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Decreased Concentration of Serum Apolipoprotein C-III in Cows with Fatty Liver, Ketosis, Left Displacement of the Abomasum, Milk Fever and Retained Placenta

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ABSTRACT. Apolipoprotein (apo) C-III is a low molecular mass protein mainly distributed in the high-density lipoprotein (HDL) fraction. In cows with postparturient diseases such as ketosis, concentrations of cholesterol, phospholipids and apoA-I and the activity of lecithin:cholesterol acyltransferase, which are mainly distributed in or functionally associated with HDL, are reduced. The purpose of the present study was to examine whether the serum concentration of apoC-III was similarly decreased in the postparturient diseases. Compared with healthy controls, the apoC-III concentration was significantly ($P<0.01$) decreased in cows with fatty liver, ketosis, left displacement of the abomasum, milk fever and retained placenta. Concentrations of apoC-III in the HDL fractions from diseased cows were also lower than in controls. Of the diseased cows, the decreased apoC-III concentration was particularly distinct in cows with milk fever. Increased nonesterified fatty acid and reduced free cholesterol, cholesteryl ester and phospholipid concentrations were observed in cows with milk fever, as in the other diseased cows. The decrease in the apoC-III concentration is suggested to be closely associated with the postparturient disorders, in particular with milk fever.

KEY WORDS: apolipoprotein C-III, bovine, fatty liver, high-density lipoprotein, postparturient disease.

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Apolipoprotein (apo) C-III is the low-molecular-mass protein component mainly distributed in the high-density lipoprotein (HDL) fraction in cows [36]. The serum concentration of this protein in lactating cows was recently determined, and was lower in the nonlactating than lactating stages [37]. In high-yielding dairy cows, fatty liver develops during the nonlactating stage, and cows with severe fatty liver have high incidences of ketosis, left displacement of the abomasum (LDA), milk fever and retained placenta [21, 30], suggesting that fatty liver is the major causal factor for the postparturient disorders.

In cows with fatty liver, concentrations of triglycerides (TG) and apoB-100 distributed largely in very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) fractions, are decreased [6, 12, 18, 28, 32]. The decrease in the apoB-100 concentration is similarly observed in cows with ketosis [27], LDA [27] and retained placenta [26]. In addition to TG and apoB-100, concentrations of free cholesterol (FC), cholesteryl esters (CE) and phospholipids (PL) and apoA-I and the activity of lecithin:cholesterol acyltransferse (LCAT), which are mainly distributed in or functionally associated with HDL, are reduced in cows with fatty liver, ketosis, LDA and retained placenta [18, 22, 23, 25–27, 32, 33]. Because of the presence in HDL and the decrease in the concentration during the nonlactating stage, it is possible to assume that the apoC-III concentration is also decreased in cows with fatty liver and the fatty liver-associated postparturient disorders. The purpose of the present study was to examine whether the apoC-III concentration was decreased in cows with fatty liver, ketosis, LDA, milk fever and retained placenta.

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MATERIALS AND METHODS

Cows: Sera of Holstein cows (n=77; 2 to 8 years old) in early lactation (1 to 37 days after parturition) were collected from farms of Fukushima, Ishikawa and Iwate Prefectures. Milk yields were approximately 8,000 kg/year/cow and were similar in healthy and diseased cows. The diets were as described previously [23, 25]. Of the cows, 12 were apparently healthy and the remaining 65 had fatty liver (n=4), ketosis (n=29), LDA (n=17), milk fever (n=5), or retained placenta (n=10). The healthy cows used as controls were without urine ketone reaction, LDA, hypocalcemia, recumbency or retained placenta. Fatty liver was defined as liver more than 30 mg TG/g liver (wet weight), and the mean value of the 4 cows with fatty liver was 69.7 mg TG/g while that of healthy control cows was 21.5 mg/g. The 4 fatty liver cows showed no apparent clinical signs. Cows with ketosis and those with LDA were diagnosed as described previously [22, 27]. Cows characterized by sternal recumbency that occurred within 2 days after parturition and by quick standing in response to calcium treatment were considered to have milk fever [2]. Downer cows that did not respond to calcium treatment were not involved. Retained placenta was defined as a placenta not expelled within 24 hr after delivery [26]. Cows with more than 2 diseases (eg, ketosis and LDA) and those with apparent inflammatory diseases were not included. Blood samples were collected

Table 1. Lipid, Mg, Ca and IP concentrations in sera from healthy control cows and cows with fatty liver, ketosis, LDA, milk fever and retained placenta

	Control	Fatty liver	Ketosis	LDA	Milk fever	Retained placenta
N	12	4	29	17	5	10
TG	10.9 ± 3.4	7.5 ± 3.0	3.4 ± 1.6 ‡	5.8 ± 1.8 ‡	11.2 ± 2.8	4.5 ± 2.2 ‡
FC	21.2 ± 1.6	14.3 ± 9.9	11.4 ± 6.9 ‡	15.5 ± 8.5	11.7 ± 5.4 ‡	11.5 ± 5.4 ‡
CE	137 ± 36	60.3 ± 32.6 ‡	74.6 ± 25.3 ‡	75.2 ± 37.4 ‡	53.7 ± 19.7 ‡	62.8 ± 21.8 ‡
PL	179 ± 8.4	90.0 ± 29.5 ‡	102 ± 35 ‡	104 ± 41 ‡	65.6 ± 23.3 ‡	92.2 ± 27.4 ‡
NEFA	307 ± 118	1,005 ± 85 ‡	1,393 ± 647 ‡	865 ± 318 ‡	860 ± 295 ‡	1,007 ± 386 ‡
Mg	2.2 ± 0.2	ND	ND	ND	3.3 ± 0.9 ‡	ND
Ca	9.5 ± 0.3	ND	ND	ND	4.9 ± 1.5 ‡	ND
IP	6.7 ± 0.4	ND	ND	ND	2.4 ± 1.6 ‡	ND

Except for NEFA (μEq/L), unit is mg/100 m*l*. ‡, $P<0.01$, compared with respective values for healthy controls. ND, not determined.

in the morning (before feeding); those from diseased cows were taken before treatment.

ApoC-III analysis: ApoC-III was purified from cow serum [36]. Immunoblot analysis and enzyme-linked immunosorbent assay (ELISA) were performed as described previously [37].

Other methods: Chylomicrons (CM)-VLDL ($d<1.006$), LDL ($d<1.063$), HDL ($d<1.21$), very high-density lipoprotein (VHDL; $d<1.25$) and the lipoprotein-deficient fractions ($d>1.25$) were prepared [13]. The lipoprotein fractions were dialyzed against phosphate-buffered saline (PBS). The hepatic TG content was determined [11]. Serum concentrations of TG, total cholesterol, FC, PL, nonesterified fatty acids (NEFA), magnesium (Mg), calcium (Ca) and inorganic phosphate (IP) were measured by use of kits (Wako Pure Chemicals, Osaka, Japan). The concentration of CE was calculated by subtracting the FC concentration from that of total cholesterol. All assays were done in duplicate. Data were analyzed, using one-way ANOVA and Scheff's F test. Values are expressed as mean ± SD.

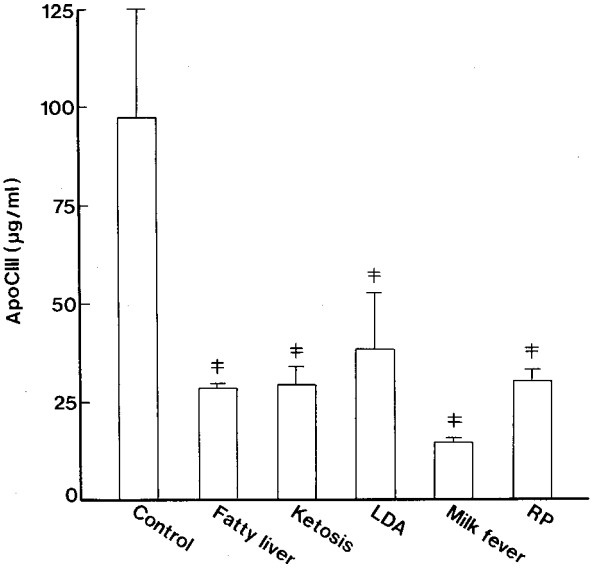

Fig. 1. Concentrations of apoC-III in sera from healthy control cows and cows with fatty liver, ketosis, LDA, milk fever and retained placenta (RP). ‡, $P<0.01$, compared with controls.

RESULTS

Though not in cows with fatty liver and milk fever, the serum TG concentration was decreased in cows with ketosis, LDA and retained placenta, compared with that in healthy control cows (Table 1). Concentrations of FC in cows with ketosis, milk fever and retained placenta were lower than those in controls, whereas the decrease in cows with fatty liver and LDA did not reach statistical significance. Decreases in CE and PL and an increase in NEFA concentrations were distinctly observed in all diseased cows. The serum Mg concentration was slightly but significantly higher in cows with milk fever than in controls. Concentrations of Ca and IP were decreased in cows with milk fever.

Serum apoC-III concentrations in healthy control cows during early lactation were in the range of 48.2 to 134 μg/m*l* (97.6 ± 26.9 μg/m*l*), and were comparable to the previously reported concentration during the same lactating stage [37]. The apoC-III concentration was significantly decreased in cows with fatty liver, ketosis, LDA, milk fever and retained placenta, compared with controls (Fig. 1). In particular, cows with milk fever had extremely low apoC-III concentration; the value in cows with milk fever was lower than in those with ketosis ($P=0.0079$), LDA ($P<0.0001$) and retained placenta ($P=0.0176$). The insignificant difference from cows with fatty liver ($P=0.1633$) appeared to be attributable to the small sample sizes of both groups.

Decreases of apoC-III concentrations in diseased cows were also detected by immunoblot analysis (Fig. 2). Extremely low densities of apoC-III bands in cows with milk fever compared with those in cows with ketosis and LDA were again shown by the analysis. Decreased densities of apoC-III bands compared with controls were similarly detected by the analysis of sera from cows with fatty liver and retained placenta (figure not shown). In the

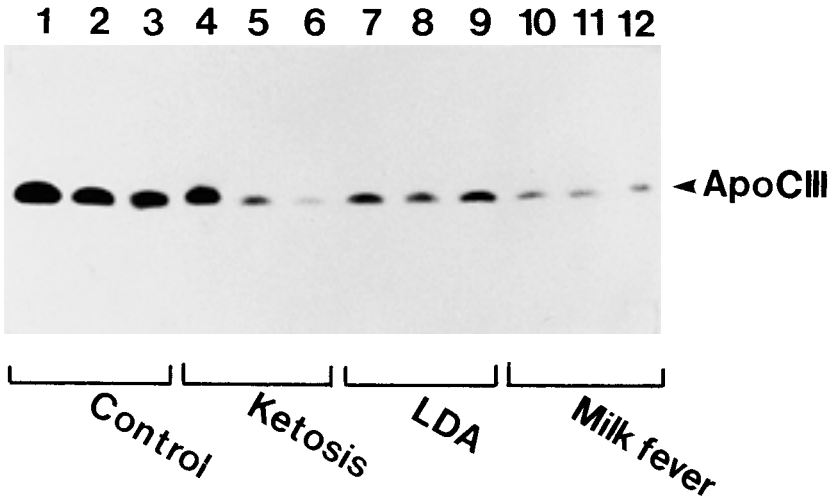

Fig. 2. Immunoblot analysis of apoC-III in sera from healthy control cows and cows with ketosis, LDA and milk fever. Sera were diluted 20-fold with PBS, and a 3.3 μl aliquot was applied per well. Relative densities (from lanes 1 to 12) were: 100, 70, 63 (control); 65, 27, 11 (ketosis); 36, 30, 38 (LDA); 20, 19, and 13 (milk fever).

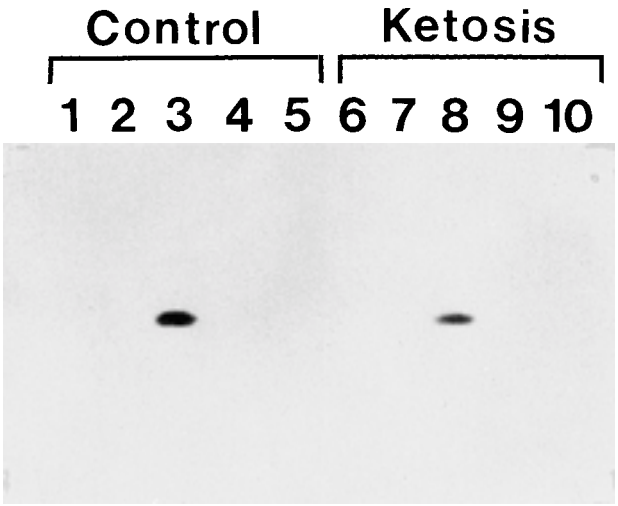

Fig. 3. Immunoblot analysis of apoC-III in lipoprotein fractions from sera of healthy control cows and cows with ketosis. Sera from the 3 control cows and 3 cows with ketosis used in Fig. 2 were combined, and CM-VLDL (lanes 1 and 6), LDL (2 and 7), HDL (3 and 8), VHDL (4 and 9), and the lipoprotein-deficient fractions (5 and 10) were prepared. A 2 μl aliquot of the 10-fold-diluted CM-VLDL, LDL or HDL fraction and a 6.7 μl aliquot of the 20-fold-diluted lipoprotein-deficient fraction were applied per well.

healthy control cows, most apoC-III was found in the HDL fraction, and it was not or only faintly detected in the other lipoprotein fractions (Fig. 3). The predominant distribution of apoC-III in HDL did not change in cows with ketosis (Fig. 3) and milk fever (not shown), and densities of HDL apoC-III bands were lower in cows with ketosis and milk fever than in controls.

DISCUSSION

We have previously shown that the apoA-I concentration [25–27, 32] and LCAT activity [22, 23, 33] are decreased in

cows with fatty liver, ketosis, LDA and retained placenta. ApoA-I is mainly distributed in HDL, and LCAT is activated by apoA-I and esterifies HDL FC [9]. The present study indicated that the concentration of apoC-III, the apoprotein largely distributed in HDL, was decreased in cows with fatty liver, ketosis, LDA, milk fever and retained placenta. This study also demonstrated decreases of FC, CE and PL concentrations in cows with milk fever.

The decreased apoC-III concentrations (15% in controls in milk fever to 39% in LDA) were more distinct than those of apoA-I in fatty liver [18, 25, 32], ketosis [27], LDA [27] and retained placenta [26] (60 to 80% of controls in all diseased cows). The greater decreases appear to be explained by the lower serum concentration (approximately 0.1 mg/m*l* [37]) and shorter half-time (30 hr or less [24]) of apoC-III than those of apoA-I (0.5 to 1.5 mg/m*l* [18, 25] and 4 days [17]). The decreased apoC-III concentrations were also larger than those of LCAT activity (60 to 70% of controls in fatty liver [23, 33], ketosis [22] and LDA [22]). This difference may be attributable to the different distributions; most apoC-III is present in HDL whereas LCAT is distributed in different proportions in HDL, LDL and the lipoprotein-deficient fraction [9]. The apoC-III concentration was found to be most sensitively decreased in the HDL constituents examined.

In rats, apoC-III is synthesized largely in the liver, and the small intestine contributes less than 10% of the total plasma apoC-III [17, 35]. ApoC-III is secreted as a constituent of both VLDL and HDL by rat hepatocytes [3]. In cattle, contributions by CM (the small intestinal origin) and VLDL to the serum apoC-III concentration appear to be negligible, because apoC-III concentrations in the CM and VLDL fractions were extremely low (1/1,100 and 1/650 of the HDL apoC-III concentration) [10]. It is conceivable that the majority of cattle apoC-III is secreted by the liver as a constituent of HDL. The predominant distribution of apoC-III in HDL was not changed in pathologic states such as ketosis. Excess NEFA taken up by or TG accumulated in the liver may impair the synthesis of apoC-III, the assembly of HDL, or its secretion by the liver, and in turn decrease the serum apoC-III concentration.

Occurrences of ketosis, LDA, milk fever and retained placenta are interrelated [5, 8, 19–21, 30, 34] and are believed to be connected to overfeeding during the nonlactating stage [5, 19]. The overfeeding before calving is one of the major causal factors for fatty liver, and the liver developed during the nonlactating stage is suggested to be involved in the occurrence of the postparturient disorders [21, 30]. The decreases in the apoC-III concentration in all diseased cows further supported the hypothesis that fatty liver is closely associated with postparturient diseases. In cows with milk fever, an increased NEFA concentration [15, 16] and fatty infiltration of the liver [7] have been reported. In addition to the increase in NEFA concentration, decreases in FC, CE and PL concentrations were observed in cows with milk fever. The changes in lipid concentrations in cows with milk fever were quite similar to those in cows with fatty liver [23, 28, 32], ketosis [1, 4, 22], LDA [14, 22] and retained placenta [26]. The decrease in the apoC-III concentration, coupled with the changes in lipid concentrations, strongly suggests that milk fever is one of the fatty liver-associated postparturient diseases, as described in the early reports on fatty liver [21, 30]. Compared with the other diseases, the apoC-III concentration was more distinctly decreased in cows with milk fever. ApoC-III gene transcription is regulated by mitogen-activated protein kinase [29]. Phosphorylation by this kinase of retinoid X receptor α, a member of the nuclear receptor family, results in the inhibition of 1,25-dihydroxyvitamin D_3-dependent signal transduction [31]. This vitamin D metabolite is involved in the regulation of the Ca metabolism and is used for the prevention of milk fever [2]. The regulatory mechanism for apoC-III synthesis may be related to the distinct decrease in the apoC-III concentration in cows with milk fever.

In conclusion, the apoC-III concentration is decreased in cows with fatty liver, ketosis, LDA, milk fever and retained placenta. The decreased rate of the apoC-III concentration was most distinct in milk fever, suggesting the involvement of apoC-III and the apoC-III-related HDL lipids in the development of milk fever. Monitoring of the apoC-III concentration during the peripartum period may be helpful for detecting cows susceptible to the fatty liver-associated disorders including milk fever.

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