Utilization of Intestinal Triglyceride-rich Lipoproteins in Mammary Gland of Cows

Tsuyoshi UCHIDE, Ken ONDA, Makoto BONKOBARA, Boonrit THONGSONG, Naoaki MATSUKI, Mutsumi INABA and Kenichiro ONO*

Laboratory of Veterinary Clinical Pathobiology, Department of Veterinary Medical Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

(Received 10 March 1999/Accepted 1 July 1999)

ABSTRACT. Elution profiles of total lipoproteins, apolipoprotein B (apoB) concentrations in lipoproteins, and plasma triglyceride (TG) levels were examined in early-, late-, and non-lactating cows. Additionally, arteriovenous (A-V) differences were also measured to elucidate the uptake of TG and apoB-containing lipoproteins in mammary gland. Non-lactating cows showed three major peaks corresponding to triglyceride-rich lipoprotein (TRL), low density lipoprotein (LDL), and high density lipoprotein (HDL) fraction, whereas both early- and late-lactating cows revealed two peaks corresponding to TRL and HDL. The peak area of TRL in early- and late-lactating cows were significantly ($p < 0.05$) smaller than that in non-lactating cows. The plasma TG levels and apoB-48 concentrations of TRL in early- and late-lactating cows were also significantly ($p < 0.01$) lower. Furthermore, early lactating cows showed significantly ($p < 0.05$) larger A-V differences in both plasma TG and apoB-48 concentration of TRL than those in late- and non-lactating cows. These results suggested that TG in exogenous (intestinal) TRL was utilized for milk fat synthesis in lactating mammary gland of cows by the receptor-mediated uptake.—**KEY WORDS:** apolipoprotein B, cattle, lipoprotein profile, mammary gland, triglyceride-rich lipoprotein.

J. Vet. Med. Sci. 61(10): 1143–1146, 1999

High milk-yield lactating cows synthesize and secrete a large amount of milk fat consisting predominantly of triglyceride (TG) [3]. Apolipoprotein B (apoB)-containing lipoproteins such as chylomicron (CM, Sf >400 , d <1.006 g/ml), very low density lipoprotein (VLDL, Sf <400 , d <1.006 g/ml), intermediate density lipoprotein (IDL, d $=1.006$ – 1.019 g/ml) and low density lipoprotein (LDL, d $=1.019$ – 1.063 g/ml) have been considered to transport TG to mammary gland for milk fat formation in cows [1, 7]. Among them, CM and VLDL called as triglyceride-rich lipoproteins (TRL) play an important role in the transport of exogenous and endogenous TG derived from intestine and liver, respectively [1, 13, 16].

ApoB is one of the major protein components of apoB-containing lipoproteins, and serves as an essential apolipoprotein that maintains the lipoprotein structure [12] as well as the ligand to receptors that regulate lipoprotein uptake to cells [4]. Bovine apoB shows two molecular isoforms, apoB-100 and apoB-48 [2, 10]. The former is synthesized and secreted as VLDL from liver, while the latter as CM from intestine [1, 8]. Therefore, the analysis of apoB isoforms provides the origin of lipoproteins from liver or intestine.

However, there are few informations on the origin of lipoproteins to transport TG to mammary gland for milk fat formation. In our previous report [19], apoB-48-containing lipoprotein derived from intestine might be a major

lipoprotein in TG metabolism in cows.

In this paper, elution profiles of lipoproteins, apoB concentrations in lipoproteins, and arteriovenous (A-V) differences in lactating and non-lactating Holstein cows were examined, and discussed in relation to the role of apoB-containing lipoproteins as a transporter of TG to mammary gland.

MATERIALS AND METHODS

Animals and chemicals: Twenty four Holstein cows kept in Animal Husbandry Experimental Station of Tokyo University were used in this study. The cows were divided into three groups dependent on their lactating periods: early-lactating (1 to 23 weeks postpartum, 25.6 ± 3.2 kg milk yield/day), late-lactating (42 to 70 weeks postpartum, 13.0 ± 3.0 kg milk yield/day), and non-lactating. The cows were fundamentally fed haylage (17 kg/day) and hay (4 kg/day), and supplementally concentrates twice a day at the rate of 3.5 kg per 10 kg of milk yield in lactating cows.

All chemical reagents were obtained from Wako Pure Chemical (Osaka, Japan) unless indicated.

Blood samples: Blood samples were collected from jugular vein for the analyses of elution profiles, apoB concentrations in lipoproteins, and plasma TG levels. For A-V difference studies, blood samples were collected from mammary vein and artery.

Preparation of lipoproteins: Plasma collected was added with 100 μ g/ml sodium azide, 50 μ g/ml gentamycin (Schering-Plough, Osaka, Japan), 10 U/ml aprotinin, 1 mM benzamidine, and 1 mM phenylmethylsulfonyl fluoride (Sigma, St. Louis, MO, U.S.A.). Total lipoproteins (TLP, d <1.21 g/ml), consisting CM, VLDL, IDL, LDL, and high

* CORRESPONDENCE TO: ONO, K., Laboratory of Veterinary Clinical Pathobiology, Department of Veterinary Medical Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan.

density lipoprotein (HDL), were separated from the plasma by a ultracentrifugation (50,000 rpm, 15 hr, 20°C) at density 1.21 g/ml using an angle rotor (RP 80, Hitachi, Tokyo, Japan).

Elution profiles of lipoproteins by gel permeation chromatography: Fractions of TRL, IDL, LDL, and HDL were separated from TLP by gel permeation chromatography as previously described [19]. Briefly, TLP was applied to Superose 6 HR 10/30 column (Pharmacia, Uppsala, Sweden) and eluted with 1 mM ethylenediamine-tetraacetic acid in phosphate buffer saline, pH 7.4 at 20°C.

Measurement of total apoB and apoB-100 concentrations: The concentrations of total apoB and apoB-100 in each lipoprotein fraction separated by gel permeation chromatography were determined by an enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody which detected apoB-100 alone or both apoB-48 and apoB-100 [19]. The concentration of apoB-48 was calculated by subtracting apoB-100 from total apoB.

Measurement of plasma TG: Plasma TG levels were determined by an enzymatic method as previously reported [11] using an automatic analyzer (model 736-20, Hitachi, Tokyo, Japan).

Statistical analysis: Significant differences were analyzed by Student's *t*-test.

RESULTS

Elution profiles of lipoproteins by gel permeation chromatography: Non-lactating cows showed three major peaks, indicated as (a), (b), and (c), corresponding to TRL, LDL, and HDL fraction, respectively, whereas both early- and late-lactating cows revealed two peaks, corresponding to TRL and HDL fraction (Fig. 1). The area of Peak (a) calculated from these elution profiles in early- and late-lactating cows were significantly ($p < 0.05$) smaller than that in non-lactating cows, whereas the Peak area (c) was significantly ($p < 0.01$) larger (Table 1).

ApoB-48 and apoB-100 concentrations in TRL, LDL, and HDL, and plasma TG levels: The concentrations of apoB-48 and apoB-100 in TRL, IDL, and LDL, and plasma TG levels in early-, late-, and non-lactating cows are shown in Fig. 2. In TRL, apoB-48 concentrations indicating CM, by which exogenous TG was transported from intestine, in both early- and late-lactating cows were significantly ($p < 0.01$) lower than that in non-lactating cows. The concentration of apoB-100 in TRL were below the assay limit. No significant difference in apoB-100 level in both IDL and LDL was observed among three cow groups. The plasma TG levels in early- and late-lactating cows were significantly ($p < 0.01$) lower than that in non-lactating cows.

Arteriovenous differences: To elucidate the uptake of TRL by mammary gland, the A-V difference in plasma TG and apoB-48 levels of TRL from mammary vein and artery were examined. In plasma TG, early-lactating cows showed significantly ($p < 0.05$) larger A-V differences compared with late- and non-lactating cows. ApoB-48 concentrations of

Fig. 1. Elution profiles of TLP in early-, late-, and non-lactating cows. Value represents the mean (broken line) $\pm$ SD (solid line) of 4 cows for each group.

Table 1. Peak area of elution profiles in early-, late-, and non-lactating cows

Peak	Lactating		Non-lactating
	Early	Late	
a	37.3 $\pm$ 14.4	30.4 $\pm$ 7.1	97.6 $\pm$ 44.2*
b	—	—	28.8 $\pm$ 11.2
c	1010 $\pm$ 101	957 $\pm$ 33.6	513 $\pm$ 69.6**

Each value represents the mean $\pm$ SD of 4 cows for each group. * and **: Significant difference between lactating and non-lactating cows (*: $p < 0.05$, **: $p < 0.01$).

TRL in both early- and late-lactating cows were significantly ($p < 0.05$) larger than that in non-lactating cows. No significant A-V differences in both apoB-48 and apoB-100 concentrations in IDL and LDL were observed among three cow groups (Fig. 3).

DISCUSSION

To investigate bovine lipoproteins and lipid metabolism, ultracentrifugation, electrophoresis, and gel permeation chromatography techniques have been commonly used [6, 15, 16]. Among these techniques, gel permeation chromatography is useful for both the analysis and preparation of bovine lipoproteins. Grummer *et al.* [9] reported that three major peaks (TRL, LDL, and HDL) were observed on the elution profiles of TLP in both lactating

Fig. 2. ApoB-48 () and apoB-100 () concentrations in 3 lipoprotein fractions and plasma TG levels (▨) in early-, late-, and non-lactating cows. Significant ($p < 0.01$) low levels of apoB-48 in TRL, and of plasma TG levels were observed in lactating cows compared with those in non-lactating cows. Each group contains 4 cows.

and non-lactating cows by gel permeation chromatography, however, only two peaks (TRL and HDL) were observed in lactating cows in this study. Ferreri and Gleockler [6] also detected two peaks in lactating cows and speculated that the lack of LDL peak was resulted from large HDL particles eluted broadly at the same position in LDL. No significant difference of apoB concentrations in LDL between lactating cows and non-lactating cows observed in this study supported that LDL peak was involved in HDL peak on the elution profile.

Many researches reported on apoB-containing lipoproteins as a transporter of TG to mammary gland [7, 16], however, it has been uncertain whether the major source of TG is a liver- or intestine-derived lipoprotein for milk fat formation. In our preceding report [19], significant low levels of plasma TG and apoB-48 in TRL were observed in Holstein cows compared with those in Japanese black cows,

Fig. 3. Arteriovenous (A-V) differences of apoB-48 () and apoB-100 () in 3 lipoprotein fractions, and plasma TG levels (▨) in early-, late-, and non-lactating cows. Significant ($p < 0.05$) large differences of apoB-48 in TRL, and of plasma TG levels were observed in lactating cows compared with those in non-lactating cows. Each group contains 4 cows.

suggesting that the major source of TG for milk fat was the apoB-48-containing lipoprotein derived from intestine. In this study, plasma TG level, TRL peak area, and apoB-48 concentration in TRL were lower in lactating cows, and A-V differences of apoB-48 concentration in TRL and plasma TG level were larger than those in non-lactating cows. These results suggested that intestinal TG was utilized in mammary gland for milk fat formation, as speculated by Palmquist and Mattos [14].

The utilization of TG in TRL was considered to be initiated by hydrolysis of TG with lipoprotein lipase (LPL) resulting in fatty acid uptake in mammary gland [5]. The activity of LPL was reported to increase in mammary gland of cows during lactating period [17]. However, significant large A-V difference in both apoB concentration in TRL and TG level observed in early-lactating cows in this study indicated that TG uptake is closely related to apoB-48 uptake and suggested the presence of receptor-mediated uptake in

lactating mammary gland. Takahashi *et al.* [18] reported that the receptor, called as VLDL receptor was detected in heart, muscle, and adipose tissue in rabbit. Therefore, the uptake mechanism for TRL derived from intestine was considered to be a crucial route for TG uptake in mammary gland of lactating cows. Further investigations on apolipoproteins in TRL and also on the receptor are necessary to elucidate the mechanism of TRL uptake in mammary gland.

REFERENCES

1. Bauchart, D. 1993. Lipid absorption and transport in ruminants. *J. Dairy Sci.* 76: 3864–3881.
2. Bauchart, D., Durand, D., Laplaud, P. M., Forgez, P., Goulinet, S. and Champman, M. J. 1989. Plasma lipoproteins and apolipoproteins in the preruminant calf, *Bos spp.*: density distribution, physicochemical properties, and the *in vivo* evaluation of the contribution of the liver to lipoprotein homeostasis. *J. Lipid Res.* 30: 1499–1514.
3. Bauman, D. E. and Davis, C. L. 1974. Biosynthesis of milk fat. pp. 31–75. In: *Lactation* (Larson, B. L. and Smith, V. R. eds.), Academic Press, New York and London.
4. Brown, M. S. and Goldstein, J. L. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science* 232: 34–47.
5. Cryer, A. 1981. Tissue lipoprotein lipase and its action in lipoprotein metabolism. *Int. J. Biochem.* 13: 525–541.
6. Ferreri, L. F. and Gleockler, D. H. 1979. Electrophoretic characterization of bovine lipoprotein subfractions isolated by agarose gel chromatography. *J. Dairy Sci.* 62: 1577–1582.
7. Glascock, R. F. and Welch, V. A. 1974. Contribution of the fatty acids of three low density serum lipoproteins to bovine milk fat. *J. Dairy Sci.* 57: 1364–1370.
8. Greeve, J., Altkemper, I., Dieterich, J.-H., Geten, H. and Windler, E. 1993. Apolipoprotein B mRNA editing in 12 different mammalian species: hepatic expression is reflected in low concentrations of apoB-containing plasma lipoproteins. *J. Lipid Res.* 34: 1367–1383.
9. Grummer, R. R., Davis, C. L. and Hegarty, H. M. 1983. Comparison of ultracentrifugation and gel filtration for the isolation of bovine lipoproteins. *Lipids* 18: 795–802.
10. Laplaud, P. M., Bauchart, D., Durand, D. and Champman, M. J. 1990. Lipoproteins and apolipoproteins in intestinal lymph of the preruminant calf, *Bos spp.*, at peak lipid absorption. *J. Lipid Res.* 31: 1781–1792.
11. McGowan, M. W., Artiss, J. D., Strandbergh, D. R. and Zak, B. 1983. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin. Chem.* 29: 538–542.
12. Olofsson, S.-O., Bjursell, G., Boström, K., Carlsson, P., Elovson, J., Protter, A. A., Reuben, M. A. and Bondjers, G. 1987. Apolipoprotein B: structure, biosynthesis and role in the lipoprotein assembly process. *Atherosclerosis* 68: 1–17.
13. Palmquist, D. L. 1975. A kinetic concept of lipid transport in ruminants. A review. *J. Dairy Sci.* 59: 355–363.
14. Palmquist, D. L. and Mattos, W. 1978. Turnover of lipoproteins and transfer to milk fat of dietary (1-carbon-14) linoleic acid in lactating cows. *J. Dairy Sci.* 61: 561–565.
15. Quincey, D., Goff, D. L., Fresnel, J. and Nouvelot, A. 1987. Qualitative and quantitative alterations of bovine serum lipoproteins with ageing. *Comp. Biochem. Physiol.* 88(B): 929–937.
16. Raphael, B. C., Dimick, P. S. and Puppione, D. L. 1972. Lipid characterization of bovine serum lipoproteins throughout gestation and lactation. *J. Dairy Sci.* 56: 1025–1032.
17. Shirley, J. E., Emery, R. S., Convey, E. M. and Oxender, W. O. 1972. Enzymic changes in bovine adipose and mammary tissue, serum and mammary tissue hormonal changes with initiation of lactation. *J. Dairy Sci.* 56: 569–574.
18. Takahashi, S., Kawarabayashi, Y., Nakai, T., Sakai, J. and Yamamoto, T. 1992. Rabbit very low density lipoprotein receptor: A low density lipoprotein receptor-like protein with distinct ligand specificity. *Proc. Natl. Acad. Sci. U.S.A.* 89: 9252–9256.
19. Uchide, T., Toya, Y., Onda, K., Matsuki, N., Inaba, M. and Ono, K. 1997. Apolipoprotein B (apoB) concentrations in lipoproteins in cows. *J. Vet. Med. Sci.* 59: 711–714.