Analysis of cholesterol lipoprotein separations in Holstein dairy cattle by anion-exchange high-performance liquid chromatography

Summary of Doctoral Thesis

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Lipid metabolism in dairy cows are consistently active for milk production. Especially, lipoprotein metabolism is significantly changed during the transition period in dairy cows for supply of nutrient to the fetus or milk production. Abnormal lipid metabolism during transition period induces fatty liver and its intercurrent illness. As such, we focused on lipoprotein fraction which plays a key role on bovine lipid metabolism. Measurement of serum lipoproteins in dairy cows has been performed using ultracentrifugation methods, which is considered the "gold standard". However, the ultracentrifugation method has disadvantages given that it is too cumbersome and time-consuming. It is reported that there is problem of measurement accuracy, since density region between HDL and LDL is overlapped. Recently, anion-exchange (AEX) high-performance liquid chromatography (HPLC) methods are recently developed and can measure serum lipoproteins such as total cholesterol, HDL-C, LDL-C, IDL-C, and VLDL-C with rapid, high isolation efficiency in humans and rabbits. However, AEX-HPLC analysis has not been applied in dairy cows.

Therefore, the current study investigated the basis examination whether AEX-HPLC methods could measure bovine lipoprotein fraction in chapter 2. Next, we compared the lipoprotein fraction of dairy cows with different lactation stages between fineness daily farmer (superior of milk quality performance and reproduction performance) and poor daily farmer in chapter 3. Finally, we investigated the changes in lipoprotein fraction in healthy daily cows during transition period and compared it to those in dairy cows with perinatal diseasein chapter 4.

The objective of the chapter 2 was to investigate ①the reproducibility, precision and linearity of AEX-HPLC methods and ② the correlation between AEX-HPLC and other analytical methods (ultracentrifugation methods and gel permeation HPLC [GP-HPLC]) using serum samples from healthy dairy cows. GP-HPLC could be useful for the analysis of lipoproteins in humans, dogs and cats. GP-HPLC separates lipoproteins on the basis of size.

Chromatogram of lipoprotein fraction by AEX-HPLC method showed apparent wave profile in HDL-C and LDL-C, however, minimal wave profile was observed in IDL-C and VLDL-C. Analytical evaluation of the lipoprotein assay of total cholesterol, HDL-C, LDL-C, IDL-C and VLDL-C using AEX-HPLC methods indicated acceptable analytical precision, with intra-assay coefficients of variation (CVs) not exceeding the commonly accepted 10% limit. Furthermore, analytical evaluation of total cholesterol, HDL-C and LDL-C with inter-assay CVs were below 10 %, respectively. However, with regard to concentrations of IDL-C and VLDL-C, we could not evaluate the inter-assay CVs because some measurement results were under the detection limit. Excellent linearity was demonstrated with total cholesterol, HDL-C and LDL-C of the dilutions tested. However, some values of IDL-C and VLDL-C were under the detection limit. Therefore, linearity was not assessed. Furthermore, positive correlation coefficients between the values of total cholesterol, HDL-C, and LDL-C were determined between AEX-HPLC and ultracentrifugation methods and between AEX-HPLC and GP-HPLC. However, definite separation between HDL and LDL could not be performed using ultracentrifugation methods and GP-HPLC in dairy cow, since density region between HDL and LDL is overlapped. Therefore, AEX-HPLC methods can measure lipoprotein fraction with accuracy and reproduction as regards HDL-C and LDL-C. Furthermore, AEX-HPLC methods ① was not affected overlapping of specific gravity between HDL-C and LDL-C as ultracentrifugation methods and ② was not affected overlapping of grain size as GP-HPLC methods. Therefore, these results suggest that AEX-HPLC would be a useful method for evaluating the lipoprotein fraction in dairy cows.

The lipoprotein fraction in dairy cows with different lactation stages measured by AEX-HPLC methods were compared between fineness S daily farmer (superior of milk quality performance and reproduction performance) and poor I daily farmer in Miyagi prefecture. No significant difference was

observed in changes in Total-C、HDL-C、LDL-C、IDL-C and VLDL-C between 2 dairy farmers. Total cholesterol, HDL-C and LDL-C increased from early lactation to mid lactation, and thereafter decreased from late lactation to dry lactation in both dairy farmers. Significant difference was observed in changes in HDL-C/Total-C (%) between 2 daily farmers. HDL-C/Total-C (%) decreased from early lactation to mid lactation, and thereafter increased from mid lactation to late lactation in S dairy farmers. HDL-C/Total-C (%) of I dairy farmer in mid lactation is higher than that of S dairy farmer. Significant difference was also observed in changes in LDL-C/Total-C (%) between 2 daily farmers. LDL-C/Total-C (%) of peak, mid and late lactation in S dairy farmer is significantly higher than that in I dairy farmer. LDL-C/Total-C (%) increased from early lactation to mid lactation, and thereafter decreased from mid lactation to late lactation in S dairy farmers. Meanwhile, LDL-C/Total-C (%) did not show any significant changes during lactation stages in I dairy farmers. These results suggested that metabolic pathway of VLDL→IDL→LDL is activated by transferring the triglyceride to mammary gland for plenty milk production in S dairy farmer. Meanwhile, milk production of I dairy farmer is lower than that of S dairy farmer. As such, LDL-C might be decreased in I dairy farmer, since transportation of VLDL-C from liver to mammary gland was decreased. For the above reason, measurement of LDL-C/Total-C(%) might be able to pick out between superior dairy farmer and poor dairy farmer.

We focused on the lipid metabolism during transition period. Firstly, for creating a reference value, the changes in lipoprotein fraction in 10 healthy daily cows during transition period were evaluated. Secondarily, we examined associations of lipoprotein profile between healthy cows (reference value) and dairy cows with perinatal disease. Lipoprotein profile of Total-C and HDL-C in healthy cows decreased from 4 weeks before from expected date of delivery to calving date, and thereafter increased. LDL-C in healthy cows decreased from 4 weeks before from expected date of delivery to 1 week

after from expected date of delivery, and thereafter increased. LDL-C/Total-C(%) in healthy cows decreased from 4 weeks before from expected date of delivery to 2 weeks after from expected date of delivery, and thereafter increased. Next, we compared lipoprotein fraction between healthy dairy cows and 19 dairy cows with perinatal disease. In the current study, lower values of LDL-C and LDL-C/Total-C(%) was observed in fat liver group and milk fever group as compared to healthy dairy cows. It was considered that inactivation of metabolic pathway of VLDL→IDL→LDL was occurred caused by decreased appetite and nutrient malabsorption. Therefore, measurement of LDL-C during transition period might be reflect metabolic status in dairy cows and used for clinical application.