J. Dairy Sci. 99:1–15
http://dx.doi.org/10.3168/jds.2015-10042

Conjugated linoleic acids influence fatty acid metabolism in ovine ruminal epithelial cells

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ABSTRACT

Conjugated linoleic acids (CLA), particularly *cis*-9,*trans*-11 (c9t11) and *trans*-10,*cis*-12 (t10c12), are used as feed additives to adapt to constantly increasing demands on the performance of lactating cows. Under these feeding conditions, the rumen wall, and the rumen epithelial cells (REC) in particular, are directly exposed to high amounts of CLA. This study determined the effect of CLA on the fatty acid (FA) metabolism of REC and expression of genes known to be modulated by FA. Cultured REC were incubated with c9t11, t10c12, and the structurally similar FA linoleic acid (LA), oleic acid (OA), and *trans*-vaccenic acid (TVA) for 48 h at a concentration of 100 μ*M*. Cellular FA levels were determined by gas chromatography. Messenger RNA expression levels of stearoyl-CoA desaturase (*SCD*) and monocarboxylate transporter (*MCT*) 1 and 4 were quantified by reverse transcription-quantitative PCR. Fatty acid evaluation revealed significant effects of CLA, LA, OA, and TVA on the amount of FA metabolites of β-oxidation and elongation and of metabolites related to desaturation by SCD. The observed changes in FA content point (among others) to the ability of REC to synthesize c9t11 from TVA endogenously. The mRNA expression levels of *SCD* identified a decrease after CLA, LA, OA, or TVA treatment. In line with the changes in mRNA expression, we found reduced amounts of C16:1n-7 *cis*-9 and C18:1n-9 *cis*-9, the main products of SCD. The expression of *MCT1* mRNA increased after c9t11 and t10c12 treatment, and CLA c9t11 induced an upregulation of *MCT4*. Application of peroxisome proliferator-activated receptor (PPAR) α antagonist suggested that activation of PPARα is involved in the changes of *MCT1*, *MCT4*, and *SCD* mRNA expression induced by c9t11. Participation of PPARγ in the changes of *MCT1* and *SCD* mRNA expression was shown by the application of the respective antagonist. The study demonstrates that exposure to CLA affects both FA metabolism and regulatory pathways within REC.

Key words: conjugated linoleic acid, rumen epithelium, stearoyl-CoA desaturase, monocarboxylate transporter, peroxisome proliferator-activated receptor

INTRODUCTION

Conjugated linoleic acids are known to have a wide range of physiological effects (Bhattacharya et al., 2006). They affect energy metabolism not only in humans (Salas-Salvadó et al., 2006) but also in ruminants (Odens et al., 2007). Thus, CLA have gained importance as feed additives in ruminants and, in particular, in lactating cows. As reviewed by Bauman et al. (2008), CLA *trans*-10,*cis*-12 (**t10c12**) in particular reduces milk fat synthesis in dairy cows, stabilizing the energy balance of the animal (Odens et al., 2007). The CLA *cis*-9,*trans*-11 (**c9t11**) isomer has been intensively studied because it is the predominant CLA in dairy products (Chin et al., 1992; Parodi, 1997) due to endogenous synthesis of this CLA (Corl et al., 2001). Commercially available mixtures of CLA, offered as feed additives for cows, mainly contain rumen-protected c9t11 and t10c12, and are aimed at initiating milk fat depression and thus stabilizing the health status of the cow at least at the onset of lactation. However, the study of Pappritz et al. (2011) revealed that only 5 to 40% of rumen-protected CLA reach the duodenum, depending on the protection method. Thus, it must be assumed that a large part of the CLA taken up orally are already released in the rumen. When CLA are released into the rumen fluid, their subsequent reduction (biohydrogenation) to *trans*-vaccenic acid (**TVA**; C18:1 *trans*-11) and stearic acid (C18:0; Harfoot and Hazlewood, 1997) may last up to 24 h (Troegeler-Meynadier et al., 2006). Thus, under these feeding conditions, the rumen epithelium may be exposed to a high CLA concentration, which may lead not only to CLA accumulation within the epithelium or release of CLA metabolization products but also to functional changes induced by CLA or their metabolites.

Received July 1, 2015.
Accepted December 11, 2015.
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To date, CLA accumulation and metabolization have been described in human vascular endothelial cells and smooth muscle cells (Müller et al., 2005; Ringseis et al., 2006; Eder and Ringseis, 2010). Regarding functional effects after CLA exposure, changes in stearoyl-CoA desaturase (**SCD**) status have been frequently described (Choi et al., 2001, 2002; Smith et al., 2002). In the rumen epithelium, SCD has not been detected to date. Stearoyl-CoA desaturase catalyzes the conversion of TVA to CLA (Griinari et al., 2000). Therefore, if the rumen epithelium could produce CLA endogenously from TVA catalyzed by SCD, this process would increase the amount of CLA that accumulates in rumen epithelial cells (**REC**) when CLA are added to the food. Presuming a modulation of SCD by CLA, accumulation of CLA in REC may also affect the amount of other products catalyzed by SCD.

We assumed that the accumulation of CLA might also have an effect on monocarboxylate transporter (**MCT**). In the rumen epithelium, the MCT1 isoform has been detected at the molecular and protein levels and has been shown to play a central role in mediating transruminal fluxes of short-chain fatty acids, including acetate, ketone bodies, and lactate (Müller et al., 2002; Graham et al., 2007; Dengler et al., 2014). Furthermore, activation of the peroxisome proliferator-activated receptor (**PPAR**) α pathway was demonstrated to lead to the effective upregulation of the *MCT1* isoform in REC (König et al., 2008; Benesch et al., 2014). In turn, PPARα is modulated by CLA, with c9t11 being the most active isomer (Moya-Camarena et al., 1999; Benjamin et al., 2005). In addition to the MCT1 isoform, the MCT4 isoform is also present in the rumen (Kirat et al., 2007). Although MCT4 regulatory pathways are not completely understood, research suggests that PPAR may play a role (Benesch et al., 2014).

Our objective was to expose cultured REC to CLA isoforms to better understand whether and how CLA accumulate in and exert effects on the cells. Cells were further exposed to linoleic acid (**LA**), oleic acid (**OA**), or TVA to distinguish between the effects of CLA and fatty acids (**FA**), which are structurally similar FA or precursors of endogenous CLA synthesis (Griinari et al., 2000). A further aim of our study was to investigate whether REC themselves express SCD and thus have the ability to desaturate the appropriate substrates, TVA in particular. Expression of SCD mRNA was determined by reverse-transcription quantitative PCR (**RT-qPCR**) to test if it can be influenced by CLA, LA, OA, or TVA. Monocarboxylate transporter 1 and MCT4 were seen as potential targets of CLA treatment and were therefore examined in detail. Finally, we attempted to elucidate the putative regulatory pathway of CLA on *SCD*, *MCT1*, and *MCT4* through the application of PPARα and PPARγ antagonists.

MATERIALS AND METHODS

Animals

Female sheep (<1 yr) were fed with grass hay and water ad libitum for at least 2 wk. The animals were killed by captive-bolt stunning and subsequent exsanguination. These experiments were in accordance with German legislation on the protection of animals and were reported to the Landesdirektion Leipzig as T 88/13. After euthanasia, the abdomen was opened, and the atrium ruminis was excised and subjected to the cell isolation procedure.

Cell Culture and Treatment Procedure

Cultivation of REC followed the procedure described by Müller et al. (2000) and Benesch et al. (2014). Because all experiments were conducted on subcultured cells obtained from primarily isolated cells, the cells had to be isolated and cultured anew from each sheep slaughtered.

For that purpose, pieces of the excised atrium ruminis were kept on ice in Ca^{2+}- and Mg^{2+}-free Dulbecco's PBS supplemented with 400 U/mL penicillin and 100 U/mL nystatin for 1 h. Thereafter, ruminal papillae were removed and subjected to fractional trypsinization as described by Gálfi et al. (1981). Division-active cells were found in fractions 3 and 4 (1 h of enzyme digestion per fraction). Isolated cells of those fractions were suspended in medium 199 containing 15% fetal bovine serum (**FBS**), 20 m*M* HEPES, 50 μg/mL gentamicin, and 2 m*M* L-glutamine and seeded at a density of 10^5 cells/cm^2 in cell culture flasks (75 cm^2; Greiner Bio One, Frickenhausen, Germany). Incubation proceeded in a humidified 5% CO_2 air atmosphere at 37°C. Forty-eight hours after seeding, medium 199 was replaced with minimum essential Eagle's nutrient medium supplemented with 10% FBS, 20 m*M* HEPES, 50 μg/mL gentamicin, and 2 m*M* L-glutamine. Six to 9 d after seeding, primary cultured cells were subcultured to obtain consistent cell densities for mRNA and GC analysis. After detaching with trypsin-EDTA, cells were resuspended and seeded (seeding density: 2–3 × 10^4 cells/cm^2) in cell culture flasks (75 cm^2; Greiner Bio One) for GC analysis, 12-well plates coated with collagen type I (Greiner Bio One) for mRNA analysis, and collagen type I-coated coverslips for immunocytochemical staining. The immunocytochemical staining was performed about 2 d after seeding using mouse-