

Original Research Paper

# Serum Ornithine Carbamoyltransferase Activity and Correlation with Fatty Liver in Dairy Cows with Displaced Abomasum

<sup>1</sup>Ken Onda, <sup>1</sup>Reiichiro Sato, <sup>2</sup>Yosuke Sasaki, <sup>1</sup>Hiroo Madarame, <sup>1</sup>Hideharu Ochiai, <sup>1</sup>Kazuhiro Kawai and <sup>1</sup>Fujiko Sunaga

<sup>1</sup>Azabu University, School of Veterinary Medicine, 17-71 Fuchinobe 1-Chome, Chuo-ku, Sagamihara 229-8501, Japan

<sup>2</sup>Organization for Promotion of Tenure Track,

University of Miyazaki, 1-1 Gakuen Kibanadainishi, Miyazaki 889-2192, Japan

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## Corresponding Author:

Ken Onda

Azabu University, School of Veterinary Medicine, 17-71 Fuchinobe 1-Chome, Chuo-ku, Sagamihara 229-8501, Japan  
Tel: +81-42-754-7111 (ext. 658)18  
Fax: +81-42-850-2464  
Email: onda@azabu-u.ac.jp

**Abstract:** Ornithine Carbamoyltransferase (OCT) is a hepatocyte-specific enzyme; elevated serum activity indicates liver damage. Displaced abomasum frequently occurs around the time of parturition in dairy cattle and is associated with fatty liver. We analyzed serum OCT activities to investigate the relationship between displaced abomasum and fatty liver. Lactating Holstein cows, 75 clinically healthy and 22 with displaced abomasum, were used. Serum OCT activities were compared between healthy cows and those with displaced abomasum. Liver biopsies of cows with displaced abomasum were classified by the severity of fat accumulation. Blood samples were collected preoperatively and at 4 and 8 days after surgery and analyzed for serum OCT, aspartate aminotransferase, alkaline phosphatase,  $\gamma$ -glutamyltransferase, urea nitrogen, total protein and hematocrit. Serum OCT activity was significantly higher in cows with displaced abomasum than in healthy cows. The degree of fatty liver in cows with displaced abomasum was classified as mild (13 cows), moderate (5 cows), or severe (4 cows). Statistical analysis revealed no significant differences in blood test results among cows with different degrees of fatty liver. However, our results indicated significant differences in serum OCT, aspartate aminotransferase, urea nitrogen, total protein and hematocrit when stratified by the number of days after surgical treatment for displaced abomasum. Serum OCT activity is increased in dairy cows with displaced abomasum and the influence of the degree of fatty liver on serum OCT activity is unclear. Therefore, OCT should be reconsidered as a marker of fatty liver in cows around the time of parturition, when displaced abomasum frequently occurs. Fatty liver should be evaluated by various clinical parameters, not by one hepatic enzyme.

**Keywords:** Dehydration, Hepatic Enzyme, Metabolic Disorders, Periparturient Disease

## Introduction

Fatty Liver (FL) frequently occurs around the time of parturition in dairy cows because of an increase in blood non-esterified fatty acids caused by negative energy balance (White, 2015). A mild grade of fat infiltration into the liver is expected even in healthy cows after calving; however, liver disorders including

FL affect cows' health and inhibit milk production and reproduction (Bobe *et al.*, 2004). Displaced Abomasum (DA), another common periparturient disease, may coincide with FL and is associated with pathogenic factors including negative energy balance, hypocalcemia, metritis and nutritional factors (Esposito *et al.*, 2014). Accurate diagnosis, treatment and assessment of the prognosis of FL may prevent

decreased milk production and reproductive difficulties (Farid *et al.*, 2013).

Diagnosis of FL in cows may be difficult. Liver biopsy is the most reliable diagnostic method but requires surgical preparation and is somewhat invasive. Ultrasound examination has recently been widely employed but does not provide a quantitative evaluation of liver function (Haudum *et al.*, 2011). For this reason, blood testing is often used in the field. Recently, Kalaitzakis *et al.* (2006; 2007) reported that serum levels of Ornithine Carbamoyltransferase (OCT), Aspartate Aminotransferase (AST) and total bilirubin concentration may be used to diagnose FL and evaluate the degree of fat infiltration into the liver. Serum OCT and iditol (sorbitol) dehydrogenase can also differentiate healthy cows from cows with FL and severe ketosis (Gröhn *et al.*, 1983).

OCT, which is expressed almost exclusively in hepatocellular mitochondria, is a liver-specific marker. The serum level of this enzyme is increased in patients with hepatic disorders including hepatitis, non-alcoholic steatohepatitis and carcinoma (Maekawa, 1995). The OCT level differs among patients with different chronic liver diseases and it is a useful surrogate marker of cirrhosis (Matsushita *et al.*, 2014). In humans with FL, OCT levels are reportedly in or slightly higher than the reference range (Watanabe *et al.*, 1994-1995). In dairy cows, the results of blood tests performed prior to surgical repair of DA could be influenced by dehydration, metabolic alkalosis and hypokalemia secondary to DA. Analysis of blood examinations performed around the time of parturition in dairy cows must also take into consideration the significant changes produced by calving and the initiation of lactation.

In the present study, we collected blood samples from cows with DA prior to surgical repair and 4 and 8 days postoperatively. We evaluated the usefulness of blood tests, including serum OCT activity, for distinguishing the degrees of FL.

## Materials and Methods

### Animals

Twenty-two lactating Holstein cows with DA (14 left-sided and 8 right-sided) were hospitalized at the Veterinary Teaching Hospital of Azabu University, School of Veterinary Medicine, from September 1998 through April 2000. None of the cows with DA had concurrent diseases such as metritis or milk fever. All cows recovered fully after the operations and finally left the hospital. The cows with DA ranged in age from 2 years 2 months to 9 years 1 month. Their number of days post parturition were as follows: 21 or fewer days,  $n = 13$ ; 22 through 42 days,  $n = 3$ ; 43 or more days,  $n = 6$ . Seventy-five lactating Holstein cows from 4 farms were

clinically healthy controls as evaluated by daily physical check-ups by farm crew from December 1999 through May 2002. All animal experiments were performed in compliance with the Guide for the Animal Care and Use Committee at Azabu University, School of Veterinary Medicine (No. 65).

### Blood Examinations

Blood samples were collected from the jugular vein. Samples for blood chemistry were incubated at 37°C to allow clotting, centrifuged at 1200× g for 20 min at 4°C, then the isolated serum was stored at -80°C prior to analysis. From the cows with DA, samples were collected at 3 time points: Immediately before the surgical operation (Pre), 4 days after the operation (Day 4) and 8 days after the operation (Day 8). Serum OCT activity was measured using a commercial kit (OCT-Test Wako, Wako Pure Chemical, Osaka, Japan) based on the method of Ohshita *et al.* (1976). Serum levels of AST, Alkaline Phosphatase (ALP),  $\gamma$ -Glutamyltransferase (GGT) and Serum Urea Nitrogen (SUN) were analyzed using an automated blood chemical analyzer (Cobas Integra, Roche Diagnostics Japan, Tokyo, Japan). Serum concentration of Total Protein (TP) was measured by refractometry and Hematocrit (HT) was measured by the microhematocrit method.

### Histopathological Analysis

Liver biopsies were performed on DA cows during surgical operations (right-flank omentopexies) using a Silverman needle and the liver samples were immediately fixed in 10% neutral buffered formalin. The fixed tissues were embedded in paraffin wax, sectioned and stained with hematoxylin-eosin. The degree of fat accumulation in the liver was classified according to the percentage of hepatocytes in a single hepatic lobule with fat accumulation: Mild, less than 20%; moderate, 20 to 40%; or severe, more than 40% (Reid *et al.*, 1977; Reid, 1980).

### Statistical Analysis

Differences in serum OCT activity between healthy and DA cows were determined by Welch's *t* test using statistical software (Statcel, 2nd edition, OMS Publishing, Saitama, Japan). Repeated measures of mixed linear models were used to analyze OCT and other blood examinations (MIXED procedure, SAS version 9.4; SAS Institute, Cary, NC). The statistical models included grade of fat accumulation in the liver (mild, moderate and severe), collection time of blood samples (Pre, Day 4 and Day 8) and the interaction between the grade and the time as fixed effects. The repeated measure was time and the model was tested using the cow as the subject term. All significant main

effects and interactions were tested by Tukey-Kramer multiple comparisons. A p value <0.05 was considered significant. All data represent mean ± Standard Error (SE).

## Results

### OCT in Healthy and DA Cows

Serum OCT activity in Holstein cows with DA before operation was significantly higher than that in clinically healthy cows (p<0.05) (Fig. 1).

### Histopathological Analysis

Histopathological analyses of the liver samples showed no abnormalities other than hepatic fat accumulation in all

22 cows with DA. Fat accumulation was mild in 13 cows, moderate in 5 and severe in 4 (Fig. 2).

### Changes in Blood Tests in Cows with DA

There is no missing data in this experiment and no interaction between grade and time (Table 1). There were no significant differences in blood test results among cows with different grades of hepatic fat accumulation (Table 1 and 2). There were significant differences in OCT, AST, HT, TP and SUN by the number of days after the surgical operation (p<0.05) (Table 1 and 3). Activities of serum OCT and AST were higher preoperatively than 8 days postoperatively. Hematocrit, TP and SUN were higher preoperatively than 4 days postoperatively.

Table 1. Least squares means of blood examinations in 22 dairy cows with displaced abomasum. N: Number of data points (22 cows with 3 blood collection times); Grade: Fat accumulation in the liver (mild, moderate and severe); Time: The day relative to the surgical procedure (preoperative, day 4 after the procedure and day 8 after the procedure); NS: Not significant; OCT: Ornithine Carbamoyltransferase; AST: Aspartate aminotransferase; GGT:  $\gamma$ -glutamyltransferase; ALP: Alkaline Phosphatase; HT: Hematocrit; TP: Total Protein; SUN: Serum Urea Nitrogen

Item	N	Mean ± SE	P		
			Grade	Time	Grade × time
OCT, IU/L	66	12.7±2.6	NS	p<0.01	NS
AST, IU/L	66	136.1±9.3	NS	p<0.01	NS
GGT, IU/L	66	42.1±3.4	NS	NS	NS
ALP, IU/L	66	48.1±1.8	NS	NS	NS
HT, %	66	31.1±0.5	NS	p<0.01	NS
TP, mg/dL	66	6.5±0.1	NS	p<0.01	NS
SUN, mg/dL	66	7.4±0.8	NS	p<0.01	NS

Table 2. Effect of grade of hepatic fat accumulation on blood tests. Mild, n = 13; Moderate, n = 5; Severe, n = 4. N: Number of data points; OCT: Ornithine Carbamoyltransferase; AST: aspartate aminotransferase; GGT:  $\gamma$ -Glutamyltransferase; ALP: Alkaline Phosphatase; HT: Hematocrit; TP: Total Protein; SUN: Serum Urea Nitrogen

Item	Mild		Moderate		Severe	
	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE
OCT, IU/L	39	12.1±3.4	15	14.5±6.9	12	12.5±4.5
AST, IU/L	39	119.2±9.6	15	144.9±20.9	12	180.4±28.8
GGT, IU/L	39	40.7±4.4	15	42.1±9.1	12	46.5±5.1
ALP, IU/L	39	47.1±2.1	15	49.1±3.9	12	50.2±5.1
HT, %	39	31.4±0.7	15	32.2±0.9	12	28.9±1.0
TP, mg/dL	39	6.6±0.1	15	6.6±0.2	12	6.0±0.2
SUN, mg/dL	39	8.6±1.12	15	5.6±0.8	12	5.9±1.6

Table 3. Effect of the day relative to surgical procedure on blood tests. Pre, preoperative; Day-4, 4 days after procedure; Day-8, 8 days after procedure. N: Number of data points; OCT: Ornithine Carbamoyltransferase; AST: Aspartate aminotransferase; GGT:  $\gamma$ -glutamyltransferase; ALP: Alkaline Phosphatase; HT: Hematocrit; TP: Total Protein; SUN: Serum urea nitrogen; a and b: Different characters in a row mean significant difference (p<0.05)

Item	Pre		Day-4		Day-8	
	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE
OCT, IU/L	22	25.1±6.8 <sup>a</sup>	22	8.7±2.6 <sup>ab</sup>	22	4.3±0.5 <sup>b</sup>
AST, IU/L	22	170.2±20.7 <sup>a</sup>	22	136.8±14.6 <sup>a</sup>	22	101.5±7.4 <sup>b</sup>
GGT, IU/L	22	44.5±6.7	22	40.6±5.7	22	41.1±5.4
ALP, IU/L	22	52.0±3.4	22	45.8±2.8	22	46.5±2.9
HT, %	22	33.9±1.0 <sup>a</sup>	22	29.7±0.7 <sup>b</sup>	22	29.7±0.7 <sup>b</sup>
TP, mg/dL	22	6.7±0.2 <sup>a</sup>	22	6.2±0.2 <sup>b</sup>	22	6.6±0.1 <sup>a</sup>
SUN, mg/dL	22	12.7±1.7 <sup>a</sup>	22	4.0±0.4 <sup>b</sup>	22	5.6±0.7 <sup>b</sup>

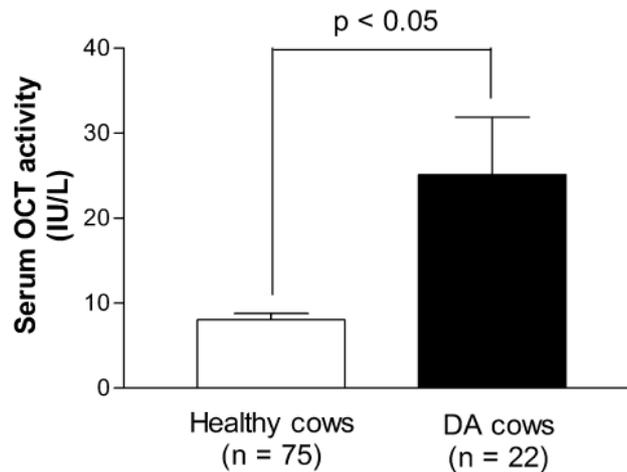


Fig.1. Serum Ornithine Carbamoyltransferase (OCT) activity in Holstein cows. Data represent means  $\pm$  SE in samples from 75 clinically healthy cows ( $8.1 \pm 0.7$  IU/L) and from 22 cows with Displaced Abomasum (DA) ( $25.1 \pm 6.8$  IU/L).  $p < 0.05$  was considered significant

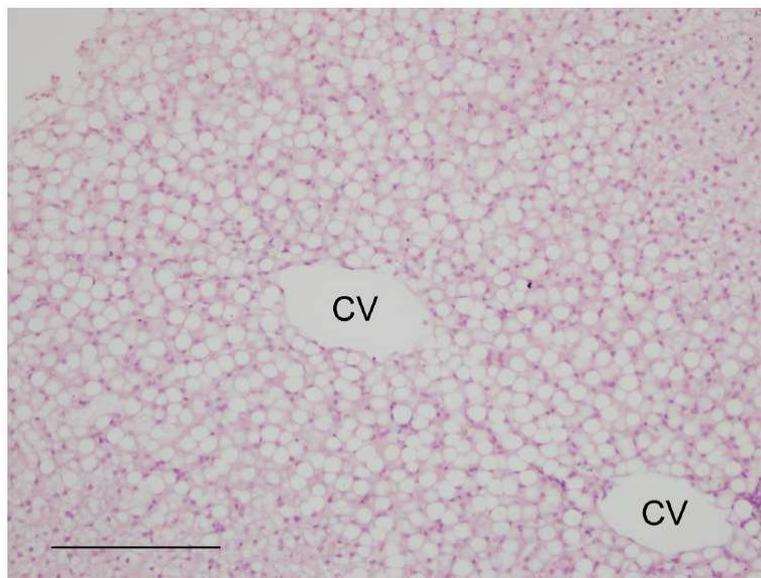


Fig. 2. Histopathology of a liver with severe fat accumulation. This photomicrograph shows large droplets within the cytoplasm's of most hepatocytes around the central vein (CV) in a cow with severe fatty liver. Bar = 200 mm

## Discussion

Our results indicate that serum OCT activity is higher in cows with DA than in healthy cows, which is in agreement with previous studies (Kalaitzakis *et al.*, 2006). High OCT activity may be caused by an influx of enzyme into the bloodstream as a result of progressive liver damage or by a reduction in the rate of metabolic elimination of OCT. Because we found no histopathological abnormalities in hepatocytes other than FL and because serum levels of OCT were not affected by the degree of fat accumulation in the liver, we think that the serum OCT activity of cows with

DA may be influenced by factors that cause DA. The levels of HT, TP and SUN were higher before the operation than 4 days afterward. The levels of SUN did not increase by the eighth day after the surgical procedure, which could indicate that the cows' protein intake did not increase. Dehydration induced by DA may have contributed to the high preoperative serum OCT levels. We did not collect liver samples on postoperative days 4 and 8 and thus did not assess the decrease of fat infiltration into the liver. Therefore, the decrease of serum OCT activity on postoperative day 8 in the cows with DA may not indicate that FL was resolved.

Cows in our study with DA exhibited no complications other than FL, which was classified as mild in 13 of the 22 patients. Kalaitzakis *et al.* (2006) reported significant positive correlations of serum OCT activity with serum AST and GGT activities but not with serum ALP activity. In our study, serum activities of these 4 enzymes did not differ significantly with levels of hepatic fat accumulation. However, levels of OCT and AST did change significantly with postoperative time, which appears to indicate the influence of DA on these 2 enzymes.

Our OCT assay procedure using a commercial kit was less sensitive than the modified method used by Kalaitzakis *et al.* (2006). However, the correlation between these 2 measurements was high and thus did not affect the interpretation of our results (Tsuchiya *et al.*, 1994). Moreover, because our liver biopsy samples were collected with Silverman needles, the specimens were small. We could not evaluate the grade of FL with the method used by Kalaitzakis *et al.* (2006), because our specimens had insufficient numbers of hepatic lobules. In a previous study of humans with FL, the concentration of serum OCT was in the reference range or slightly elevated (Watanabe *et al.*, 1994-1995). A recently-developed sensitive ELISA for measuring serum OCT has been shown to be useful for evaluating liver damage (Murayama *et al.*, 2006). However, the usefulness of serum OCT activity for evaluation of FL severity, even in humans, has yet to be established.

In conclusion, serum OCT activity in cows with DA was elevated preoperatively and decreased by the eighth postoperative day, regardless of the severity of hepatic fat accumulation. The high OCT activity was therefore influenced not only by fat accumulation but also by dehydration secondary to DA. Serum OCT activity should be reconsidered as a marker of FL in cows with DA, especially around the time of parturition, when DA frequently occurs.

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## Author Contribution

All authors have read and approved the final manuscript.

**Ken Onda:** Study conception, coordination and preparation of the manuscript.

**Reiichiro Sato and Hideharu Ochiai:** Sample collection and processing.

**Yosuke Sasaki:** Statistical analysis and preparation of the manuscript.

**Hiroo Madarame:** Histopathological evaluation of liver tissues.

**Kazuhiro Kawai and Fujiko Sunaga:** Study design and analysing the samples.

## Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

## Conflict of Interest

None of the authors has any conflict of interest to declare.

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