7–97.8)</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>55.5–140</td>
<td>82.28 ± 4.56 (68.5–81)</td>
<td>80.99 ± 6.99 (53–116)</td>
<td>70.86 ± 6.72 (37–121)</td>
<td>83.76 ± 7.98 (41–190)</td>
<td>63.04 ± 3.60 (40–98)</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.0–5.1</td>
<td>3.35 ± 0.50 (2.9–4.0)</td>
<td>3.30 ± 0.18 (2.4–3.8)</td>
<td>3.17 ± 0.11 (2.4–4.2)</td>
<td>3.21 ± 0.13 (2.9–4.2)</td>
<td>2.97 ± 0.09 (2.0–4.2)</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>0.5–12.5</td>
<td>17.25 ± 1.44 (14–21)</td>
<td>15.60 ± 1.54 (8–32)</td>
<td>23.92 ± 2.65 (16–46)</td>
<td>22.00 ± 1.19 (9–65)</td>
<td>27.95 ± 6.25 (7–121)</td>
</tr>
<tr>
<td>SLIPO (mg/g)</td>
<td>8.56–22.17</td>
<td>168.58 ± 37.09 (81.2–322)</td>
<td>216.05 ± 11.99 (169–240)</td>
<td>379.78 ± 15.88 (98–432)</td>
<td>311.52 ± 11.85 (98–410)</td>
<td>352.46 ± 9.52 (220–480)</td>
</tr>
<tr>
<td>TG (mg/g)</td>
<td>6.4–24.7</td>
<td>17.38 ± 0.37 (16.8–18.2)</td>
<td>27.77 ± 1.47 (18.3–39.1)</td>
<td>40.41 ± 4.55 (27–88.9)</td>
<td>75.15 ± 7.12 (81–137)</td>
<td>114.24 ± 8.89 (47–219)</td>
</tr>
<tr>
<td>Age (m)</td>
<td>2.5–10.2</td>
<td>4.00</td>
<td>3.60 ± 0.54 (2–7)</td>
<td>4.05 ± 0.52 (2–7)</td>
<td>3.70 ± 0.38 (2–9)</td>
<td>4.95 ± 0.49 (2–9)</td>
</tr>
<tr>
<td>Body score</td>
<td>2.4</td>
<td>2.75 ± 0.25 (2–3)</td>
<td>2.50 ± 0.18 (2–3)</td>
<td>3.00 ± 0.17 (2.5–4)</td>
<td>2.50 ± 0.12 (3.5–2.5)</td>
<td>2.20 ± 0.19 (1.5–4)</td>
</tr>
</tbody>
</table>

*Reference intervals represent values in the 2.5 to 97.5 percentiles.
**Within a row, different superscripts denote significantly (P < 0.05) different values among groups.

Figure 1—Photomicrograph of liver biopsy specimen revealing fat accumulation in small droplets in the cytoplasm of most hepatocytes in a cow with moderate fatty liver (GdL 3). Notice that cell nuclei are not displaced. CV = Central vein. PT = Periporal triad. H&E stain; bar = 100 µm.

In the 4 cows with mild fatty liver (GdL 2), small vacuoles were visible around the central vein but no other lesions were observed. In cows with moderate fatty liver (GdL 3), small fat vacuoles and lipid droplets were apparent in all areas of the hepatic lobule; ≥ 1 small vacuole was detected in the hepatocyte cytoplasm, but there was no alteration in cellular morphology or displacement of the cell nuclei (Figure 1). In cows with moderate-to-severe fatty liver (GdL 4), hepatic cells with large vacuoles were detected only around the central vein. In more peripheral areas of the lobule, hepatocytes with ≥ 1 small vacuole were observed. The large vacuoles in the cells around central veins were likely the result of fusion of smaller
droplets. Cell nuclei were displaced by fat to the periphery, and a few cells were distorted (Figure 2).

In cows with severe fatty liver (GdL 5), there was panlobular distribution of large lipid drops. All hepatocytes had vacuoles of various sizes. Larger lipid drops were observed primarily in cells around the centrallobular vein. In those cells, cell contour was altered and nuclei were displaced, creating so-called stamp or signet-ring-shaped cells; however, few nuclei were distorted (Figures 3 and 4). In 4 cows, small foci of parenchymal necrosis with polymorphonuclear cell infiltration were noticed. Among the cows that died were some with severe fatty liver with hepatic tissue that was heavily infiltrated with lipids to the extent that it resembled adipose tissue. In those cows, biopsy specimens were macroscopically whitish in appearance and of friable consistency.

Liver biochemical analysis—Total lipid and triglyceride concentrations in hepatic tissue increased in proportion to the severity of fatty liver and parallel with increasing GdL ($P < 0.05$; Table 1). Especially in the GdL 5 group, triglyceride concentrations in cows that died were significantly ($P < 0.05$) higher than concentrations in those that recovered. A strong correlation ($r = 0.801; P = 0.01$) between GdL and triglyceride content was detected, whereas a weaker but satisfactory correlation ($r = 0.586; P = 0.01$) was observed between GdL and total lipid concentration (Table 2). As expected, total lipid and triglyceride content were also satisfactorily correlated ($r = 0.598; P = 0.01$).

Left-displaced abomasum was diagnosed in all cows during the first 5 weeks after calving. Some cows (6/54 [11%]) with moderate-to-severe and severe fatty liver were detected in the fifth postpartum week, but 82% (36/68) of all cases arose within the first 4 weeks after the beginning of lactation. Left-displaced abomasum was most frequently (25/68 [31%]) diagnosed during the second postpartum week, which was also when most of the cows with severe fatty liver were noticed (16 cows in the GdL 5 group and 1 cow in the GdL 4 group). All 9 cows with an LDA in the first week after calving already had severe fatty liver.

Serum biochemical analysis—Serum OCT activity was significantly higher in cows with moderate-to-severe and severe fatty liver and with GdL of 4 and 5, and activities were higher than the upper reference limit even in those with mild or moderate fatty liver (Table 1). Especially in cows in the GdL 5 group, OCT activities in cows that died were significantly ($P < 0.05$) higher than in those that recovered. Serum OCT activity increased proportionate to liver lipid content and was significantly higher in cows with severe fatty liver. Strong correlations between OCT activity and total lipid concentration ($r = 0.694; P = 0.01$), triglyceride concentration ($r = 0.647; P = 0.01$), and GdL ($r = 0.752; P = 0.01$; Table 2) were detected. Correlations between OCT and GDH, AST, ALT, and GGT activities and total bilirubin concentration were also detected. In cows with severe fatty liver that died, serum OCT activity was positively correlated with ALP activity and serum bile acids concentration and negatively correlated with BUN concentration, whereas the correlation with GGT activity was even stronger (Table 3).
Serum AST activity in cows in the GdL 5 group was significantly higher than in cows in other GdL groups. Serum enzyme activities were very high in cows that died. Serum AST activity was > 250 U/L in several cows and as high as 850 U/L in 1 cow, resulting in a significantly different mean value (191.97 U/L) in cows in that group, compared with the mean (106.52 U/L) in cows in the GdL 5-died group. Strong correlations were detected between AST activity and triglyceride and total lipid concentrations; GdL; and ALT, GDH, and OCT activities, and weaker correlations were detected between AST activity and GGT activity and with β-HB and total bilirubin concentrations. Serum ALT activity was increased proportionate to increases in liver lipid concentration.

Serum GDH activity in cows with mild and moderate disease was significantly higher than in cows without disease, and the increases in GDH activity were proportionate to the increase in liver lipid content. Differences between GdL degrees were significant, with the highest values detected in cows with severe fatty liver (Table 1). Serum GDH activity was correlated with serum AST and OCT activities, triglyceride and total lipid concentrations, and GdL (Table 2).

Many cows had significantly high values for serum SDH activity, but increases were not proportionate to the degree of fatty liver because high values were obtained in cows with mild fatty liver and in those with severe fatty liver. Values for SDH activity were correlated with OCT and GGT activity and with β-HB and total lipid concentrations (Table 2).

No association between serum ALP activity and liver lipid content was detected, and only a weak correlation between ALP activity and GGT activity was observed (r = 0.343; P = 0.01; Table 2). The correlation between serum activities of ALP and GGT was stronger (r = 0.687; P = 0.01) and AST (r = 0.616; P = 0.01) activities were also detected. The correlations between GGT activity and OCT activity (r = 0.616; P = 0.01; Table 2) and serum bilirubin concentrations (r = 0.500; P = 0.01) were stronger in the cows in the GdL 5-died group. In general, serum GGT activity was significantly higher in cows with severe fatty liver that died and was strongly correlated with OCT, AST, and SDH activities and β-HB concentration.

Serum bile acids concentration did not increase in proportion to the increase in GdL (Table 1). In cows in which total lipid concentration was very high (such as in the GdL 5-died group), markedly high bile acids concentrations were detected (maximum, 263.5 µmol/L) along with very low values (minimum, 9 µmol/L). In the same group, serum bile acids concentrations were correlated with OCT, GDH, and GGT activities (Table 3). In all cows, weaker correlations were detected with serum NEFA (r = 0.277; P = 0.05) and OCT (r = 0.442; P = 0.01) concentrations.

Serum total bilirubin concentration was significantly high only in cows with severe fatty liver, in which mean values were > 1 mg/dL (Table 1). Serum total bilirubin concentration was correlated with triglyceride concentration (r = 0.516; P = 0.01) and GdL (r = 0.530; P = 0.05) and was weakly correlated with total lipid concentration (r = 0.450; P = 0.01) and OCT (r = 0.443; P = 0.01) and AST (r = 0.440; P = 0.01) activities (Table 2).

Compared with reference values, serum NEFA concentration was high in all cows, but was consis-

---

**Table 2**—Spearman correlation coefficients (r) for serum, liver, and clinical variables in the same 68 cattle with fatty liver and LDA as in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>AST</th>
<th>OCT</th>
<th>GDH</th>
<th>ALT</th>
<th>SDH</th>
<th>GGT</th>
<th>ALP</th>
<th>tBIL</th>
<th>SBA</th>
<th>NEFA</th>
<th>β-HB</th>
<th>tLPD</th>
<th>TG</th>
<th>GdL</th>
<th>Glucose</th>
<th>Albumin</th>
<th>BUN</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCT</td>
<td>1</td>
<td>0.68*</td>
<td>0.63*</td>
<td>0.52*</td>
<td>0.50*</td>
<td>0.44*</td>
<td>0.41*</td>
<td>0.40*</td>
<td>0.36*</td>
<td>0.35*</td>
<td>0.34*</td>
<td>0.33*</td>
<td>0.34*</td>
<td>0.34*</td>
<td>0.33*</td>
<td>0.34*</td>
<td>0.34*</td>
</tr>
<tr>
<td>SDH</td>
<td>0.68*</td>
<td>1</td>
<td>0.59*</td>
<td>0.41*</td>
<td>0.40*</td>
<td>0.35*</td>
<td>0.34*</td>
<td>0.33*</td>
<td>0.32*</td>
<td>0.31*</td>
<td>0.30*</td>
<td>0.30*</td>
<td>0.30*</td>
<td>0.30*</td>
<td>0.30*</td>
<td>0.30*</td>
<td>0.30*</td>
</tr>
<tr>
<td>GGT</td>
<td>0.50*</td>
<td>0.59*</td>
<td>1</td>
<td>0.45*</td>
<td>0.44*</td>
<td>0.43*</td>
<td>0.42*</td>
<td>0.41*</td>
<td>0.40*</td>
<td>0.39*</td>
<td>0.38*</td>
<td>0.38*</td>
<td>0.38*</td>
<td>0.38*</td>
<td>0.38*</td>
<td>0.38*</td>
<td>0.38*</td>
</tr>
<tr>
<td>ALP</td>
<td>0.44*</td>
<td>0.41*</td>
<td>0.44*</td>
<td>1</td>
<td>0.44*</td>
<td>0.44*</td>
<td>0.44*</td>
<td>0.44*</td>
<td>0.44*</td>
<td>0.44*</td>
<td>0.44*</td>
<td>0.44*</td>
<td>0.44*</td>
<td>0.44*</td>
<td>0.44*</td>
<td>0.44*</td>
<td>0.44*</td>
</tr>
<tr>
<td>tBIL</td>
<td>0.40*</td>
<td>0.36*</td>
<td>0.44*</td>
<td>0.44*</td>
<td>1</td>
<td>0.44*</td>
<td>0.44*</td>
<td>0.44*</td>
<td>0.44*</td>
<td>0.44*</td>
<td>0.44*</td>
<td>0.44*</td>
<td>0.44*</td>
<td>0.44*</td>
<td>0.44*</td>
<td>0.44*</td>
<td>0.44*</td>
</tr>
<tr>
<td>SBA</td>
<td>0.36*</td>
<td>0.34*</td>
<td>0.34*</td>
<td>0.34*</td>
<td>0.34*</td>
<td>1</td>
<td>0.40*</td>
<td>0.40*</td>
<td>0.40*</td>
<td>0.40*</td>
<td>0.40*</td>
<td>0.40*</td>
<td>0.40*</td>
<td>0.40*</td>
<td>0.40*</td>
<td>0.40*</td>
<td>0.40*</td>
</tr>
<tr>
<td>NEFA</td>
<td>0.35*</td>
<td>0.34*</td>
<td>0.34*</td>
<td>0.34*</td>
<td>0.34*</td>
<td>0.34*</td>
<td>1</td>
<td>0.34*</td>
<td>0.34*</td>
<td>0.34*</td>
<td>0.34*</td>
<td>0.34*</td>
<td>0.34*</td>
<td>0.34*</td>
<td>0.34*</td>
<td>0.34*</td>
<td>0.34*</td>
</tr>
<tr>
<td>β-HB</td>
<td>0.43*</td>
<td>0.43*</td>
<td>0.43*</td>
<td>0.43*</td>
<td>0.43*</td>
<td>0.43*</td>
<td>0.43*</td>
<td>1</td>
<td>0.43*</td>
<td>0.43*</td>
<td>0.43*</td>
<td>0.43*</td>
<td>0.43*</td>
<td>0.43*</td>
<td>0.43*</td>
<td>0.43*</td>
<td>0.43*</td>
</tr>
</tbody>
</table>

See Table 1 for remainder of key.

*Correlation was significant (P = 0.01). †Correlation was significant (P < 0.05).
tently > 1 mmol/L in those that died with severe fatty liver (Table 1). Serum NEFA concentration was positively correlated with β-HB ($r = 0.514; P = 0.01$) and total bilirubin ($r = 0.448; P = 0.01$) concentrations and negatively correlated with glucose concentration ($r = -0.469; P = 0.01$). Serum glucose concentration was not significantly different among the degrees of GdL, and hypoglycemia (serum glucose concentration < 40 mg/dL) was recorded only in animals with severe fatty liver. Generally, glucose concentration was within the reference interval and was negatively correlated with total bilirubin ($r = -0.466; P = 0.01$), NEFA ($r = -0.469; P = 0.01$), and β-HB ($r = -0.481; P = 0.01$) concentrations (Table 2). The β-HB concentration increased in proportion to GdL, with higher values measured in the GdL 5-died group, which had concentrations > 1.19 mmol/L. Correlations between β-HB concentration and GGT activity and total bilirubin and NEFA concentrations were strong.

**Discussion**

One of our study aims was to investigate the occurrence and severity of fatty liver in cows with LDA. Results indicated that 13.2% of the cows studied had severe fatty liver even in the first week after parturition, indicating that fatty infiltration of the liver had commenced prior to parturition and that mobilization of body reserves had been initiated at least 3 weeks before calving, a finding that has been reported before. In the present study, nearly half (25/54 [46%]) of the cows with moderate-to-severe and severe fatty liver (ie, cows in the GdL 4 and GdL 5 groups) developed LDA in the first 2 weeks after calving, a finding that was in accordance with earlier observations that 25% of dairy cows have some degree of fatty liver during the first 2 weeks after calving and that severe fatty liver can develop in cows with LDA. Most cows with severe fatty liver were detected in the first 4 weeks of lactation (when LDA is also most frequently diagnosed) because excessive lipomobilization and liver fat accumulation are favored by hormonal and metabolic factors during that period. Therefore, the risk of severe fatty liver in cows with LDA decreases as lactation progresses.

Results also indicated that a proportion of the cattle with LDA were first-call heifers, a finding that was in accordance with earlier observations. Among heifers, 9 had severe fatty liver, which was similar to previous findings. Moreover, 4 of those 9 heifers died, supporting the conclusion that first-call heifers with LDA are also vulnerable to severe fatty infiltration of the liver. Apart from the heifers, most LDAs were diagnosed in cows 3 to 7 years of age, another finding supported by previous observations. On the basis of fatty liver severity, age was not significantly different among the various groups, but cows with severe fatty liver that did not recover had a mean age of 4.95 years. This may be because liver metabolism in cows that have had 2 or 3 lactations is already affected and fatty liver is more common.

Most cows with severe fatty liver that eventually died had a body condition score of 1.5 to 2, indicating that cows with less stored body fat had a less favorable postsurgical prognosis. A possible explanation for this could be that advanced lipomobilization in those cows developed before displacement of the abomasum, so that by the time LDA was detected, the liver was already heavily infiltrated by fat and body energy reserves were depleted. The development of LDA and consequent appetite loss aggravates severe fatty liver, which in turn causes continued poor appetite after the operation and leads ultimately to liver failure and death. In the present study, liver failure was regarded as the primary cause of death in cows that did not recover because all were in the severe fatty liver group, confirming that the course of postoperative convalescence in cows with LDA is related to the extent and severity of fatty liver.

In dairy cows, lipid accumulates in the liver chiefly in the form of triglyceride. In the present study, liver triglyceride concentration increased significantly with increasing GdL degree, and many of the cows that did not survive had markedly high concentrations of triglyceride. In some instances, triglyceride concentrations were > 7 times the reference values. In the GdL 5 group, the significantly higher concentrations of triglyceride in cows that died, compared with those in cows that recovered, emphasized the influence of fatty liver in the recovery of cows with LDA. The differences in triglyceride concentration in the cows with severe fatty liver were attributed to the fact that the histologic classification scheme used in this study is a semiquantitative scale that, despite the advantage of having a basis in histologic findings, cannot differentiate among various gradations of high triglyceride concentrations.

Accumulation of triglyceride in the cytoplasm of hepatocytes causes disturbances in hepatic structure and function that are likely to have particular clinical importance when they coexist with aggravating factors such as LDA. Enzyme leakage from hepatocytes is 1 manifestation of these disturbances.

Results indicated that OCT, a mitochondrial enzyme, was useful for detection of even mild fatty liver and differentiation of various Gdls. Most cows with fatty liver, whether mild, moderate, or severe, also had high serum OCT activity, likely as a result of lipid accumulation in hepatocytes that caused dilatation and dysfunction of mitochondria. Although not widely studied, this enzyme is considered to be useful in the diagnosis of fatty liver, especially when the condition is severe, and may have even more importance given the correlation with AST activity. The significantly higher serum activity in cows with fatty liver, as well as the high maximum values in cows that did not recover after corrective LDA surgery, strengthens support for the diagnostic value of OCT for fatty liver and makes it useful prognostically for the postoperative convalescence of affected cows.

Serum OCT activity was determined by use of a described method, which was a modification of the method Oshtita et al adapted to bovine serum. Results indicated that determination of serum OCT activity via this method was sensitive and reliable. The wide range of values and increased serum activity of OCT in cows with severe liver damage enabled evaluation of this enzyme for differentiating among different Gdls. The fact that small increases in OCT activity accurately reflected mild fatty liver suggests that OCT
activity may be a reliable index for predicting postoperative outcome in cows undergoing corrective surgery for LDA.

Determination of serum AST activity has diagnostic value in cows with LDA. It is known that AST activity is high in cows with LDA, a finding attributed to concurrent liver damage. However, serum AST activity has been reported to have predictive value for consequent LDA occurrence. Although serum AST activity is high in most cows after parturition, in cows in the present study, a significant increase in activity proportionate to the increase in liver triglyceride and serum total lipid concentrations was evident, with extremely high values (850 U/L) detected in severely affected cows that died after surgery. The strong correlation between serum AST activity and triglyceride and total lipid concentrations and with GdL suggests that determination of serum AST activity may be useful for diagnosis of fatty liver. The fact that AST activity was correlated with OCT, GDH, and ALT activities and total bilirubin concentration may add to its diagnostic usefulness.

Increases in serum GDH activity also paralleled increases in GdL, allowing differentiation of fatty liver into degrees of severity. Serum GDH activity is a preferred indicator for use in detecting liver cell necrosis. In the present study, GDH activity was useful in detection of liver damage caused by lipid accumulation, and the magnitude of increase in values was correlated with the degree of fatty liver infiltration, especially in cows with mild to moderate fatty liver. The fact that mildly high serum GDH activity reflected mild fatty liver might be used to predict a positive postoperative outcome in cows that undergo LDA surgery. Serum GDH activity does not typically change in association with calving, so detection of high activity may be of diagnostic importance. The correlation between serum GDH activity and hepatic fat infiltration, as well as the correlation between serum OCT and AST activities, was indicative of the type of hepatocellular damage induced by fat accumulation. These findings were in agreement with those from some studies but in contrast with those of others.

Sorbitol dehydrogenase is another enzyme that is used as an indicator of acute hepatocellular damage. In the present study, serum SDH activity was significantly higher in cows with severe liver damage that did not recover. High values were also recorded in cows with lower liver fat content, and the correlation between serum SDH activity and triglyceride content was not strong. The correlation of serum SDH activity with serum OCT activity and with total lipid concentration confirmed that SDH activity increased with liver damage caused by fat accumulation, an observation that has been reported. It is noteworthy, however, that serum SDH activity did not reflect the degree of fatty liver change.

In the cholestasis-indicating enzymes ALP and GGT, no difference in serum ALP activity among GdL degrees was detected, indicating that this analyte is not useful for diagnosis of fatty liver in cows with LDA. However, serum GGT activity was significantly higher in cows with severe fatty liver that did not recover. Significant correlations existed between GGT activity and triglyceride and total lipid concentrations and between GGT activity and OCT and AST activities, with the latter 2 being higher in cows that did not recover. Those correlations confirmed that in cows with severe fatty liver, intracellular accumulation of lipid and the consequent pressure that results cause obstruction of bile ducts or biliary epithelium. We concluded that high serum GGT activity was correlated with severe fatty liver and a grave postsurgical prognosis in cows with LDA.

In the present study, serum ALT activity, which is not considered to be specific for liver damage in ruminants and thus is not often measured, was increased with increasing degrees of GdL, a finding that was in contrast to findings from another report. Serum ALT activity was, however, strongly correlated with AST, the diagnostic value of which is greater; this finding has also been reported.

Data from the present study indicate that high serum bile acids concentration was not an indicator of fatty liver. A possible explanation may be variation in serum bile acids concentration in cows according to time of day, interindividual differences, stage of lactation, milk yield, time since last feeding, and age. Another explanation could be overlap among groups with different degrees of fatty liver. In the present study, even in cows with low liver triglyceride concentration, bile acids concentrations as high as 120 μmol/L were recorded, whereas values as low as 9 μmol/L were detected in cows with severe fatty liver that died. As a consequence, the reference range of values for serum bile acids (5 to 73 μmol/L) was wide and was calculated from values for the 110 healthy cows that were older than 2.5 years of age and from which food had been withheld for approximately 8 hours. As was evident from the data, although all cows with LDA were inappetant, the fluctuation in serum bile acids concentration was not dampened, even in cows in a severe state of inappetence and energy deficit as indicated by serum NEFA and β-HB concentrations. In the present study, serum bile acids concentrations as high as 263 U/L were recorded. These extreme values were only detected in cows with severe fatty liver that did not recover, but in that state, liver damage is already severe. Consequently, determination of serum bile acids concentration may aid in diagnosis of fatty liver but is of limited value because there are more sensitive indicators of liver damage, such as OCT and GDH activity. In contrast to other reports, no significant correlation was found between serum bile acids concentration and enzymes that assess liver function except for GGT; and that correlation was significant only in the 21 cows that died. Furthermore, our results indicated that there was no significant correlation between serum bile acids concentration and the degree of fatty liver, a finding that was in accordance with those of Garry et al. Therefore, our results do not support use of serum bile acids concentration for diagnosis of fatty liver, unlike recommendations made in other reports, or for rendering a postoperative prognosis in cattle with LDA.

Another important finding in the present study was the usefulness of serum total bilirubin determination in diagnosis of fatty liver in cows with LDA. Serum bilirubin values were significantly higher in cows with severe fatty liver than in control cows; however, the increase in liver triglyceride and serum total lipid concentrations was not considered to be specific for liver damage in ruminants and thus is not often measured, was increased with increasing degrees of GdL, a finding that was in contrast to findings from another report. Serum ALT activity was, however, strongly correlated with AST, the diagnostic value of which is greater; this finding has also been reported.
total bilirubin concentration is typically high in cows with LDA because serum total bilirubin concentrations increase during periods of anorexia and often increase after calving. Therefore, serum total bilirubin concentration would be expected to be higher in cows with LDA and concurrent liver damage from fatty liver. In the present study, serum total bilirubin concentration was significantly higher in cows with severe fatty liver, especially in those that died, in which nearly all had concentrations > 1 mg/dL. Moreover, the strong correlations between total bilirubin and triglyceride concentration and GdL may make total bilirubin determination useful in diagnosis of fatty liver, especially if the condition is severe; in that instance, substantial increases in values are considered to be a poor prognostic sign.

Cows with LDA often also have fatty liver and ketosis. In cows with LDA, inappetence results in energy deficit and decreased availability of gluconeogenic precursors; as a consequence, intrahepatic metabolism of NEFAs shifts to favor production of ketones and reesterification of NEFAs to triglycerides. Ketosis develops and lipomobilization becomes more intense with increased NEFA concentration, which leads to fatty liver. In the present study, all cows that did not recover had β-HB concentrations > 1.1 mmol/L, indicating clinically important ketosis. Cows classified as GdL 5 had significantly higher serum NEFA concentrations. Moreover, correlations between β-HB and serum concentrations of NEFAs, total lipid, triglyceride, and total bilirubin and between β-HB and serum activities of GGT, OCT, and AST support usefulness of β-HB determination in diagnosis of fatty liver in cows with LDA. In addition, some investigators have proposed that β-HB concentration can be used as a prognostic indicator for subsequent occurrence of LDA. The complication of LDA by high serum β-HB concentrations in cows in the present study supported the fact that both ketosis and LDA are associated with fatty liver, making diagnosis of fatty liver in cows with LDA more important.

It has been reported that glucose concentrations decrease in the first month of lactation. Although ketosis is associated with hypoglycemia and serum glucose concentration reportedly decreases as liver triglyceride increases, the finding of hypoglycemia was sporadic, even in cows that died and had severe fatty liver and high β-HB concentrations. The fact that cows with LDA, despite possible concurrent ketosis, had glucose concentrations within the reference interval has been observed by many researchers and may be a result of insulin resistance. Glucose concentration was not diagnostically useful in evaluating fatty liver in cows with LDA in the present study.

The postsurgical outcome of cows with LDA was related to the extent of fatty liver. In all cows with LDA, some degree of fatty liver developed, and all cows that died had severe fatty liver. Because no single serum biochemical variable serves as an absolute indicator of fatty liver, a combination of variables is necessary for diagnosis of the condition. Assessment of the activity of enzymes in descending order of OCT, AST, and GDH and total bilirubin concentration is recommended for diagnosis of fatty liver in dairy cows with LDA. The higher sensitivity of OCT activity, in addition to the specificity for detection of hepatocellular damage, supports determination of this enzyme for classifying various degrees of fatty liver and for predicting the course of postsurgical convalescence related to fatty liver.

References
20. Scaniszlo F, Kasrai F. Cholsäurespiegel im Blutserum von...