Decreases in serum apolipoprotein B-100 and A-I concentrations in cows with milk fever and downer cows

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Abstract

Milk fever occurring during the peripartum period has been suggested to be caused by fatty liver developed during the non-lactating stage because diseased cows have increased serum concentrations of non-esterified fatty acids (NEFA) and show hepatic lipidosis. In cows with fatty liver and related diseases such as ketosis, serum concentrations of apolipoprotein (apo) B-100 and apoA-I are decreased. The purpose of the present study was to examine whether apoB-100 and apoA-I concentrations are similarly decreased in cows with milk fever. Apolipoprotein concentrations were also measured in cows with downer syndrome, which has been suggested to be related, at least in part, to milk fever. Compared with healthy cows during early lactation, apoB-100 and apoA-I concentrations were decreased in cows with milk fever and also in downer cows. In cows with milk fever, the decreases in apoB-100 and apoA-I concentrations were associated with increased NEFA and decreased cholesterol and phospholipid concentrations. However, in downer cows, serum lipid concentration changes were not as distinct as in cows with milk fever. These results, coupled with previous findings on the decreases in apoB-100 and apoA-I concentrations of cows with fatty liver-related diseases, suggest that fatty liver is involved in the development of milk fever and partly in that of downer cow syndrome.

Résumé

Il a été suggéré que la fièvre vitulaire survenant durant la période péripartum résultait du développement d'un foie gras durant la période de tarissement étant donné que chez les vaches atteintes il y a une augmentation de la concentration sérique d'acides gras non-estérifiés (AGNE) et une lipidose hépatique. Chez des vaches avec un foie gras et présentant des maladies reliées, telle une cétose, les concentrations sériques d'apolipoprotéine (apo) B-100 et apoA-I sont diminuées. Le but de l'étude était d'évaluer si les concentrations d'apoB-100 et d'apoA-I étaient également diminuées chez les vaches avec une fièvre vitulaire. Les concentrations d'apolipoprotéine furent également mesurées chez des vaches affectées du syndrome de la vache à terre, étant donné qu'il a été suggéré que ce syndrome serait relié, du moins en partie, à la fièvre vitulaire. Comparativement à des vaches en santé en début de lactation, les concentrations d'apoB-100 et d'apoA-I étaient diminuées chez des vaches avec une fièvre vitulaire et chez des vaches avec le syndrome de la vache à terre. Chez des vaches avec une fièvre vitulaire, les diminutions des concentrations d'apoB-100 et d'apoA-I étaient associées avec une augmentation d'AGNE et une réduction des concentrations de cholestérol et de phospholipide. Toutefois, chez les vaches avec le syndrome de la vache à terre, les changements des concentrations sériques de lipide n'étaient pas aussi marquées que chez des vaches avec fièvre vitulaire. Ces résultats, combinés aux trouvailles précédentes sur la diminution des concentrations d'apoB-100 et d'apoA-I chez des vaches avec des maladies reliées à un foie gras, suggèrent qu'un foie gras est impliqué dans le développement de la fièvre vitulaire et partiellement dans le syndrome de la vache à terre.

(Traduit par Docteur Serge Messier)

Introduction

High-yielding dairy cows are susceptible to several peripartum diseases, including ketosis, left displacement of the abomasum (LDA), retained placenta, milk fever, and downer cow syndrome; these diseases are suggested to originate from fatty liver developed during the non-lactating stage (1–4). Overfeeding during the non-lactating stage and reduced feed intake and stress near parturition accelerate the release of non-esterified fatty acids (NEFA) from adipose tissues, resulting in an excess uptake of NEFA by the liver. The incorporated NEFA are esterified into triglycerides (TG). Although the mechanism of outbreak in each disease is unknown except for ketosis (5,6), suppressed liver functions due to fatty infiltration of the liver are thought to be closely associated with the development of peripartum diseases.

The most prominent changes in cows with fatty liver and related diseases are decreases in concentrations of lipoproteins, most of which are synthesized by the liver. Apolipoprotein (apo) B-100 is a protein distributed in very low-density lipoprotein (VLDL) and low-density lipoprotein fractions and is essential for the transport of TG

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Table I. Serum metabolite concentrations in healthy cows (C), cows with milk fever (MF), milk fever and ketosis (MF + K), downer cows (DC), and downer cows with ketosis (DC + K)

	Control (n = 12)	MF (n = 17)	MF + K (n = 13)	DC (n = 12)	DC + K (n = 13)
Ca (mmol/L)	2.30 ± 0.32	1.08 ± 0.38[b]	1.08 ± 0.20[b]	2.09 ± 0.24	2.09 ± 0.22
IP (mmol/L)	2.03 ± 0.40	0.76 ± 0.53[b]	0.65 ± 0.26[b]	1.59 ± 0.53	1.50 ± 0.48[a]
BHB (mmol/L)	0.81 ± 0.28	0.66 ± 0.12	1.10 ± 0.20[b]	0.65 ± 0.12	2.95 ± 2.24[b]
TB (mg/dL)	0.23 ± 0.11	0.42 ± 0.33	0.65 ± 0.35[b]	0.33 ± 0.21	0.75 ± 0.44[b]
NEFA (μEq/L)	415 ± 203	794 ± 335[b]	1231 ± 289[b]	667 ± 194[b]	1347 ± 422[b]
TC (mg/dL)	88.2 ± 15.1	69.0 ± 18.1[a]	72.4 ± 12.0[a]	71.9 ± 21.1	67.9 ± 23.2
PL (mg/dL)	107 ± 26.1	70.7 ± 24.7[b]	77.8 ± 15.9[b]	86.2 ± 17.8	75.4 ± 23.7[a]
AST (IU/L)	76.6 ± 16.5	63.7 ± 32.0	92.2 ± 43.6	107 ± 78.4	153 ± 101[a]
CPK (IU/L)	86.0 ± 60.8	838 ± 1428	1009 ± 1050	1934 ± 2643	1849 ± 2613

Ca — calcium; IP — inorganic phosphates; BHB — β-hydroxybutyrate; TB — total bilirubin; NEFA — non-esterified fatty acids; TC — total cholesterol; PL — phospholipids; AST — aspartate transaminase; CPK — creatine phosphokinase
[a] $P < 0.05$, [b] $P < 0.01$, compared with respective values for healthy controls

from the liver to extrahepatic tissues. ApoA-I is a major apoprotein in the high-density lipoprotein (HDL) fraction, has a role for the activation of lecithin:cholesterol acyltransferase (LCAT), and is thereby involved in the regulation of cholesterol esterification. The apoB-100 and apoA-I concentrations are decreased in experimentally induced (7,8) and naturally acquired fatty liver (9,10) and in ketosis (11), LDA (11), and retained placenta (12). Decreases in apoB-100 and apoA-I concentrations appear to be directly related to suppressed VLDL TG secretion (3,7,8) and reduced cholesterol esterification (10,13–15), respectively, that are observed in diseased cows. Milk fever has also been suggested to be a fatty liver-related disease because of its association with an elevated NEFA concentration (16,17), fatty infiltration of the liver (18), and decreased cholesterol, phospholipid (PL), and apoC-III concentrations (19). The purpose of the present study was to determine whether apoB-100 and apoA-I concentrations were decreased in cows with milk fever, to obtain data further supporting the hypothesis that milk fever is a fatty liver-related disease. The apoB-100 and apoA-I concentrations were also determined in downer cows, because fatty liver has been proposed to be one of the major causes of downer cow syndrome (20).

Materials and methods

Cows

Holstein cows (2 to 8 y old, n = 67) during early lactation (1 to 9 d after parturition) from farms of Iwate Prefecture were used. Their milk yields and diet were as described previously (11,12). Of the 67 cows, 12 were clinically healthy and the remaining 55 had milk fever (n = 17), milk fever with ketosis (n = 13), downer cow syndrome (n = 12) and downer cow syndrome with ketosis (n = 13). Milk fever was diagnosed by hypocalcemia, recumbency, and quick standing in response to calcium (Ca) treatment (20). The borderline value for hypocalcemia was obtained from the mean − 2SD value for serum Ca concentration in healthy cows (2.30 ± 0.32 mmol/L), and was calculated to be 1.66 mmol/L. Ketosis was detected by the urine ketone reaction (11). Downer cows were defined by the following criteria: recumbency, with Ca concentrations higher than the borderline value, and no response to Ca treatment. Cows with fracture, dislocation, arthritis, dystocia, and apparent inflammatory diseases, such as mastitis, were not included in the downer cow group. Healthy cows were chosen as controls by the scrutinies of clinical signs, including udders and negative urine ketone reaction. Blood samples were collected in the morning (before feeding); those from diseased cows were taken in the morning after the day that clinical signs of disease had been initially noticed, and prior to first treatment. All procedures involving cows and their care were conducted in accordance with the Animal Care Standards of Rakuno Gakuen University.

Apolipoprotein analysis

Serum concentrations of apoB-100 (21) and apoA-I (22) were evaluated by enzyme-linked immunosorbent assays.

Other methods

Serum Ca concentration was determined using an atomic absorption spectrophotometer (Model AA-670; Shimadzu, Kyoto, Japan). The β-hydroxybutyrate (BHB) concentration was estimated by use of a kit (Sanwa Kagaku, Nagoya, Japan). Concentrations of inorganic phosphate (IP), magnesium (Mg), total bilirubin (TB), NEFA, total cholesterol (TC), and PL, and activities of aspartate transaminase (AST), creatine phosphokinase (CPK), and lactate dehydrogenase (LDH) were measured by automated analysis (Model 7450; Hitachi, Hitachi, Japan). Data were analyzed, using one-way analysis of variance and Scheffe's F-test. Values were expressed as mean ± SD.

Results

Serum Ca concentration was decreased in cows with milk fever, but not in downer cows, as compared with controls (Table I). The association with ketosis had little effect on the Ca concentration. The IP concentration in cows with milk fever was remarkably lower than that in downer cows. Significant changes in the Mg concentration was not detected in cows with milk fever or in downer cows (data not shown). Increases in the BHB concentration were significant in ketotic cows with milk fever and ketotic downer cows, but did

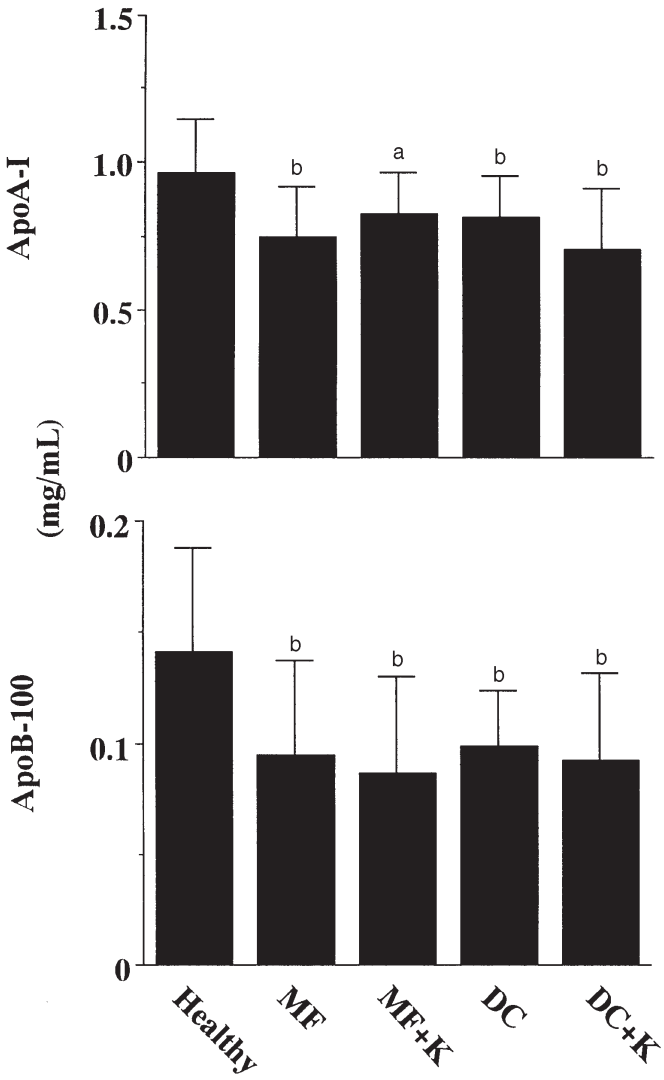

Figure 1. ApoB-100 and apoA-I concentrations in healthy controls, cows with milk fever (MF), cows with milk fever and ketosis (MF + K), downer cows (DC) and downer cows with ketosis (DC + K). [a] $P < 0.05$, [b], $P < 0.01$, compared with controls.

not reach significance in cows with milk fever alone ($P = 0.0748$) or downer cow syndrome alone ($P = 0.175$). The TB concentration was increased in both ketotic milk fever cows and ketotic downer cows. The NEFA concentration was increased, particularly in ketotic cows. The TC concentration was decreased in cows with milk fever alone, as described previously (19), and those with milk fever and ketosis, but not in downer cows alone ($P = 0.115$) or in those with ketosis ($P = 0.0695$). Changes in the PL concentration were similar to those in the TC concentration. The AST activity was significantly increased in downer cows with ketosis. Creatine phosphokinase (Table I) and LDH (not shown) activities were markedly increased particularly in downer cows, but their differences were not significant because of large deviations.

ApoB-100 and apoA-I concentrations in healthy cows during early lactation were 0.141 ± 0.050 mg/mL and 1.089 ± 0.141 mg/mL, respectively, and were comparable to values previously reported (10–12,21) (Figure 1). The apoB-100 concentration was decreased in cows with milk fever, milk fever and ketosis, downer cows, and downer cows with ketosis. Differences among the 4 disease groups were not significant (58% in controls in milk fever with ketosis to 71% in downer cows). The apoA-I concentration was similarly decreased in the 4 diseased groups. As in apoB-100, differences among the groups were not significant (64% in downer cows with ketosis to 76% in milk fever with ketosis).

Discussion

The present study indicated that apoB-100 and apoA-I concentrations were decreased in cows with milk fever and also in downer cows. An increase in NEFA and decreases in TC and PL concentrations were distinct in cows with milk fever, but not in downer cows. Occurrences of milk fever, downer cow syndrome, ketosis, LDA and retained placenta during peripartum period are interrelated (23,24). In the present study, we found an association of milk fever with ketosis at a high rate (13/30 in Table I) and also one of downer cows with ketosis (13/25). The intimate associations between ketosis and LDA (11) and between ketosis and retained placenta (12) have been previously observed. The interrelated occurrences of peripartum diseases are thought to be due to overfeeding during the nonlactating stage (4). The overfeeding, together with reduced feed intake near parturition, results in an excess hepatic uptake of NEFA. The fatty liver is suggested to act as a trigger for development of peripartum diseases, including milk fever (1–3,6,16–18). ApoB-100 and apoA-I concentrations are decreased in cows with fatty liver (7–10), ketosis (11), LDA (11), and retained placenta (12). The apoC-III concentration (19) and LCAT activity (10,13–15) are similarly decreased in common in fatty liver and fatty liver-related diseases, including milk fever. Taken in conjunction with previous results, the present findings suggest support to the hypothesis that milk fever is one of the fatty liver-related diseases.

Fatty liver is also inferred to be one of the causal factors for downer cow syndrome (20). Sixty to 70% of downer cows investigated in the slaughterhouse had fatty liver (more than 30 mg of TG/g of liver; Oikawa, unpublished results). This preliminary study may indicate that the decreases in apoB-100 and apoA-I concentrations of downer cows were primarily caused by fatty liver. In the downer cows without ketosis, TB, TC, and PL concentrations were not significantly different from those in controls (Table I). Moreover, the increase in NEFA concentration was not distinctly observed. The indistinct changes in lipid concentrations may reflect the presence of at least 2 different groups of downer cows; one induced by fatty liver, the other caused by factors other than fatty liver, as suggested from the data on slaughtered downer cows.

The mechanism leading to the decreases in apoA-I and apoB-100 concentrations in cows with milk fever is unknown. One possible mechanism is the impairment of signal transduction mediated by nuclear receptors. The serum Ca concentration is mainly regulated by 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$; the active form of vitamin D] (20,25). The 1,25-$(OH)_2D_3$ exerts its function by binding with nuclear vitamin D receptor (VDR). The ligand-bound VDR becomes active when it forms a heterodimer with retinoid X receptor (RXR), which also belongs to the nuclear receptor family

(26). The RXR, other than with VDR, forms a heterodimer with peroxisome proliferator-activated receptor (PPAR). The PPAR, another nuclear receptor, is activated by peroxisome proliferators such as fibrates (hypolipidemic drugs) and by fatty acids (26). Activation of PPAR by fatty acids results in decreases of hepatic expressions of apoA-I, apoB-100, and apoC-III (27). In cows with fatty liver, impairment of the PPAR- and RXR-mediated signal transduction, probably their overexpression, may be induced by an elevated NEFA concentration or accumulated TG and, as a consequence, decrease these apolipoprotein concentrations. Impaired functions of PPAR and RXR may further reduce the 1,25-$(OH)_2D_3$-binding activity of VDR, leading to the development of milk fever.

In conclusion, apoB-100, and apoA-I concentrations were decreased in cows with milk fever and downer cows, as observed in cows with fatty liver and fatty liver-related peripartum diseases, such as ketosis, suggesting the involvement of the fatty liver in the development of milk fever and, at least in part, of downer cow syndrome.

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