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Conjugated linoleic acid-induced milk fat depression in lactating ewes is accompanied by reduced expression of mammary genes involved in lipid synthesis[1]

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ABSTRACT

Conjugated linoleic acids (CLA) are produced during rumen biohydrogenation and exert a range of biological effects. The *trans*-10,*cis*-12 CLA isomer is a potent inhibitor of milk fat synthesis in lactating dairy cows and some aspects of the mechanism have been established. Conjugated linoleic acid-induced milk fat depression has also been observed in small ruminants and our objective was to examine the molecular mechanism in lactating ewes. Multiparous lactating ewes were fed a basal ration (0.55:0.45 concentrate-to-forage ratio; dry matter basis) and randomly allocated to 2 dietary CLA levels (n = 8 ewes/treatment). Treatments were zero CLA (control) or 15 g/d of lipid-encapsulated CLA supplement containing *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA isomers in equal proportions. Treatments were fed for 10 wk and the CLA supplement provided 1.5 g of *trans*-10,*cis*-12/d. No treatment effects were observed on milk yield or milk composition for protein or lactose at wk 10 of the study. In contrast, CLA treatment significantly decreased both milk fat percentage and milk fat yield (g/d) by about 23%. The de novo synthesized fatty acids (FA; <C16) were significantly decreased in proportion (15%) and daily yield (27%), and the proportion of preformed FA (>C16) was increased (10%) for the CLA treatment. In agreement with the reduced de novo FA synthesis, mRNA abundance of acetyl-coenzyme A carboxylase α, FA synthase, stearoyl-CoA desaturase 1, and glycerol-3-phosphate acyltransferase 6 decreased by 25 to 40% in the CLA-treated group. Conjugated linoleic acid treatment did not significantly reduce the mRNA abundance of enzymes involved in NADPH production, but the mRNA abundance for sterol regulatory element-binding factor 1 and insulin-induced gene 1, genes involved in regulation of transcription of lipogenic enzymes, was decreased by almost 30 and 55%, respectively, with CLA treatment. Furthermore, mRNA abundance of lipoprotein lipase decreased by almost 40% due to CLA treatment. In conclusion, the mechanism for CLA-induced milk fat depression in lactating ewes involved the sterol regulatory element-binding protein transcription factor family and a coordinated downregulation in transcript abundance for lipogenic enzymes involved in mammary lipid synthesis.
Key words: conjugated linoleic acid, milk fat, lipogenesis, mammary

INTRODUCTION

Conjugated linoleic acid (**CLA**) is a generic term used to describe positional and geometric isomers of linoleic acid. Several CLA isomers are naturally produced by rumen bacteria as intermediates in the biohydrogenation of dietary PUFA, with *cis*-9,*trans*-11 CLA being the predominant isomer found in ruminant-sourced foods (Bauman and Lock, 2006). Conjugated linoleic acid isomers also originate from industrial hydrogenation and *cis*-9,*trans*-11 and *trans*-10,*cis*-12 are the 2 isomers that have been most extensively studied. Research over the last decade has established CLA as unusual bioactive FA that exert a range of biological effects in different tissues and species, including antiobesity, anticarcinogenic, antidiabetogenic, and antiatherogenic effects (Belury, 2002; Bhattacharya et al., 2006). Baumgard et al. (2000) were the first to demonstrate that *trans*-10,*cis*-12 CLA resulted in a reduction in milk fat synthesis in lactating dairy cows, and this discovery provided a basis to explain the cause of diet-induced milk fat depression (**MFD**), a syndrome in lactating cows that had perplexed dairy producers and scientists

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for over a century (Bauman and Griinari, 2003; Bauman et al., 2011).

The molecular mechanism behind CLA-induced MFD is not completely resolved; however, the phenotypic characterization provides insight into the functional mechanism. In CLA-induced MFD in dairy cows, fat is the only milk component inhibited with *trans*-10,*cis*-12 CLA treatment. Furthermore, the reduction in milk fat secretion involves FA of all chain lengths, but effects are particularly pronounced for de novo synthesized FA (Bauman and Griinari, 2003). Cellular level investigations have clearly shown a coordinated downregulation in transcript abundance and (or) enzymatic activity for lipogenic enzymes involved in milk fat synthesis in the mammary gland of lactating cows and rodent models (Bauman et al., 2011). Molecular mechanisms mediating the inhibitory effect of *trans*-10,*cis*-12 CLA on mammary lipogenesis have not been extensively investigated, but results support a central role for sterol regulatory element-binding transcription factor family (Peterson et al., 2004; Harvatine and Bauman, 2006; Gervais et al., 2009).

Conjugated linoleic acid-induced MFD has also been observed in small ruminants including sheep (Lock et al., 2006; Sinclair et al., 2007; Weerasinghe et al., 2012) and goats (Lock et al., 2008; Shingfield et al., 2009). Although ruminants share similarities in many aspects, distinct differences exist related to ruminal lipid metabolism and the relative sensitivity of mammary lipogenic processes (Chilliard et al., 2003; Shingfield et al. 2010), and this might modify the mammary response to CLA treatment. For example, in lactating goats, plant oil supplements result in a reduction in milk fat secretion of de novo FA that is independent of mammary expression or activity of acetyl-CoA carboxylase α (**ACACA**) and FA synthase (**FASN**; Bernard et al., 2009).

Dairy ewes may represent a good model to examine the mechanism of CLA-induced MFD. They are relatively available, cost effective, manageable in size, and daily milking allows a quantitative evaluation of treatment effects on milk fat yield and FA composition. Furthermore, the relationship between *trans*-10,*cis*-12 CLA dose and the reduction in milk fat output is similar to cows when dose is expressed on a metabolic BW basis (Lock et al., 2006; Sinclair et al., 2007). To date, the molecular basis for MFD, whether induced by diet or CLA supplements, has not been investigated in lactating ewes. Therefore, the objective of the current study was to investigate the molecular mechanism mediating MFD in lactating ewes fed a CLA supplement containing *trans*-10,*cis*-12 CLA. For this purpose, we used tissue samples obtained from lactating ewes that were fed a rumen-protected CLA supplement for 10 wk. Results for CLA effects on performance, organ weight, and carcass composition are reported in a companion paper (Sinclair et al., 2010).

MATERIALS AND METHODS

Animals and Treatments

All experimental procedures involving lactating ewes were conducted at Harper Adams University (Newport, Shropshire, UK) in accordance with the UK Animals (Scientific Procedures) Act 1986, with details reported in the companion publication (Sinclair et al., 2010). Briefly, at d 16 ± 1.6 (mean ± SE) postpartum, 16 multiparous Friesland and British Milk Sheep ewes were randomly allocated to 2 treatments (randomized block design) based on breed, milk yield and milk fat yield as measured in the previous 7 d, BW, and BCS. Ewes were milked twice daily and fed a basal ration (0.55:0.45 concentrate-to-forage ratio, DM basis) that was composed mainly of hay, rolled barley, and dried molassed sugar beet feed (Sinclair et al., 2010). Dietary ME and CP averaged (per kilogram of DM) 10.9 MJ and 156 g, respectively, with fresh feed offered once per day at 1.05 × ad libitum intake.

Treatments for the present study involved diets that were supplemented with CLA at 2 levels: no CLA (control, **CON**) or 15 g of CLA supplement/d (**+CLA**). The supplement, a lipid-encapsulated CLA that contained 2 CLA isomers in equal proportions, provided 1.5 g of *trans*-10,*cis*-12/d and an equal amount of *cis*-9,*trans*-11 CLA (Lutrell; BASF SE, Ludwigshafen, Germany). Ewes received the 2 experimental treatments throughout a 10-wk period. With the exception of milk fat, the phenotype between the CON and +CLA treatments was comparable after 10 wk of treatment. A third treatment (40 g of CLA supplement/d) reported in the companion paper (Sinclair et al., 2010) was not included in the present study. The 40 g/d CLA group differed in milk yield, milk protein yield, and BW change. Similar off-target effects of CLA have been previously reported in lactating cows receiving high doses of CLA (Bell and Kennelly, 2003).

At the end of the experimental period, ewes were slaughtered over a 72-h period by stunning and exsanguination. Subsamples of mammary secretory tissues from the left side of the mammary gland were immediately dissected and cubes were prepared. The cubes (≤0.5 cm) were immediately placed in a 15-mL disposable sample tube and immersed in 5 mL of RNAlater solution (RNAlater tissue collection: RNA stabilization solution; Ambion Inc., Austin, TX). After sample tubes