J. Dairy Sci. 88:3851–3859

Carnitine Palmitoyltransferase I in Liver of Periparturient Dairy Cows: Effects of Prepartum Intake, Postpartum Induction of Ketosis, and Periparturient Disorders*

H. M. Dann† and J. K. Drackley
Department of Animal Sciences, University of Illinois, Urbana 61801

ABSTRACT

Thirty-five multiparous Holstein cows were used to determine the role of mitochondrial carnitine palmitoyltransferase I (CPT I) in liver on peripartal adaptations of fatty acid metabolism. From dry-off to parturition, cows were fed a diet at either ad libitum (n = 17) or restricted intake (RI, 80% of calculated requirements for net energy; n = 18). After parturition, all cows were fed a lactation diet. At 4 d in milk (DIM), cows underwent a physical examination and were classified as healthy (n = 15) or having at least one periparturient disorder (PD; n = 17). Cows in the healthy group were assigned to either a control (n = 6) group or a ketosis induction (KI; n = 9) group. Cows with periparturient disorders were assigned to a third (PDC; n = 17) group. Cows in control and PDC groups were fed for ad libitum intake. Cows in KI were fed at 50% of their respective intake at d 4 postpartum starting from 5 DIM and continuing to signs of clinical ketosis or until 14 DIM; cows then were returned to ad libitum intake. Liver was biopsied at –30 d, 1 d, at signs of clinical ketosis or 14 d, and 28 d relative to parturition. Mitochondria were isolated by differential centrifugation. Activity of CPT I was 5.4 and 7.6 nmol of palmitoylcarnitine formed per min/mg of protein for ad libitum and RI, respectively, at –30 DIM. Sensitivity of CPT I to its inhibitor, malonyl CoA, did not differ between ad libitum and RI cows. Differences in CPT I activity between ad libitum and RI were no longer significant at 1 DIM. Postpartum CPT I activity and malonyl CoA sensitivity at 1 DIM, onset of clinical ketosis or 14 DIM, and 28 DIM were not affected by prepartum intake (ad libitum vs. RI), postpartum health status (healthy vs. PD), or ketosis induction status (control vs. KI vs. PDC). Activity of CPT I was positively correlated with liver total lipid, liver triglyceride, liver triglyceride to glycogen ratio, and serum nonesterified fatty acids. Activity of CPT I and dry matter intake were not correlated. Prepartum intake affected prepartum CPT I activity but not malonyl CoA sensitivity. Neither induction of primary ketosis nor periparturient disorders greatly affected CPT I activity or sensitivity, which indicates that alterations of CPT I may not be a major factor in the etiology of primary ketosis or other periparturient disorders.
(**Key words:** carnitine palmitoyltransferase I, liver, ketosis, lipid metabolism)

Abbreviation key: CPT = carnitine palmitoyltransferase, $\mathbf{IC_{50}}$ = concentration at which activity is reduced by 50%, **KI** = ketosis induction, **PD** = periparturient disorder, **PDC** = periparturient disorder control, **RI** = restricted intake.

INTRODUCTION

Dairy cows are susceptible to a number of metabolic disorders and infectious diseases during the periparturient period (Goff and Horst, 1997). An increased understanding of lipid metabolism, in particular fatty acid oxidation, may allow the development of nutritional and management approaches to prevent the development of metabolic disorders, such as hepatic lipidosis and ketosis, in dairy cows.

Hepatic oxidation of long-chain fatty acids occurs in mitochondria and peroxisomes (Drackley et al., 2001). Mitochondrial fatty acid oxidation, the subject of this investigation, involves 4 key steps (Louet et al., 2001): 1) uptake and activation of fatty acids to fatty acyl-CoA, 2) translocation of the fatty acyl-CoA into the mitochondria, 3) β-oxidation of fatty acyl-CoA, and 4) ketogenesis. The carnitine palmitoyltransferase (**CPT**) system allows fatty acids to be translocated into the mitochondria (McGarry and Brown, 1997). The CPT system is composed of 3 enzymes: CPT I (EC 2.3.1.21), carni-

Received June 14, 2005.
Accepted July 26, 2005.
Corresponding author: James K. Drackley; e-mail: drackley@uiuc.edu.
*Supported by USDA-CSREES Section 1433 Animal Health and Disease Funds appropriated to the Illinois Agricultural Experiment Station (project number 35-925). Heather M. Dann was supported by a Jonathan Baldwin Turner graduate fellowship from the College of Agricultural, Consumer and Environmental Sciences, University of Illinois.
†Present address: William H. Miner Agricultural Research Institute, Chazy, NY 12921.

tine-acylcarnitine translocase, and CPT II (McGarry and Brown, 1997). Carnitine palmitoyltransferase I, an integral protein located on the outer mitochondrial membrane, catalyzes the formation of fatty acyl-carnitine from fatty acyl-CoA and carnitine and is believed to be a key regulatory step in metabolism of long-chain fatty acids (McGarry and Brown, 1997).

Long-chain fatty acid oxidation is primarily controlled by changes in CPT I activity, changes in malonyl-CoA concentration, and changes in sensitivity of CPT I to inhibition by malonyl-CoA (Kerner and Hoppel, 2000; Louet et al., 2001). Methylmalonyl-CoA can inhibit CPT I in sheep (Brindle et al., 1985) and cattle (Jesse et al., 1986; Knapp, 1990). Nutritional and hormonal status of the animal affects CPT I. Gene expression of CPT I is increased by glucagon, cAMP, 3,3′,5-triiodothyronine, and long-chain fatty acids; CPT I gene expression is decreased by insulin (Park et al., 1995; Zammit, 1996; Kerner and Hoppel, 2000; Louet et al., 2001). Activity of CPT I also is controlled by interactions between mitochondria and cytoskeletal components (Guzmán et al., 2000).

The regulatory role of CPT I on fatty acid oxidation has been studied extensively in nonruminants during different physiological and pathological states. In rodents during the fed state, plasma glucose concentration is high, plasma NEFA concentration is low, the glucagon to insulin ratio is low, fatty acid synthesis is high, and hepatic malonyl-CoA concentration is high, resulting in low hepatic CPT I activity, low CPT I expression, and high sensitivity to inhibition by malonyl-CoA (Eaton et al., 1996; Kerner and Hoppel, 2000). In catabolic states, such as starvation and diabetes, the malonyl-CoA concentration decreases and the glucagon to insulin ratio increases, resulting in an increase in hepatic fatty acid oxidation through increased substrate (NEFA) availability and changes in CPT I activity and sensitivity to malonyl-CoA (Bremer, 1981; Park et al., 1995; Eaton et al., 1996; Kerner and Hoppel, 2000).

In ruminants, activity of CPT I and sensitivity of CPT I to malonyl-CoA and methylmaloyl-CoA inhibiton have not been thoroughly evaluated during different physiological and pathological states. Aiello et al. (1984) showed that CPT I activity in dairy cows was greater at d 30 than at d 60, 90, or 180 of lactation. The higher activity of CPT I in early lactation was associated with higher rates of gluconeogenesis and ketogenesis, possibly due to a greater negative energy balance in early lactation. Similar to Aiello et al. (1984), Dann et al. (2000) showed that CPT I activity peaked at 1 DIM and decreased at 21 and 65 DIM. Mizutani et al. (1999) compared CPT I activity of cows in early (0 to 110 DIM), mid (111 to 220 DIM), and late (>220 DIM) lactation and found no difference among stages of lactation. Energy status of the cows at the various stages was not reported by Mizutani et al. (1999); energy balance among groups may have been similar and therefore no difference in CPT I activity would be expected.

Knapp (1990) compared nonketotic nonlactating and lactating dairy cows and found no difference in CPT I activity. No information was provided about DIM or gestation status. In contrast to Knapp (1990), Mizutani et al. (1999) and Dann et al. (2000) showed that CPT I activity for lactating dairy cows was higher than that for nonlactating dairy cows. In ewes, total CPT (CPT I plus CPT II) activity was not altered by physiological state (fed nonpregnant, fasted nonpregnant, fasted pregnant; Butler et al., 1988).

Limited information is available concerning how CPT I activity and its regulation is affected by metabolic disorders in ruminants. Mizutani et al. (1999) found that CPT I activity in dairy cows with hepatic lipidosis was lower than that in dairy cows without hepatic lipidosis outside the periparturient period. The authors suggested that the development of hepatic lipidosis in nonperiparturient cows might be related to low CPT I activity but that development of hepatic lipidosis during the periparturient period may be caused by another mechanism. The authors were unable to determine CPT I activity in healthy cows during the periparturient period.

The activity and function of CPT I are important for understanding metabolic changes in ketosis and hepatic lipidosis. Despite the probable role of CPT I in controlling oxidative flux of NEFA within the ruminant liver (Aiello et al., 1984; Jesse et al., 1986; Chow and Jesse, 1992; Drackley, 1999), little is known about its activity, expression, and regulation in periparturient cows. Thus, investigation of CPT I is warranted. Our objective was to determine how prepartum nutrient intake and postpartum health status affects hepatic CPT I activity and sensitivity of CPT I to inhibition by malonyl-CoA in multiparous periparturient Holstein cows. The hypothesis was that CPT I activity would increase, and sensitivity of CPT I to inhibition by malonyl-CoA would decrease when cows experience negative energy balance around parturition.

MATERIALS AND METHODS

Experimental Design and Management of Cows

All procedures were conducted under protocols approved by the University of Illinois Institutional Animal Care and Use Committee. The experiment from which samples were obtained for this study has been reported previously (Dann et al., 2005). Thirty-five multiparous Holstein cows were fed a diet (Table 1) in the form of