J. Dairy Sci. 100:1–13
https://doi.org/10.3168/jds.2016-12369

Effect of conjugated linoleic acid and acetate on milk fat synthesis and adipose lipogenesis in lactating dairy cows

N. Urrutia[1] and K. J. Harvatine[2]
Department of Animal Science, The Pennsylvania State University, University Park 16802

ABSTRACT

During biohydrogenation-induced milk fat depression (MFD), nutrients are spared from milk fat synthesis and are available for other metabolic uses. Acetate is the major carbon source spared and it may increase lipid synthesis in adipose tissue during MFD. The objective of this study was to compare the effect of *trans*-10, *cis*-12 conjugated linoleic acid (CLA) and the amount of acetate spared during CLA-induced MFD on adipose tissue lipogenesis. Nine multiparous, lactating, ruminally cannulated Holstein cows (244 ± 107 d in milk; 25 ± 8.4 kg of milk/d; mean ± standard deviation) were randomly assigned to treatments in a 3 × 3 Latin square design. Experimental periods were 4 d followed by a 10-d washout. Treatments were control (CON), ruminal infusion of acetate (AC; continuous infusion of 7 mol/d adjusted to pH 6.1 with sodium hydroxide), or abomasal infusion of CLA (10 g/d of both *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA). Dry matter intake, milk yield, and milk protein yield and percentage were not affected by treatments. Compared with CON, milk fat yield decreased 23% and fat percent decreased 28% in CLA, and milk fat yield increased 20% in AC. Concentration and yield of milk de novo synthesized fatty acids (<C16) were reduced and concentration of preformed fatty acids (>C16) was increased by CLA, compared with CON. Yield of de novo synthesized fatty acids and palmitic acid was increased by AC, compared with CON. Lipogenesis capacity of adipose tissue explants was decreased 72% by CLA, but was not affected by AC. Acetate oxidation by adipose explants was not affected by treatments. Treatments had no effect on expression of key lipogenic factors, lipogenic enzymes, and leptin; however, expression of fatty acid binding protein 4 was reduced in CLA compared with CON. Additionally, hormone-sensitive lipase and perilipin 1 were decreased by CLA and acetate. Plasma glucose and glucagon concentrations were not affected by treatments; however, CLA increased nonesterified fatty acids 17.7%, β-hydroxybutyrate 16.1%, and insulin 27.8% compared with CON, and AC increased plasma β-hydroxybutyrate 18%. In conclusion, during CLA-induced MFD in low-producing cow adipose tissue was sensitive to the anti-lipogenic effects of CLA, while spared acetate did not stimulate adipose lipogenesis. However, acetate may play an important role in stimulating lipogenesis and improving energy status in the mammary gland under normal conditions.
Key words: acetate, conjugated linoleic acid, milk fat, spared nutrient

INTRODUCTION

Biohydrogenation (**BH**)-induced milk fat depression (**MFD**) is a condition where milk fat yield is reduced to up to 50%, with no reduction in milk yield or other milk components. Biohydrogenation-induced MFD occurs in response to bioactive fatty acids (**FA**) formed in the rumen during altered fermentation from feeding highly fermentable or high unsaturated FA diets or diets containing several other risk factors (reviewed by Bauman and Griinari, 2001). Although multiple *trans* FA formed during altered fermentation reduce milk fat synthesis, *trans*-10, *cis*-12 CLA was the first bioactive FA identified and is the most extensively studied. Abomasal infusion of CLA has provided invaluable insights into the mechanism of BH-induced MFD including the downregulation of key lipogenic enzyme [i.e., fatty acid synthase (*FASN*) and acetyl-CoA carboxylase α (*ACCα*)] and lipogenic factors [sterol regulatory element binding protein 1 (*SREBP1*) and thyroid hormone responsive spot 14 (*S14*)] in the lactating mammary gland (Harvatine and Bauman, 2006).

During BH-induced MFD, yield of both de novo synthesized and preformed FA are decreased in milk, but a greater reduction occurs in de novo synthesized FA. The main precursor for de novo synthesis of FA in the bovine mammary gland is the 2-carbon VFA acetate.

Received November 28, 2016.
Accepted March 16, 2017.
[1]Current address: Instituto de Investigaciones Agropecuarias, INIA Remehue, Osorno, Chile, 5290000.
[2]Corresponding author: kjh182@psu.edu

Acetate provides the majority of the carbon and approximately half of the reducing equivalents (**NADPH**) needed for de novo lipogenesis; the remaining NAPDH comes from glucose metabolism through the pentose phosphate pathway (Bauman et al., 1970; Ingle et al., 1973; Smith, 1983). Therefore, the reduction in milk fat synthesis during BH-induced MFD results in a large reduction in mammary utilization of acetate in addition to a decrease in the use of glucose and preformed FA for de novo lipogenesis and triglyceride assembly.

Nutrients spared from milk fat synthesis are expected to reduce intake in the long term. However, a meta-analysis of intake during short-term CLA infusion (3–5 d) observed a small decrease in intake that explained only a portion of the energy spared by the reduction in milk fat (Harvatine et al., 2009b). Therefore, it is possible that during CLA-induced MFD, spared nutrients might be partitioned to other tissues or metabolic uses. In support of this, Harvatine et al. (2009b) observed increased expression of lipogenic enzymes and regulators in adipose tissue after 3 d of CLA-induced MFD and proposed that the observed response was due to nutrients spared from milk fat synthesis rather than a direct effect of CLA on adipose tissue. In other animal models, including rodents and pigs, it is well known that *trans*-10, *cis*-12 CLA directly reduces body fatness (Ostrowska et al., 1999; Park and Pariza, 2007; Foote et al., 2010). In the bovine, CLA decreased lipid synthesis in adipocyte cell culture (Kadegowda et al., 2013) and in adipose tissue explants (Choi et al., 2014). However, most of the work in other animal and culture models use higher doses of CLA than what adipose tissue is exposed to during BH-induced MFD.

The objective of this study was to compare the effect of *trans*-10, *cis*-12 CLA and the amount of acetate spared during CLA-induced MFD on adipose tissue lipogenesis. We hypothesized that in the lactating cow, acetate alone would stimulate adipose tissue lipogenesis and therefore explain the contrasting observation that CLA-induced MFD reduced mammary lipogenesis, but increased adipose lipogenesis.

MATERIALS AND METHODS

Experimental Design and Treatments

All experimental procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee (#41727). Nine ruminally cannulated multiparous Holstein cows (244 ± 107 DIM; 25 ± 8.4 kg of milk/d) were randomly assigned to 1 of 6 treatment sequences in a 3 × 3 Latin square design. Three treatment sequences were allocated 2 times each and 3 treatment sequences were allocated once (Supplemental Table S1; https://doi.org/10.3168/jds.2016-12369). Cows were housed in a tie-stall barn located at the Pennsylvania State University Dairy Production Research and Teaching Center. Experimental periods were 4 d of treatment followed by a 10-d washout. Treatments were control (**CON**), intraruminal infusion of 7 mol/d acetate adjusted to pH 6.1 with sodium hydroxide (**AC**), and abomasal infusion of 10 g/d of *trans*-10, *cis*-12 CLA (**CLA**).

The acetate infusate was prepared by diluting glacial acetic acid in 7 L of distilled water followed by adjustment to pH 6.1 using sodium hydroxide pellets (J.T. Baker, Center Valley, PA). The control and CLA treatments received a 5.5% sodium chloride solution, which provided the same moles of sodium and volume of the acetate infusate. Acetate and control infusates were infused 22 h/d through the rumen cannula using acid-resistant tubing (Norprene L/S 14, Cole-Parmer, Vernon Hills, IL) and peristaltic pumps (Masterflex L/S drive 7520–35, Cole-Parmer) similar to Sheperd and Combs (1998). Pumps were turned off and lines detached for 1 h twice per day to allow milking in the parlor (0500 and 1700 h). The CLA treatment consisted of abomasal infusion of 34 g/d of a CLA methyl ester stock (Lutalin, BASF, Lampertheim, Germany) that contained 30% *trans*-10, *cis*-12 CLA and 30% *cis*-9, *trans*-11 CLA to provide 10 g/d of *trans*-10, *cis*-12 CLA. The CLA stock was infused in equal doses every 6 h through an abomasal infusion line [0.5 cm (i.d.) polyvinyl chloride tubing (Spires et al., 1975)] placed through the rumen cannula. The infusion lines were inserted the day before initiation of treatments and placement in the abomasum was checked daily. The lines were rinsed with 50 mL of warm water before and after CLA infusion and with 20 mL of 70% ethanol after the last water rinse. Control and acetate treatments received 100 mL of warm water and 20 mL of 70% ethanol as a handling control.

Feed Sampling and Analysis

Cows were fed individually the same TMR once daily (0800 h) at 110% of expected intake and intake was recorded daily. Forage and base diet DM concentration was determined weekly for diet adjustment and DMI determination (72 h in a forced-air oven at 55°C). All individual feed ingredients were sampled by period and composited for analysis of CP, NDF, and ADF by wet chemistry procedures (Cumberland Valley Analytical Services Inc., Maugansville, MD). Briefly, CP and ADF was determined according to AOAC International (2000), and NDF according to Van Soest et al. (1991)