

脂肪肝牛の血清中 35K タンパク質

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Specific Increase of a 35K Protein in the Sera of Cows with Fatty Liver

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ABSTRACT. Sera and liver extracts of cows with fatty liver were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis to demonstrate the specific changes in protein profile of fatty liver cows. In the sera from cows with fatty liver, a 35K protein was found to be increased as compared to that of sera from normal cows. On the other hand, the increase of 35K protein was not observed in sera from cows with other diseases we so far tested. The increase of 35K protein in sera from fatty liver cows was further confirmed by gel filtration of the sera using Sephadex G-100. The increase of 35K protein was also observed in liver extracts from fatty liver cows. These results suggested that the increase of this protein was specifically associated with fatty liver of cows.—**KEY WORDS:** fatty liver, SDS-PAGE, 35K-protein.

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Fatty liver syndrome is one of the major post parturient disorders of particularly high yielding cows. Recently, the clinical significance of fatty liver has been re-evaluated because the liver may be accompanied by several complications, including ketosis and reduced fertility [6, 12]. The diagnosis of fatty liver can be achieved principally by either estimating the fat content histologically or measuring the triglyceride content of liver obtained by needle biopsy [1, 5]. In view of the clinical importance of fatty liver, studies on differential diagnosis using simple clinico-biochemical methods are required, and many attempts have been reported [1, 10, 11, 13]. However, a specific method suitable for diagnostic purposes has not yet been developed.

In the experimental fatty liver induced by ethionine, it has been reported that protein synthesis in the liver is inhibited, thereby affecting the protein dynamics of the liver and serum in the ethionine-treated animals [3]. This finding prompted us to investigate

the protein dynamics of serum and liver obtained from cows with fatty liver. In the present paper, we report that a 35K protein (a protein having a molecular weight of 35,000 dalton) is increased in both sera and livers of fatty liver cows. We also report that the increase of this 35K protein is not observed in other diseases we so far tested, suggesting that the increase is specifically associated with fatty liver of dairy cows.

MATERIALS AND METHODS

Marker proteins for molecular weight determination on Sodium Dodecyl Sulfate (SDS)-polyacrylamide gel electrophoresis and for gel filtration using Sephadex G-100 column were purchased from Boehringer Mannheim Biochemicals (Tokyo, Japan). Sephadex G-100 (superfine) and blue dextran 2000 were from Pharmacia Fine Chemicals (Uppsala, Sweden). Triolein was from Sigma Chemical Co. (St. Louis, MO, USA). Acrylamide, N,N'-methylene-bis(acrylamide) and SDS were from Nakarai Chemicals (Kyoto, Japan). All other reagents were of analytical grade.

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Fatty livers from 13 Holstein cows were obtained from Tamamura slaughter house, Gumma Prefecture. Venous blood corresponding to each fatty liver cow was obtained before sacrifice and the serum was immediately collected. Livers and sera from Holstein cows with cholangitis (7 cows), fat necrosis (3 cows), liver abscess (4 cows), liver exhibited cloudy swelling and fragility (4 cows), and normal liver (6 cows) were similarly collected.

Polyacrylamide gel electrophoresis in the presence of SDS followed the method of Rudolph and Krueger [14] using a 10% polyacrylamide separating slab gel, with slight modifications [7, 8]. Standards used to calibrate gels for molecular weight determination were phosphorylase b (94,000 dalton), bovine serum albumin (68,000 dalton), ovalbumin (43,000 dalton), α -chymotrypsinogen (26,000 dalton), myoglobin (18,000 dalton) and cytochrome C (12,500 dalton).

Liver triglycerides were extracted by *n*-heptane [2], and ester groups in triglycerides were determined spectrophotometrically [15]. Triglyceride content in the liver was calculated using triolein as the standard. Protein content was determined by the method of Lowry *et al.* [9] using bovine serum albumin as the standard.

RESULTS

At first, liver triglyceride content was measured to confirm biochemically that the materials used in the present study were from fatty liver cows. In normal livers, triglyceride content was 8.3 ± 2.2 mg per g tissue (wet weight, $n=6$), whereas in fatty livers the value was 63.1 ± 41.4 mg per g tissue ($n=13$). The difference in contents between normal and fatty livers was shown to be significant by student *t* test analysis ($p < 0.01$).

Fig. 1 compares SDS-polyacrylamide gel

electrophoretic patterns of sera from normal cows with those from fatty liver cows. Three major protein bands were observed in both groups of sera. A 68K protein (a protein having a molecular weight of 68,000 dalton) had the same electrophoretic mobility as that of bovine serum albumin used as a marker protein. Another two major proteins, a protein migrating between 68K and 43K and a protein having a molecular weight of about 26,000 dalton were designated to be immunoglobulin heavy and light chains, respectively. The protein pattern including those of the three major proteins were principally similar for both groups of sera. However, several protein bands were distinctly different. In fatty liver sera, 47K and 30K proteins decreased as compared to those in normal serum. In contrast, a 35K protein and a 23K protein increased significantly in fatty liver sera and in particular, the increase of 35K protein was seen in all fatty liver sera tested. Triglyceride contents of fatty livers used in Fig. 1 (F 1-4) were 70, 63, 53, 38 mg per g tissue, respectively. A direct correlation was not observed between the triglyceride content in livers and the extent of the decrease or increase of the four serum proteins.

SDS-polyacrylamide gel electrophoretic patterns of sera obtained from cows with liver exhibited cloudy swelling and fragility, cholangitis, fat necrosis and liver abscess are shown in Fig. 2. There was a decrease in the band corresponding to 47K protein in all the liver or liver-related diseases, suggesting that the decrease seen in Fig. 1 was not specific to fatty liver. Also, the decrease of 30K protein was observed in the cases of cholangitis (No. 3, 4) and fat necrosis (No. 5). The 23K protein, which had not appeared in the normal serum used in Fig. 1 was detected in the lane of normal serum in Fig. 2, and seemed to be split into two bands. The 23K protein appeared to be labile and inadequate to use as a marker

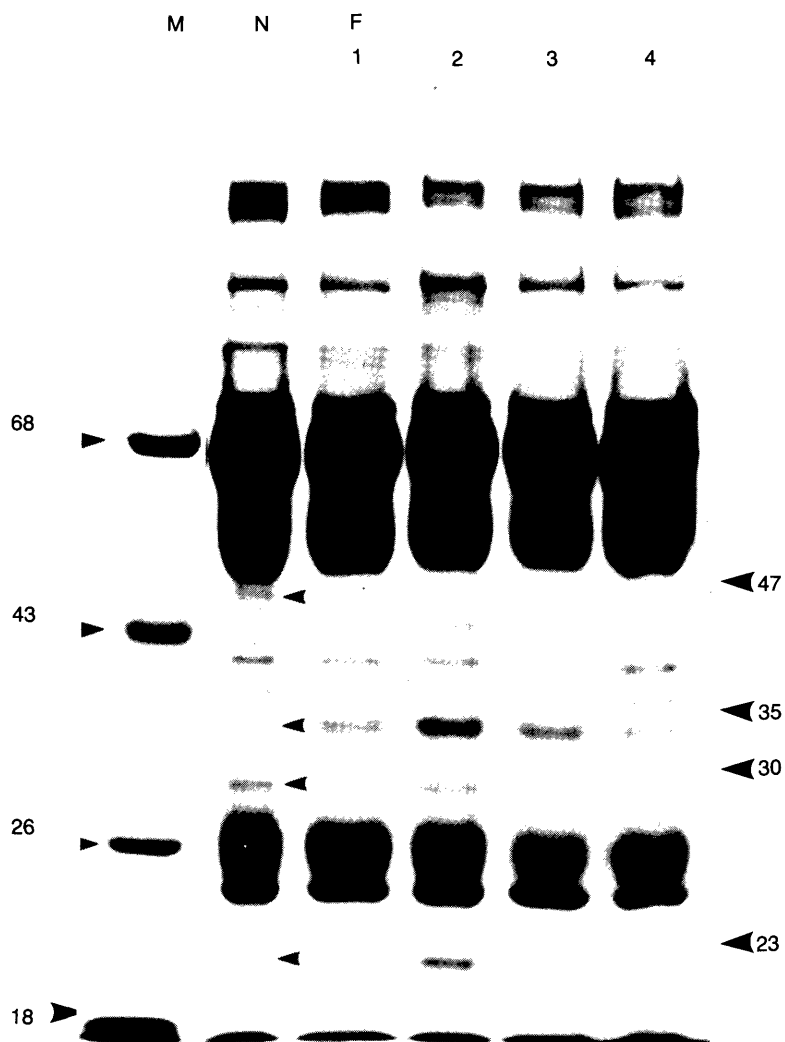


Fig. 1. SDS-polyacrylamide gel electrophoresis of sera from normal and fatty liver cows. Each serum was adjusted to 3 mg/ml protein with 20 mM Tris/Cl (pH 7.5). The diluted serum (0.2 ml) was mixed with 0.1 ml of a solution containing 9% SDS, 15% glycerol, 6% 2-mercaptoethanol, 0.05% bromophenol blue and 50 mM Tris/Cl (pH 7.5), and boiled for 2 min. A 50 μ l aliquot (containing 100 μ g of protein) was applied to each lane. After electrophoresis, the gel was stained overnight with 0.18% Coomassie brilliant blue, 45% ethanol and 10% acetic acid, destained with 25% ethanol and 10% acetic acid, rinsed with 3% glycerol and 10% acetic acid, and then dried. M, marker proteins; N, normal serum; F, fatty liver serum. Molecular weights are shown as $\times 10^{-3}$.

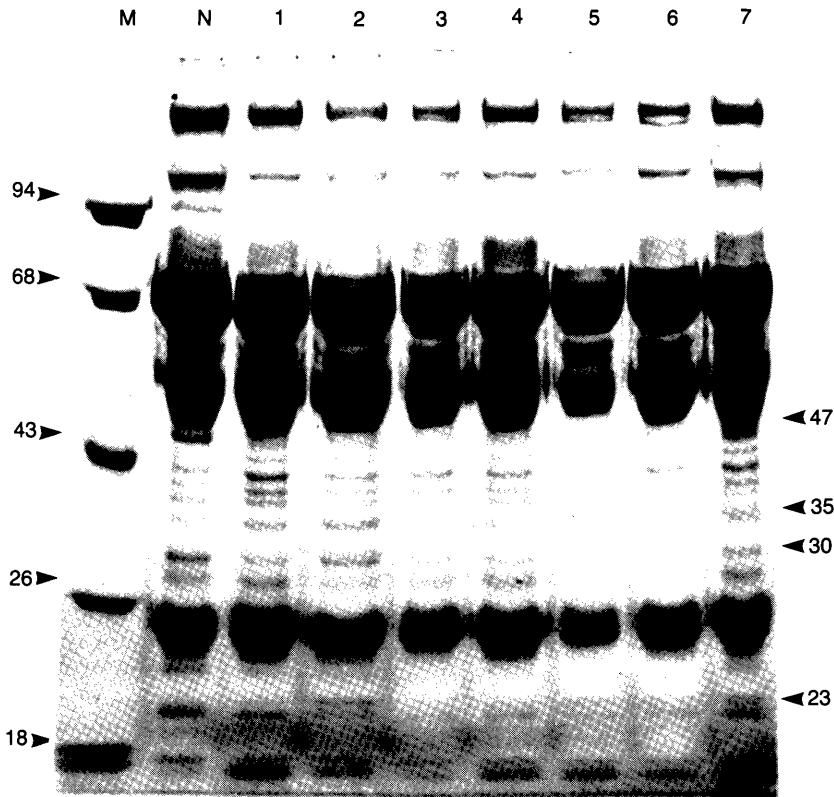


Fig. 2. SDS-polyacrylamide gel electrophoresis of sera from cows with liver exhibited cloudy swelling and fragility (No. 1, 2), cholangitis (No. 3, 4), fat necrosis (No. 5) and liver abscess (No. 6, 7). M, marker protein; N, normal serum. Other conditions were as described in Fig. 1.

proteins for specifying fatty liver. As compared to the 47K, 30K and 23K proteins, the 35 protein did not change apparently in all sera tested. The 35K protein appeared to be associated specifically with fatty liver.

To verify the increase of 35K protein in the sera from fatty liver cows, a serum (a pool of sera nos. 1-4 in Fig. 1) was fractionated using gel filtration on Sephadex G-100, and the elution pattern was compared with that of normal serum. Sephadex G-100 chromatography produced two major peaks with the elution profile of the fatty liver serum being similar to that of the normal serum (Fig. 3a). All eluted fractions were analyzed by SDS-polyacrylamide gel electrophoresis, and the 35K protein was

detected in fractions nos. 30 and 31 of fatty liver serum (Fig. 3b). In normal serum, the 35K protein could not be detected in all the fractions even in the corresponding nos. 30 and 31, suggesting that the 35K protein amount was too small to detect its band because the 35K protein is a minor component in normal serum. The 35K protein of serum from fatty liver cows was eluted between the positions of blue dextran (void volume) and catalase (240,000 dalton), showing that the molecular weight of the protein in its native form was over 240,000 dalton and the value was much higher than that of its denatured form in the presence of SDS.

In addition to serum, liver extracts from

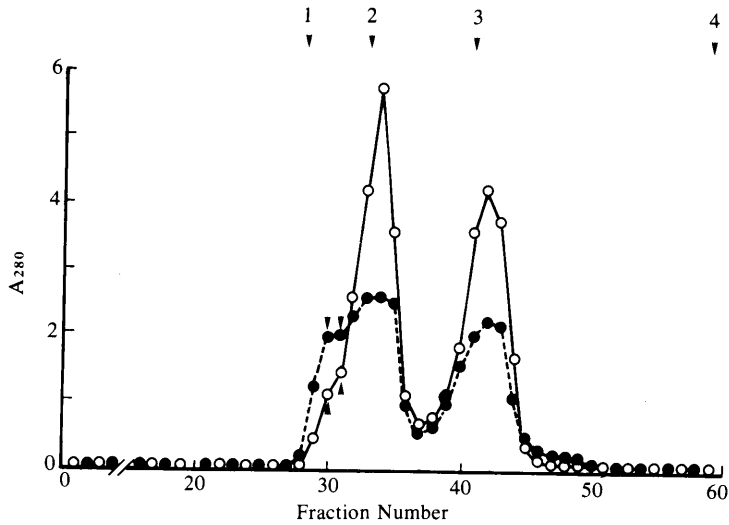
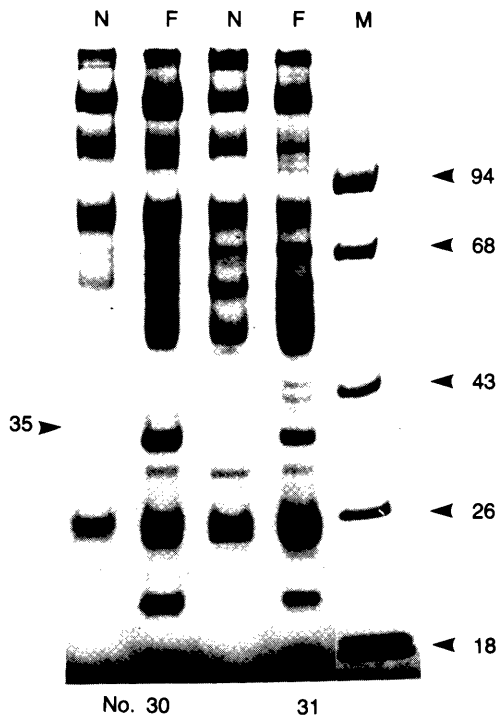


Fig. 3a. Gel filtration on Sephadex G-100 of sera from normal and fatty liver cows. Three ml of normal serum (○, 201 mg protein) and 2.2 ml of serum from liver cows (●, 139 mg protein) were applied separately to a Sephadex G-100 column (2.6×80 cm) which had been previously equilibrated with 20 mM sodium phosphate buffer (pH 7.0). Serum from fatty liver cows used in this experiment were a pool of sera nos. 1-4 (Fig. 1). The flow rate was 20 ml/hr and the fraction size was 5 ml. Numbers indicated by arrow show the positions of molecular weight markers: 1, blue dextran 2000 (mean molecular weight, 2,000,000 dalton); 2, catalase (240,000 dalton); 3, bovine serum albumin (68,000 dalton); 4, myoglobin (18,000 dalton).



normal and fatty livers were also analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 4). When compared to the sera, liver extracts were richer in protein amount and species, and their electrophoretic patterns were very complicated. Nevertheless, the increase of 35K protein was clearly demonstrated in the fatty liver extracts, like in the corresponding sera.

DISCUSSION

In the present paper, we have shown that the 35K protein is increased both in serum and liver of cows with fatty liver. This

Fig. 3b. SDS-polyacrylamide gel electrophoresis of the fractions from the Sephadex G-100 chromatograph in Fig. 3a. The electrophoretic patterns of fraction nos. 30 and 31 (indicated by arrow in Fig. 3a) are shown in this figure. The electrophoretic conditions were as described in Fig. 1.

increase was not observed at least in sera from cows with liver exhibited cloudy swelling and fragility, cholangitis, fat necrosis and liver abscess, thereby strongly suggesting that the increase of the protein is specific to fatty liver.

Ethionine, an analogue of the amino acid methionine, induces experimental fatty liver in the rat and several other species. Ethionine traps ATP as S-adenosylethionine and inhibits ATP-linked protein synthesis, and results in the change of protein dynamics including apolipoproteins in the liver [4]. Although we used only one cow, we have succeeded in inducing fatty liver in a cow by abdominal administration of ethionine (82 mg triglycerides per g tissue). Analysis by SDS-polyacrylamide gel electrophoresis of serum and liver obtained from the cow gave similar results with those presented in this paper (unpublished results). The result obtained using ethionine-induced fatty liver also suggests that the increase of 35K protein is specific to fatty liver, irrespective of experimental or naturally occurring fatty liver.

At present, the 35K protein has not yet been identified, and the characteristics of the protein are unknown. However, an important information can be obtained from the gel filtration data shown in Fig. 3. The molecular weight of the protein is determined to be 35,000 dalton by polyacrylamide gel electrophoresis in the presence of SDS. On the other hand, the molecular weight in the native form is estimated to be over 240,000 dalton by gel filtration on Sephadex G-100. The difference between native and denatured forms can be explained by the assumption that the 35K protein is one of the components of a large protein molecule. Another possibility is that the 35K protein is a apoprotein moiety of apolipoprotein, because the apparent molecular weight would be estimated to be more than 35,000 dalton if the protein was

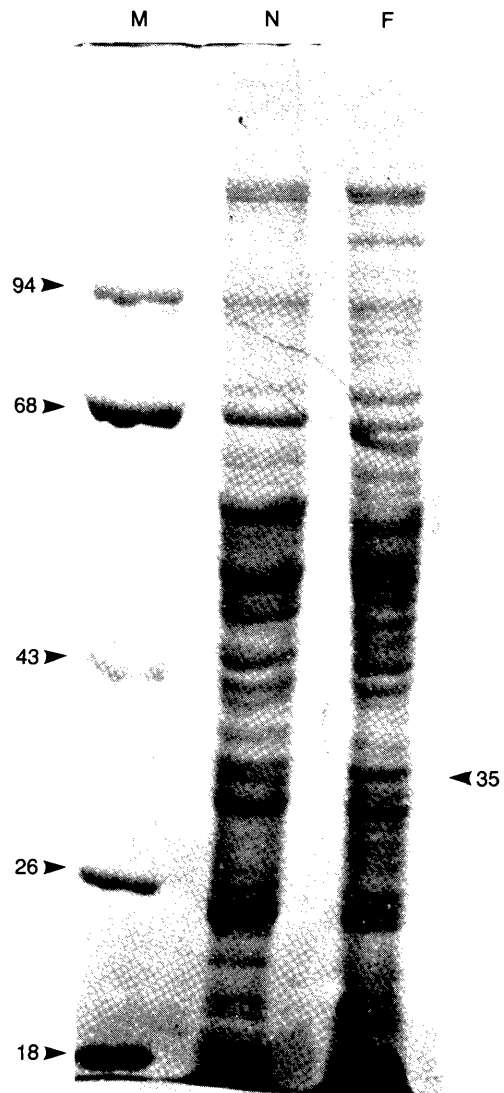


Fig. 4. SDS-polyacrylamide gel electrophoresis of liver extracts from normal and fatty liver cows. Liver (corresponding to No. 2 serum in Fig. 1) was homogenized with a Waring blender in 9 volumes of 20 mM Tris/Cl (pH 7.5) and centrifuged at $10,000 \times g$ for 10 min. The supernatant obtained was used as the liver extract. Other conditions were as described in Fig. 1.

bound to lipids. The 35K protein content is increased in the liver as well as in serum of cows with fatty liver. This suggests that the protein is of liver origin, as is apolipoprotein, and may be shed to the blood stream.

To our knowledge, this study is the first clinico-biochemical manifestation indicating that a 35K protein in serum, presumably of liver origin, is specifically increased in cows with fatty liver. This finding may constitute a basis for investigating the correlation of protein dynamics in serum and the mechanism of fatty liver, and further provide a useful tool for differential diagnosis of fatty liver syndrome in cows.

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要 約

脂肪肝牛の血清中35K タンパク質：関口邦彦・加藤憲夫¹⁾・元井葎子¹⁾（東京都家畜保健衛生所，¹⁾家畜衛生試験場）——脂肪肝牛の血清と肝臓のタンパク質動態を SDS-ポリアクリルアミドゲル電気泳動で解析した。脂肪肝牛血清では他の肝疾患牛にくらべて35K タンパク質のレベルが高かった。このことは血清をセファデックス G-100でゲルろ過することにより確認された。肝臓においても35K タンパク質は正常牛肝臓にくらべて高かった。