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Variable liver fat concentration as a proxy for body fat mobilization postpartum has minor effects on insulin-induced changes in hepatic gene expression related to energy metabolism in dairy cows

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ABSTRACT

The liver plays a central role in adaptation for energy requirements around calving, and changes in the effects of insulin on hepatic energy metabolism contribute to metabolic adaptation in dairy cows. Hepatic insulin effects may depend on body fat mobilization. The objective of this study was to investigate the effects of insulin on the hepatic gene expression of enzymes involved in energy metabolism and factors related to nutrition partitioning in cows with high and low total liver fat concentration (LFC) after calving. Holstein cows were retrospectively grouped according to their LFC after calving as a proxy for body fat mobilization. Cows were classified as low (LLFC; LFC <24% fat/dry matter; n = 9) and high (HLFC; LFC >24.4% fat/dry matter; n = 10) fat-mobilizing after calving. Euglycemic-hyperinsulinemic clamps [6 mU/(kg × min) of insulin for 6 h] were performed in wk 5 antepartum (ap) and wk 3 postpartum (pp). Before and at the end of the euglycemic-hyperinsulinemic clamps, liver biopsies were taken to measure the mRNA abundance of enzymes involved in carbohydrate and lipid metabolism, expression related to the somatotropic axis, and adrenergic and glucocorticoid receptors. The mRNA abundance of pyruvate carboxylase, cytosolic phosphoenolpyruvate carboxykinase (PEPCK; *PCK1*), acyl-CoA-dehydrogenase very long chain (*ACADVL*), and hydroxyl-methyl-glutaryl-CoA-synthase 1 increased, but the mRNA abundance of solute carrier family 2 (*SLC2A2* and *SLC2A4*), growth hormone receptor 1A (*GHR1A*), insulin-like growth factor 1 (*IGF1*), sterol regulatory element binding factor 1, adrenoceptor α 1A, and glucocorticoid receptor decreased from ap to pp. Insulin treatment was associated with decreased *PCK1,* mitochondrial PEPCK, glucose-6-phosphatase, propionyl-CoA-carboxylase α, carnitine-palmitoyltransferase 1A, *ACADVL*, and insulin receptor mRNA, but increased *IGF1* and *SLC2A4* mRNA ap and pp and *GHR1A* mRNA pp. The mRNA abundance of *SLC2A4* was greater, and the mRNA abundance of *GHR1A* and *IGF1* tended to be lower in LLFC than in HLFC. Administration of insulin, albeit at a supraphysiological dose, was associated with inhibition of gene expression related to glucose production and β-oxidation, but we observed variable effects in the degree of insulin depression of individual genes. Insulin status is important for regulation of nutrient partitioning, but different LFC pp had very little influence on changes in hepatic gene expression following administration of insulin.
Key words: dairy cow, insulin, hepatic gene expression, energy metabolism, somatotropic axis

INTRODUCTION

During the transition from late pregnancy to lactation, marked changes occur in carbohydrate and lipid metabolism to ensure nutrient supply for milk production in high-yielding dairy cows (Bauman, 2000; Ingvartsen and Andersen, 2000; Drackley et al., 2001). Nutrient partitioning is under homeorhetic control, resulting in several endocrine changes during the transition period (Bauman, 2000; Ingvartsen and Andersen, 2000; Drackley et al., 2001). Insulin plays a pivotal role in energy partitioning during this period, and pancreatic insulin release and insulin sensitivity also change with the onset of lactation (McDowell, 1983; Brockman and Laarveld, 1986; De Koster and Opsomer, 2013). Insulin affects hepatic glucose and lipid metabolism and influences the gene expression of key enzymes of hepatic gluconeogenesis, fatty acid oxidation, ketogenesis, and cholesterol synthesis (Donkin, 1999; Nguyen et al., 2008; Loor, 2010). However, due to changes in insulin release and sensitivity around the time of calving, the

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effects of insulin on the gene expression of enzymes in the liver may depend on lactation stage (Sano et al., 1993; Hayirli, 2006; Aschenbach et al., 2010).

Insulin action in cows also depends on body condition, and impaired insulin sensitivity has been observed primarily in overconditioned dairy cows before and after calving (De Koster and Opsomer, 2013; De Koster et al., 2015). Lipid mobilization in early lactation varies greatly among individual cows (Hammon et al., 2009; Weber et al., 2013b); thus, the liver has to cope with a variable substrate flow for energy utilization. Interestingly, cows with elevated body condition and greater body fat mobilization during the transition period show greater body fat oxidation as measured by indirect calorimetry in a respiration chamber before calving, when body fat mobilization and an increase in the release of nonesterified fatty acids (**NEFA**) into the plasma is not occurring (Börner et al., 2013). We speculate that the regulation of hepatic energy metabolism by insulin may be involved in variable hepatic fuel oxidation.

In this study, we investigated insulin effects on hepatic energy metabolism using the euglycemic-hyperinsulinemic clamp (**EGHIC**) in dairy cows in late pregnancy and early lactation with high and low body fat mobilization indicated by their mean total liver fat concentration (**LFC**; Schäff et al., 2012). Investigations of the liver focused on 19 candidate genes involved in carbohydrate and lipid metabolism and on the regulation of energy partitioning by the somatotropic axis and adrenergic and glucocorticoid receptors. It is well known that changes in the hepatic somatotropic axis occur around the time of calving in dairy cows (Etherton and Bauman, 1998), and the adrenergic and glucocorticoid systems are known to counteract the effects of insulin (Mc Dowell, 1983; Brockman and Laarveld, 1986).

The dairy cows investigated in this study were grouped according to their mean total LFC postpartum (**pp**), which indicated differences in body fat mobilization around the time of calving in the present study (Schäff et al., 2012), as well as in previous studies (Hammon et al., 2009; Weber et al., 2013b). Performance data from the cows in wk 5 antepartum (**ap**) and wk 3 pp, as well as studies on insulin responsiveness, pancreatic insulin release, and postpartum insulin-dependent glucose metabolism around the time of calving, were recently published in a companion paper by Weber et al. (2016). The cows showed similar DMI, milk yield, and energy balance during the clamp studies, but they differed with respect to body condition and whole-body fuel oxidation (Börner et al., 2013; Weber et al., 2016).

Focusing on individual variation in the regulation of hepatic energy metabolism, the present study aimed to determine insulin-dependent differences in the hepatic gene expression of key enzymes involved in carbohydrate and lipid metabolism and factors related to nutrient partitioning in high-yield dairy cows that varied in their fat mobilization around the time of calving (Weber et al., 2016). Because the present study was based on a study of the measurement of maximal insulin response using the EGHIC (Weber et al., 2016), we used a supraphysiological insulin dose. We hypothesized that the regulation of gene expression with regard to hepatic carbohydrate and lipid metabolism, as well as nutrient partitioning by insulin, may vary before and after calving and with respect to elevated hepatic fat content. These findings will contribute to the understanding of the adaptation of hepatic energy metabolism and the effect of insulin on hepatic regulation in the livers of high-yield dairy cows.

MATERIALS AND METHODS

Animals, Husbandry, and Feeding

All treatments were conducted in accordance with the guidelines for the use of animals as experimental subjects of the State Government of Mecklenburg-Western Pomerania (Registration No. LALLF M-V/TSD/7221.3-2.1-021/09). For the present study, 20 multiparous German Holstein cows from a local farm were chosen based on their milk yield during their previous lactation (>10,000 kg/305 d) and age (second to fourth lactation). To minimize genetically based variations in fat metabolism, all cows were selected for heterozygosity at a polymorphic locus in the acyl-CoA-diacylglycerol acyltransferase 1 gene (*DGAT1* K232A: lysine or alanine at position 232), which affects fat metabolism in muscle and the mammary glands (Thaller et al., 2003).

We focused our investigations on transcriptional response in the liver to insulin treatment using the EGHIC at wk 5 ap and wk 3 pp. These studies were embedded in a comprehensive project on energy metabolism and regulation of feed intake in dairy cows during the transition period. The results of the clamp studies on insulin-dependent glucose metabolism have recently been published by Weber et al. (2016). Dairy cows investigated in this study were observed from wk 7 ap until wk 5 pp; animal management and performance data have been published previously (Schäff et al., 2012; Börner et al., 2013). All cows were kept in a tie stall and fed a TMR (ad libitum, twice daily) adapted for the dry period (a far-off diet from wk 7 to wk 4 ap and a close-up diet from wk 3 ap until calving) or lactation. The ingredients and chemical compositions of the different diets were determined according to the recommendations of the German Society of Nutritional