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Short communication: Circulating fatty acylcarnitines are elevated in overweight periparturient dairy cows in association with sphingolipid biomarkers of insulin resistance

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ABSTRACT

Defects in mitochondrial fatty acid processing are associated with the development of fatty liver disease, inflammation, and insulin resistance in overweight nonruminants. Surplus fatty acids (FA) and defects in FA oxidation favor the accumulation of fatty acylcarnitines (FAC) and the sphingolipid ceramide. Moreover, elevated circulating FAC and ceramide concentrations are inversely related to insulin action. Because we have previously demonstrated that plasma ceramide levels increase during the transition from gestation to lactation, our aim was to determine whether changes in plasma medium- and long-chain FAC levels are related to circulating FA and sphingolipids in peripartal dairy cows. We hypothesized that plasma FAC levels would be higher in overweight cows experiencing increased lipolysis, and that FAC levels would be positively associated with elevations in plasma ceramides. Twenty-one multiparous Holstein cows were grouped according to body condition score (BCS) at d −30 prepartum as lean (BCS <3.0; n = 10) or overweight (BCS >4.0; n = 11). Blood was collected at d −30, −15, −7, and 4, relative to parturition. Circulating FAC and ceramide levels were determined using liquid chromatography and tandem mass spectrometry. To investigate the potential contributions of sphingomyelin (SM) hydrolysis to ceramide accrual, we also determined plasma SM levels during the peripartum period. Data were analyzed under a mixed model with the fixed effects of adiposity and time, and the random effect of cow. Relative to lean cows, overweight cows had elevated FAC during the transition from gestation to lactation. Circulating FAC levels were positively associated with FA, ceramide, and dihydro-SM levels. Although circulating FAC levels increased in all cows during the peripartum, enhanced prepartum adiposity contributed to a greater rise in plasma FA and FAC. Our results support on-going efforts to determine whether altered mitochondrial FA processing promotes the accumulation of the insulin resistance biomarker ceramide in blood and liver.
Key words: ceramide, fatty acylcarnitine, lipotoxicity, sphingomyelin

Short Communication

Elevated genetic merit for milk production imposes heightened energy requirements for modern dairy cows, resulting in severe negative energy balance that is prolonged over early lactation (Coppock and Wilks, 1991; Grummer et al., 2004). Although lipomobilization from adipose tissue constitutes a key response for maintaining energy homeostasis during early lactation, excessive levels of circulating free fatty acids (**FA**) can cause metabolic stress and increase the propensity of cows to develop peripartal metabolic diseases (e.g., ketosis and fatty liver; Dechow et al., 2004; Ospina et al., 2010). Moreover, rampant lipolysis is directly associated with gestational adiposity (Kim and Suh, 2003; Rico et al., 2015) and the extent of adipose tissue-specific insulin antagonism during peripartum (Zachut et al., 2013). Consequently, body fat accumulation is a risk factor for metabolic disease in nonruminants (Grundy, 2004), as well as the hyperlipidemic peripartal dairy cow (Roche et al., 2009). Furthermore, overweight individuals exhibit increased sedentary time, which increases their risk for developing insulin resistance (Hamilton et al., 2007), and low physical activity is positively associated with health disorders in dairy cows transitioning from gestation to lactation (Edwards and Tozer, 2004).

Although the association between elevated circulating FA concentrations and metabolic impairment is well established (i.e., lipotoxicity; DeFronzo, 2004;

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Summers, 2006; Ospina et al., 2010), our understanding of the factors underlying the propensity for peripartal disease in hyperlipidemic overweight cows remains incomplete. Studies in rodents and humans indicate that alterations in FA processing (e.g., catabolism vs. anabolism) can play an important role in the onset of lipid-associated metabolic dysfunction. In dairy cows, circulating FA levels increase immediately postpartum, particularly in overweight cows (Rico et al., 2015), and omics has revealed peripartal alterations in lipid metabolism (Hailemariam et al., 2014; Imhasly et al., 2014; Rico et al., 2017). During this critical period, the capacity to completely oxidize FA (i.e., palmitate) to CO_2 is reduced, whereas incomplete β-oxidation to acid-soluble products (i.e., ketones and TCA cycle intermediates) is enhanced (Litherland et al., 2011). Moreover, these conditions favor the hepatic synthesis of esterified products, such as triacylglycerols (**TAG**). In nonruminants, increased hepatic FA influx also promotes the accumulation of fatty acylcarnitines (**FAC**; Schooneman et al., 2013). Fatty acylcarnitines are FA esters formed when fatty acyl-CoA are shuttled into the mitochondrion for β-oxidation, a process facilitated by carnitine palmitoyltransferase 1 (McGarry and Brown, 1997). Because FAC are formed when activated FA are targeted for oxidation, they are regarded as potential indicators of defects in FA processing (Adams et al., 2009; Ramos-Roman et al., 2012). The hepatic accumulation of FAC can promote their accrual in circulation, a process potentially facilitated by organic cation/carnitine transporter 2 (Schooneman et al., 2013). Indeed, hepatic FA influx is accompanied by elevations in circulating FAC in obese subjects diagnosed with type 2 diabetes (Mihalik et al., 2010), as well as in individuals with nonalcoholic fatty liver disease (Kalhan et al., 2011). In dairy cows, short-chain propionyl-carnitine has been reported to be elevated in cows with compromised health (i.e., mastitis, metritis, laminitis, and retained placenta; Hailemariam et al., 2014). However, we recognize that elevations in long-chain FAC can occur in mid-lactation cows with healthy liver function (Huber et al., 2016).

Although hepatic lipid accumulation is characterized by TAG deposition and FAC accumulation, intracellular FA can be partitioned to other metabolic fates, including the synthesis of the insulin resistance biomarker ceramide (Haus et al., 2009; Watt et al., 2012; Schooneman et al., 2013). We recently reported that plasma ceramides are positively associated with circulating FA in overweight dairy cows during the peripartum period (Rico et al., 2015, 2017). We postulated that ceramide accrual may be due to enhanced de novo ceramide synthesis from FA, as observed in hyperlipidemic nonruminants with fatty liver disease (Raichur et al., 2014). Alternatively, ceramide generation from proinflammatory tumor necrosis factor-α–provoked sphingomyelin (**SM**) hydrolysis may be the cause (Peraldi et al., 1996). To further characterize the potential relationship between FA oxidative metabolism and ceramide metabolism, our goal was to characterize changes in plasma FAC levels in peripartal lean and overweight dairy cows, and to delineate their relationship to FA and sphingolipid supply. We hypothesized that plasma FAC levels would increase in overweight cows experiencing enhanced lipolysis, and FAC levels would be positively associated with elevations in plasma ceramides.

To test our hypothesis, an experiment was completed at Dovan Farms (Berlin, PA), West Virginia University Agricultural Research and Education Partner, and a 700-Holstein cow commercial dairy farm (Berlin, PA). Nonlactating, pregnant, multiparous Holstein cows (n = 21) were enrolled in the study 45 d before expected parturition and allocated to 1 of 2 groups according to their adiposity at d −30 relative to calving, as either lean (BCS = 2.9 ± 0.22, n = 10) or overweight (i.e., overconditioned; BCS = 4.1 ± 0.10, n = 11), as reported in Rico et al. (2015). Parity was not different between BCS groups ($P > 0.9$), averaging 3.5 ± 0.82 and 3.45 ± 1.08 for lean and overweight cows, respectively. Experimental procedures were approved by the West Virginia University Institutional Animal Care and Use Committee.

Diets were formulated to meet nutrient recommendations and were primarily composed of corn silage and grass haylage, as described previously (Rico et al., 2015). Contents of NDF, CP, and starch were 48, 12.2, and 15% for the prepartum diet, and 33.4, 16.5, and 25% for the lactation diet, respectively. Animals were fed for ad libitum intake with free access to water. Weekly TMR samples were analyzed for nutrient composition by near-infrared spectroscopy (Cumberland Valley, Cumberland, MD; AOAC International, 1995; method 989.03). Body weights and BCS (1.0 to 5.0 scale; Wildman et al., 1982) were recorded weekly. Three trained investigators independently recorded BCS for all cows and data were averaged for each cow at d −30, −15, −7, 4, 14, and 21 relative to expected parturition. Cows were fitted with collar-mounted activity meters for hourly activity data collection, transmitted to an antenna, and recorded using ALPRO software (DeLaval, Kansas City, MO). Prior to morning feeding, blood samples (10 mL) were collected by coccygeal venipuncture at d −30, −15, −7, and 4 relative to expected parturition. Blood was kept on ice for 30 min until centrifugation at 3,400 × g for 10 min at 4°C. Plasma containing EDTA was removed, immediately snap-frozen in liquid nitrogen, and stored at −80°C. Postpartum blood sampling at d 4 was selected because