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Dietary supplement of conjugated linoleic acids or polyunsaturated fatty acids suppressed the mobilization of body fat reserves in dairy cows at early lactation through different pathways

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ABSTRACT

To investigate the metabolic changes in the adipose tissue (AT) of dairy cows under milk fat depression (MFD), 30 cows were randomly allocated to a control diet, a conjugated linoleic acid (CLA)-supplemented diet, or a high-starch diet supplemented with a mixture of sunflower and fish oil (2:1; as HSO diet) from 1 to 112 d in milk. Performance of animals, milk yield, milk composition, energy balance, and blood metabolites were measured during lactation. Quantitative PCR analyses were conducted on the AT samples collected at wk 3 and 15 of lactation. The CLA and HSO diets considerably depressed milk fat yield and milk fat content at both wk 3 and 15 in the absence of significant changes in milk protein and lactose contents. In addition, the HSO diet lowered milk yield at wk 15 and decreased dry matter intake of cows from wk 3 to 15. Compared with the control, both CLA and HSO groups showed reduced body weight loss, improved energy balance, and decreased plasma concentrations of nonesterified fatty acids and β-hydroxybutyrate at early lactation. The gene expression analyses reflected suppressed lipolysis in AT of the CLA and HSO groups compared with the control at wk 3, as suggested by the downregulation of hormone-sensitive lipase and fatty acid binding protein 4 and the upregulation of perilipin 2. In addition, the HSO diet promoted lipogenesis in AT at wk 15 through the upregulation of 1-acylglycerol-3-phosphate O-acyltransferase 2, mitochondrial glycerol-3-phosphate acyltransferase, perilipin 2, and peroxisome proliferator-activated receptor γ. The CLA diet likely regulated insulin sensitivity in AT as it upregulated the transcription of various genes involved in insulin signaling, inflammatory responses, and ceramide metabolism, including protein kinase B2, nuclear factor κ B1, toll-like receptor 4, caveolin 1, serine palmitoyltransferase long chain base subunit 1, and *N*-acylsphingosine amidohydrolase 1. In contrast, the HSO diet resulted in little or no change in the pathways relevant to insulin sensitivity. In conclusion, the CLA and HSO diets induced a shift in energy partitioning toward AT instead of mammary gland during lactation through the regulation of different pathways.
Key words: milk fat depression, lipolysis, insulin resistance, gene expression, ceramide

INTRODUCTION

High-producing dairy cows undergo negative energy balance (**NEB**) during the early stage of lactation due to insufficient DMI and the increasing nutrient requirement for lactation (Drackley et al., 2005). Adaptation to NEB may increase the risk of metabolic disorders, resulting from excessive mobilization of nutrients from body reserves (Ingvartsen, 2006). The NEB at early lactation can be attenuated by milk fat depression (**MFD**), as fat serves as the major energy component in milk (Moore et al., 2004). The MFD can be induced by dietary managements, for instance, by increasing the dietary concentrate/forage ratio, by increasing dietary starch level, or by dietary PUFA supplement (Kalscheur et al., 1997; Shingfield et al., 2006; Piccioli-Cappelli et al., 2014). Dietary starch level varies with the grain composition and therefore can be increased, for instance, by replacing barley with wheat (Bernard et al., 2016). A feeding strategy combining high starch level and PUFA supplement was found to effectively depress milk fat content (Zened et al., 2012). These MFD diets modify the ruminal biohydrogenation and increase the production of *trans* fatty acids that inhibit mammary lipogenesis (Shingfield et al., 2010; Ventto

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et al., 2017). Of these *trans* fatty acids, *trans*-10,*cis*-12 CLA is the only isomer whose role in MFD has been clearly established (Baumgard et al., 2000). *Cis*-9, *trans*-11 CLA is another CLA isomer that is often used in mixture with *trans*-10, *cis*-12 CLA because industrial synthesis results in a 1:1 mix of both isomers. However, evidence has been found suggesting that *cis*-9, *trans*-11 CLA has no effect on milk fat synthesis (Baumgard et al., 2000). The mechanisms underlying the MFD induced by *trans*-10, *cis*-12 CLA include the coordinated reduction in the expression of various lipogenic genes in the mammary gland (Harvatine and Bauman, 2006; Gervais et al., 2009). In addition, *trans*-10,*cis*-12 CLA treatment and other milk fat depression diets also influence the metabolism in nonmammary tissues, for instance, increase the expression of lipogenic genes of the adipose tissue (**AT**) during mid lactation (Harvatine et al., 2009; Thering et al., 2009). The coordination of metabolic changes in different tissues results in nutrient partitioning toward nonmammary tissues, particularly AT (Shingfield et al., 2010). During the periparturient period, nutrient partitioning is partly regulated by naturally occurring maternal insulin resistance (**IR**), which induces increased lipolysis and decreased lipogenesis in AT and thus leads to the mobilization of body fat reserves (Rico et al., 2015; Selim et al., 2015). Human and rodent studies suggest that IR in AT is affected differently by CLA isomers. *Trans*-10,*cis*-12 CLA was reported to reduce triglyceride (**TG**) stores in adipocytes by increasing IR (Kennedy et al., 2009), whereas *cis*-9,*trans*-11 CLA was claimed to have antidiabetic effects (Moloney et al., 2007).

Insulin resistance is triggered by ceramide, a sphingolipid abundant in cell membrane that blocks phosphoinositide 3-kinase (**PI3K**) insulin signaling pathway in rodents (Chavez et al., 2003). Moreover, IR is associated with SFA-induced inflammatory signaling that promotes the synthesis of ceramides (Chavez and Summers, 2012). In dairy cows, the mechanisms underlying IR remain uncertain. However, evidence has suggested that IR is associated with increased plasma, hepatic, and AT ceramide concentrations (Qin et al., 2017; Rico et al., 2017) and with upregulated inflammatory responses at gene expression level (Vailati-Riboni et al., 2016).

In the present study, we aimed to induce MFD in dairy cows by either applying dietary rumen-protected CLA supplement or a high-starch diet with PUFA supplement and investigate the effects of these diets on the lipid metabolism in AT. We hypothesized that these diets promote lipogenesis and inhibit lipolysis in AT during early lactation through the regulation of IR and the IR-related inflammatory responses and ceramide metabolism pathways.

MATERIALS AND METHODS

Animals, Experimental Design, and Diets

The National Ethics Committee (ESAVI/4997/04.10.03/2012, Hämeenlinna, Finland) approved all experimental procedures in accordance with the guidelines established by the European Community Council Directives 86/609/EEC (European Council, 1986). Thirty multiparous Nordic Red dairy cows of 792 ± 72 kg (mean ± SD) BW during the last 2 wk before calving and 2.9 ± 1.0 (mean ± SD) parity were recruited from 28 d before expected calving until 112 d postpartum but received the experimental diets only after calving. Cows were assigned to the diet groups to be comparable according to parity, BCS, BW, and Nordic total merit (http://www.nordicebv.info/ntm-nordic-total-merit-2). Cows were housed in an experimental barn with a loose housing system, had free access to water and salt block, and were milked twice daily at 0700 and 1645 h.

During the transition period, cows were offered a standard prepartum concentrate supplement (Supplemental Table S1; https://doi.org/10.3168/jds.2017-14298). Treatments comprised a basal diet based on grass silage (**CON**), the same basal diet supplemented with CLA supplement (CLA diet/group), or a grass silage-based diet containing high-starch concentrate and supplemented with 40 g/kg of DM of a mixture of sunflower oil (EBM Grupp AS, Tallinn, Estonia) and fish oil (BASF, Ludwigshafen, Germany) in a 2:1 ratio (wt/wt) to induce MFD (**HSO**; Table 1). The CLA and HSO diets were formulated to induce a decrease in milk fat content during the first 112 d of lactation. The forage was restrictively fermented grass silage prepared from primary growth of mixed timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) swards, grown at Jokioinen (60°49′N, 23°28′E) treated with a formic acid-based ensiling additive (5 L per t, AIV 2 Plus, Valio Ltd., Finland). The CLA supplement was given as rumen-protected lipid encapsulate (780 g of fatty acids/kg), containing both *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers. Both CLA isomers were present at 100 g/kg of supplement (Lutrell Pure, BASF, Ludwigshafen, Germany). Higher doses of CLA were used in early lactation to overcome the lower sensitivity of the mammary gland to CLA (Moore et al., 2004). Supplementation levels were 150 g/d during 1 to 14 DIM, 125 g/d during 15 to 21 DIM, and 100 g/d from 22 DIM to the end of study which resulted in providing 15.0, 12.5, and 10.0 g of each isomer per day.

The diets were offered as a TMR with a forage:concentrate ratio of 55:45 on DM basis and were formulated to be iso-nitrogenous. The CLA supplement was