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Time-dependent effect of *trans*-10,*cis*-12 conjugated linoleic acid on gene expression of lipogenic enzymes and regulators in mammary tissue of dairy cows

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ABSTRACT

Trans-10,*cis*-12 conjugated linoleic acid (CLA) has been identified as an intermediate of rumen fatty acid biohydrogenation that caused milk fat depression (MFD) in the dairy cow. Previous studies in cows experiencing CLA- and diet-induced MFD have identified reduced mammary expression of the master lipogenic regulator sterol response element transcription factor 1 (*SREBF1*) and many of its dependent genes. To distinguish between primary mechanisms regulating milk fat synthesis and secondary adaptations to the reduction in milk fat, we conducted a time-course experiment. Eleven dairy cows received by abomasal infusion an initial priming dose of 6.25 g of CLA followed by 12.5 g/d delivered in multiple pulses per day for 5 d. Cows were milked 3×/d and mammary biopsies were obtained under basal condition (prebolus control) and 12, 30, and 120 h relative to initiation of CLA infusion. Milk fat concentration and yield decreased progressively reaching a nadir at 69 h (1.82% and 38.2 g/h) and averaged 2.03 ± 0.19% and 42.1 ± 4.10 g/h on the last day of treatment (±standard deviation). Expression of fatty acid synthase (*FASN*) and lipoprotein lipase (*LPL*) were decreased at 30 and 120 h compared with control. Expression of *SREBF1* and *THRSP* were also decreased at 30 and 120 h compared with control. Additionally, we failed to observe changes in other factors, including peroxisome proliferator-activated receptor γ and liver x receptor β and milk fat globular membrane proteins, during CLA treatment. However, expression of milk fat globular membrane proteins were decreased after 14 d of diet-induced MFD in samples from a previous experiment, indicating a possible long-term response. The rapid decrease in lipogenic enzymes, *SREBF1*, and *THRSP* provide strong support for their transcriptional regulation as a primary mechanism of milk fat depression.

Key words: milk fat, conjugated linoleic acid, SREBF1, THRSP, milk fat depression

INTRODUCTION

Milk fat synthesis is variable in dairy cows and responsive to nutritional factors. Specifically, diet-induced milk fat depression (**MFD**) represents a decrease in milk fat yield with no change in yield of milk or other milk components (see review by Bauman and Griinari, 2003). Milk fat depression is caused by unique intermediates from ruminal fatty acid (**FA**) biohydrogenation; *trans*-10,*cis*-12 CLA was the first of these unique biohydrogenation intermediates to be identified and is the most well studied (Baumgard et al., 2000).

During MFD, mammary lipogenic capacity is decreased and transcription of mammary genes involved in milk fat synthesis are coordinately downregulated (Baumgard et al., 2002). Decreased expression of sterol response element transcription factor 1 (*SREBF1*) and thyroid hormone responsive spot 14 (*THRSP*) have been reported during *trans*-10,*cis*-12 CLA and diet-induced MFD (Harvatine and Bauman, 2006a). The protein SREBF1 is a master regulator of lipid synthesis and has been shown to be repressed by PUFA in liver of rodents and humans (Jump et al., 2005). Although its exact biochemical function is not known, spot 14 (*THRPS*) is nuclear protein that is closely associated with the regulation of FA synthesis in lipogenic tissues, including the mammary gland during MFD, and may function as a transcriptional co-activator (Cunningham et al., 1998; Chou et al., 2007, 2008). Additionally, studies performed in mammary epithelial cells have suggested roles for liver x receptor β (*LXRB*; Oppi-Williams et al., 2013; Harvatine et al., 2014) and peroxisome proliferator-activated receptor γ (**PPARγ**; Shi et al., 2014) in regulation of milk fat synthesis (reviewed by Bionaz et al., 2015).

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Virtually all previous gene expression studies have been performed in the context of fully established MFD and, accordingly, cannot distinguish causative mechanisms from secondary physiological adaptations to reduced milk fat synthesis (i.e., responses causing reduced lipogenesis vs. those only associated with reduced mammary lipogenesis). Our objective was to investigate the temporal expression of lipogenic enzymes, *SREBF1*, and *THRSP* in mammary tissue of lactating cows during CLA-induced MFD and our hypothesis was that SREBF1 and THRSP are primary responses during induction of MFD.

MATERIALS AND METHODS

Animals and Treatments

All experimental procedures were approved by the Cornell University Institutional Animal Care and Use Committee. Samples were collected from 11 ruminally fistulated lactating dairy cows from the Cornell University Teaching and Research Center in 2 blocks. In the first block, 8 midlactation cows were assigned randomly to sampling sequence in a 2-period design. Mammary biopsies before the CLA bolus (**CON**) and 12 h after the abomasal CLA bolus infusion were collected during 1 period and mammary biopsies were collected 30 and 120 h after the bolus in the other period. Biopsies within period were taken from alternate rear quarters and periods were separated by 14 d to allow recovery. In the second block, 3 midlactation cows had biopsies collected sequentially from alternate rear quarters in a single period, with biopsies of the same quarter being at least 4 d apart.

Trans-10,*cis*-12 CLA was infused into the abomasum using a 0.5-cm i.d. line placed through the rumen cannula. An initial priming dose of 6.25 g of CLA was given at time zero, followed by 2.1 g every 4 h for 36 h and then 3.1 g every 6 h until 120 h. This provided 12.5 g/d of *trans*-10,*cis*-12 CLA, which is slightly over the maximally effective dose (~10 g/d; de Veth et al., 2004). The *trans*-10,*cis*-12 CLA methyl ester stock (BASF Corporation, Ludwigshafen, Germany) contained 88.3% total CLA (98% *trans*-10,*cis*-12 isomer), 6.8% palmitic acid, 2.7% oleic acid, and 2.0% stearic acid. Previous investigations have shown that the methyl ester and free FA forms of *trans*-10,*cis*-12 CLA are equally effective in inhibiting milk fat synthesis (de Veth et al., 2004).

Cows received a TMR fed once per day in the first block and twice per day in the second block. Feed ingredients were dried (55°C forced-air oven for 72 h), ground (Wiley mill with 1-mm screen; Arthur H. Thomas, Swedesboro, NJ), and nutrient composition determined by wet chemistry procedures (AOCS, 2000; at Dairy One Cooperative, Ithaca, NY). The diet contained 31.1 and 33.2% NDF and 16.6 and 16.7% CP in the first and second block, respectively (Supplemental Table S1; https://doi.org/10.3168/jds.2017-13935).

Samples from a previous experiment (Harvatine and Bauman, 2006a) were used to further investigate the regulation of the milk fat globular membrane (**MFGM**) proteins during MFD. Briefly, mammary biopsies were collected as described above from control (normal milk fat) and after 3 d of intravenous infusion of 10 g/d of *trans*-10,*cis*-12 CLA that resulted in a 24% decrease in milk fat yield, and 10 d of a low-forage and high-oil diet (3.0% soybean oil and 1.5% fish oil) that resulted in a 38% reduction in milk fat yield.

Sampling and Analysis

Cows were milked 3×/d (0800, 1600, and 2400 h) and samples were taken at times shown and analyzed for fat and true protein using a mid-infrared spectrophotometer (AOCS, 2000; at Dairy One Cooperative). Additionally, milk lipids were extracted in hexane-isopropanol, transmethylated with sodium methoxide, and FAME were quantified by GC according to Perfield et al. (2006). Transfer of CLA to milk was calculated as yield in milk divided by amount infused.

Mammary biopsies were performed at approximately 1030 h according to (Harvatine and Bauman, 2006a) using a needle biopsy tool (Magnum Biopsy Gun system; Bard Biopsy Systems, Tempe, AZ). Tissues were snap-frozen in liquid nitrogen and stored at −80°C. Total RNA was isolated from approximately 30 mg of mammary tissue using the RNeasy Lipid Kit with on column Dnase I (Qiagen, Valencia, CA). The RNA concentration and integrity were determined by an Agilent 2100 BioAnalyzer [RNA integrity number 8.4 ± 0.46 (mean ± SD); Agilent Technologies, Santa Clara, CA]. Total RNA was reverse transcribed using the SuperScript III First Strand Synthesis kit (Invitrogen, Carlsbad, CA) with random primers (3 μg of total RNA in a 20 μL total reaction with 2.5 ng/μL of primers). Quantitative real-time reverse transcriptase PCR (**qRT-PCR**) assays were developed and validated for genes of interest (Supplemental Table S2; https://doi.org/10.3168/jds.2017-13935), as described by Harvatine and Bauman (2006a). The qRT-PCR reactions included ABI Power SYBR with ROX (Applied Biosystems, Foster City, CA), 400 n*M* gene-specific forward and reverse primers (Invitrogen), and 5 to 25 ng of cDNA in a 2-step amplification program (95°C for 15 s and 60°C for 60 s) with an ABI PRISM 7000 Sequence Detection System