

Concentrations of Apolipoprotein C-III in Healthy Cows during the Peripartum Period and Cows with Milk Fever

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ABSTRACT. Apolipoprotein (apo) C-III is a low-molecular-mass protein mainly distributed in the high-density lipoprotein fraction in cattle serum. We have recently shown that the apoC-III concentration is decreased in cows with fatty liver, ketosis, left displacement of the abomasum, retained placenta and milk fever. The decrease was most distinct in milk fever, thereby suggesting that apoC-III is particularly relevant to the development of milk fever and also that apoC-III is a candidate diagnostic marker for this disease. The purpose of the present study was to examine whether the apoC-III concentration in healthy cows is altered during the peripartum period, to assess the usefulness of apoC-III as a marker for milk fever. ApoC-III concentrations in 17 cows were monitored during the peripartum period (-48 to +12 days from parturition). Of the 17 cows, 14 were apparently healthy during the period. The apoC-III concentrations in the 14 healthy cows were unaltered during the period from -48 to -21 days, but thereafter showed individual variations. Compared with values during the period from -48 to -21 days, the apoC-III concentration was increased (137%) in 5 cows during the period from +1 to +12 days, whereas it decreased (60.7%) in 9 cows. Three cows suffered from milk fever at -3 to +10 days. Decreased apoC-III concentrations in diseased cows (15 to 37% of controls) were more distinct than in the 9 healthy cows. The apoC-III concentration was correlated with lecithin:cholesterol acyltransferase activity in cows with milk fever, but not in healthy cows. Correlation analysis also indicated that apoC-III and apoB-100 concentrations were negatively correlated in 5 healthy cows with increased apoC-III concentrations, but positively in 9 healthy cows with decreased concentrations and cows with milk fever. Determination of the apoC-III concentration during the peripartum period is suggested to be helpful in diagnosing milk fever. The possible relevance of apoC-III and apoB-100 in the development of milk fever is also implied.

KEY WORDS: apoB-100, apoC-III, bovine, milk fever, peripartum period.

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Most metabolic diseases of dairy cows develop in the peripartum period, during which the transition from the pregnant, nonlactating state to the nonpregnant, lactating state occurs [1]. The periparturient metabolic diseases include ketosis, left displacement of the abomasum, retained placenta and milk fever. Such diseases are suggested to be derived from fatty liver developed during the nonlactating stage [9, 16]. The major biochemical characteristics of cows with fatty liver and fatty liver-related diseases are decreases in lipoprotein lipid and apoprotein concentrations. Thus far, decreases in concentrations of apolipoprotein (apo) B-100 [6, 8, 15, 17], apoA-I [8, 14, 15, 17] and cholesterol esters (CE) [10-12, 18] and a reduction in activity of lecithin:cholesterol acyltransferase (LCAT) [10-12, 18] have been used in diagnosing fatty liver and fatty liver-related diseases.

ApoC-III is the low-molecular-mass protein mainly distributed in the high-density lipoprotein (HDL) fraction in cattle serum [20, 21]. We have recently shown that the apoC-III concentration is decreased in cows with fatty liver, ketosis, left displacement of the abomasum, retained placenta and milk fever [22]. Among these diseases, the decreased apoC-III concentration was most distinct in milk fever (less than 30% of controls), suggesting particular involvement of apoC-III in the pathogenesis of milk fever. Of the lipoprotein constituents, LCAT activity has been shown to be the most useful marker for detection of fatty

liver and fatty liver-related diseases, because the activity is unaltered in healthy cows during the peripartum period [12]. A disadvantage of LCAT activity is that the decreased rates of activity in diseased cows are not so distinct (60 to 70% of controls). If the apoC-III concentration in healthy cows is unaltered during the peripartum period, apoC-III can be substituted for LCAT in diagnosing milk fever because of its distinct decrease in this disease. The purpose of the present study was to determine apoC-III concentrations in healthy cows during the peripartum period and, moreover, to compare their concentrations with those in cows with milk fever.

MATERIALS AND METHODS

Cows: Holstein cows (n=17, 2 to 7 years old) during the peripartum period (-48 to +12 days from parturition) in farms of Ishikawa Prefecture were used. Their milk yields and diet were as described previously [12]. Blood samples from 17 cows were collected at approximately 10-day intervals during the peripartum period. Of the 17 cows, 14 had no apparent clinical signs during the observed period, whereas 3 had milk fever at -3 to +10 days. Milk fever was diagnosed by recumbency and by quick standing in response to calcium treatment.

Apolipoprotein analysis: ApoC-III [20] was purified from cow serum. Immunoblot analysis and enzyme-linked immunosorbent assay (ELISA) of apoC-III were performed

as described [21]. The serum apoB-100 concentration was determined by ELISA [24].

Other methods: LCAT activity was evaluated as described [18]. Serum concentrations of triglycerides (TG), total cholesterol, free cholesterol (FC), phospholipids (PL), nonesterified fatty acids (NEFA), calcium (Ca), magnesium (Mg) and inorganic phosphate (IP) and the activity of aspartate aminotransferase (AST) were measured enzymatically. The concentration of CE was calculated by subtracting the FC concentration from that of total cholesterol. The data were analyzed, using one-way ANOVA and Scheffe's F-test. Values are expressed as mean \pm SD.

RESULTS

ApoC-III concentrations, determined by ELISA, of 17 cows from -48 to -21 days were in the range of 38.7 to 83.2 $\mu\text{g/ml}$ ($57.7 \pm 14.9 \mu\text{g/ml}$). The concentration during the period of -48 to -31 days ($58.5 \pm 16.3 \mu\text{g/ml}$) was nearly identical to that during a period from -30 to -21 days ($56.9 \pm 15.1 \mu\text{g/ml}$), and differences in each cow between the two periods were within 20%. Values obtained by immunoblot analysis were proportional to those obtained by ELISA, as described previously [22, 23]. The apoC-III concentrations during the peripartum period were therefore determined by immunoblot analysis and expressed as the percent of those at the initial sampling days (from -48 to -21 days). In some cows, sera were initially collected at day -18. In these cases, values at day -18 were used as controls.

Of the 17 cows monitored, 14 were apparently healthy and did not show distinct signs of disease until 12 days after parturition. From -20 to +12 days, apoC-III concentrations of the 14 healthy cows showed individual variations. Compared with values from -48 to -21 days, apoC-III concentrations were increased more than 20% in 5 cows at from -20 to +12 days, whereas they were decreased more than 20% in 9 cows. The borderline value (20%) was deduced by the individual variations of each cow during -48 to -21 days (see above). The apoC-III concentrations (% of values from -48 to -21 days) in the 5 cows were: $97.4 \pm 10.4\%$ (-20 to -11 days), $123 \pm 16.8\%$ (-10 to 0 days) and $137 \pm 22.5\%$ (+1 to +12 days); whereas those in the 9 cows were $91.2 \pm 21.0\%$ (-20 to -11 days), $63.0 \pm 30.8\%$ (-10 to 0 days) and $60.7 \pm 11.8\%$ (+1 to +12 days). The apoC-III concentration in 5 cows during the period of +1 to +12 days was significantly ($P < 0.05$) higher than that from -48 to -21 days. The increase during the period from -10 to 0 days, compared with the period from -48 to -21 days, was not significant ($P = 0.288$). The decreased apoC-III concentration in the 9 cows from +1 to +12 days was significant ($P < 0.05$), compared with -48 to -21 days. The decrease during the period of -10 to 0 days did not reach significance ($P = 0.0651$).

We have previously shown that healthy cows during the peripartum period can be classified into two groups with respect to LCAT activity; healthy group 1 (LCAT activity is unaltered during the period) and healthy group 2 (the activity is reduced as in cows with ketosis and milk fever) [12].

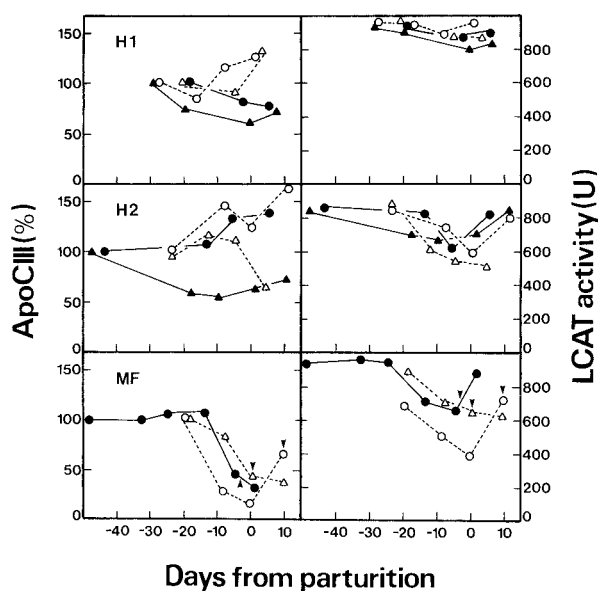


Fig. 1. Changes during the peripartum period of the apoC-III concentration and LCAT activity of healthy group 1 (H1), healthy group 2 (H2) and cows with milk fever (MF). Of the 7 each H1 and H2 healthy cows, results of 4 each are depicted. Milk fever occurred at days -3 (), 1 () and 10 ().

By evaluating LCAT activity, the 14 cows were retrospectively allotted into healthy group 1 ($n=7$) and healthy group 2 ($n=7$, Fig. 1). The 5 cows with increased apoC-III concentrations were allocated into both healthy group 1 ($n=3$) and healthy group 2 ($n=2$). Likewise, of the 9 cows with decreased apoC-III concentrations, 4 cows were classified into healthy group 1 and 5 into healthy group 2, indicating that cows with increased and decreased apoC-III concentrations did not correspond to healthy groups 1 and 2, respectively. The apoC-III concentration and LCAT activity were not correlated in healthy group 1 ($r = -0.133$, $P = 0.656$) or in healthy group 2 ($r = 0.181$, $P = 0.493$). The concentration of CE, the product of LCAT reaction, is also different in the healthy groups 1 and 2 and is lower in healthy group 2 [12]. The CE concentrations were not lower in the 9 cows with decreased apoC-III concentrations than in the 5 cows with increased apoC-III concentrations (data not shown). Concentrations of TG, FC, PL, NEFA, Ca, Mg and IP and activity of AST were not different between cows with increased and decreased apoC-III concentrations.

The apoB-100 concentration physiologically decreases during the peripartum period [24], but in cows with fatty liver and fatty liver-related diseases, decreases are more distinct than those in healthy cows [15]. ApoB-100 concentrations during periods from -10 to 0 days and +1 to +12 days were significantly lower in the 9 cows with decreased apoC-III concentrations than in the 5 cows with increased apoC-III concentrations (Table 1). The difference during the period from -20 to -11 days did not reach significance ($P = 0.0528$). ApoB-100 and apoC-III concentrations were

Table 1. Serum apoB-100 concentrations in healthy cows with increased apoC-III concentrations, those with decreased apoC-III concentrations and cows with milk fever

| Group/ Day | ApoC-III increased (n=5) | ApoC-III decreased (n=9) | Milk fever (n=3) |
|---------------|-----------------------------|-----------------------------|---------------------|
| -48--21 | 417 ± 148 | 357 ± 122 | 295 ± 30 |
| -20--11 | 378 ± 90 | 273 ± 37 | 186 ± 74 |
| -10-0 | 291 ± 45 | 189 ± 74* | 128 ± 82* |
| +1--12 | 227 ± 72 | 163 ± 38* | 167 ± 48 |

Unit is $\mu\text{g/ml}$. *, $P < 0.05$ compared with respective values for the group of ApoC-III increased.

positively correlated in the 9 cows with decreased apoC-III concentrations ($r=0.494$, $P=0.0110$), and negatively in the 5 cows with increased apoC-III concentrations ($r=-0.558$, $P=0.0147$). No correlation between apoB-100 and LCAT activity was observed in the 5 cows with increased apoC-III concentrations or in the 9 cows with decreased concentrations (data not shown).

Of the 17 cows monitored, 3 cows developed milk fever at -3 to +10 days from parturition. Concentrations in the 3 diseased cows of lipids, Ca and other metabolites were as described in a previous paper [22]. Maximal decreased rates of apoC-III concentrations in the 3 diseased cows were 15 to 37%, and were more distinct than those of the 9 healthy cows with decreased apoC-III concentrations (Fig. 1). Except for 1 cow, decreased apoC-III concentrations were observed prior to the appearance of clinical signs such as recumbency. The apoC-III concentration and LCAT activity in the 3 diseased cows were significantly correlated ($r=0.672$, $P=0.0069$), suggesting the association of the apoC-III concentration with LCAT activity, at least in cows with milk fever. The apoB-100 concentration during the period from -10 to 0 days was lower in the 3 diseased cows than in 5 cows from the increased apoC-III concentrations (Table 1). The difference during the same period between cows with milk fever and the 9 cows with decreased apoC-III concentrations was not significant ($P=0.325$). The apoB-100 concentration was positively correlated with the apoC-III concentration in cows with milk fever ($r=0.591$, $P=0.0242$).

DISCUSSION

The present study indicated that the apoC-III concentration in healthy cows was increased (5/14 in this study) or decreased (9/14) during the peripartum period. The apoC-III concentration in healthy cows did not correlate with LCAT activity, but did with apoB-100 concentration negatively in cows with increased apoC-III concentrations or positively in cows with decreased apoC-III concentrations. In cows with milk fever, the apoC-III concentration was more distinctly decreased than in healthy cows, and the apoC-III concentration was positively correlated with both LCAT activity and the apoB-100 concentration.

In the lactation cycle, the apoC-III concentration shows

the lowest value during the nonlactating stage, gradually increases during early lactation, and reaches the maximum concentration during midlactation [21]. The increased apoC-III concentration in the 5 healthy cows from +1 to +12 days, compared to -48 to -21 days, appears to reflect the transition of the apoC-III concentration from the nonlactating to midlactating stages.

We have previously shown that there are two types of cows during the peripartum period with respect to LCAT activity; one has unaltered LCAT activity compared with those during the nonlactating stage, and the other has reduced LCAT activity [10]. None the cows with unaltered LCAT activity had any postparturient diseases during early lactation, whereas cows with reduced LCAT activity had high incidences of diseases such as ketosis. We also found that cows which were free from postparturient diseases can be allotted into two groups [12]; healthy groups 1 (LCAT activity is unaltered) and 2 (the activity is reduced). The reduced LCAT activity in healthy group 2 is suggested to be due to fatty liver, because in cows with fatty liver, LCAT activity is reduced [11, 18]. The healthy cows with decreased apoC-III concentrations did not correspond to healthy group 2. LCAT activity and the CE concentration in the cows with decreased apoC-III concentrations did not differ from those in the cows with increased apoC-III concentrations. The other serum lipid concentrations such as NEFA or AST activity were similarly not different between cows with increased and decreased apoC-III concentrations. The only difference observed in cows with decreased apoC-III concentrations was lower apoB-100 concentrations than in cows with increased apoC-III concentrations. In cows with fatty liver, the apoB-100 concentration is also decreased [6, 8, 17]. Reduced LCAT activity (together with a decreased CE concentration) and decreased concentrations of apoC-III and apoB-100 may reflect different aspects (probably different stages) of fatty liver development. LCAT functionally associates with HDL by esterifying FC on the surface of the HDL [3], whereas apoB-100 is distributed in very low-density lipoprotein (VLDL) and low-density lipoprotein fractions [6, 17]. The VLDL apoB-100 and HDL LCAT may independently relate to differential stages of fatty liver development.

ApoC-III is distributed in human plasma in chylomicrons (CM), and the VLDL and HDL fractions [7, 13]. The apoC-

III is associated with HDL in the fasting state, but it is transferred to the CM and VLDL fractions after the plasma TG concentration is increased by absorption of dietary fat [2]. The total plasma apoC-III concentration increases in hypertriglyceridemia [4], suggesting the involvement of apoC-III in the regulation of TG metabolism. By comparison, except for a calf with hyperlipidemia [23], most apoC-III in normolipidemic cattle is detected in HDL [5, 19, 20, 22]. The redistribution by feeding from HDL to the CM and VLDL fractions is not observed [20]. Moreover, the apoC-III and TG concentrations are negatively correlated in lactating cows [20, 21]. These characteristics of cow apoC-III appear to reflect the extremely low TG concentration (approximately 1/10 of humans). Compared with cows during lactation, those during the prepartum period have higher TG concentrations. It is also noteworthy that cows with milk fever show relatively high TG concentrations [22]. The increased TG concentration induced by nonlactation and milk fever may indicate the functional relevance of apoC-III in the TG metabolism, which is not apparent in lactating cows having extremely low TG concentration. The apoC-III-regulated TG metabolism is thus inferred to be involved in the pathogenesis of milk fever. The TG-transport protein apoB-100 may be relevant to this apoC-III regulation.

From the practical aspect, determination of the apoC-III concentration is less useful than LCAT activity to detect cows susceptible to milk fever, because a decreased apoC-III concentration was not associated with decreased lipid concentrations. However, the decreased concentration of apoC-III was more distinct than that of LCAT activity. In addition to LCAT and apoB-100, determination of the apoC-III concentration during the peripartum period appears to be helpful in diagnosing periparturient diseases, in particular in milk fever.

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