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Adaptations of hepatic lipid metabolism and mitochondria in dairy cows with mild fatty liver

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ABSTRACT

The inevitable deficiency in nutrients and energy at the onset of lactation requires an optimal adaptation of the hepatic metabolism to overcome metabolic stress. Fatty liver is one of the main health disorders after parturition. Therefore, to investigate changes in hepatic lipid metabolic status and mitochondria in dairy cows with mild fatty liver, liver and blood samples were collected from healthy cows (n = 15) and cows with mild fatty liver (n = 15). To determine the effects of palmitic acids (PA), one of the major component of fatty acids, on lipid metabolism and mitochondria in vitro, calf hepatocytes were isolated from healthy calves and treated with various concentrations of PA (0, 50, 100, and 200 μM). Dairy cows with mild fatty liver displayed hepatic lipid accumulation. The protein levels of sterol regulatory element-binding protein 1c (SREBP-1c) and peroxisome proliferator-activated receptor-α (PPARα) and mRNA levels of acetyl CoA carboxylase 1 (*ACC1*), fatty acid synthase (*FAS*), acyl-CoA oxidase (*ACO*), and carnitine palmitoyltransferase 1A (*CPT1A*) were significantly higher in dairy cows with mild fatty liver than in control cows. The hepatic mitochondrial DNA content, mRNA levels of oxidative phosphorylation complexes I to V (*CO 1-V*), protein levels of cytochrome c oxidase subunit IV (COX IV), voltage dependent anion channel 1 (VDAC1), peroxisome proliferator activated receptor-γ coactivator-1α (PGC-1α) and nuclear respiratory factor 1 (NRF1), and adenosine triphosphate (ATP) content were all markedly increased in the liver of dairy cows with mild fatty liver compared with healthy cows. The PA treatment significantly increased lipid accumulation; protein levels of SREBP-1c and PPARα; and mRNA levels of *ACC1*, *FAS*, *ACO*, and *CPT1A* in calf hepatocytes. Moreover, the mitochondrial DNA content, mRNA levels of *CO 1-V*, protein levels of COX IV, VDAC1, PGC-1α, NRF1, mitochondrial transcription factor A, and ATP content were significantly increased in PA-treated hepatocytes compared with control hepatocytes. The protein level of mitofusin-2 was significantly decreased in PA-treated groups. In conclusion, lipid synthesis and oxidation, number of mitochondria, and ATP production were increased in the liver of dairy cows with mild fatty liver and PA-treated calf hepatocytes. These changes in hepatic mitochondria and lipid metabolism may be the adaptive mechanism of dairy cows with mild fatty liver.

Key words: mild fatty liver, lipid metabolism, mitochondria, adaptation, dairy cow

INTRODUCTION

The transition period in dairy cows, which spans from 3 wk before to 3 wk after calving, is defined as the changes from late pregnancy to the adaptation phase of early lactation. Voluntary DMI reduction around the time of parturition along with increases in energy requirements to meet the needs of gravidity and lactogenesis lead to cows entering a state of negative energy balance (Weber et al., 2013; Song et al., 2016). Drackley et al. (2001) reported that energy demands increased approximately 3-fold in early-lactating cows compared with pregnant and nonlactating cows. Therefore, cows mobilize fat depots to provide fatty acids as an energy fuel to meet energy requirements.

During the transition period, circulating fatty acid concentration and blood flow to the liver are both increased, and excessive amounts of fatty acids are trans-

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ported to the liver (Reynolds et al., 2003). Palmitic acid (**PA**) is an important representative of fatty acids released during lipolysis (Rukkwamsuk et al., 2000). Although many peripheral tissues remove fatty acids from the blood, up to 25% of fatty acids are removed by the liver and subsequently oxidized or re-esterified and stored as triglycerides (**TG**; Sejersen et al., 2012). High liver fat content can result in metabolic imbalances that are related to clinical diseases such as ketosis and fatty liver syndrome (Bobe et al., 2004). In the first month after calving, 5 to 10% of dairy cows have severe fatty liver, and 30 to 40% have mild and moderate fatty liver (Bobe et al., 2004). More important, fatty liver is associated with increased veterinary costs, decreased milk production, and longer calving intervals (Grummer, 2008). Therefore, the rapid adaptation of key metabolic pathways in the liver to support lactation is central to the ability of cows to make an uneventful transition.

The regulation of hepatic lipid metabolism is largely dependent on mitochondria, the primary organelle of cellular adenosine triphosphate (**ATP**) production, using the energy released from the respiratory chain mediated by oxidative phosphorylation complexes (CO I, CO II, CO III, CO IV, and CO V; ***CO 1-V***; Begriche et al., 2013; Yamaguchi et al., 2016). Mitochondrial impairment can contribute to the development of fatty liver or insulin resistance in mice (Rovira-Llopis et al., 2017). More important, amelioration of mitochondrial function prevents hepatic steatosis in obese mice (Lin et al., 2014). Interestingly, Koliaki and Roden (2013) showed that obese individuals with fatty liver had increased mitochondrial respiratory rates compared with lean ones, suggesting hepatic mitochondrial flexibility at the early stages of fatty liver. Moreover, several studies reported that adaptations of mitochondrial function and lipid metabolism were observed in mice or patients with nonalcoholic fatty liver (Begriche et al., 2013; Franko et al., 2014; Sunny et al., 2017). Dairy cows during the periparturient period also displayed hepatic metabolic adaptations (Drackley et al., 2001; Ha et al., 2017), even though the cellular and molecular mechanisms have not yet been elucidated. We investigated the changes in hepatic lipid metabolism and mitochondria in cows with moderate and severe fatty liver in our previous studies (Li et al., 2015; Du et al., 2017a; Gao et al., 2018). Nevertheless, to our knowledge, few studies have evaluated the status of mitochondria in dairy cows with mild fatty liver and the adaptive mechanism of calf hepatocytes in vitro (Wathes, 2012; Laubenthal et al., 2016). In addition, the effects of PA on lipid metabolism and mitochondria of calf hepatocytes are unclear. Therefore, the aim of this study was to investigate (1) the hepatic lipid metabolic status in dairy cows with mild fatty liver, (2) the hepatic mitochondria status in dairy cows with fatty liver, and (3) the effects of PA on lipid metabolism and mitochondria in calf hepatocytes.

MATERIALS AND METHODS

Animals

The Ethics Committee on the Use and Care of Animals at Jilin University (Changchun, China) approved the study protocol [2015 clinical trial (2015-121)]. To guarantee that the cows did not have any other comorbidities, all cows received a routine physical examination. Cows were fed ad libitum, and all cows received the same diet. The basal diet formulation is shown in Table 1. We chose lactating Holstein cows with similar numbers of lactations (median = 3, range = 2–4) and DIM (median = 6 d, range = 3–9 d) from a 1,000-cow dairy farm located in Changchun, Jilin Province, China. Measurement of liver TG is the gold standard for diagnosing and staging fatty liver in dairy cows (Bobe et al., 2004). According to a review by Bobe et al. (2004), fatty liver can be categorized into normal liver and mild, moderate, and severe fatty liver. In healthy cows, hepatic TG content is less than 1% (% g/g of wet weight). In cows with mild fatty liver, hepatic TG content is higher than 1% but less than 5%. Fifteen healthy Holstein cows and 15 cows with mild fatty liver were selected for the experiments. Table 2 provides a basic description of the cows with mild fatty liver and the healthy cows.

Table 1. Nutrient composition of the diets

Item	Amount
Ingredient (%)	
Corn silage	40.00
Corn	35.00
Wheat bran	8.00
Soybean meal	5.00
Sunflower	8.00
NaCl	1.00
Premix[1]	1.80
$NaHCO_3$	1.20
Total	100.00
Nutrient composition (% of DM, unless noted)	
NE_L (MJ/kg)	6.70
CP	15.20
NDF	33.45
ADF	17.20
NFC	40.40
Ca	0.70
P	0.50

[1]Provided the following per kilogram of diet: vitamin A 200,000 IU, vitamin D 70,000 IU, vitamin E 1,000 IU, Fe 2,000 mg, Cu 600 mg, Zn 2,400 mg, Mn 1,300 mg, I 6 mg, Co 7 mg.