J. Dairy Sci. 98:1–10
http://dx.doi.org/10.3168/jds.2015-9695

Associations between hepatic metabolism of propionate and palmitate in liver slices from transition dairy cows

M. M. McCarthy, M. S. Piepenbrink, and T. R. Overton[1]
Department of Animal Science, Cornell University, Ithaca, NY 14853

ABSTRACT

Multiparous Holstein cows (n = 95) were used to evaluate changes in hepatic propionate and palmitate metabolism and liver composition over time during the transition period, along with the relationships of these variables with cumulative increases in nonesterified fatty acids and β-hydroxybutyrate during the periparturient period. Data from 3 previous experiments were used to address the study objectives, accounting for a total of 95 multiparous Holstein cows. Liver slices from biopsies on d −21, 1, and 21 relative to parturition were used to determine conversion of [1-^{14}C]palmitate to CO_2 and esterified products (EP) and the conversion of [1-^{14}C]propionate to CO_2 and glucose. Hepatic glycogen content was highest on d −21 and was 26.9 and 36.5% of prepartum values on d 1 and 21, respectively. Liver triglyceride content was lowest at d −21 and was 271 and 446% of prepartum values on d 1 and 21, respectively. We detected no difference in the capacity for the liver to oxidize [1-^{14}C]palmitate to CO_2 between d −21 and d 1; however, on d 21, oxidation was 84% of prepartum values. The capacity of the liver to convert [1-^{14}C]palmitate to EP was 148 and 139% of prepartum values on d 1 and 21, respectively. The capacity of liver to convert [1-^{14}C]propionate to CO_2 was 127 and 83% of prepartum values on d 1 and 21, and the capacity of liver to convert [1-^{14}C]propionate to glucose was 126 and 85% of prepartum values on d 1 and 21, respectively. Correlation relationships suggest that overall, cows with elevated prepartum liver triglyceride content had elevated triglycerides throughout the transition period along with increased [1-^{14}C]palmitate oxidation and conversion to EP and a decreased propensity to convert [1-^{14}C]propionate to glucose. Cows with increased [1-^{14}C]propionate oxidation had increased conversion of [1-^{14}C]propionate to glucose throughout the transition period. Overall, conditions that lead to impairments in fatty acid metabolism during the transition period appear to be associated with impaired postpartum hepatic propionate metabolism.

Key words: early lactation, hepatic metabolism, propionate, palmitate

INTRODUCTION

The metabolic demand of lactation during the immediate postpartum period nearly doubles energy requirements compared with prepartum requirements and results in a state of negative energy balance (Drackley et al., 2001). One of the increases in this metabolic demand of lactation is the need to support milk lactose synthesis in the mammary gland (Bell and Bauman, 1997). At 4 d postpartum, glucose utilization by the mammary gland is estimated to be 2 times greater than that of the gravid uterus during late gestation (Bell, 1995). This rapid increase in glucose demand requires careful orchestration of the metabolic utilization of nutrients for other tissues (Bauman and Currie, 1980).

To accommodate this increase in glucose demand, the liver increases its metabolic activity (Reynolds et al., 2003, 2004). Propionate that is produced from ruminal fermentation is quantitatively the greatest contributor to gluconeogenesis during the periparturient period (Reynolds et al., 2003). However, during the postpartum period, there is a large increase in hepatic utilization of other gluconeogenic precursors (amino acids, lactate, and glycerol) to help meet glucose needs during early lactation (Reynolds et al., 2003). Postpartum endocrine changes and alterations in responses of insulin-dependent tissues to insulin stimulation lead to the sparing of glucose for mammary gland lactose synthesis (Bell, 1995). Because of these glucose-sparing mechanisms, there is decreased lipogenesis and increased lipolysis in adipose tissue (Bell and Bauman, 1997). During the periparturient period, this increase in net lipid mobilization increases the circulating pool of NEFA in the blood stream that can be incorporated into milk fat by the mammary gland, and the proportional uptake of NEFA by the liver also increases (Reynolds et al., 2003). In the liver, these NEFA can

Received April 9, 2015.
Accepted June 14, 2015.
[1]Corresponding author: tro2@cornell.edu

be oxidized completely to CO_2 or partially oxidized to ketone bodies, including BHBA, that can be exported into the circulation for further de novo fatty acid synthesis in the mammary gland and to help meet the energetic needs of peripheral tissues, thereby sparing glucose for mammary lactose synthesis. Hepatic ketone synthesis depends on energy balance and availability of intermediates in the Krebs cycle, and may also be related to individual differences in hepatic gluconeogenic capacity. When there is a large supply of propionate, hepatic ketone body synthesis is reduced (Zammit, 1990; Drackley et al., 2001). When rates of lipid mobilization are high, uptake of NEFA by the liver can exceed the rate of oxidation and also the ability of the liver to export reesterified triglycerides in the form of very low density lipoproteins (**VLDL**), leading to liver triglyceride accumulation (Drackley, 1999). Excess liver triglyceride accumulation has been associated with a decreased ability for the hepatocyte to synthesize urea (Strang et al., 1998). The consequent increase in liver ammonia may decrease glucose synthesis from propionate (Overton and Drackley, 1999). As such, there are many potential relationships between hepatic gluconeogenesis and FA metabolism. The objective of this study was to further elucidate how these relationships of energy metabolism change during the transition to lactation. We were interested in evaluating changes in hepatic propionate and palmitate metabolism and liver composition over time during the transition period and the relationships between these variables, along with their relationships with the cumulative increases in circulating NEFA and BHBA concentrations during the periparturient period.

MATERIALS AND METHODS

Experimental Animals and Procedures

All procedures involving animals were approved by the Cornell University Institutional Animal Care and Use Committee (Ithaca, NY) before the onset of the experiments. Data from 95 Holstein cows entering second lactation or greater from the Cornell University Teaching and Research Center Dairy were used for this data set from 3 separate experiments (Piepenbrink, 2003; Piepenbrink and Overton, 2003; Piepenbrink et al., 2004). Briefly, cows were housed in individual tiestalls and fed the same prepartum and postpartum basal rations within each experiment. Cows received either no dietary treatment (Piepenbrink, 2003), varying amounts of rumen-protected choline (Piepenbrink and Overton, 2003), or varying amounts of 2-hydroxy-4-(methylthio)-butanoic acid (Piepenbrink et al., 2004) beginning at 21 d before expected calving and continuing through either d 63 (Piepenbrink, 2003; Piepenbrink and Overton, 2003) or d 84 (Piepenbrink et al., 2004) postpartum.

Plasma samples were obtained 3 times per week via venipuncture of the coccygeal vessels from d −21 through d 21 relative to parturition and analyzed for NEFA and BHBA. Plasma concentrations of NEFA and BHBA were analyzed by enzymatic analyses (NEFA-C; Wako Pure Chemical Industries, Osaka, Japan; and BHBA dehydrogenase; kit no. 310, Sigma Chemical, St. Louis, MO).

Liver tissue was sampled via percutaneous trocar biopsy (Veenhuizen et al., 1991) from cows under local anesthesia on d −21, 1, and 21 relative to parturition. After blotting the liver sample to remove excess blood and connective tissue, a portion of the sample was immersed in ice-cold PBS (0.015 *M*; 0.9% NaCl, pH 7.4) and transported to the laboratory within 45 min of tissue collection for measurement of in vitro metabolism of [1-^{14}C]propionate and [1-^{14}C]palmitate as previously described (Piepenbrink, 2003; Piepenbrink and Overton, 2003; Piepenbrink et al., 2004). The remaining portion of liver tissue that was collected was snap-frozen in liquid nitrogen and stored at −80°C until analysis for triglyceride and glycogen content as previously described (Piepenbrink, 2003; Piepenbrink and Overton, 2003; Piepenbrink et al., 2004).

Statistical Analysis

All statistical computations were performed using SAS software (version 9.3; SAS Institute Inc., Cary, NC). Data for hepatic glycogen and triglyceride content, in vitro conversion of [1-^{14}C]palmitate to CO_2 and esterified products (**EP**), and conversion of [1-^{14}C]propionate to CO_2 and glucose and the ratio of glucose to CO_2 were subjected to repeated-measures ANOVA using PROC MIXED of SAS and the REPEATED statement. The fixed effect was time and the random effect was cow, and the PDIFF option was used to identify differences within time. Four covariance structures were tested: compound symmetry, heterogeneous compound symmetry, first-order autoregressive, and heterogeneous first-order autoregressive, and the covariance structure that resulted in the smallest Akaike information criterion was used. The degrees of freedom for PROC MIXED were estimated using the Kenward-Roger option in the model statement. Least squares means and standard errors of the mean were reported. Statistical significance was declared at $P \leq 0.05$ and trends were discussed at $0.05 < P \leq 0.10$.

Thirteen variables (Table 1) were chosen to test in Pearson correlation by SAS (2000; SAS Institute Inc., Cary, NC). The area under the curve (**AUC**) was