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Cholesterol metabolism, transport, and hepatic regulation in dairy cows during transition and early lactation

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ABSTRACT

The transition from the nonlactating to the lactating state represents a critical period for dairy cow lipid metabolism because body reserves have to be mobilized to meet the increasing energy requirements for the initiation of milk production. The purpose of this study was to provide a comprehensive overview on cholesterol homeostasis in transition dairy cows by assessing in parallel plasma, milk, and hepatic tissue for key factors of cholesterol metabolism, transport, and regulation. Blood samples and liver biopsies were taken from 50 multiparous Holstein dairy cows in wk 3 antepartum (a.p.), wk 1 postpartum (p.p.), wk 4 p.p., and wk 14 p.p. Milk sampling was performed in wk 1, 4, and 14 p.p. Blood and milk lipid concentrations [triglycerides (TG), cholesterol, and lipoproteins], enzyme activities (phospholipid transfer protein and lecithin:cholesterol acyltransferase) were analyzed using enzymatic assays. Hepatic gene expression patterns of 3-hydroxy-3-methylglutaryl-coenzyme A (HMGC) synthase 1 (*HMGCS1*) and HMGC reductase (*HMGCR*), sterol regulatory element-binding factor (*SREBF*)*-1* and *-2*, microsomal triglyceride transfer protein (*MTTP*), ATP-binding cassette transporter (*ABC*) *A1* and *ABCG1*, liver X receptor (*LXR*) α and peroxisome proliferator activated receptor (*PPAR*) α and γ were measured using quantitative RT-PCR. Plasma TG, cholesterol, and lipoprotein concentrations decreased from wk 3 a.p. to a minimum in wk 1 p.p., and then gradually increased until wk 14 p.p. Compared with wk 4 p.p., phospholipid transfer protein activity was increased in wk 1 p.p., whereas lecithin:cholesterol acyltransferase activity was lowest at this period. Total cholesterol concentration and mass, and cholesterol concentration in the milk fat fraction decreased from wk 1 p.p. to wk 4 p.p. Both total and milk fat cholesterol concentration were decreased in wk 4 p.p. compared with wk 1 and 14 p.p. The mRNA abundance of genes involved in cholesterol synthesis (*SREBF-2*, *HMGCS1*, and *HMGCR*) markedly increased from wk 3 a.p. to wk 1 p.p., whereas *SREBF-1* was downregulated. The expression of *ABCA1* increased from wk 3 a.p. to wk 1 p.p., whereas *ABCG1* was increased in wk 14 p.p. compared with other time points. In conclusion, hepatic expression of genes involved in the biosynthesis of cholesterol as well as the *ABCA1* transporter were upregulated at the onset of lactation, whereas plasma concentrations of total cholesterol, phospholipids, lipoprotein-cholesterol, and TG were at a minimum. Thus, at the gene expression level, the liver seems to react to the increased demand for cholesterol after parturition. Whether the low plasma cholesterol and TG levels are due to impaired hepatic export mechanisms or reflect an enhanced transfer of these compounds into the milk to provide essential nutrients for the newborn remains to be elucidated.

Key words: cholesterol metabolism, lipoprotein, dairy cow

INTRODUCTION

Cholesterol and lipid metabolism have been widely investigated in humans and in rodent animal models as important factors contributing to lipid-associated diseases, such as atherosclerosis or Tangier disease (Maxfield and Tabas, 2005). In dairy cows, an important lipid-related metabolic disorder is fatty liver (Grummer, 1993). Particularly during the periparturient period lipid metabolism is challenged to ensure the increasing energy demands. Thus, adipose tissue is mobilized and the liver has to cope with an increasing supply of NEFA either by oxidation or by reesterification to triglycerides (**TG**; Grummer, 1993). Fatty liver develops when TG synthesis exceeds the export of TG as very low density lipoproteins (**VLDL**; Goff and Horst, 1997).

Lipoproteins are composed of a core of hydrophobic lipids (TG and cholesteryl esters) and an envelope constituted of apoproteins and amphiphilic lipids (phospholipids and free cholesterol). Hepatocytes secrete

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cholesterol and TG in VLDL, which are processed in the circulation into intermediate-density lipoproteins by hydrolysis of the triglycerides. Intermediate-density lipoproteins are rich in cholesteryl esters and are either metabolized to low density lipoproteins (**LDL**) or taken up by the liver. In humans, LDL are the main carriers of cholesterol in blood and deliver cholesterol from the liver to the peripheral tissues. Cholesterol is returned from extrahepatic tissues to the liver in high density lipoproteins (**HDL**) by reverse cholesterol transport.

Selective aspects of cholesterol metabolism in dairy cows have been a matter of intense investigation. Thus, bovine serum lipids and lipoproteins during gestation and lactation have been determined (Raphael et al., 1973; Puppione et al., 1980) as well as the percentage of cholesterol in milk (Bitman and Wood, 1990). Genes involved in cholesterol synthesis, such as 3-hydroxy-3-methylglutaryl-CoA reductase (**HMGCR**) and 3-hydroxy-3-methylglutaryl-CoA synthase (**HMGCS**), and the regulation of the latter by sterol regulatory element-binding proteins (SREBP) in dairy cows have been recently assessed (Viturro et al., 2009; Schlegel et al., 2012). However, in those studies, only specific parameters of the complex network of cholesterol metabolism were selectively highlighted or analysis was restricted to specific compartments (e.g., plasma), or both. Moreover, potential associations between the hepatic activity and resulting effects on both plasma and milk cholesterol levels have been neglected so far.

Therefore, the purpose of the present study was to give a comprehensive overview of cholesterol metabolism, transport, and hepatic regulatory genes in dairy cows during the transition period and until mid lactation at different regulatory and functional levels. To get insights into potential interactions between the liver and the mammary gland, plasma and milk cholesterol contents were assessed in parallel in relation to endocrine factors, enzyme activities, and hepatic gene expressions of regulatory factors and enzymes.

MATERIALS AND METHODS

Animal Trial

The animal experiments were carried out at the Agricultural Experimental Unit Hirschau of the Technical University of Munich (Munich, Germany) and were approved by the responsible department for animal welfare affairs. Fifty multiparous Holstein dairy cows (parity 3.2 ± 0.2; mean ± SEM) were studied during a period from wk 3 antepartum (**a.p.**) to wk 14 postpartum (**p.p.**). Cows were fed a mixed ration based on hay, grass, and corn silage for ad libitum intake and additional cereal-based concentrate according to milk production. The energy content of the mixed ration was 6.53 ± 0.08 and 7.96 ± 0.04 MJ of NE_L/kg of DM for the concentrates, respectively. The partial mixed ration had a crude fat content of 32 ± 6 and concentrates of 24 ± 6 g/kg of DM, respectively. Details on the animal trial and the feeding regimen were described previously (Gross et al., 2011a).

Blood and Milk Sampling

Blood samples were collected from the jugular vein between 0730 and 0900 h before feeding in wk 3 a.p., wk 1 p.p., wk 4 p.p., and wk 14 p.p. The EDTA blood samples were immediately cooled down on wet ice, centrifuged at 2,000 × g for 15 min at 4°C and then aliquoted. Plasma samples were stored at −20°C until analysis.

Morning milk samples were collected once weekly at 0500 h in wk 1 p.p., wk 4 p.p., and wk 14 p.p. and stored at −20°C until analysis.

Plasma Lipids

Concentrations of total cholesterol (**TC**) and TG were measured with enzymatic kits from bioMérieux SA (Geneva, Switzerland; no. 61219 for TC; no. 61236 for TG). Concentrations of phospholipids (**PL**) and free cholesterol (**FC**) were measured with enzymatic kits from Wako Pure Chemical Industries Ltd. (Osaka, Japan; no. 296-63801 for PL; no. 435-35801 for FC). High density lipoprotein cholesterol (**HDL-C**) was measured with an immunoinhibition method (kit no. 412-72395) and LDL cholesterol (**LDL-C**) with an enzymatic kit (no. 419-24017) from Wako Chemical GmbH (Neuss, Germany).

Concentrations of cholesteryl esters (**CE**), VLDL cholesterol (**VLDL-C**), and LDL-C were calculated using the following equations:

$$\text{Cholesteryl esters} = \text{total cholesterol} - \text{free cholesterol};$$

$$\text{VLDL-C} = \text{triglycerides}/5 \text{ (Friedewald et al., 1972)};$$

$$\text{LDL-C} = \text{total cholesterol} - (\text{HDL-C} + \text{triglycerides}/5) \text{ (Friedewald et al., 1972)}.$$

The LDL-C was additionally measured with an enzymatic kit (no. 419-24017) from Wako Chemical GmbH. The correlation between enzymatically measured and calculated LDL-C was 0.99 ($P < 0.001$).