J. Dairy Sci. 99:1–14
http://dx.doi.org/10.3168/jds.2016-11296

Palmitic acid feeding increases ceramide supply in association with increased milk yield, circulating nonesterified fatty acids, and adipose tissue responsiveness to a glucose challenge

J. E. Rico,* A. T. Mathews,* J. Lovett,† N. J. Haughey,† and J. W. McFadden*[1]
*Division of Animal and Nutritional Sciences, West Virginia University, Morgantown 26506
†Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21287

ABSTRACT

Reduced insulin action is a key adaptation that facilitates glucose partitioning to the mammary gland for milk synthesis and enhances adipose tissue lipolysis during early lactation. The progressive recovery of insulin sensitivity as cows advance toward late lactation is accompanied by reductions in circulating nonesterified fatty acids (NEFA) and milk yield. Because palmitic acid can promote insulin resistance in monogastrics through sphingolipid ceramide-dependent mechanisms, palmitic acid (C16:0) feeding may enhance milk production by restoring homeorhetic responses. We hypothesized that feeding C16:0 to mid-lactation cows would enhance ceramide supply and ceramide would be positively associated with milk yield. Twenty multiparous mid-lactation Holstein cows were enrolled in a study consisting of a 5-d covariate, 49-d treatment, and 14-d posttreatment period. All cows were randomly assigned to a sorghum silage-based diet containing no supplemental fat (control; n = 10; 138 ± 45 d in milk) or C16:0 at 4% of ration dry matter (PALM; 98% C16:0; n = 10; 136 ± 44 d in milk). Blood and milk were collected at routine intervals. Liver and skeletal muscle tissue were biopsied at d 47 of treatment. Intravenous glucose tolerance tests (300 mg/kg of body weight) were performed at d −1, 24, and 49 relative to start of treatment. The plasma and tissue concentrations of ceramide and glycosylated ceramide were determined using liquid chromatography coupled with tandem mass spectrometry. Data were analyzed as repeated measures using a mixed model with fixed effects of treatment and time, and milk yield served as a covariate. The PALM increased milk yield, energy-corrected milk, and milk fat yield. The most abundant plasma and tissue sphingolipids detected were C24:0-ceramide, C24:0-monohexosylceramide (GlcCer), and C16:0-lactosylceramide. Plasma concentrations of total ceramide and GlcCer decreased as lactation advanced, and ceramide and GlcCer were elevated in cows fed PALM. The PALM increased hepatic ceramide levels, a response not observed in skeletal muscle tissue. Plasma ceramides (e.g., C24:0-ceramide) were positively correlated with plasma NEFA and milk yield, and positively correlated with NEFA levels following a glucose challenge. Our data demonstrate a remodeled plasma and hepatic sphingolipidome in mid-lactation dairy cows fed PALM. The potential involvement in ceramide in homeorhetic nutrient partitioning to support lactation requires further consideration.
Key words: ceramide, insulin resistance, lactation

INTRODUCTION

The development of insulin resistance in adipose and skeletal muscle tissues enables the dairy cow to partition glucose toward the mammary gland during early lactation (Bell, 1995; Bell and Bauman, 1997). As a consequence, a decrease in insulin sensitivity can enhance the mobilization of nonesterified fatty acids (**NEFA**) from adipose tissue (Contreras et al., 2010; Zachut et al., 2013). Nonesterified fatty acids can undergo mitochondrial β-oxidation in peripheral tissues, and re-esterification in the mammary gland to contribute to milk fat synthesis. These coordinated metabolic adaptations support energy demand as milk production rapidly increases following parturition. Beyond peak milk yield, insulin sensitivity improves and the lipolytic release of NEFA decreases as the cow progresses toward late lactation and milk production steadily declines (McNamara and Hillers, 1986; Bell and Bauman, 1997).

A causal relationship between enhanced fatty acid supply and the development of insulin resistance in dairy cattle has been reported (Pires et al., 2007a). Specifically, the intravenous infusion of tallow emulsion can improve insulin responsiveness (Pires et al., 2007a). In support, suppressing adipose tissue lipolysis during feed restriction can improve insulin sensitivity (Pires

Received April 8, 2016.
Accepted August 1, 2016.
[1]Corresponding author: JWMcFadden@mail.wvu.edu

et al., 2007b). In type 2 diabetic monogastric animal models, sustained hyperlipidemia shifts fatty acid processing toward the hepatic synthesis and lipoprotein secretion of sphingolipid ceramide (Watt et al., 2012; Boon et al., 2013). Interestingly, de novo ceramide synthesis is controlled by the availability of saturated palmitoyl-CoA and the activation of serine palmitoyltransferase-1 (**SPT1**; Figure 1; Watt et al., 2012). The accrual of ceramide can antagonize insulin-stimulated glucose uptake by inhibiting the phosphorylation of protein kinase B (Summers et al., 1998; Chavez et al., 2003). With the advent of mass spectrometry-based lipidomics, sphingolipid ceramide and monohexosylceramide (glucosylceramide or galactosylceramide; **GlcCer**) have emerged as predicative biomarkers for the development of insulin resistance (Chavez et al., 2014). Similar to the progression of the diabetic state in humans (Kautzky-Willer et al., 1997), we recently discovered that the decline in insulin sensitivity with the onset of lactation occurs concomitantly with the accumulation of ceramide in plasma (Rico et al., 2015). For example, we and others have observed a positive relationship between the concentration of NEFA and the sphingolipid C24:0-ceramide in plasma, and the severity of insulin resistance (Haus et al., 2009; Rico et al., 2015); however, the relationships between NEFA, ceramide supply, and milk production in context of homeorhetic nutrient partitioning have not been evaluated in the mid-lactation dairy cow.

Supplementing diets with SFA is a common nutritional approach on farm to increase energy intake. Although production responses to SFA feeding are inconsistent (Loften et al., 2014), some evidence supports increased milk yield or conversion of feed to milk with SFA supplementation (Mosley et al., 2007; Lock, et al., 2013; Piantoni et al., 2013). Highly enriched SFA supplements are used by dairy producers because of their minimal effects on rumen microbial activity (Palmquist and Jenkins, 1980; Jenkins, 1993; Maia et al., 2010) and because of their beneficial milk production response as compared with unsaturated fatty acid supplementation (Christensen et al., 1994; Relling and Reynolds, 2007), particularly in high-producing dairy cows (Rico et al., 2014a). Although SFA feeding increases dietary energy, favorable lactation outcomes may not completely depend on the energy content of the SFA. For instance, feeding palmitic acid (C16:0) increases ECM and milk fat yield when compared with C18:0 supplementation (Rico et al., 2014b). It is clear that long-chain SFA are antagonists of insulin action in monogastric animals (Boden, 1997; Funaki, 2009), a response that is mediated by ceramide-dependent protein kinase B inactivation (Summers et al., 1998; Chavez et al., 2003). Because SPT1 upregulation is dependent upon palmitoyl-CoA availability (Figure 1), feeding mid-lactation dairy cows C16:0 may upregulate de novo ceramide synthesis. Humans consuming a diet high in C16:0, relative to oleic acid, display increased circulating levels of ceramide (Kien et al., 2013), resembling upregulated ceramide synthesis observed in subjects diagnosed with type 2 diabetes (Haus et al., 2009). Although we and others have not observed changes in systemic glucose tolerance in dairy cows fed C16:0 when compared with unsupplemented control cows (Piantoni et al., 2013; Mathews et al., 2016), we have observed a decrease in glucose-stimulated NEFA disappearance in mid-lactation cows fed C16:0 by wk 7 (Mathews et al., 2016), suggesting the possibility of localized adipose tissue insulin resistance with prolonged C16:0 feeding. The unexplored metabolic fates for absorbed C16:0, and the associative and functional role of ceramide to modify insulin sensitivity in response to C16:0 supplementation merits attention.

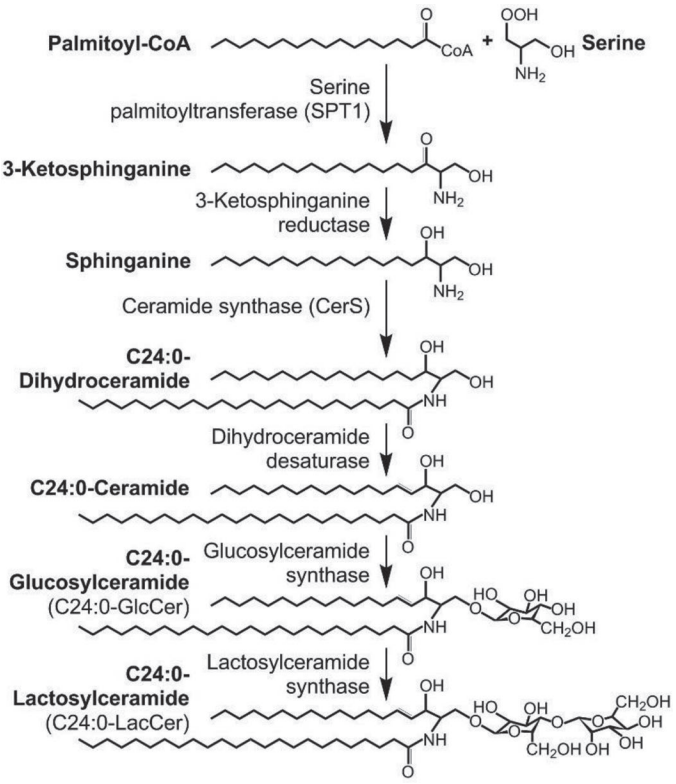

Figure 1. De novo ceramide synthesis initiated by the condensation of palmitoyl-CoA and serine to form 3-ketosphinganine. For simplicity, C24:0-linked sphingolipids and glycosphingolipids are illustrated. Monohexosylceramides (GlcCer) consist of a single sugar residue, either glucose or galactose (galactosylceramide is not shown).

The objective for the current study was to employ a targeted lipidomics approach to quantify ceramides, GlcCer, and lactosylceramides (**LacCer**) in plasma, and liver and skeletal muscle tissues collected from mid-