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Internal Medicine

Transient elastography: a novel, non-invasive method for the evaluation of liver stiffness and controlled attenuation parameter in cows

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ABSTRACT. This study investigated the use of transient elastography (TE) as a tool for the non-invasive evaluation of liver stiffness and controlled attenuation parameter (CAP) in cows. A total of 85 cows were used for this study. After the liver was located and subcutaneous tissue thickness was checked by means of ultrasonography, liver stiffness and CAP were measured using TE. At least 10 measurements were taken per individual cow. In addition, intraclass correlation coefficients were calculated for intra-rater and two-rater inter-rater reliability of liver stiffness and CAP measurement, and were evaluated. Of the 85 cows, 61 (71.8%) were measurable owing to their significantly less body weight and subcutaneous tissue thickness compared with those of unmeasurable cows. Liver stiffness showed no significant differences among sexes, ages, or breeds. CAP showed no significant differences among sexes and breeds. Intra- and inter-rater reliabilities for liver stiffness and CAP were almost perfect. Both liver stiffness and CAP could be quantitatively evaluated with good reproducibility in cows using TE, and CAP increased with the growth of the cows. However, evaluation was not possible in obese cows or cows with high values for subcutaneous tissue thickness or body weight.

KEY WORDS: controlled attenuation parameter, cow, liver stiffness measurement, transient elastography

Liver function tests for cows that are currently in general use include blood biochemical analysis [1, 26, 27], clearance tests for foreign substances such as bromosulfophthalein (BSP) [14] and indocyanine green (ICG) [19, 24], liver biopsy [1, 10, 12, 26], and ultrasound examination [1, 26].

Liver biopsy is considered to be the most reliable method of evaluating fibrosis or fatty changes in the liver [10, 12, 26]; however, the procedure is highly invasive and can lead to complications [28]. A previous study had reported that the incidence of complications associated with liver biopsy in human medicine is 1–5%, and the mortality rate from complications of liver biopsy is 0–0.4% [20]. Non-invasive evaluation of liver function is, therefore, essential. Ultrasound examination is the most common imaging technique for diagnosing liver diseases [23]. It is noninvasive, easy to perform, and provides immediate results. However, the drawbacks of this technique include a) its inaccuracy in differentiating fibrosis from steatosis and b) its inaccuracy in reproducibility and in exact quantification of fat accumulation [25].

In human medicine, unidimensional transient elastography (TE) is a noninvasive technique, which has been increasingly used in the assessment of diffuse liver diseases. The advantage of TE is that it is non-invasive, reproducible, gives immediate results, is less influenced by sampling errors compared with liver biopsy, and is an operator- and machine-independent technique [23]. Fibroscan® (Echosens, Paris, France) is an ultrasound-based, vibration-controlled, transient elastography (VCTETM) device used to evaluate liver stiffness and hepatic fat deposition in the absence of liver biopsy. To date, many studies have demonstrated the feasibility and usefulness of Fibroscan methodology in patients with various liver conditions, including chronic hepatitis B [3] and C [3, 29] and non-alcoholic steatohepatitis (NASH) [9].

Details of the technical description and examination procedure of Fibroscan have been previously described [22, 29]. Briefly, TE measures stiffness of the liver by generating shear waves and ultrasound, and measures the shear-wave velocity from the shallow part to the deep part of the liver. The harder the tissue, the faster the shear wave propagates. In addition, knowing that the

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fat affects ultrasound propagation, controlled attenuation parameter (CAP), specifically targeting the liver, has been developed to detect and quantify steatosis [23]. CAP estimates the attenuation of the ultrasound waves at the central frequency of the probe of the Fibroscan device, and is guided by vibration-controlled transient elastography, ensuring that the operator automatically obtains the attenuation values of the liver. CAP was computed only when the associated liver stiffness measurement was valid and used the same signals as the ones used to measure liver stiffness [6, 23]. CAP is not influenced by liver fibrosis and cirrhosis and it has the ability to quantify and detect hepatic steatosis from 10% of fatty infiltration [23].

Liver fibrosis in cow is mainly caused by persistent inflammation of the liver or necrosis of hepatocytes. It is a pathological condition that is secondary to infection with parasites such as hepatic fasciolosis, liver congestion, and cholestasis [26], and its incidence is low. In contrast, fatty liver (hepatic lipidosis) is a major metabolic disorder of many dairy cows during their early lactation period and is associated with decreased health status and reproductive performance [2]. In dairy cows, fatty liver occurs primarily in the first 4 weeks after calving, when up to 50% of all cows have some accumulation of triacylglycerol in the liver.

Therefore, non-invasive evaluation of liver function is essential in cows. No reports have described the use of the FibroScan® in farm animals, and whether this device is applicable to cattle remains unclear. Moreover, there are no reports quantitatively evaluating the liver stiffness or liver steatosis in cow. In the present study, TE was used to measure liver stiffness and CAP in cows, to investigate the use and reproducibility of TE in bovine liver tests.

MATERIALS AND METHODS

All experiments were carried out with the approval of the committee for animal clinical research and clinical trial at Animal Medical Center, Nihon University.

Animals

A total of 85 cows were used for this study. Fifty-three calves and dairy cows were kept at a dairy farm in Kanagawa Prefecture (age, 5–2,585 days) and 32 beef cows were kept at a fattening farm in Kanagawa Prefecture (age, 100–322 days).

Location of the liver and measurement of subcutaneous tissue thickness

Cows were physically retained by pushing against the wall in a standing position without anesthesia. The liver was identified with ultrasound examination from the 9th to 12th intercostal spaces on the right side and the thickness of the subcutaneous tissue was measured from the ultrasound findings.

Measurement of liver stiffness and CAP by TE

After the liver was identified with ultrasound examination, liver stiffness and CAP were measured using a Fibroscan 502 with the 3.5 MHz standard M-sized probe. The M-sized probe has a transducer with a diameter of 7 mm and can measure the liver at the depth of 2.5–6.5 cm underneath the skin surface. The device estimates liver stiffness in kilopascal (kPa) and liver steatosis in decibel/meter (dB/m). The principles of CAP have been described following previous information [23]. Measurements were performed by first holding the probe vertically against the body surface and locating the liver that was free of large vascular structures using M- and A-mode ultrasound images. Next, stiffness of the liver and CAP were measured by shear waves produced by the probe. Stiffness measurements >8.0 kPa were considered to show that the liver had not been correctly measured, and stiffness measurements ≤8.0 kPa and CAP measurement values were considered valid. An individual cow from which valid values could be obtained ≥10 times was considered measurable, and an individual cow from which <10 valid measurements were obtained was considered unmeasurable. The measurement success rate was taken as the proportion of measurable cows from the total number of measured cows.

Body measurements

After measuring liver stiffness and CAP using TE, all cows were measured for chest girth and withers height. Body weight was measured using a weighing tape for Wagyu or dairy cow (Fujihira Industry, Tokyo, Japan). Withers height was measured using a stable gauge (Fujihira Industry) to measure height from the ground to the withers.

Blood tests

Blood of the measurable cows by TE was taken from the jugular vein or coccygeal vein. Blood samples were collected in a plain vacuum blood collection tube (Terumo Corp., Tokyo, Japan) for biochemical tests and in an ethylenediaminetetraacetic acid disodium salt vacuum blood collection tube (Terumo Corp.) for complete blood cell count (CBC) and total protein (TP) measurement. The plain vacuum blood collection tube was coagulated at room temperature, centrifuged at 1,700 × *g* for 15 min at 4°C and stored at −20°C, until used for biochemical tests. CBC was measured using the Celltac Alpha (Nihon Kohden Corporation, Tokyo, Japan), and TP was measured using a refractometer. Blood biochemical tests were performed by Fujifilm Monolith (Tokyo, Japan) and measured albumin (ALB), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (γ-GTP), total cholesterol (T-Chol), and triglycerides (TG).

Evaluation of intra- and inter-rater reliabilities

For evaluations of reliability, 5 lactating cows and 5 calves were used. Intra-rater reliability was evaluated by having the same examiner measure the same part of each cow 3 times, and intraclass correlation coefficient was determined from the resulting values. Inter-rater reliability was evaluated by having two examiners measure the same part of each of the 10 cows, and the intraclass correlation coefficient was determined from the values thus obtained. Intra- and inter-rater reliabilities were evaluated from intraclass correlation coefficients on the basis of the classification of Landis and Koch [17], to investigate the reproducibility of liver stiffness and CAP measurement by TE.

Analysis and statistical processing

Data were analyzed using the JMP®12 statistical analysis software (SAS Institute Japan Ltd., Tokyo, Japan). The Fisher's exact probability test was used to compare the body weight and the subcutaneous tissue thickness between the measurable and unmeasurable groups in each farm. The Wilcoxon signed-rank test was used to compare the measured items among the measurable and unmeasurable groups in each farm. For comparison among the three groups, Tukey-Kramer's HSD test was performed after one-way analysis of variance. Spearman's rank correlation coefficient was used to analyze correlations of liver stiffness and CAP with each of the measured blood test items. Intra- and inter-rater reliabilities were tested using the intraclass correlation coefficient software (KTS&C, Osaki, Japan). A level of significance less than 0.05 was considered statistically significant. All data were expressed as means ± standard deviation (SD).

RESULTS

Measurement success rate

The measurement success rate was 71.8% (61/85) overall, 77.4% (41/53) in the dairy farm, and 62.5% (20/32) in the fattening farm (Fig. 1).

Body weight and subcutaneous tissue thickness

In the dairy farm, mean body weight was 355.1 (± 267.8) kg in the measurable group (n=41) and 572.5 (± 161.5) kg in the unmeasurable group (n=12; P<0.01). In the fattening farm, mean body weight was 197.3 (± 65.3) kg in the measurable group (n=20) and 314.4 (± 72.7) kg in the unmeasurable group (n=12; P<0.01). In both dairy and fattening farms, the number of measurable cows was larger in cows with body weight <250 kg than in those with body weight ≥250 kg (P<0.001, Table 1).

In the dairy farm, the number of cows with body weight <250 kg was 20 of 41 cows (48.8%) in the measurable group of cows and 0 of 12 cows (0%) in the unmeasurable group (P<0.001). In the fattening farm, the number of cows with body weight <250 kg was 16 of 20 cows (80.0%) in the measurable group and 2 of 12 cows (16.7%) in the unmeasurable group (P<0.001, Table 1).

Distribution of subcutaneous tissue thickness in all tested cows of each farms are shown in Fig. 2. In the dairy farm, mean subcutaneous tissue thickness was 1.4 (± 0.4) cm in the measurable group (n=41) and 2.5 (± 0.3) cm in the unmeasurable group (n=12; P<0.001). In the fattening farm, mean subcutaneous tissue thickness was 1.8 (± 0.4) cm in the measurable group (n=20) and 2.8 (± 0.3) cm in the unmeasurable group (n=12; P<0.001). In both dairy and fattening farms, the number of measurable cows was larger in cows with a subcutaneous tissue thickness <2.5 cm than in those with a subcutaneous tissue thickness ≥2.5 cm (P<0.001, Table 2).

Differences in liver stiffness according to various factors

Of all cows in the measurable group (n=61), mean liver stiffness was 3.6 (± 1.0) kPa. Mean liver stiffness was 3.6 (± 1.0) kPa

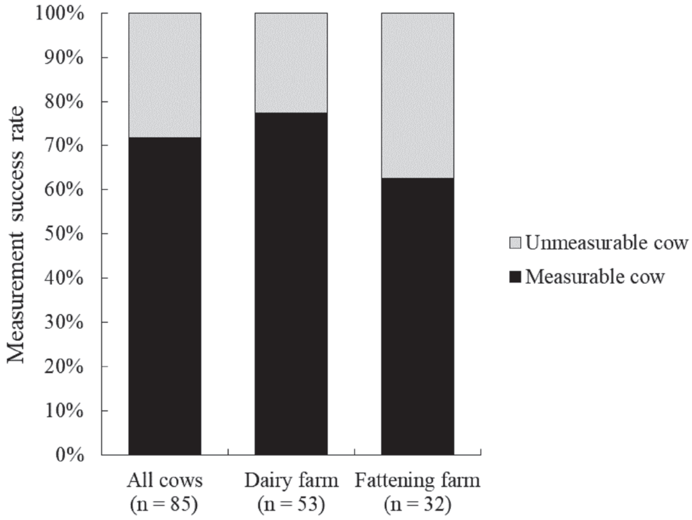

Fig. 1. Measurement success rate of all tested cows, the dairy farm, and the fattening farm.

Table 1. Absolute and relative frequency of cows categorized into measurable and unmeasurable groups according to the body weight

	Dairy farm (n=53)*			Fattening farm (n=32)*		
	Body weight		Total	Body weight		Total
	<250 kg	≥250 kg		<250 kg	≥250 kg	
Measurable [a)]	20 [c)] (48.8)	21 (51.2)	41 (100)	16 [c)] (80.0)	4 (20.0)	20 (100)
Unmeasurable [b)]	0 (0.0)	12 (100)	12 (100)	2 (16.7)	10 (83.3)	12 (100)
Total	20 (86.8)	33 (62.3)	53 (100)	18 (56.3)	14 (43.8)	32 (100)

**P*<0.001. a) ≥10 measurements made and ≥10 valid values obtained. b) ≥10 measurements made, but <10 valid values obtained. c) Numbers represent number of cows (percentage of total).

Table 2. Absolute and relative frequency of cows categorized into measurable and unmeasurable groups according to the subcutaneous tissue thickness

	Dairy farm (n=53)*			Fattening farm (n=32)*		
	Subcutaneous tissue thickness		Total	Subcutaneous tissue thickness		Total
	<2.5 cm	≥2.5 cm		<2.5 cm	≥2.5 cm	
Measurable [a)]	41 [c)] (100)	0 (0)	41 (100)	20 [c)] (100)	0 (0)	20 (100)
Unmeasurable [b)]	5 (41.7)	7 (58.3)	12 (100)	1 (8.3)	11 (91.7)	12 (100)
Total	46 (86.8)	7 (13.2)	53 (100)	21 (65.6)	11 (34.3)	32 (100)

**P*<0.001. a) ≥10 measurements made and ≥10 valid values obtained. b) ≥10 measurements made, but <10 valid values obtained. c) Numbers represent number of cows (percentage of total).

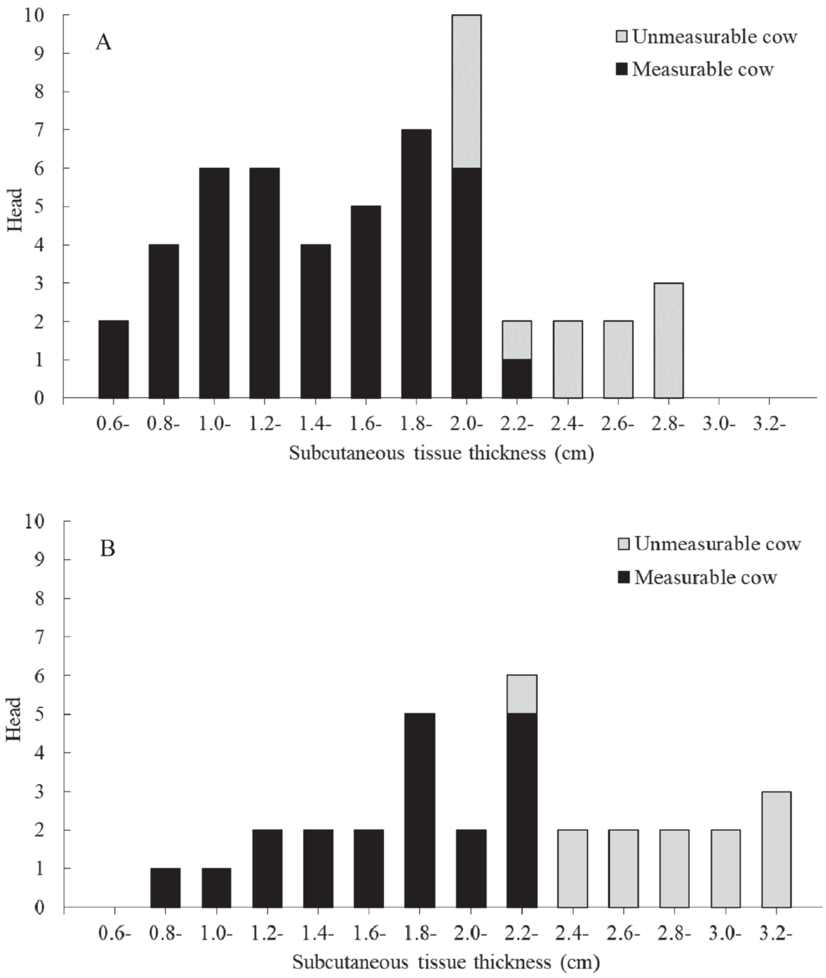

Fig. 2. Distribution of subcutaneous tissue thickness in all tested cows from each farm. A: the dairy farm (n=53). B: the fattening farm (n=32).

in females (n=42) and 3.5 (± 1.0) kPa in males (n=19), with no significant difference among sexes. There was also no difference among breeds, ages, and farms.

Differences in CAP according to various factors

Of all cows in the measurable group (n=61), mean CAP was 162.7 (± 42.7) dB/m. Mean CAP was 164.4 (± 44.0) dB/m in females (n=42) and 158.9 (± 41.0) dB/m in males (n=19), with no significant difference among sexes. There was no difference among breeds.

The measurable cows were categorized according to their age and farms, as follows: dairy calves aged <350 days, dairy cows aged >350 days, and fattening cattle aged <350 days. Dairy calves aged <350 days (n=22, 129.4 [± 24.3] dB/m) had lower CAP than dairy cows aged >350 days (n=19, 184.9 [± 43.9] dB/m) and fattening cattle aged <350 days (n=20, 178.3 [± 35.0] dB/m) (P<0.001, Fig. 3).

Blood tests

Blood test results for all cows in the measurable group (n=61) were approximately within normal ranges (Table 3). Liver stiffness and CAP showed no correlation with CBC or any of the blood biochemistry test items.

Intra- and inter-rater reliabilities

Intra-rater intraclass correlation coefficient was 0.92 for liver stiffness and 0.85 for CAP, indicating high agreement for both measurements according to the classification of Landis and Koch [17]. The two-rater intraclass correlation coefficient was 0.86 for liver stiffness and 0.92 for CAP, indicating almost perfect agreement for both measurements, according to the classification of Landis and Koch.

DISCUSSION

The present study represents the first investigation of the use of TE as a non-invasive tool for evaluating liver stiffness and CAP in cows. In this study, 71.8% of cows tested were measurable, and both intra- and inter-rater reliability were high, indicating that liver stiffness and CAP could be quantitatively measured in cows with good reproducibility using TE.

This study showed that FibroScan can be performed successfully in cows representing a wide age range (5 days to 2,585 days). However, 22.6% of measurements were invalid in the dairy farm and 37.5% of measurements were invalid in the fattening farm. Previous studies using FibroScan have reported a failure rate of 2.1–7.5% in adults [3, 4, 7, 15, 21] and that of 6.1–15% in children [5, 8, 11]. The failure rate with cows in this study was higher than those with adults and children in previous studies. The major reasons for measurement failure include the excessive thickness of the subcutaneous tissue due to fattening and a higher body weight than humans. Factors associated with FibroScan measurement failure in adults include female gender, high BMI, and metabolic syndrome [7].

Cows in the unmeasurable group showed subcutaneous tissue thickness ≥2.5 cm and body weight ≥250 kg, representing

Table 3. Serum chemical values in measurable cows (n=61)

		Results
TP	(g/d*l*)	6.7 ± 0.8
ALB	(g/d*l*)	3.2 ± 0.4
AST	(U/*l*)	66.0 ± 15.0
γ-GTP	(U/*l*)	26.2 ± 20.3
T-Chol	(mg/d*l*)	135.1 ± 66.0
TG	(mg/d*l*)	13.0 ± 6.7

Data are expressed as means ± SD. TP, total protein; ALB, albumin; AST, aspartate transaminase; γ-GTP, g-glutamyl transpeptidase; T-Chol, total cholesterol; TG, triglyceride.

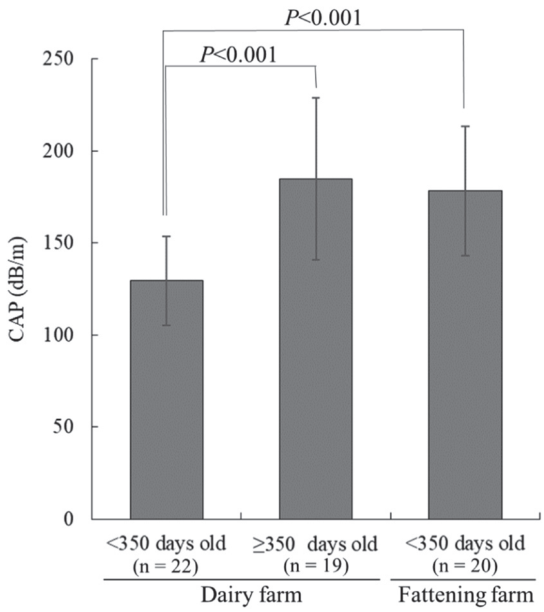

Fig. 3. Mean controlled attenuation parameter (CAP) categorized by age and farm.

characteristics of obese cows. In this study, all cows with subcutaneous tissue thickness ≥2.5 cm were unmeasurable. Because the M-sized probe used in this study can measure depths at 2.5 cm underneath the skin surface, shear waves and ultrasound do not reach the liver with the subcutaneous tissue ≥2.5 cm [5]. Body weight and subcutaneous tissue thickness were greater in the unmeasurable group than in the measurable group, likely because fat gets deposited in the subcutaneous tissue as obesity progresses, thereby increasing the body weight. The device used in this study has an L-sized probe for measuring depths of 3.5–7.5 cm underneath the skin. The use of different probes according to subcutaneous tissue thickness and the width of the intercostal spaces in subjects is recommended in human medicine [11, 16, 18]; using L-sized probes for the present measurements in cows may be expected to improve the measurement success rate.

Hepatic lipidosis is reportedly reversible and neither inflammation nor fibrosis are usually seen in cattle [10]. The cows used in this study did not have liver fibrosis or fatty liver and there was no difference in liver stiffness. In this study, mean liver stiffness of all cows was 3.7 ± 1.0 kPa. Liver stiffness was reported as 5.4 kPa for adult patients with no fibrosis and slight portal fibrosis [15], whereas it was 3.9–4.7 kPa for healthy children [5, 8, 11]. Liver stiffness in healthy cows was similar to that in adults or children. There were no significant differences by age or sex. These results agree with those reported by Kettaneh *et al*. [15] and Goldschmidt *et al*. [11]. But some reports reveal age- or gender-dependent reference values [8, 16, 21].

CAP is well correlated to liver steatosis and can be used to detect and quantify steatosis [23]. CAP is only influenced by the steatosis stage. Several reports have stated cut-off values for CAP [13]. The cut-off value of CAP for detecting hepatic steatosis of ≥5% is 247–250 dB/m, and that of ≥10% is 210–283 dB/m; this value differs depending on the reports. The cutoff value of CAP for the diagnosis of hepatic steatosis is still controversial, with it varying with the study population and steatosis criteria. The mean CAP (162.7 dB/m) of all cows in this study was lower than the cut-off value of CAP for liver steatosis in humans, and the liver was normal from the absence of fatty liver based on ultrasonography and blood tests. The reason that dairy calves had lower CAP than dairy cows is probably because of less fat accumulation in the liver of the calves compared with that of the adult cows. In addition, fattening cows had a higher CAP than dairy calves, and were comparable to dairy cows. This suggests that fattening cows have more fat deposits in the liver than dairy cows due to differences in the feeding conditions.

TE reportedly shows good reproducibility in adult patients [22] and children [11]. In the present study, intra- and inter-rater reliabilities of liver stiffness and CAP measurement by TE were high, confirming good reproducibility of TE in cows.

These findings indicate that liver stiffness and CAP can be evaluated quantitatively in cows using TE, with good reproducibility. Further research aimed at the application of TE to clinical settings with cows needs to investigate measurement conditions, such as the use of different types of probe, depending on the individual cow. It also needs to investigate how results from TE compare with results from liver biopsy, the current gold standard for diagnosing liver diseases.

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