J. Dairy Sci. 98:1–14
http://dx.doi.org/10.3168/jds.2015-9519

Plasma ceramides are elevated in overweight Holstein dairy cows experiencing greater lipolysis and insulin resistance during the transition from late pregnancy to early lactation

J. E. Rico,* V. V. R. Bandaru,† J. M. Dorskind,† N. J. Haughey,† and J. W. McFadden*[1]
*Division of Animal and Nutritional Sciences, West Virginia University, Morgantown 26505
†Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21287

ABSTRACT

Insulin resistance is a homeorhetic adaptation to parturition in dairy cows transitioning from late pregnancy to early lactation. An increase in prepartum adiposity can predispose periparturient cows to greater lipolysis and insulin resistance, thus increasing the risk for metabolic disease. Mechanisms mediating the development of insulin resistance in overweight peripartal dairy cows may depend on ceramide metabolism. The sphingolipid ceramide accumulates in plasma and tissues of overweight monogastric animals, and facilitates saturated fatty acid-induced insulin resistance. Considering this evidence, we hypothesized that plasma ceramides would be elevated in periparturient dairy cattle and that these sphingolipids would correlate with the magnitude of lipolysis and insulin resistance. To test our central hypothesis, multiparous Holstein cows were allocated into 2 groups according to their body condition score (BCS) at d −30 prepartum: lean (BCS <3.0; n = 10) or overweight (BCS >4.0; n = 11). Blood samples were collected at d −45, −30, −15, and −7, relative to expected parturition, and at d 4 postpartum. Plasma glucose, insulin, nonesterified fatty acids (NEFA), and β-hydroxybutyrate (BHBA) concentrations were measured, and insulin sensitivity was estimated. The concentrations of individual plasma ceramide and glycosylated ceramide were determined using liquid chromatography-based mass spectrometry. Results demonstrated that greater adiposity was associated with a greater loss in body condition during late pregnancy. Overweight cows had greater circulating concentrations of glucose, insulin, and NEFA, and lower insulin sensitivity relative to lean cows. We detected 30 different sphingolipids across 6 lipid classes with acyl chains ranging from 16 to 26 carbons. The most abundant plasma sphingolipids detected were C24:0-ceramide, C24:0-monohexosylceramide, and C16:0-lactosylceramide. Plasma concentrations of total ceramide and monohexosylceramide increased as lactation approached, and saturated ceramide and monohexosylceramide were elevated in cows with greater adiposity relative to those with a lean phenotype. Plasma ceramides (e.g., C24:0-ceramide) were positively correlated with plasma NEFA and inversely correlated with insulin sensitivity. Our data demonstrate a remodeled plasma sphingolipidome in dairy cows transitioning from late pregnancy to lactation characterized by a concomitant increase in plasma ceramides with the development of peripartal insulin resistance.
Key words: ceramide, insulin resistance, periparturient dairy cow

INTRODUCTION

Periparturient dairy cows experience an elevated demand for glucose due to an increased requirement by the mammary gland for lactose synthesis (Bell, 1995). Dairy cattle transitioning from late pregnancy to early lactation develop insulin resistance (Bell and Bauman, 1997) as a metabolic adaptation to energy deficit. Insulin resistance facilitates an increase in hepatic gluconeogenesis and a decrease in glucose uptake by skeletal muscle and adipose tissue (Bell, 1995; Spachmann et al., 2013). Consequently, circulating glucose is spared for lactose synthesis, a major osmotic regulator of milk secretion. To increase NEFA availability for β-oxidation in peripheral tissues and re-esterification in the mammary gland, the periparturient dairy cow mobilizes triacylglycerol stores in adipose tissue (Drackley et al., 2005; Drackley and Andersen, 2006). Because insulin is an antilipolytic hormone, insulin resistance can further increase adipose tissue lipolysis (Pires et al., 2007a,b). Collectively, the transition dairy cow adapts to energy insufficiency by modifying peripheral insulin action to augment glucose economy.

An increase in prepartum adiposity can predispose dairy cows to a greater magnitude of insulin resis-

Received March 2, 2015.
Accepted July 12, 2015.
[1]Corresponding author: JWMcFadden@mail.wvu.edu

tance during early lactation (Holtenius et al., 2003; Holtenius and Holtenius, 2007). The greater extent of insulin resistance in overweight peripartal dairy cows can contribute to excessive adipose tissue lipolysis and thus greater metabolic disease risk (e.g., hepatic steatosis and ketosis). Although insulin resistance in dairy cattle transitioning from pregnancy to lactation is mediated in part by growth hormone (Bell and Bauman, 1997), considerable evidence supports a causative role of long-chain saturated fatty acids as antagonists of whole-body insulin sensitivity (Boden, 1997; Funaki, 2009). The ability of surplus saturated fatty acyl-CoA to inhibit insulin action appears to be mediated by the structurally diverse sphingolipid ceramide (Chavez and Summers, 2012).

Although ceramides were previously believed to be merely structural elements of cell membranes, recent discoveries demonstrate that ceramides are directly implicated in evolutionarily conserved cellular processes, such as cell cycle arrest, apoptosis, and stress responses (Hannun and Obeid, 2008). Ceramides can be (1) formed by de novo synthesis initiated by the condensation of palmitoyl-CoA via action of serine palmitoyltransferase, (2) formed by hydrolysis of sphingomyelin by either acid or neutral sphingomyelinases, or (3) salvaged from complex sphingolipids that are broken down into sphingosine and reacylated (Figure 1; Hirabayashi et al., 2006). An increase in palmitoyl-CoA availability upregulates de novo ceramide synthesis and sphingomyelin hydrolysis in peripheral tissues of obese insulin resistant mice with hepatic steatosis, responses that contribute to ceramide accumulation in tissues and plasma (Cuschieri et al., 2007; Holland et al., 2011a).

The extent of insulin resistance correlates with the concentrations of ceramide in plasma collected from obese humans with type 2 diabetes, most notably C24:0-ceramide, which is the most abundant ceramide species in human circulation (Haus et al., 2009). Plasma ceramide is transported as components of low-density lipoproteins (**LDL**) of hepatic origin that contribute to the pathophysiology of whole-body insulin resistance. Although the mechanisms are not completely

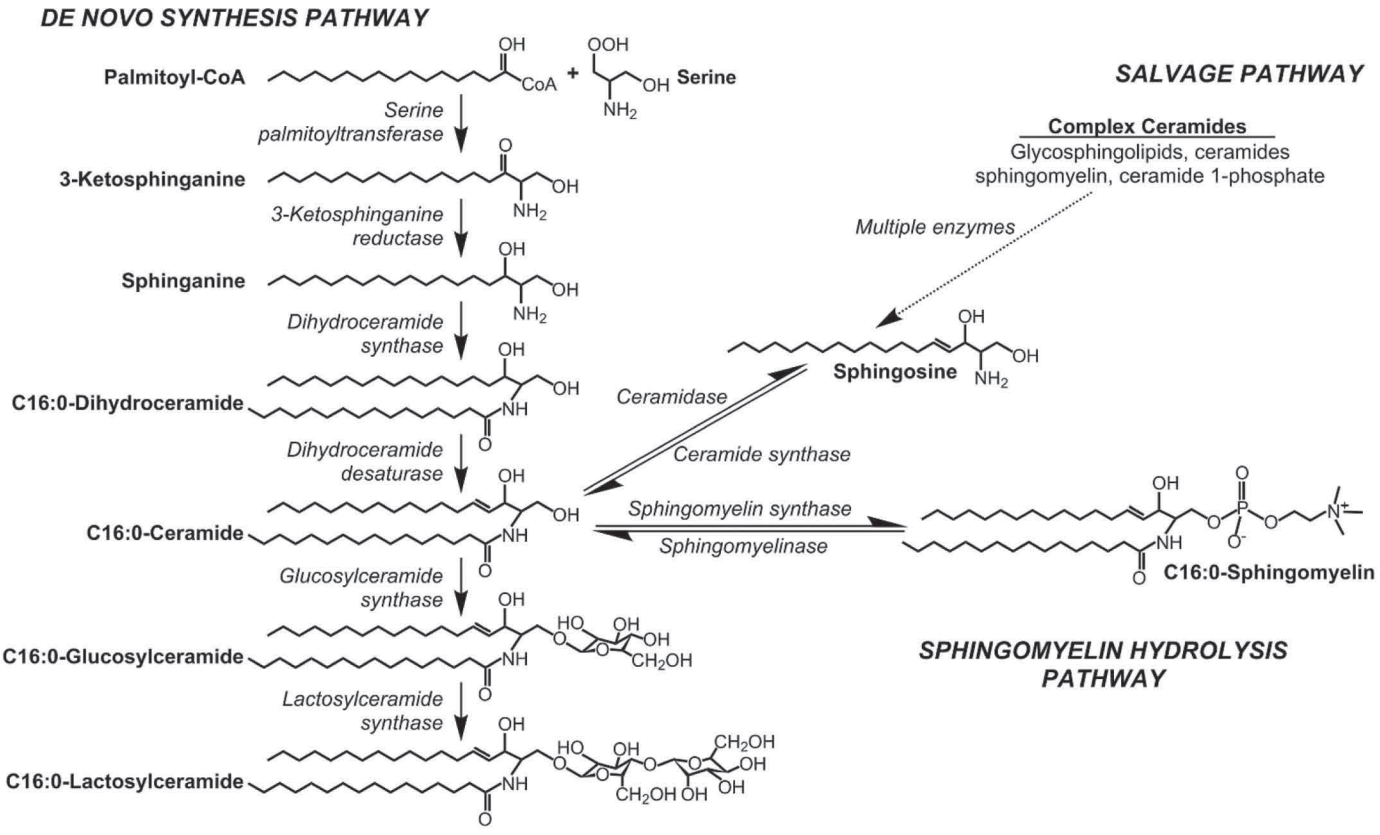

Figure 1. Metabolic pathways of ceramide synthesis. Ceramide can be generated by 3 pathways: de novo synthesis initiated by the condensation of palmitoyl-CoA and serine to form 3-ketosphinganine; hydrolysis of sphingomyelin; or breakdown of complex sphingolipids via a series of reactions referred to as the salvage pathway. Complex sphingolipids have various acyl chain lengths varying in carbon length and degree of saturation. For simplicity, only C16:0-linked sphingolipids and glycosphingolipids are illustrated. Monohexosylceramides consist of a single sugar residue, either glucose or galactose (galactosylceramide is not shown).