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

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ABSTRACT

Thirty-five multiparous Holstein cows were used to determine the role of mitochondrial carnitine palmitoyltransferase I (CPT I) in liver on periparturient 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, IC₅₀ = 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.

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*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.

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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 methylmalonyl-CoA inhibition 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

Table 1. Ingredient and chemical composition (mean $\pm$ SE) of diets fed to multiparous Holstein cows during the dry and lactating periods.

Item	Diet ¹		
	Far-off dry	Close-up dry	Lactation
	% of DM		
Ingredient			
Alfalfa hay	14.30	13.12	—
Alfalfa silage	15.30	14.03	20.00
Corn silage	53.20	48.80	25.00
Cottonseed	2.00	1.83	10.00
Ground shelled corn	5.00	4.59	26.65
Soybean meal	4.50	4.13	13.50
Soyhulls	—	—	1.50
Oathulls	4.47	4.10	—
Mineral and vitamin mix ²	0.20	0.18	0.25
Vitamin E ³	0.25	0.23	—
Sodium chloride	0.53	0.49	0.25
Magnesium oxide	0.25	0.23	0.14
Calcium carbonate	—	1.17	—
Close-up premix ⁴	—	7.10	—
Sodium bicarbonate	—	—	1.00
Dicalcium phosphate	—	—	0.45
Limestone	—	—	1.10
Sodium sulfate	—	—	0.16
Chemical composition ⁵			
Number of samples ⁶	8	8	8
CP	15.2 $\pm$ 0.4	14.6 $\pm$ 0.4	18.7 $\pm$ 0.4
ADF	31.8 $\pm$ 0.4	29.2 $\pm$ 0.4	20.7 $\pm$ 0.4
NDF	45.6 $\pm$ 0.7	41.8 $\pm$ 0.6	29.5 $\pm$ 0.4
NE _L , Mcal/kg ⁷	1.60 $\pm$ 0.04	1.47 $\pm$ 0.03	1.77 $\pm$ 0.02
Ca	0.58 $\pm$ 0.02	1.44 $\pm$ 0.02	0.99 $\pm$ 0.05
P	0.38 $\pm$ 0.01	0.38 $\pm$ 0.01	0.50 $\pm$ 0.02
Mg	0.37 $\pm$ 0.01	0.52 $\pm$ 0.01	0.32 $\pm$ 0.01
K	1.56 $\pm$ 0.06	1.46 $\pm$ 0.05	1.22 $\pm$ 0.05
Na	0.30 $\pm$ 0.01	0.27 $\pm$ 0.01	0.49 $\pm$ 0.04

¹Far-off dry period diet fed from dry-off until -22 d before expected parturition; Close-up dry period diet fed from -21 d before expected parturition to parturition; Alfalfa hay (~2 kg of DM) was top-dressed on the lactation diet from parturition to 14 DIM.

²Contained a minimum of 5% Mg, 10% S, 7.5% K, 2.0% Fe (from ferrous sulfate monohydrate), 3.0% Zn (from zinc oxide), 3.0% Mn (from manganous oxide), 5000 mg/kg Cu (from cupric sulfate pentahydrate), 250 mg/kg I (from calcium iodate), 40 mg/kg Co (from cobalt carbonate), 150 mg/kg Se (from sodium selenite), 2200 kIU/kg vitamin A, 660 kIU/kg vitamin D₃, and 7700 IU/kg vitamin E.

³Contained 44,000 IU/kg.

⁴Contained 10% CP, 1% fat, 3% crude fiber, 6.3% Ca, 0.3% P, 2.5% Mg, 3.2% S, 0.43% K, 11% Cl, 6.6 mg/kg Se, 110 kIU/kg vitamin A, 44 kIU/kg vitamin D₃, 1320 IU/kg vitamin E, and 13,200 mg/kg niacin.

⁵Calculated from monthly analyses of individual ingredients in diets.

⁶Number of samples used to determine the chemical composition of the diet.

⁷Calculated by Dairy One (Ithaca, NY) using the NRC (1989) energy equations for concentrates and the Van Soest variable discount method for forages.

a TMR from dry-off (approximately -60 d relative to expected parturition) to parturition at either ad libitum (n = 17) or restricted (**RI**; n = 18) feeding rates. Intake was restricted to 80% of calculated NE_L requirements based on NRC (1989). A close-up premix (Table 1) and calcium carbonate were added to the prepartum diet beginning -21 d relative to expected parturition; the amount of this TMR offered continued to be restricted to the same amount. After parturition, all cows were fed a lactation diet (Table 1). Alfalfa hay (~2 kg of DM) was top-dressed on the lactation TMR from parturition through 14 DIM.

At 4 DIM, cows underwent a thorough physical examination (described in Dann et al., 2005) and were classified as healthy (n = 15) or having at least one periparturient disorder (**PD**; n = 17). Healthy cows were assigned to either the control (n = 6) group or the ketosis induction (**KI**; n = 9) group. Cows with periparturient disorders were assigned to the periparturient disorder control (**PDC**; n = 17) group; no cows with PD were assigned to KI. Three cows were excluded from the postpartum data set for reasons unrelated to this study. Cows in control and PDC groups were fed for ad libitum intake. Ketosis induction was by feed restriction (Bahaa et al.,

1997). Beginning at 5 DIM, cows in KI were fed at 50% of their intake at 4 DIM until signs of clinical ketosis (anorexia, ataxia, or abnormal behavior) or until 14 DIM and then were returned to ad libitum intake and treated, if necessary. Health records were maintained for all cows. Of the 17 PD cows, 16 cows had retained placenta (failure to expel the placenta within 24 h of parturition) and metritis and 1 cow had subclinical ketosis at the 4 DIM physical examination. Four of 9 KI cows showed signs of clinical ketosis and were treated with 500 mL of 50% dextrose intravenously and 500 mL of propylene glycol per os. The other KI cows had subclinical ketosis based on a positive urine ketone test (Labstix; Bayer Corporation, Elkhart, IN), but did not require treatment. Concentration of BHBA in serum was used to confirm subclinical ketosis (Dann et al., 2005), which was set at a threshold concentration of 1400 $\mu\text{mol/L}$ BHBA (Duffield, 2000). Four of the 5 subclinically ketotic cows with a positive urine ketone test had serum BHBA greater than 1400 $\mu\text{mol/L}$.

Liver was sampled via puncture biopsy (Hughes, 1962) from cows under local anesthesia at -30 d, 1 d, at signs of clinical ketosis or 14 d, and 28 d relative to parturition. Approximately 2 g of liver was frozen immediately in liquid nitrogen and used for analysis of total lipid, triglyceride, and glycogen as reported elsewhere (Dann et al., 2005). Approximately 1 g of liver to be used for determination of CPT I activity and CPT I sensitivity to its inhibitor malonyl-CoA was placed in isolation buffer that contained 220 mM mannitol, 70 mM sucrose, 2 mM HEPES, and 0.1 mM EDTA at pH 7.4 and 4°C (Mersmann et al., 1972). All subsequent processing steps were conducted at 4°C. The liver was removed from the isolation buffer, minced with a razor blade, weighed, suspended in isolation buffer, and homogenized with a motor-driven Potter-Elvehjem tissue homogenizer. Mitochondria were isolated by differential centrifugation of the homogenate (Greenawalt, 1974; Jesse et al., 1986). The homogenate was centrifuged at $650 \times g$ for 10 min, and the supernatant was removed; the pellet was resuspended in isolation buffer and recentrifuged at $650 \times g$ for 10 min. The supernatant was removed and combined with the previous supernatant. The pooled supernatant was centrifuged at $7000 \times g$ for 15 min. The mitochondrial pellet was resuspended in isolation buffer and centrifuged at $7000 \times g$ for 15 min. The final mitochondrial pellet was resuspended in isolation buffer for measurement of CPT I activity. Mitochondrial protein concentration was determined (Lowry et al., 1951) and averaged 17 mg of protein/g of liver with a range from 8 to 32 mg of protein/g of liver.

Activity of CPT I from freshly isolated mitochondria was assayed using a modification of the method of

Bremer (1981) at 30°C as the formation of palmitoyl-[^3H]-carnitine from palmitoyl-CoA and [^3H]-carnitine (American Radiolabeled Chemicals Inc., St. Louis, MO). Briefly, the mitochondrial suspension (~1.6 mg of protein/mL) was incubated for 3 min alone or in the presence of malonyl-CoA (0 to 110 μM) in reaction medium (75 mM KCl, 50 mM mannitol, 25 mM HEPES, 2 mM KCN, 0.2 mM EGTA (ethylene glycol-bis(2-amino ethyl ether)-N,N,N',N'-tetraacetic acid), 1% bovine albumin, 1 mM dithiothreitol, and 120 μM palmitoyl-CoA; pH 7.4) and then incubated for 6 min with [^3H]-carnitine solution (1000 μM L-carnitine, ~1.5 $\mu\text{Ci}/\mu\text{mol}$). The total assay volume was 1 mL. The reaction was terminated by the addition of 4 mL of 6% HClO_4 . A butanol extraction method was used to extract palmitoyl-[^3H]-carnitine; 0.4 mL of the butanol phase was transferred to a scintillation vial and mixed with 10 mL of scintillation cocktail (Scintisafe Econo 2; Fisher Scientific, Atlanta, GA), and radioactivity was determined in a liquid scintillation spectrophotometer (Beckman LS 6000IC; Beckman Instruments Inc., Fullerton, CA).

The CPT I activity was expressed as nanomoles of palmitoylcarnitine formed per minute per milligram of protein. The sensitivity of CPT I to malonyl-CoA inhibition was determined by measuring the concentration of malonyl-CoA required for 50% inhibition of the enzyme activity (IC_{50}). The concentration of malonyl-CoA used ranged from 0 to 110 μM . Activity of CPT I with no addition of malonyl-CoA was set to 100%. Activity of CPT I was reduced to $14.58 \pm 0.53\%$ (mean $\pm$ SE) in the presence of 110 μM malonyl-CoA. Additional methods and data from the production portion of this study were presented in Dann et al. (2005) and are referenced when appropriate.

Statistical Analyses

Statistical analysis was conducted as described previously (Dann et al., 2005). Three cows were not included in the postpartum period datasets because one cow had complications associated with surgery for displaced abomasum and was euthanized, one cow had an intestinal volvulus and was euthanized, and one cow was mistakenly allowed ad libitum access to feed during a portion of the ketosis induction period and its data were deemed unreliable.

Statistical computations were performed using the Statistical Analysis System (release 8.2; SAS Institute Inc., Cary, NC). The NLIN procedure of SAS was used to determine the amount of malonyl-CoA needed to decrease CPT I activity by 50%. Hepatic CPT I activity and sensitivity to malonyl-CoA data were analyzed using the MIXED procedure of SAS (Littell et al., 1996). For each variable analyzed, 4 covariance structures

were evaluated: compound symmetry, autoregressive order 1, autoregressive heterogeneous order 1, and unstructured covariance. The covariance structure that resulted in the Akaike's information criterion closest to zero was used (Littell et al., 1996). The Kenward-Roger degrees of freedom method was used with the MIXED procedure. Malonyl-CoA IC_{50} data violated model assumptions and were $\log_{10}$ transformed before statistical analysis. Least squares means are reported. Significance was declared at $P < 0.10$ and trends discussed when $P > 0.10$ and < 0.15 .

Prepartum data were analyzed as a randomized design. The model contained the effect of prepartum intake (ad libitum or RI). Cow was designated as a random effect. Postpartum data before the start of ketosis induction were analyzed using a 2×2 factorial arrangement of main effects. The model contained the effects of prepartum intake (ad libitum or RI), postpartum health status (healthy or PD), and the interaction of prepartum intake and postpartum health status. Cow was designated as a random effect. The number of cows to be assigned to healthy or PD groups within ad libitum and RI prepartum intake was expected to be similar. To verify that assumption, an odds ratio test was conducted using the LOGISTIC procedure of SAS.

Postpartum data obtained during the ketosis induction period (5 DIM to signs of clinical ketosis or 14 DIM) and after return of all cows to ad libitum intake (15 to 42 DIM) were analyzed as an incomplete $2 \times 2 \times 2$ factorial arrangement of main effects (prepartum intake $\times$ postpartum health status $\times$ ketosis induction status) with ketosis induction randomized within healthy cows. The model contained the effects of prepartum intake (ad libitum or RI), postpartum health status (healthy or PD), ketosis induction status (control, KI, or PDC), the interaction of prepartum intake and postpartum health status, and the interaction of prepartum intake and ketosis induction status. Tukey's procedure for multiple means comparisons was used to separate treatment means for postpartum analyses after 5 DIM (control vs. KI vs. PDC).

The CORR procedure of SAS with the Spearman option (Hatcher and Stepanski, 1994) was used to determine correlations between CPT I and liver total lipid content, liver triglyceride content, serum NEFA and BHBA concentrations, and DMI.

RESULTS AND DISCUSSION

The mean CPT I activity at 30 d before expected parturition was 5.44 and 7.60 nmol of palmitoylcarnitine formed per min/mg of protein ($P = 0.03$) for ad libitum and RI cows, respectively (Table 2). As expected, the activity was higher for RI cows that had lower DMI

and were in negative energy balance. During the entire dry period, daily DMI averaged 1.82 and 1.12% of BW for ad libitum and RI cows ($P < 0.001$; Dann et al., 2005). Thus, cows fed ad libitum consumed on average 142% of NE_L requirements and RI cows consumed 85% of NE_L requirements ($P < 0.001$; Dann et al., 2005). Corresponding to the negative energy balance, RI cows had higher serum NEFA (292 vs. 167 $\mu\text{Eq/L}$; $P < 0.001$), lower serum glucose (58 vs. 61 mg/dL; $P = 0.01$), and lower serum insulin (4.84 vs. 8.19 $\mu\text{IU/mL}$; $P < 0.001$) concentrations than did cows fed ad libitum (Dann et al., 2005). Serum BHBA concentration tended ($P = 0.13$) to be higher for RI (4.2 vs. 3.5 mg/dL; Dann et al., 2005). There was no difference in liver content of total lipid (4.3%; $P = 0.38$) or triglyceride (0.27%; $P = 0.64$) between groups at 30 d before expected parturition (Dann et al., 2005).

Chow and Jesse (1992) found that mitochondrial CPT I activity in isolated sheep hepatocytes was lower in hepatocytes preincubated with insulin than in control hepatocytes. In agreement with an effect of insulin on CPT I, in vitro oxidation of palmitate by liver explants was lower for tissue biopsied during a hyperinsulinemic, euglycemic clamp in dairy cows (Andersen et al., 2002). These findings suggested that, although hepatic concentration of malonyl-CoA serves as the primary regulator of CPT I activity, circulating concentrations of insulin and glucagon can modify CPT I activity and sensitivity to malonyl-CoA. In nonruminants, malonyl-CoA is typically considered the main regulator of CPT I activity. However, Guzmán et al. (2000) presented evidence that CPT I activity is controlled also by interactions between mitochondria and cytoskeletal components. They proposed that Ca^{2+} calmodulin-dependent protein kinase II or AMP-activated protein kinase phosphorylates cytokeratins 8 and 18, causing disruption of intermediate filaments and consequently removing the inhibitory interactions between the cytoskeleton and mitochondrial components and thereby yielding a more active CPT I. The potential role of this mechanism in ruminants has not been investigated.

Sensitivity of CPT I to malonyl-CoA was not affected ($P = 0.81$) by prepartum intake (Table 2). The mean IC_{50} was 1.03 μM . An increase in IC_{50} indicates a decrease in sensitivity of CPT I to inhibition by malonyl-CoA. Drynan et al. (1996) demonstrated in starved rats that the decrease in sensitivity of CPT I to malonyl-CoA inhibition coincided with the increase in blood ketone bodies and that those changes occurred well after decreases in serum insulin and hepatic malonyl-CoA concentrations. In our experiment, the modest negative energy balance induced during the dry period (85% of NE_L requirement) did not cause a marked increase in serum BHBA concentration; consequently, the degree

Table 2. Hepatic carnitine palmitoyltransferase I (CPT I) activity and sensitivity to malonyl-CoA for multiparous Holstein cows with different prepartum intake (PRE), postpartum health status (POST), and ketosis induction status (IND).

Variable	Prepartum intake ¹			Postpartum health status ²			Ketosis induction status ³				<i>P</i> -value				
	AL	RI	SE	Healthy	PD	SE	Healthy		PD	SE	PRE	POST	IND	PRE ×	PRE ×
							Control	KI	PDC					POST	IND
CPT I activity ⁴															
–30 d	5.44	7.60	0.66	—	—	—	—	—	—	—	0.03	—	—	—	—
1 d	7.93	9.09	0.62	8.29	8.73	0.60	—	—	—	—	0.19	0.61	—	0.95	—
14 d or ketosis	8.33	8.97	0.72	8.80	8.50	0.71	8.04	9.56	8.50	1.08	0.50	0.77	0.29	0.78	0.24
28 d	7.71	7.38	0.83	7.64	7.46	0.83	6.98	8.29	7.46	1.30	0.83	0.87	0.44	0.60	0.20
Malonyl-CoA IC ₅₀ ⁵															
–30 d	0.98	1.08	0.28	—	—	—	—	—	—	—	—	—	—	—	—
1 d	1.15	1.13	0.25	1.08	1.20	0.24	—	—	—	—	—	—	—	—	—
14 d or ketosis	1.25	1.78	0.33	1.75	1.27	0.33	1.58	1.92	1.27	0.50	—	—	—	—	—
28 d	1.87	1.49	0.50	1.88	1.48	0.50	1.57	2.20	1.48	0.78	—	—	—	—	—
Malonyl-CoA IC ₅₀ ⁶															
–30 d	–0.26	–0.22	0.11	—	—	—	—	—	—	—	0.81	—	—	—	—
1 d	–0.13	–0.05	0.11	–0.16	–0.02	0.11	—	—	—	—	0.60	0.36	—	0.23	—
14 d or ketosis	–0.05	0.11	0.11	0.05	0.01	0.11	–0.06	0.16	0.01	0.17	0.23	0.80	0.32	0.15	0.89
28 d	0.13	0.11	0.11	0.18	0.06	0.11	0.16	0.20	0.06	0.17	0.81	0.40	0.83	0.40	0.66

¹AL = Ad libitum intake; RI = restricted intake.²PD = Periparturient disorder.³KI = Ketosis induction; PDC = periparturient disorder control.⁴CPT I activity expressed in nmol of palmitoylcarnitine per min per mg of protein.⁵Concentration (μ M) of malonyl-CoA needed to inhibit CPT I activity by 50% under defined assay conditions.⁶Malonyl-CoA IC₅₀ data violated model assumptions and were log10 transformed for statistical analysis.

of energy deficit may not have been extreme enough to affect malonyl-CoA sensitivity.

Postpartum CPT I activity and sensitivity to malonyl-CoA in general were minimally affected by prepartum intake, postpartum health status, or ketosis induction status (Table 2). Although the difference in CPT activity between ad libitum and RI cows was no longer significant ($P = 0.19$) at d 1 postpartum, the slightly higher mean activity for cows fed RI prepartum corresponds to the lower content of total lipid in liver at d 1 postpartum for those cows (Dann et al., 2005). Greater oxidative capacity could decrease the amount of long-chain fatty acids available for esterification. At the onset of clinical ketosis or 14 DIM, sensitivity to malonyl-CoA tended ($P = 0.15$) to be affected by the interaction of prepartum intake and postpartum health status. Healthy cows previously fed RI had the lowest sensitivity to malonyl-CoA (2.39 ± 0.46), compared with IC₅₀ values of 1.12 ± 0.44 , 1.37 ± 0.50 , and 1.17 ± 0.43 for healthy cows fed ad libitum, cows with PD fed ad libitum, and cows with PD fed RI, respectively. At 14 DIM or onset of clinical ketosis, the non-PD cows that were fed RI during the dry period had the numerically lowest contents of total lipid and triglyceride in liver.

Activity of CPT I was higher at 1 DIM compared with –30 DIM (Table 2). The increase in CPT I activity coincided with the peak in serum NEFA and an increase in hepatic total lipid and triglyceride contents immedi-

ately after parturition (Dann et al., 2005). The increase in CPT I activity from the pregnant, nonlactating state to the nonpregnant, lactating state was expected and has been observed previously in dairy cows (Mizutani et al., 1999; Dann et al., 2000). Higher activity of CPT I coincided with higher rates of ketogenesis and gluconeogenesis (Aiello et al., 1984). The rates of ketogenesis and gluconeogenesis are expected to increase with the onset of lactation and the associated negative energy balance.

From 1 DIM to onset of clinical ketosis or 14 DIM, CPT I activity decreased ~3% for controls and for PDC but increased ~15% for KI. However, differences were not significant ($P > 0.29$) between postpartum health status groups or among ketosis induction status groups for CPT I activity at the onset of clinical ketosis or 14 DIM (Table 2). There also was no difference ($P = 0.23$) among groups for the sensitivity of CPT I to malonyl-CoA at the onset of clinical ketosis or 14 DIM (Table 2). Of the 9 KI cows, the 4 cows with clinical ketosis had a range in CPT activity of 7.5 to 13.2 nmol of palmitoylcarnitine formed per min/mg of protein (mean 9.4 nmol of palmitoylcarnitine formed per min/mg of protein) and a range in IC₅₀ of 0.6 to 1.8 μ M (mean 1.1 μ M). The 5 cows with subclinical ketosis had a range in CPT activity of 7.4 to 13.8 nmol of palmitoylcarnitine formed per min/mg of protein (mean 9.8 nmol of palmitoylcarnitine formed per min/mg of protein) and a range

in IC_{50} of 0.8 to 6.4 μM (mean 2.4 μM). Consequently, no clear relationship existed between degree of ketosis and CPT I activity or malonyl-CoA sensitivity.

We expected that CPT I activity would be higher, and sensitivity to malonyl-CoA lower for KI cows because of the severe negative energy balance (53% of NE_L requirement) that was imposed. In contrast, energy balance was 93 and 88% of NE_L requirement for control and PDC. During the induction period, KI cows had higher serum NEFA ($P < 0.001$) and BHBA ($P < 0.001$) and lower serum glucose ($P < 0.001$) and insulin ($P = 0.07$) concentrations than did control and PDC cows (Dann et al., 2005). Liver total lipid and triglyceride contents at the onset of clinical ketosis or 14 DIM were higher ($P < 0.001$) for KI cows than for control and PDC cows (Dann et al., 2005).

Nonruminants in a ketotic state have increased expression of CPT I, decreased malonyl-CoA concentration, and decreased sensitivity of CPT I to inhibition by malonyl-CoA compared with the fed state (Grantham and Zammit, 1988; Drynan et al., 1996). We expected that KI cows would have higher CPT I activity and be less sensitive to inhibition by malonyl-CoA than control and PDC cows because of the lower serum insulin concentration in KI cows (Zammit, 1990, 1996). Zammit (1990) suggested that hypoinsulinemia in early lactation is likely to result in a lower malonyl-CoA concentration and activation of CPT I, leading to increased rates of fatty acid oxidation and ketogenesis. It is unclear why CPT I activity and sensitivity of CPT I to malonyl-CoA were unaffected by ketosis induction in our study. Perhaps CPT I activity already is increased to near maximal values, and its sensitivity to malonyl-CoA minimized, as a part of the normal adaptive process to lactation in dairy cows. Consequently, further changes in response to ketosis induction would not be necessary and increased ketogenesis may be controlled primarily by greater NEFA delivery to the liver. It would be interesting to evaluate sensitivity to CPT I to inhibition by methylmalonyl-CoA, which might directly link propionate supply to fatty acid oxidation (Zammit, 1990).

During the period of 15 to 42 DIM, the effects of ketosis induction and periparturient disorders on metabolism were diminished. No carryover effects of postpartum health or ketosis induction were detected for energy balance, DMI, milk yield, serum BHBA, glucose, insulin, and NEFA concentrations, or liver total lipid, triglyceride, and glycogen contents (Dann et al., 2005). As expected, at 28 DIM there were no differences ($P > 0.65$) between postpartum health status groups or among ketosis induction status groups for CPT I activity or CPT I sensitivity to malonyl-CoA (Table 2).

Comparison of CPT I activity among studies in the literature is difficult because of the vast differences among assay procedures (radioactive, spectrophotometric, or fluorometric assays), concentration of substrates used (carnitine and palmitoyl-CoA), and the manner in which data are presented (percentage of control, amount of palmitoylcarnitine formed per unit time per amount of protein, or amount of CoA released per unit time per amount of protein). In our study, CPT I activity averaged 7.74 ± 0.23 nmol of palmitoylcarnitine formed per min/mg of protein, which is similar to values reported by Knapp (1990) for lactating and nonlactating cows at slaughter. Knapp (1990) reported CPT I activity values of 8.01 and 7.73 nmol per min/mg of protein using assays based on 3H -carnitine incorporation and 5,5'-dithio-(2-nitrobenzoic acid) reaction, respectively. Maximal activity observed in our study was 16.47 nmol of palmitoylcarnitine formed per min/mg of protein, and that in the Knapp (1990) study was 17.5 nmol per min/mg of protein. Activity of CPT I reported by Aiello et al. (1984) using a ^{14}C -carnitine incorporation method was much lower than in our study and ranged from ~1 to 4 nmol per mg of protein per 5 min during 30 to 180 DIM. Mizutani et al. (1997, 1999) used a fluorometric assay and reported large differences in CPT I activities: 33.6 nmol of CoA release per min/mg of protein in healthy beef cattle, 169 nmol of CoA release per min/mg of protein in nonlactating dairy cows, and 304 nmol of CoA release per min/mg of protein in lactating dairy cows. The reason for the wider range and higher values for CPT I reported by Mizutani et al. (1997, 1999) is not known.

The average concentration of malonyl-CoA needed to inhibit CPT I activity by 50% in our study was $1.33 \pm 0.11 \mu M$. Others reported an inhibition constant calculated for malonyl-CoA inhibition of CPT I activity of ~0.3 μM in Holstein cows and crossbred beef cattle in the fed state (Jesse et al., 1986). Activity of CPT I in liver from dairy cows was reduced 44 and 50% by 10 μM malonyl-CoA and 10 μM methylmalonyl-CoA, respectively (Knapp, 1990).

Data for several variables (CPT I activity, liver total lipid content, liver triglyceride content, liver triglyceride to glycogen ratio, serum NEFA and BHBA, and DMI) from 30 d before expected parturition, 1 DIM, the onset of clinical ketosis or 14 DIM, and 28 DIM (Dann et al., 2005) were pooled for correlation analysis. When a blood sample was not collected the same day as the liver sample, the blood sample collected closest to the liver sample day was used for correlation analysis. Activity of CPT I was positively correlated ($P < 0.001$; Figure 1) with serum NEFA (Spearman $\rho = 0.49$; $n = 119$), serum BHBA (Spearman $\rho = 0.40$; $n = 119$), liver total lipid (Spearman $\rho = 0.37$; $n = 115$), and liver tri-

Figure 1. Correlations of carnitine palmitoyltransferase I (CPT I) activity with serum NEFA, serum BHBA, liver total lipid, and liver triglyceride.

glyceride (Spearman $\rho = 0.45$; $n = 115$). Activity of CPT I also was correlated (not shown) with the liver triglyceride to glycogen ratio (Spearman $\rho = 0.40$; $n = 113$). There was no correlation between CPT I activity and DMI ($P = 0.86$; $n = 114$). Correlations at individual sampling points also were not significant. Mizutani et al. (1997) found no correlation between hepatic CPT I activity and serum concentrations of NEFA, phospholipid, triglycerides, or cholesterol in Japanese beef cattle ($n = 38$) and suggested that serum lipids do not directly affect CPT I activity. Based on the correlation

analyses conducted in this study and the Mizutani et al. (1997) study, the role of CPT I in regulation of lipid metabolism in ruminants outside of the periparturient period remains unclear.

CONCLUSIONS

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 of malonyl-CoA

inhibition, which indicates that alterations of CPT I may not be major factor directly involved in the etiology of primary ketosis or other periparturient disorders.

ACKNOWLEDGMENTS

The authors thank M. R. Murphy and J. Odle for assistance with determination of kinetic parameters for the inhibition curves, G. A. Bollero for advice on statistical analysis, and D. E. Morin for veterinary expertise.

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