J. Dairy Sci. 101:1–13
https://doi.org/10.3168/jds.2017-12878

Epigenetic mechanisms contribute to decrease stearoyl-CoA desaturase 1 expression in the liver of dairy cows after prolonged feeding of high-concentrate diet

T. L. Xu,*† H. M. Seyfert,† and X. Z. Shen*[1]
*College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, 210095, P.R. China
†Leibniz Institute for Farm Animal Biology, Institute for Genome Biology, 18196 Dummerstorf, Germany

ABSTRACT

Subacute ruminal acidosis (SARA) of dairy cattle is a widely occurring but not very overt metabolic disorder thought to impair milk composition. The enzyme stearoyl-CoA desaturase 1 (SCD1) is rate-limiting for the formation of Δ-9 unsaturated fatty acids and thus crucially involved in controlling lipid metabolism in the liver. It is known that SCD1 expression is downregulated during SARA, but the underlying molecular mechanisms are unknown. To study these mechanisms, we enrolled 12 healthy multiparous mid-lactation Holstein cows into a diet-induced SARA experiment. Six cows were fed a high-concentrate diet for 18 weeks (60% content of high-concentrate to 40% forage; HC group), whereas the others received a low-concentrate diet ad libitum (40% high-concentrate content to 60% forage; LC group). Sustained low ruminal pH values (pH 5.6 maintained for 4 h/d) and reduced milk yield performance (2.07 kg/d less than LC cows) verified that SARA had been induced in the HC group. Results showed a significantly decreased concentrations of *cis*-9 monounsaturated long-chain fatty acids in plasma collected from hepatic but not portal veins. This was matched by reduced *SCD1* mRNA and protein concentrations in HC livers. The expression levels of genes related to lipid formation (*DGAT1* and *PLIN2*) were downregulated during SARA, whereas those of catabolic genes (*CPT1A*, *CPT2*, and *ACOX1*) and some inflammatory genes were upregulated. Expression of *SCD1* was downregulated through reduced transcription and abundance of the transcription factor sterol regulatory element-binding protein 1 (SREBP1c).This effect was augmented by local chromatin tightening and DNA methylation at and around the SREBP1c binding site in the *SCD1* promoter. Chromatin immunoprecipitation assays confirmed that SARA reduced SREBP1c binding at the *SCD1* promoter; hence, epigenetic mechanisms are involved in regulating the expression of genes related to long-chain fatty acid modification, partially through downregulation of both SCD1 and SREBP1c in the liver. Our results suggest that in addition to inflammatory genes, SCD1 is also involved in SARA-induced epigenetic regulation and its associated metabolic changes. This knowledge might help to provide a target for intervening against the detrimental metabolic effects of SARA.
Key words: SARA, epigenetic regulation, SREBP1c, chromatin remodeling

INTRODUCTION

Overfeeding high-yielding dairy cows with high-concentrate diets is a long-standing problem. Feeding high-concentrate diets may provide enough energy to fully exploit cows' genetic potential for milk synthesis, but, in daily practice, there is a risk of eliciting nutritional deficiency diseases, such as SARA (Kleen et al., 2003). Clinically, this disease is characterized by reduced milk synthesis and induction of inflammatory responses (Bauman and Griinari, 2001; Zebeli and Ametaj, 2009; Colman et al., 2010; Loor et al., 2016). High-concentrate diets result in the accumulation of VFA and lactic acid, which decreases ruminal pH and thus induces acidosis. These perturbations ultimately cause LPS, the major cell-wall component of gram-negative bacteria, to leak from the rumen into the blood (Plaizier et al., 2008; Chen et al., 2012; Chang et al., 2015d; Zhang et al., 2016). The LPS trigger toll-like-receptor 4 (**TLR4**) signaling, which in turn activates the *NFKB* complex of transcription factors, thereby throwing a master switch of immune gene regulation (Vaure and Liu, 2014). This initiates the release of proinflammatory cytokines, such as *TNFA* and *IL1* and *IL6* (Hayden and Ghosh, 2011). Hence, SARA-mediated systemic circulation of increased LPS levels causes generalized inflammation, affecting both the liver and mammary glands. The liver is the central metabolic organ and maintains the bal-

Received March 16, 2017.
Accepted November 3, 2017.
[1]Corresponding author: xzshen@njau.edu.cn

ance between lipid utilization and synthesis (Arisqueta et al., 2013). Understanding SARA-mediated alterations of lipid metabolism in this organ is essential for understanding key aspects of SARA pathology.

The enzyme stearoyl-CoA desaturase 1 (**SCD1**) introduces a *cis*-9 bond into SFA (Rincon et al., 2012). Its activity is rate-limiting for the formation of Δ-9 UFA. This key step in the formation of triglycerides and cholesterol esters influences milk composition and milk yield in cows (Moioli et al., 2007; Maryam et al., 2016). Moreover, due to the importance of very low density lipoproteins (**VLDL**), fatty liver occurs when the rate of hepatic triglyceride (**TAG**) synthesis exceeds the rate of TAG disappearance through either hydrolysis or exportation via VLDL (Pullen et al., 1990; Drackley, 1999). The VLDL metabolism seems to be highly relevant to the expression of SCD1 in the livers of dairy cows. It has also been found that SCD1 activity has a role during inflammation and coping with stress in mice (Chen et al., 2008; Liu et al., 2011). Although some studies have reported decreased SCD1 activity in bovine livers compared with adipose tissues and mammary glands (St John et al., 1991), more recent papers have revealed that transcription of *SCD* is indeed involved in the hepatic lipid metabolism of lactating cows by supplying extra lipid nutrients (Vahmani et al., 2014). Moreover, *SCD* expression has also been associated with the effects of sunflower or linseed oil supplementation on lipid metabolism in the livers of goats (Bernard et al., 2009). These studies suggest the liver may contribute to the Δ-9 desaturation of absorbed fatty acid in ruminants.

Sterol regulatory element binding protein-1 (**SREBP1**) is a key factor controlling the expression of *SCD1* and of other genes involved in fat catabolism (Bené et al., 2001; Ren et al., 2016; Xu et al., 2016). It activates the expression of *SCD1* and other factors of lipid metabolism by binding to specific sequence motifs called sterol response elements in their promoters (Osborne, 2000). Although knowledge of this mechanism has come from research in nonruminant species, our previous study of the bovine *SCD1* promoter has identified a similar stimulatory role of SREBP1c in ruminants (Xu et al., 2016).

However, it is likely that epigenetic mechanisms also contribute to controlling *SCD1* expression in a diet-dependent fashion. It has been found that feeding mice with either high-carbohydrate or high-fat diets differentially modulated *SCD1* expression in livers. This effect was mediated in part by different degrees of CpG methylation of the *SCD1* promoter (Schwenk et al., 2013). Moreover, it has been established in mammary glands of goats and cows that SARA results in a high degree of DNA methylation within crucial CpG islands of the *SCD1* promoter, indicating the involvement of epigenetic mechanisms in its regulation (Dong et al., 2014; Tao et al., 2015). In addition, we previously showed that SARA-induced activation of immune genes in the liver is regulated through epigenetic mechanisms (Chang et al., 2015b,c).

The mechanisms for reducing *SCD1* expression in the liver during SARA are unknown. The first objective of the current study was therefore to unravel these mechanisms. Second, we wanted to determine how SARA shifts the balance between lipid anabolism and catabolism in the liver. Regarding potential mechanisms for downregulating *SCD1* transcription in the liver during SARA, we hypothesized that this may in part be accomplished by reducing SREBP1 binding at the *SCD1* promoter, perhaps through compaction of its binding site. We thus built the experimental design of this study on our previously detailed structural and functional characterization of the *SCD1* promoter from cattle, including experimental validation that SREBP1 is indeed a pivotal driver of *SCD1* expression in the liver.

MATERIALS AND METHODS

Experimental Design and Sample Collection

Twelve healthy multiparous mid-lactating Holstein cows (455 ± 28 kg of BW, 31.59 ± 3.2 kg/d of milk yield, and 106 ± 7 DIM; mean ± SD) were fitted with rumen fistula and catheters in the portal and hepatic veins before the trial began. They were randomly divided into 2 groups of 6 cows each to be fed either high-concentrate or low-concentrate diets (**HC** or **LC**, respectively). The HC diet contained 60% concentrate and 40% forage and was given as an experimental treatment for 18 wk, whereas a diet of 40% concentrate and 60% forage served as a control in the LC group. Animals were kept in individual tiestalls and were fed 3 times a day at 0400, 1200, and 2000 h. Dry matter intake was 21.7 ± 1.1 per head. Components and nutrient levels of the respective diets were as published in previous studies (Abaker et al., 2017) and are presented in Table 1. Animals were cared for through 4 wk and antibiotics were applied continuously for 3 d after surgery. Sterilized heparin saline (500 IU/mL) was used to prevent catheter blockage daily at 8-h intervals until the end of the experiment. Cows were monitored daily for feed ingestion, rectal temperature, and respiratory rate. The experiment began after all animals had made a full recovery from surgery; no cows showed any clinical signs of infection during the experiment. Sample collection and storage methods were as previously described (Abaker et al., 2017). Briefly, blood was sampled 4 h