Dietary L-Carnitine Affects Periparturient Nutrient Metabolism and Lactation in Multiparous Cows¹

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ABSTRACT

The objectives of this study were to determine the effects of dietary L-carnitine supplementation on liver lipid accumulation, hepatic nutrient metabolism, and lactation in multiparous cows during the periparturient period. Cows were assigned to treatments at d –25 relative to expected calving date and remained on the experiment until 56 d in milk. Treatments were 4 amounts of supplemental dietary carnitine: control (0 g/d of L-carnitine; n = 14); low carnitine (LC, 6 g/d; n = 11); medium carnitine (MC, 50 g/d; n = 12); and high carnitine (HC, 100 g/d; n = 12). Carnitine was supplied by mixing a feed-grade carnitine supplement with 113.5 g of ground corn and 113.5 g of dried molasses, which was then fed twice daily as a topdress to achieve desired daily carnitine intakes. Carnitine supplementation began on d –14 relative to expected calving and continued until 21 d in milk. Liver and muscle carnitine concentrations were markedly increased by MC and HC treatments. Milk carnitine concentrations were elevated by all amounts of carnitine supplementation, but were greater for MC and HC than for LC during wk 2 of lactation. Dry matter intake and milk yield were decreased by the HC treatment. The MC and HC treatments increased milk fat concentration, although milk fat yield was unaffected. All carnitine treatments decreased liver total lipid and triacylglycerol accumulation on d 10 after calving. In addition, carnitine-supplemented cows had higher liver glycogen during early lactation. In general, carnitine supplementation in-

creased in vitro palmitate β -oxidation by liver slices, with MC and HC treatments affecting in vitro palmitate metabolism more potently than did LC. In vitro conversion of Ala to glucose by liver slices was increased by carnitine supplementation independent of dose. The concentration of nonesterified fatty acids in serum was not affected by carnitine. As a result of greater hepatic fatty acid β -oxidation, plasma β -hydroxybutyric acid was higher for the MC and HC treatments. Serum insulin was greater for all carnitine treatments, although plasma glucose was unaffected. Plasma urea N was lower and plasma total protein was higher for the MC and HC treatments. By decreasing liver lipid accumulation and stimulating hepatic glucose output, carnitine supplementation might improve glucose status and diminish the risk of developing metabolic disorders during early lactation.

Key words: L-carnitine, dairy cow, hepatic metabolism, periparturient period

INTRODUCTION

Carnitine is a required cofactor for carnitine palmitoyltransferase-I (**CPT-I**; EC 2.3.1.21), which condenses carnitine with activated long-chain fatty acids (**LCFA**) for transport from cytosol into mitochondria; therefore, carnitine is essential for mitochondrial β -oxidation of LCFA. Hepatic β -oxidation of LCFA is stimulated by exogenous carnitine in several species (Drackley et al., 1991a,b; Owen et al., 2001a; Spaniol et al., 2003). Additionally, carnitine supports β -oxidation by transporting short- and medium-chain fatty acids from peroxisomes to mitochondria and by altering the intramitochondrial ratio of acetyl-coenzyme A (**CoA**):CoA (Rebouche and Seim, 1998). Further, as a result of enhanced LCFA β -oxidation, carnitine supplementation has been shown to increase hepatic glucose production by stimulating the flux of metabolites through pyruvate carboxylase (**PC**; Ji et al., 1996; Owen et al., 2001a). Adaptations in hepatic lipid and carbohydrate metabolism are required for optimal health and productivity of dairy cows (Drackley et al., 2001). The metabolic functions of carnitine suggest that carnitine

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supply might be important during the periparturient period.

Fatty liver can occur when the rate of LCFA esterification exceeds the rates of hepatic LCFA β -oxidation and triacylglycerol (TG) export. Although most cows accumulate liver TG to some degree during the periparturient period, excessive hepatic TG can negatively affect hepatic gluconeogenic and ureagenic capacity, as well as predispose cows to development of metabolic disorders and infectious diseases (Bobe et al., 2004). Ruminant liver possesses a low capacity for TG export as very low density lipoproteins (Kleppe et al., 1988), but large increases in very low density lipoprotein secretion would be needed to greatly affect liver TG accumulation during the periparturient period (Drackley and Andersen, 2006). The rate and extent of adipose tissue lipolysis and the capacity for hepatic LCFA β -oxidation might be the most important factors regulating the degree of liver TG accumulation (Drackley and Andersen, 2006).

The activity and mRNA abundance of hepatic CPT-I increases around parturition (Dann and Drackley, 2005; Loor et al., 2005) consistent with increases in hepatic β -oxidation of LCFA during early lactation (Grum et al., 1996; Andersen et al., 2002). However, CPT-I activity and mRNA abundance were positively correlated with liver TG and serum NEFA concentrations, indicating that NEFA were esterified to TG despite increased capacity for mitochondrial LCFA β -oxidation. Although hepatic carnitine concentration increases around parturition (Grum et al., 1996), it is unclear whether carnitine might be limiting for CPT-I activity during early lactation. Acute feed restriction did not affect liver carnitine concentration or in vitro hepatic LCFA β -oxidation in midlactation dairy cows, whereas abomasal L-carnitine infusion markedly increased liver carnitine and in vitro LCFA β -oxidation while decreasing liver lipid accumulation during feed restriction (Carlson et al., 2006).

From these results, we speculated that supplemental carnitine might be required for optimal hepatic metabolism of LCFA during the periparturient period. To our knowledge, carnitine supplementation to periparturient dairy cows has not been studied. Our objectives were to determine the effects of dietary carnitine on liver TG accumulation, hepatic lipid and carbohydrate metabolism, tissue carnitine concentrations, DMI, and lactation performance of Holstein cows during the periparturient period.

MATERIALS AND METHODS

Experimental Design and Management of Cows

All experimental techniques and procedures were approved by the University of Illinois Institutional Animal

Care and Use Committee. Fifty-six cows entering their second or greater lactation were stratified by parity and assigned randomly to dietary treatments in a 4×2 factorial arrangement consisting of 4 amounts of supplemental L-carnitine and 2 sources of anionic salts. Cows were moved to individual tie stalls on d -25 relative to expected calving date. Prepartum diets were similar in ingredient and nutrient composition (Table 1) but differed in source of supplemental anionic salts, minerals, and vitamins. Anionic sources were supplied either as a blend of individual anionic salts or as a commercially available anionic salt pellet (Close-Up Pellet; Dawes Laboratories, Arlington Heights, IL). Each prepartum diet was balanced to achieve a DCAD of -10 mEq/100 g of DM. Prepartum diets were fed from d -21 relative to expected calving until parturition. All cows were fed the same basal lactation diet (Table 1) from calving until 56 DIM. Dietary carnitine treatments were fed as a topdress from d -14 relative to expected calving until 21 DIM. Prepartum and lactation diets were balanced to meet or exceed nutrient requirements of multiparous Holstein cows based on NRC (2001) recommendations, except that the lactation diet was expected to be deficient in NE_L and MP during the early postpartum period. Statistical and biological interactions between anionic salt source and dietary carnitine treatments were not anticipated or realized based on statistical analysis; therefore, this report considers the effects of dietary L-carnitine supplementation only.

Cows were assigned to 1 of 4 dietary carnitine treatments: control (0 g/d of L-carnitine; $n = 14$), low carnitine (LC, 6 g/d; $n = 11$), medium carnitine (MC, 50 g/d; $n = 12$), or high carnitine (HC, 100 g/d; $n = 12$). Carnitine was supplemented by feeding 0, 12, 100, or 200 g/d of a commercial carnitine supplement (50% L-carnitine; Lonza, Inc., Allendale, NJ) for the control, LC, MC, and HC treatments, respectively. For each treatment, the appropriate amount of the commercial L-carnitine supplement was mixed with 113.5 g of ground corn and 113.5 g of dried molasses, and this mixture was topdressed twice daily to achieve desired daily carnitine intakes. Dosages for the MC and HC treatments were based on estimates of in vitro carnitine degradation in ruminal fluid from cows adapted to carnitine supplementation (LaCount et al., 1996b). We assumed that approximately 80% of dietary carnitine would be degraded in the rumen of periparturient cows, resulting in 10 and 20 g of L-carnitine available for intestinal absorption for MC and HC, respectively. Previously, we found that abomasal carnitine infusion of 20 g/d into midlactation cows decreased liver total lipid concentrations but did not adversely affect cow performance or metabolism (Carlson et al., 2006). The LC treatment (6

Table 1. Ingredient and chemical composition (DM basis) of experimental diets

Component	Diet		
	Prepartum		Lactation
	Individual	Pellet	
	% of DM		
Ingredient			
Corn silage	36.37	36.38	34.16
Alfalfa silage	13.64	13.64	12.66
Wheat straw	9.09	9.10	1.60
Corn, ground	14.64	15.33	19.21
Soybean meal, 48% CP	9.91	9.55	6.40
Soybean meal, expeller ¹	—	—	8.50
Soybean hulls	9.09	9.10	6.34
Cottonseed	—	—	6.40
Fat ²	—	—	1.49
Limestone	1.36	1.68	1.17
Dicalcium phosphate	0.68	0.68	0.32
Sodium bicarbonate	—	—	1.07
Magnesium oxide	0.14	0.27	0.21
Salt	0.23	0.23	0.21
Mineral-vitamin premix ³	0.23	—	0.21
Individual anionic salt blend ⁴	4.32	—	—
Anionic salt pellet ⁵	—	4.05	—
Vitamin A ⁶	0.03	—	—
Vitamin D ⁷	0.07	—	—
Vitamin E ⁸	0.20	—	0.02
Nutrient content ⁹			
CP	12.5 ± 0.6	13.0 ± 0.4	15.0 ± 0.5
ADF	27.7 ± 1.0	26.7 ± 0.8	23.7 ± 1.2
NDF	42.7 ± 1.4	41.3 ± 0.7	37.3 ± 2.2
Ether extract	2.5 ± 0.3	3.1 ± 0.1	5.4 ± 0.3
NE _L , Mcal/kg	1.46 ± 0.02	1.50 ± 0.02	1.68 ± 0.02
Ca	1.58 ± 0.12	1.58 ± 0.11	1.00 ± 0.08
P	0.40 ± 0.03	0.42 ± 0.02	0.40 ± 0.03
Mg	0.39 ± 0.03	0.40 ± 0.01	0.33 ± 0.02
K	1.46 ± 0.07	1.45 ± 0.05	1.38 ± 0.11
S	0.42 ± 0.04	0.45 ± 0.04	0.17 ± 0.02
Na	0.14 ± 0.04	0.13 ± 0.02	0.39 ± 0.06
Cl	0.85 ± 0.10	0.80 ± 0.13	0.27 ± 0.12
DCAD, ⁹ mEq/100 g	-9.26	-9.48	31.8

¹SoyPlus, West Central Cooperative (Ralston, IA).

²Energy Booster 100, MS Specialty Nutrition (Dundee, IL).

³Contained a minimum of 5.0% Mg, 7.5% K, 10.0% S, 2.0% Fe, 3.0% Mn, 3.0% Zn, 40 mg/kg of Co, 5,000 mg/kg of Cu, 250 mg/kg of I, 150 mg/kg of Se, 2,200 kIU/kg of vitamin A, 660 kIU/kg of vitamin D, 22,000 IU/kg of vitamin E.

⁴Contained 41.0% wheat middlings, 26.3% magnesium sulfate, 12.7% calcium sulfate, 10.0% ammonium chloride, and 10.0% calcium chloride.

⁵Close-up Pellet, Dawe's Laboratories (Arlington Heights, IL). Contained a minimum of 28.1% CP, 2.8% crude fat, 2.81% Ca, 8.7 mg/kg of Se, 370 kIU/kg of vitamin A, and 2,478 IU/kg of vitamin E; contained a maximum of 9.0% ADF and 3.9% Ca.

⁶Contained 30,000 kIU/kg.

⁷Contained 5,008.8 kIU/kg.

⁸Contained 44,000 IU/kg.

⁹Calculated from the analysis of individual ingredients sampled weekly and composited monthly. Values are presented as mean ± SD. Values for DCAD were estimated by the NRC (2001) model using analyzed composition of forages and cottonseed and default values for concentrates.

g/d) was based on findings of previous studies in which dietary supplementation (7 g/d) or ruminal infusion (6 g/d) increased carnitine concentrations in plasma (LaCount et al., 1995, 1996b) and liver (LaCount et al., 1995). The topdress mixtures were usually consumed

immediately upon feeding, although 1 cow on the MC treatment initially avoided the topdress mixture upon feeding but eventually consumed it.

Cows were housed in individual tie stalls during the entire experiment except for approximately 7 d before

expected parturition, when cows were housed in individual box stalls until d 2 after calving. All cows were allowed access to an outdoor lot from 0700 to 0900 h daily. Experimental diets were fed as TMR, which were mixed once daily at 0930 h and fed at 1000 and 1800 h. The topdress was applied to the TMR immediately after feeding. Cows were milked twice daily at 0500 and 1600 h.

Data Collection, Sampling Procedures, and Analytical Methods

Dry matter intake was determined daily throughout the experiment. Feed offered and refused was weighed and recorded daily. The DM concentration of corn silage, alfalfa silage, wheat straw, and cottonseed was assessed weekly (AOAC, 1995) and diet composition was adjusted weekly to account for DM variation. Samples of forages, cottonseed, and concentrate mixes were collected weekly, stored at -20°C , composited monthly, and analyzed for concentrations of CP, ADF, NDF, ether extract, Ca, P, Na, Cl, K, Mg, and S by wet chemistry methods (www.dairyone.com/Forage/Procedures/default.htm) at a commercial laboratory (Dairy One Cooperative Inc., Ithaca, NY). The nutrient composition of monthly composites of forages, cottonseed, and each concentrate mix was used to calculate the nutrient composition of the TMR.

Milk yield was determined for each milking and summarized daily with an electronic milk metering system (WestfaliaSurge Inc., Naperville, IL). Milk samples were collected weekly from 2 consecutive milkings and composited in proportion to milk yield. A portion of each composite was preserved (800 Broad Spectrum Microtabs II; D&F Control Systems, Inc., San Ramon, CA) and analyzed for concentrations of fat, protein, lactose, and urea N by midinfrared methods (AOAC, 1995) at a commercial laboratory (Dairy Lab Services, Dubuque, IA). The remainder of each composite was placed in polypropylene tubes and frozen at -20°C .

Body weight was measured and recorded once weekly from d -25 relative to expected calving until 56 DIM. Likewise, BCS was assigned weekly by 3 experienced individuals (Wildman et al., 1982) and averaged.

Blood was sampled from a coccygeal vessel at ~ 1 h prior to feeding on d -25 , -22 , -19 , -16 , -13 , and -10 relative to expected calving and then daily until actual calving date. During lactation, blood was sampled daily from the day of calving until d $+10$, and then on d 13, 16, 19, 21, 28, 35, 42, 49, and 56 of lactation. Blood was collected into evacuated tubes containing clot activator or sodium heparin (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Serum and plasma were

obtained by centrifugation ($1,300 \times g$ for 15 min), aliquoted into polypropylene tubes, and stored at -20°C .

For all time points, concentrations of urea N (Talke and Schubert, 1965; urea/BUN kit; Roche Diagnostics Corp., Indianapolis, IN), total protein (Weichselbaum, 1945; total protein kit; Roche), BHBA (Williamson and Mellanby, 1974; Ranbut kit; Randox Laboratories Ltd., Oceanside, CA), glucose (Peterson and Young, 1968; glucose/HK kit; Roche), and cholesterol (Allain et al., 1974; cholesterol/HP kit; Roche) were determined in plasma by an autoanalyzer (University of Illinois Clinical Pathology Laboratory, Urbana, IL). Serum samples from all sampling days were analyzed for concentration of NEFA (NEFA C kit; Wako Chemicals USA, Inc., Richmond, VA) with the modifications of Johnson and Peters (1993). Serum sampled on d -21 , -13 , -7 , $+1$, $+7$, $+13$, $+21$, $+28$, and $+35$ was analyzed for insulin (Coat-a-Count Insulin kit; Diagnostic Products Corporation, Los Angeles, CA) using the procedures of Studer et al. (1993). Serum sampled on d -7 and $+7$ relative to calving was analyzed for albumin (Doumas et al., 1971; Albumin Plus kit; Roche), alanine transaminase [ALT; Bergmeyer et al., 1986b; ALT (ALAT/GPT) kit; Roche], alkaline phosphatase (ALP; Tietz et al., 1983; Alkaline Phosphatase IFCC Liquid kit; Roche), aspartate aminotransferase (AST; Bergmeyer et al., 1986a; AST kit; Roche), total bilirubin (Wahlefeld et al., 1972; Total Bilirubin kit; Roche), γ -glutamyltransferase (GGT; Persijn and van der Slik, 1976; GGT Szasz Liquid kit; Roche), and sorbitol dehydrogenase (SDH; Rose and Henderson, 1975; SDH kit; Diagnostic Chemicals Limited, Charlottetown, Prince Edward Island, Canada) by autoanalyzer techniques.

Liver samples (~ 3 to 6 g) were collected under local lidocaine anesthesia via puncture biopsy (Hughes, 1962) on d -21 relative to expected calving date and d 2, 10, and 28 after calving. This sampling interval was selected because concentrations of liver TG peak around d 10 postpartum (Drackley et al., 2005). Tissue was blotted on sterile gauze to remove excess blood. A portion of liver (~ 1 g) was placed in ice-cold PBS (9 mM; 0.9% NaCl; pH, 7.4) and the remainder was placed into cryogenic vials that were frozen and stored in liquid N. Concentrations of total lipid (Hara and Radin, 1978), TG (Fletcher, 1968; Foster and Dunn, 1973), and glycogen (Lo et al., 1970) in liver were determined.

Within 75 min of biopsy, liver tissue was transported to the laboratory and sliced with a Krumdieck tissue slicer (Alabama Research and Development, Munford, AL) filled with ice-cold PBS. Incubations were conducted to determine rates of $[1-^{14}\text{C}]$ palmitate conversion to CO_2 , acid-soluble products (ASP; $\sim 80\%$ ketone bodies and acetate; Jesse et al., 1986), or intracellular esterified products (EP), and of $[1-^{14}\text{C}]$ L-Ala conversion

to glucose and CO₂. Experimental procedures for liver incubations were identical to those described previously (Carlson et al., 2006), except that radioactivity in CO₂ was determined by placing the hanging well and filter paper into a scintillation vial rather than rinsing the hanging well and the transferring filter paper only.

A biopsy of the semitendinosus muscle was conducted under local anesthesia immediately prior to the liver biopsy on d -21 relative to expected calving and on d 2, 10, and 28 after calving. After making a 2-cm incision using a sterile scalpel blade, a biopsy needle (Bard Magnum; 12 gauge × 16 cm; C. R. Bard, Inc., Murray Hill, NJ) was used to excise approximately 200 mg of muscle tissue. Tissue was placed in a cryovial that was frozen and stored in liquid N.

Free carnitine, short-chain acylcarnitine, and long-chain acylcarnitine concentrations were determined in all liver and muscle samples and in milk samples obtained during wk 2 (during supplementation) and wk 6 (3 wk after supplementation was stopped) of lactation. Total carnitine concentrations were determined in plasma samples collected on d -22, -7, 1, 9, and 27 relative to calving date. Analytical procedures were similar to those described previously (McGarry and Foster, 1976; Bhuiyan et al., 1992; Carlson et al., 2007), except that samples were incubated with 1 IU of carnitine acetyltransferase (EC 2.3.1.7).

Calculations and Estimates

The dietary content of NE_L was calculated as described by Dann et al. (2006). Average weekly NE_L balances were calculated individually for each cow using equations of the NRC (2001) as described by Dann et al. (2006). Dietary provision and balances of MP, Met, and Lys was estimated according to the NRC (2001) using experimentally derived mean values for all inputs (Dann et al., 2006).

Rates of [1-¹⁴C]palmitate and [1-¹⁴C]L-Ala conversion to radiolabeled end-products were calculated using known radioactivity in incubation media and the amount of radioactivity in specific end-products; values were then corrected for tissue weight, incubation duration, and amount of background radioactivity in blank flasks. Alanine conversion to glucose was corrected for column extraction efficiency using [³H]L-1-glucose (Sigma Chemical Co., St. Louis, MO) as an internal standard. Outliers among triplicate values were identified using a Q-test (90% confidence interval; Dean and Dixon, 1951) and subsequently omitted from calculations of average conversion rates.

Statistical Analysis

Fifty-six gestating Holstein cows were enrolled in the study. Seven cows were removed from the study be-

cause of conditions unrelated to dietary treatment [early calving (2), calving complications (2), mastitis (1), abomasal obstruction (1), and complications of displaced abomasum surgery (1)]; therefore, 49 cows were included in the analyses.

Daily DMI and milk yield data were averaged by week relative to calving prior to statistical analysis. Data for DMI, BW, BCS, and blood components were separated according to pre- and postpartum sampling periods and analyzed separately. Analysis of liver metabolism and composition data included both pre- and postpartum values.

Cows assigned to the HC treatment had unexpectedly low concentrations of lactose and carnitine in milk and also had a disproportionately high incidence of clinical mastitis. It is well known that mastitis decreases milk lactose concentration (Bansal et al., 2005; Nielsen et al., 2005). Less is known about the effect of mastitis on milk carnitine, but values followed a pattern similar to that of lactose. To identify whether mastitis influenced treatment effects on concentrations of lactose and carnitine in milk, DMI, milk yield, and milk components were analyzed with and without mastitic cows in the model. Analysis confirmed that clinical mastitis affected the interpretation of milk lactose and milk carnitine concentrations, but not of any other data. Therefore, cows treated for clinical mastitis were omitted from the model and only cows that did not experience clinical mastitis were included in the analysis of milk lactose and carnitine concentration [control (n = 13), LC (n = 10), MC (n = 11), and HC (n = 6)].

Data were subjected to ANOVA using PROC MIXED of SAS (SAS Institute Inc., Cary, NC). Measurements over time were analyzed using the REPEATED statement in SAS; the covariance structure resulting in the lowest Akaike's information criterion was used (Littell et al., 1998). Fixed effects in the model were prepartum diet, amount of carnitine supplementation, day relative to calving, and all associated interactions. Prepartum diet did not interact significantly with amount of carnitine supplementation or day relative to calving ($P > 0.15$); therefore, prepartum diet and associated interactions were removed from the final statistical model. The random effect of cow nested within amount of carnitine supplementation was used as the error term for testing fixed effects and as the error term in the REPEATED statement. Initial measurements (d -21) obtained for blood components, tissue composition, and in vitro liver metabolism were used for covariate adjustment; the covariable was removed if it was nonsignificant ($P > 0.10$) to the overall model. Treatment degrees of freedom were partitioned into single degree of freedom orthogonal contrasts used to test: 1) control vs. LC + MC + HC; 2) LC vs. MC + HC; and 3) MC vs. HC; therefore,

Table 2. Model inputs and estimates (NRC, 2001) used to predict MP balance (g/d) and Met and Lys as percentages of MP for multiparous Holstein cows during supplementation of different amounts of L-carnitine from d -14 relative to calving until d 21 of lactation

Variable	Treatment ¹							
	Prepartum				Lactation			
	Control	LC	MC	HC	Control	LC	MC	HC
Input								
DMI, kg	14.2	13.0	14.2	13.8	19.0	18.0	18.3	15.9
BW, kg	767	768	791	764	673	667	685	660
BCS	3.48	3.32	3.58	3.44	2.87	2.69	2.84	2.68
Day of gestation	269	269	269	269	—	—	—	—
DIM	—	—	—	—	11	11	11	11
Milk, kg/d	—	—	—	—	35.1	33.3	33.3	27.4
Fat, %	—	—	—	—	4.57	4.33	5.10	5.63
Protein, %	—	—	—	—	3.18	3.22	3.23	3.27
Lactose, %	—	—	—	—	4.73	4.70	4.68	4.58
Estimate ²								
MP required, g/d	926	888	927	913	2,369	2,273	2,289	1,944
MP supplied, g/d	1,350	1,235	1,348	1,311	2,088	1,979	2,012	1,738
MP balance, g/d	424	347	421	398	-281	-294	-277	-206
Met, % of MP	1.91	1.92	1.91	1.91	1.77	1.78	1.78	1.80
Lys, % of MP	7.06	7.13	7.08	7.08	6.50	6.53	6.52	6.62

¹Control, 0 g/d of L-carnitine; LC = low carnitine, 6 g/d; MC = medium carnitine, 50 g/d; HC = high carnitine, 100 g/d.

²Nutrient composition of monthly composites used for corn silage, alfalfa silage, wheat straw, and cottonseed; default values (NRC, 2001) used for nutrient composition of concentrates.

the overall *P*-values for treatment are not presented. Significance of orthogonal contrasts and interactions was declared at $P \leq 0.05$ and trends were discussed at $0.05 < P \leq 0.10$. In the instance of a significant treatment $\times$ time interaction ($P \leq 0.10$), the PDIFF statement in SAS was used to determine differences between treatments at specific time points ($P \leq 0.05$).

RESULTS AND DISCUSSION

Diet Composition

The nutrient composition of experimental diets is presented in Table 1. The CP content deviated from intended concentrations; prepartum diets were balanced to contain 15.0% CP, and the lactation diet was balanced for 17.0% CP (NRC, 2001). Corn silage was sampled and analyzed for CP concentration immediately prior to the experiment, but the CP concentration of corn silage fed throughout the experiment was substantially lower. The variation between expected and actual CP concentration in corn silage occurred within the same silo, and the change was not discovered until after the experiment began. Slight variations in CP content of alfalfa silage and concentrate mixes also contributed to the lower than formulated dietary CP concentrations.

Cows were in negative MP balance during the first 21 d postpartum (Table 2). Deficiency of AA or MP has been implicated as a risk factor for fatty liver development (Bell et al., 2000; Bobe et al., 2004), although

the effect is often confounded by depressed DMI and decreased intake of other nutrients (Bobe et al., 2004). The deficit in calculated MP balance was similar across treatments and would likely accentuate the ability to detect effects of supplemental carnitine because endogenous carnitine synthesis requires methyl groups originating from Met (Rebouche and Seim, 1998).

Carnitine Concentrations in Liver, Muscle, Plasma, and Milk

Concentrations of free carnitine, short-chain acylcarnitine, and long-chain acylcarnitine in liver are presented in Table 3. Free carnitine was increased by the MC and HC treatments on d 2 and 10 (treatment $\times$ time, $P < 0.01$), whereas LC did not increase free carnitine relative to the control. Short-chain acylcarnitine in liver was increased by carnitine supplementation ($P = 0.03$) irrespective of day relative to calving, but concentrations did not respond to increasing dose ($P \geq 0.11$). Long-chain acylcarnitine was substantially higher in the MC and HC treatments relative to LC on d 2 and 10 (treatment $\times$ time, $P < 0.01$); however, MC cows had higher long-chain acylcarnitine in liver than did HC on d 2. Total carnitine concentrations were increased by MC and HC treatments on d 2 and 10 (treatment $\times$ time; $P < 0.01$), and changes followed a pattern similar to that of free carnitine. Free carnitine and acylcarnitine concentrations in liver rapidly decreased after supple-

Table 3. Least squares means for concentrations of free carnitine, short-chain acylcarnitine, long-chain acylcarnitine, and total carnitine in liver from multiparous Holstein cows fed different amounts of L-carnitine from d -14 relative to calving until d 21 of lactation

Variable ¹	Treatment ²				SEM	P-value			Treatment × time
						Treatment contrast ³			
	Control	LC	MC	HC		1	2	3	
—— nmol/g of wet liver tissue ——									
Free									
–21 d (covariate)	32.3				1.4				
2 d	51.3 ^b	74.6 ^b	255.4 ^a	227.7 ^a	13.1	<0.01	<0.01	0.63	<0.01
10 d	35.8 ^b	54.4 ^b	165.0 ^a	177.7 ^a	13.1				
28 d	24.7	29.6	42.9	40.7	12.5				
Short-chain									
–21 d (covariate)	1.73				0.44				
2 d	3.17	6.11	17.91	9.49	3.31	0.03	0.11	0.66	0.25
10 d	2.97	6.74	8.23	10.22	3.31				
28 d	1.39	1.48	1.56	4.34	3.16				
Long-chain									
–21 d (covariate)	4.87				0.29				
2 d	7.21 ^c	9.22 ^c	41.74 ^a	34.75 ^b	2.34	<0.01	<0.01	0.79	<0.01
10 d	5.09 ^b	7.44 ^b	22.39 ^a	27.06 ^a	2.30				
28 d	3.37	4.51	6.06	6.45	2.24				
Total									
–21 d (covariate)	38.9				1.7				
2 d	61.9 ^b	90.3 ^b	313.1 ^a	273.8 ^a	15.6	<0.01	<0.01	0.84	<0.01
10 d	45.6 ^b	68.9 ^b	192.5 ^a	217.1 ^a	15.6				
28 d	31.1	36.0	47.5	53.6	14.9				

^{a-c}Means within a row with unlike superscripts differ ($P \leq 0.05$).

¹Data for d -21 were pooled because cows were not on treatment; postpartum data were covariately adjusted for differences in pretreatment values.

²Control, 0 g/d of L-carnitine; LC = low carnitine, 6 g/d; MC = medium carnitine, 50 g/d; HC = high carnitine, 100 g/d.

³Contrast 1 = control vs. LC + MC + HC; contrast 2 = LC vs. MC + HC; contrast 3 = MC vs. HC.

mentation stopped, as indicated by values obtained on d 28.

Hepatic carnitine concentrations for the control treatment were ~1.6-fold higher on d 2 compared with pretreatment values. Grum et al. (1996) reported that acid-soluble carnitine (free carnitine plus short-chain acylcarnitine) increased sharply around parturition but that concentrations returned to prepartum values by 21 DIM. In our study, the marked increases of hepatic carnitine concentrations for MC and HC confirm that exogenous carnitine is readily taken up by liver, as demonstrated previously in midlactation dairy cows (Carlson et al., 2006), rats (Ruff et al., 1991), and growing pigs (Heo et al., 2000a; Owen et al., 2001a,b). Dietary supplementation of 50 or 100 g/d of L-carnitine had a greater effect on long-chain acylcarnitine than did abomasal carnitine infusion (20 g/d) in midlactation cows (Carlson et al., 2007), likely because of greater NEFA supply to the liver during the periparturient period.

Carnitine supplementation increased free carnitine in muscle compared with the control ($P = 0.01$; Table 4), but MC and HC treatments further elevated muscle free carnitine vs. LC ($P = 0.01$) without interaction with

day relative to calving ($P = 0.64$). Short-chain acylcarnitine concentration was unaffected by carnitine supplementation compared with the control ($P = 0.26$). Long-chain acylcarnitine was consistently elevated by MC and HC relative to LC ($P < 0.01$). On d 2, MC and HC treatments had greater long-chain acylcarnitine than did the control, but concentrations for MC and the control tended to be similar on d 10 (treatment × time, $P = 0.06$). Interestingly, concentrations of free carnitine, long-chain acylcarnitine, and total carnitine remained elevated 28 d after calving in the MC and HC treatments, which was 7 d after supplementation ceased. Muscle carnitine concentrations did not differ between MC and HC for any carnitine fraction.

Distribution of carnitine among free carnitine and carnitine esters in muscle was similar to that reported previously for midlactation cows (Carlson et al., 2007). In cattle, abomasal infusion of 6 g/d into lactating cows (LaCount et al., 1995) and dietary supplementation of 3 g/d to growing steers (Greenwood et al., 2001) did not alter muscle carnitine concentrations. In contrast, abomasal infusion of 20 g/d increased muscle carnitine in dairy cows (Carlson et al., 2007), and MC and HC increased muscle carnitine more potently than did LC

Table 4. Least squares means for concentrations of free carnitine, short-chain acylcarnitine, long-chain acylcarnitine, and total carnitine in muscle from multiparous Holstein cows fed different amounts of L-carnitine from d -14 relative to calving until d 21 of lactation

Variable ¹	Treatment ²				SEM	P-value			Treatment × time
						Treatment contrast ³			
	Control	LC	MC	HC		1	2	3	
— nmol/g of wet muscle tissue —									
Free									
−21 d (covariate)	944				44				
2 d	861	980	1,355	1,157	99	0.01	0.01	0.30	0.64
10 d	925	979	1,199	1,257	95				
28 d	821	918	1,277	1,111	100				
Short-chain									
−21 d (covariate)	326				37				
2 d	268	285	433	435	70	0.26	0.11	0.64	0.82
10 d	375	327	435	362	67				
28 d	314	339	390	375	71				
Long-chain									
−21 d (covariate)	109				6				
2 d	99 ^b	118 ^b	183 ^a	157 ^a	13	<0.01	<0.01	0.39	0.06
10 d	115 ^{bc}	92 ^c	141 ^{ab}	157 ^a	12				
28 d	87 ^b	110 ^b	169 ^a	148 ^a	13				
Total									
−21 d (covariate)	1,379				62				
2 d	1,209	1,401	1,992	1,745	116	0.01	0.01	0.18	0.44
10 d	1,397	1,411	1,796	1,772	112				
28 d	1,201	1,359	1,863	1,631	117				

^{a-c}Means within a row with unlike superscripts differ ($P \leq 0.05$).

¹Data for d -21 were pooled because cows were not on treatment; postpartum data were covariately adjusted for differences in pretreatment values.

²Control, 0 g/d of L-carnitine; LC = low carnitine, 6 g/d; MC = medium carnitine, 50 g/d; HC = high carnitine, 100 g/d.

³Contrast 1 = control vs. LC + MC + HC; contrast 2 = LC vs. MC + HC; contrast 3 = MC vs. HC.

(Table 4). These studies indicate that muscle carnitine accumulation can be modified by supplemental carnitine in a dose-dependent manner, as noted in studies conducted with growing pigs (Heo et al., 2000a; Owen et al., 2001a,b).

Total carnitine in plasma was higher for HC than for MC at d -7 relative to calving ($P = 0.02$; Table 5), and both MC and HC treatments were higher than LC ($P < 0.01$). On d 1 and 9 postpartum, plasma total carnitine was increased by MC and HC relative to the control and LC (treatment $\times$ time, $P < 0.01$), and the control and LC had similar plasma carnitine concentrations. Treatments did not differ on d 27 after calving. Postpartum plasma carnitine concentrations were similar for MC and HC, which corresponded to observations for liver and muscle carnitine. Plasma carnitine concentrations for the control treatment were similar to those of unsupplemented midlactation cows (Carlson et al., 2007). LaCount et al. (1996b) found that dietary supplementation of 7 g/d increased plasma carnitine, although feeding 6 g/d did not significantly affect plasma carnitine in our study. Carnitine concentrations in plasma for cows fed MC and HC were similar to those of midlac-

tation cows infused with 20 g/d of L-carnitine (Carlson et al., 2007).

Milk carnitine concentrations (Table 5) were increased during carnitine supplementation (wk 2) but not after carnitine was discontinued (wk 6 of lactation). During wk 2, free carnitine concentrations were greater for the MC and HC groups than for the control, whereas LC and HC did not differ (treatment $\times$ time, $P = 0.02$). Short-chain acylcarnitine was not affected by carnitine supplementation. Long-chain acylcarnitine was elevated by the HC treatment, but not by the LC or MC treatments (treatment $\times$ time, $P = 0.03$). Total carnitine in milk was increased by the LC, MC, and HC treatments, but concentrations were not different between MC and HC (treatment $\times$ time, $P = 0.01$). During wk 2, milk carnitine output (g/d) was higher for MC cows than for LC and HC, whereas the LC and MC treatments were greater than the control (treatment $\times$ time, $P = 0.01$).

Total carnitine concentration in milk from control cows during supplementation (wk 2 in lactation) was ~2-fold greater than that of midlactation cows, but fell to similar concentrations by wk 6 of lactation (Carlson

Table 5. Least squares means for concentrations of total carnitine in plasma and free carnitine, short-chain acylcarnitine, long-chain acylcarnitine, and total carnitine in milk from multiparous Holstein cows fed different amounts of L-carnitine from d -14 relative to calving until d 21 of lactation.

Variable	Treatment ¹				SEM	P-value			Treatment × time
						Treatment contrast ²			
	Control	LC	MC	HC		1	2	3	
Plasma ³	nmol/mL								
−21 (covariate)	6.89				0.59				
−7 d	5.09	7.33	28.80	37.29	2.49	<0.01	<0.01	0.02	
1 d	6.27 ^b	5.43 ^b	27.22 ^a	27.08 ^a	2.05	<0.01	<0.01	0.70	<0.01
9 d	2.91 ^b	4.47 ^b	30.53 ^a	31.41 ^a	2.05				
27 d	2.70	2.49	5.17	6.60	2.05				
Milk ⁴									
Free						<0.01	0.02	0.55	0.02
2 wk	176.1 ^c	253.3 ^{bc}	378.3 ^a	348.0 ^{ab}	38.9				
6 wk	72.7	82.5	108.6	97.5	38.9				
Short-chain						0.15	0.84	0.59	0.46
2 wk	20.0	51.8	42.9	48.2	16.3				
6 wk	28.5	21.9	27.7	37.8	16.3				
Long-chain						0.02	0.09	0.05	0.03
2 wk	7.6 ^b	10.3 ^b	12.0 ^b	20.4 ^a	2.4				
6 wk	2.5	2.9	2.9	3.5	2.4				
Total						<0.01	0.01	0.80	0.01
2 wk	203.7 ^c	315.4 ^b	433.2 ^a	416.6 ^a	35.8				
6 wk	103.7	107.3	139.2	138.8	35.8				
Milk output	g/d								
Total						<0.01	0.06	0.01	0.01
2 wk	1.16 ^c	1.64 ^b	2.35 ^a	1.48 ^{bc}	0.16				
6 wk	0.66	0.63	0.86	0.76	0.16				

^{a-c}Means within a row with unlike superscripts differ ($P \leq 0.05$).

¹Control, 0 g/d of L-carnitine; LC = low carnitine, 6 g/d; MC = medium carnitine, 50 g/d; HC = high carnitine, 100 g/d.

²Contrast 1 = control vs. LC + MC + HC; contrast 2 = LC vs. MC + HC; contrast 3 = MC vs. HC.

³Data for d -21 were pooled because cows were not on treatment; postpartum data were covariately adjusted for differences in pretreatment values.

⁴Data from cows treated for clinical mastitis were omitted from analysis [control (n = 13), LC (n = 10), MC (n = 11), and HC (n = 6)].

et al., 2007). Carnitine secretion in milk is greater in early lactation (Erfle et al., 1974), which is probably due to a greater capacity for carnitine uptake by the mammary gland (Shennan et al., 1998) as well as a greater carnitine supply (Grum et al., 1996) during early lactation. The short-chain acylcarnitine fraction in milk did not increase because of dietary carnitine, whereas abomasal infusion of 20 g/d into midlactation cows increased this fraction by ~2.6-fold relative to unsupplemented cows (Carlson et al., 2007). Stage of lactation may have affected distribution between free carnitine and short-chain acylcarnitine. Decreased milk yield for HC likely explains the smaller milk carnitine output compared with MC because total carnitine concentrations were similar between treatments.

Supplementation of 6 g/d numerically increased carnitine and carnitine ester concentrations in liver while significantly increasing concentrations of carnitine in milk, whereas supplementation of 50 and 100 g/d markedly increased liver, muscle, plasma, and milk carni-

tine. Our results confirm that carnitine is not completely degraded by ruminal microorganisms, in agreement with previous reports (LaCount et al., 1996b; Greenwood et al., 2001), although the extent of degradation remains unclear.

Our results indicate that liver, muscle, and plasma carnitine concentrations were maximized by dietary supplementation of 50 g/d of L-carnitine. Possible reasons for a lack of further increase when 100 g/d was supplemented are that transport mechanisms were saturated or that renal excretion was increased in the HC treatment. LaCount et al. (1996a) reported no differences in plasma and milk carnitine concentrations between cows abomasally infused with either 6 or 12 g/d of L-carnitine. Additionally, abomasal carnitine infusion of 3, 6, or 12 g/d into lactating cows did not affect fecal carnitine output, but linearly increased urinary carnitine concentration and output (LaCount et al., 1996a). These results indicate that intestinal carnitine absorption was constant across treatments, but capac-

Figure 1. Least squares means for DMI for multiparous Holstein cows fed different amounts of L-carnitine from d -14 relative to calving until d 21 of lactation. Treatments: control, 0 g/d of L-carnitine; LC = low carnitine, 6 g/d; MC = medium carnitine, 50 g/d; HC = high carnitine, 100 g/d. Orthogonal contrasts were used to test overall treatment effects: 1) control vs. LC + MC + HC; 2) LC vs. MC + HC; 3) MC vs. HC. Prepartum: largest SEM = 1.6 kg/d; all contrasts, $P > 0.29$; treatment $\times$ time, $P = 0.36$. Postpartum: largest SEM = 1.0 kg/d; all contrasts, $P > 0.35$; treatment $\times$ time, $P = 0.007$. Asterisks (*) indicate ($P \leq 0.05$): wk 1, control and MC > HC; wk 2, control and LC > HC.

ity for renal reabsorption was exceeded. In humans, prolonged carnitine supplementation decreased the rate of renal reabsorption, suggesting that the kidney adapts to carnitine intake (Rebouche et al., 1993). Although fecal and urinary carnitine output were not measured in the present study, this mechanism is plausible given that HC had greater plasma carnitine concentrations 7 d before calving but that differences dissipated after calving.

Carnitine concentrations in liver, plasma, and milk samples taken after supplementation ceased were similar among treatments; however, muscle carnitine remained elevated 7 d after the end of supplementation. The muscle carnitine pool is much larger and has a much slower turnover rate than that of liver (Rebouche and Engel, 1984). The metabolic consequences of enhanced muscle carnitine concentration in lactating dairy cows are not clear.

Intake, BW, BCS, and Production

Dry matter intake did not differ among treatments at any point before calving (Figure 1). Average DMI decreased from 14.5 to 12.4 kg/d during the 3 wk prior to calving. Average BW was not altered by carnitine supplementation prior to calving, although the HC treatment gained less BW than the MC treatment ($P = 0.03$) despite similar DMI. Prepartum BCS, BCS change, and energy balance were not different among treatments prepartum (Table 6).

Average weekly DMI was similar among control, LC, and MC treatments during the 8 wk after calving (Fig-

ure 1). The DMI was significantly lower for the HC treatment during wk 1 and 2 of lactation (treatment $\times$ time, $P = 0.01$) but was similar to other treatments from wk 3 to 8. Postpartum BW and BW change were not affected by carnitine supplementation (Table 6). Average BCS was lower for HC cows than for the control and MC during wk 1 after calving (treatment $\times$ time, $P = 0.03$), but BCS did not differ among treatments at any subsequent point in lactation. The MC treatment lost more BCS than did HC ($P = 0.03$) from wk 1 to 8 of lactation, whereas BCS decreased in a similar manner for the control, LC, and MC.

Milk yield was lower for HC cows throughout the first 6 wk of lactation (treatment $\times$ time, $P = 0.05$) but milk yield did not differ among the control, LC, and MC treatments at any point (Figure 2). Net energy balance (as a percentage of energy requirement) was lower for the HC treatment during wk 1 postpartum (treatment $\times$ time, $P = 0.02$; Figure 3, panel B) as a consequence of depressed DMI. A plausible explanation for the decreased DMI and milk yield for HC cows is that increased hepatic ATP production decreased DMI (Allen et al., 2005), as discussed later.

Milk composition and 3.5% FCM yield are presented in Table 7. The MC and HC treatments increased milk fat concentration compared with the LC treatment ($P = 0.02$), although milk fat yield was unaffected by carnitine supplementation. Previously, abomasal carnitine infusion (20 g/d) during short-term feed restriction did not affect milk fat percentage (Carlson et al., 2006). Protein percentage in milk tended to be higher for HC cows than for MC cows ($P = 0.09$), although milk protein yield did not differ among treatments. Milk lactose concentration was not affected by carnitine treatments ($P \geq 0.16$); however, lactose yield tended to be decreased by HC during wk 1 to 6 (treatment $\times$ time, $P = 0.01$; data not shown) in concert with decreased milk yield. Concentration of TS in milk was greater for MC and HC than LC ($P = 0.05$), whereas TS concentration was equivalent between the control and LC. Average milk urea N concentration did not differ among treatments, but HC tended to have greater milk urea N concentration than the control, LC, and MC at wk 3 in lactation (treatment $\times$ time, $P = 0.07$; data not shown). Because milk fat concentration was greater for HC, 3.5% FCM yield did not differ among treatment groups despite decreased milk yield for HC cows.

Liver Composition

Concentrations of total lipids, TG, and glycogen in liver are presented in Table 8. The LC, MC, and HC treatments decreased postpartum liver lipid and TG accumulation compared with the control. On d 2, total

Table 6. Least squares means for BW, BCS, and energy balance for multiparous Holstein cows fed different amounts of L-carnitine from d -14 relative to calving until d 21 of lactation

Variable						P-value			
						Treatment contrast ²			Treatment × time
	Treatment ¹				1	2	3		
Control	LC	MC	HC	SEM					
Prepartum (wk -3 to calving)									
BW, kg	767	768	791	764	26	0.79	0.77	0.44	0.47
BW change ³	10	6	17	1	6	0.68	0.70	0.03	—
BCS	3.48	3.32	3.58	3.44	0.11	0.79	0.14	0.35	0.78
BCS change ³	-0.17	-0.11	-0.02	-0.13	0.09	0.38	0.80	0.34	—
Energy balance, Mcal/d	6.08	3.97	5.56	5.23	0.99	0.28	0.22	0.75	0.29
Postpartum (calving to wk 8)									
BW, kg	658	658	665	655	20	0.96	0.93	0.70	0.67
BW change ³	-46	-28	-52	-25	13	0.38	0.51	0.12	—
BCS	2.72	2.59	2.66	2.67	0.07	0.26	0.35	0.92	0.03
BCS change ³	-0.41	-0.35	-0.39	0.01	0.13	0.16	0.28	0.03	—
Energy balance, Mcal/d	-1.09	0.16	-2.42	-1.31	1.32	0.94	0.21	0.54	0.07

¹Control, 0 g/d of L-carnitine; LC = low carnitine, 6 g/d; MC = medium carnitine, 50 g/d; HC = high carnitine, 100 g/d.

²Contrast 1 = control vs. LC + MC + HC; contrast 2 = LC vs. MC + HC; contrast 3 = MC vs. HC.

³Prepartum change = wk 1 minus wk 3 precalving; postpartum change = wk 8 minus wk 1 postcalving.

lipid and TG concentrations in liver from LC were similar to those of the control. However, by d 10 after calving, values increased for the control but remained constant for LC (treatment × time, $P = 0.01$). Interestingly, liver total lipid and TG concentrations of control cows remained higher than LC and HC cows at d 28 of lactation.

Liver TG concentrations usually peak between d 7 and 14 after calving, most likely around d 10 postpar-

tum (Drackley et al., 2005). The liver TG concentration for the control (6.0%) falls in the low range of the moderate fatty liver category (5 to 10% liver TG; Bobe et al., 2004); however, this amount of TG infiltration did not appear to be detrimental to milk yield, milk composition, or cow performance relative to treatments that decreased hepatic TG. Despite small and statistically nonsignificant increases in liver carnitine concentration, the LC treatment decreased liver TG concentration, as did MC and HC on d 10, suggesting that only small increases in liver carnitine concentration are necessary to decrease liver TG accumulation. Carnitine has an indispensable function in hepatic LCFA β -oxidation and thus in the etiology of fatty liver. Systemic carnitine deficiency in rats has been shown to cause fatty liver as a result of reduced CPT-I activity, but liver lipid infiltration was markedly decreased by treatment of carnitine-deficient rats with exogenous L-carnitine (Spaniol et al., 2003).

Carnitine-supplemented cows had greater postpartum glycogen concentrations in liver than did control cows ($P = 0.01$), without interaction with day relative to calving ($P = 0.46$). The control and LC groups had a similar TG:glycogen ratio on d 2, but all carnitine treatments decreased this ratio relative to the control by d 10 (treatment × time, $P = 0.02$). Regardless of the supplementation amount, carnitine lessened liver glycogen depletion.

In Vitro Metabolism by Liver Slices

Palmitate Metabolism. Measures of in vitro palmitate metabolism by liver slices (Table 9) are useful to

Figure 2. Least squares means for milk yield for multiparous Holstein cows fed different amounts of L-carnitine from d -14 relative to calving until d 21 of lactation. Treatments: control, 0 g/d of L-carnitine; LC = low carnitine, 6 g/d; MC = medium carnitine, 50 g/d; HC = high carnitine, 100 g/d. Orthogonal contrasts were used to test overall treatment effects: 1) control vs. LC + MC + HC; 2) LC vs. MC + HC; 3) MC vs. HC. Largest SEM = 2.0 kg/d; contrast 1, $P = 0.09$; contrast 2, $P = 0.43$; contrast 3, $P = 0.08$; treatment × time, $P = 0.05$. Asterisks (*) indicate ($P \leq 0.05$): wk 1, control, LC, and MC > HC; wk 2, control, LC, and MC > HC; wk 3, control and MC > HC; wk 4, control > HC; wk 5, control > HC; wk 6, control and MC > HC.

Figure 3. Least squares means for NE_L balance expressed as Mcal/d (panel A) or as the percentage of requirement (panel B) for multiparous Holstein cows fed different amounts of L-carnitine from d -14 relative to calving until d 21 of lactation. Treatments: control, 0 g/d of L-carnitine; LC = low carnitine, 6 g/d; MC = medium carnitine, 50 g/d; HC = high carnitine, 100 g/d. Orthogonal contrasts were used to test overall treatment effects: 1) control vs. LC + MC + HC; 2) LC vs. MC + HC; 3) MC vs. HC. (A) Prepartum: largest SEM = 1.2 Mcal/d; all contrasts, $P > 0.22$; treatment $\times$ time, $P = 0.29$. Postpartum: largest SEM = 2.2 Mcal/d; all contrasts, $P > 0.21$; treatment $\times$ time, $P = 0.07$. Asterisks (*) indicate ($P \leq 0.05$): wk 2, LC > MC; wk 3, LC > HC; wk 7, LC > MC. (B) Prepartum: largest SEM = 7.9%; all contrasts, $P > 0.26$; treatment $\times$ time, $P = 0.33$. Postpartum: largest SEM = 5.3%; all contrasts, $P > 0.86$; treatment $\times$ time, $P = 0.02$. Asterisks (*) indicate ($P \leq 0.05$): wk 1, control, LC, and MC > HC; wk 2, LC > MC; wk 7, LC and HC > MC.

explain the underlying mechanisms associated with the degree of liver TG accumulation. The rate of palmitate conversion to CO_2 was unaffected by carnitine supplementation ($P = 0.97$). However, each carnitine treatment increased palmitate conversion to ASP by liver slices (treatment $\times$ time, $P = 0.01$). On d 2 after calving, the MC and HC treatments potentially increased palmitate conversion to ASP, whereas LC increased ASP production to a lesser extent. Palmitate conversion to ASP was higher for MC than either LC or HC on d 10 post-calving, whereas values for LC were similar to those of the control. Conversion of palmitate to EP was decreased by MC and HC treatments on d 2 after calving, although HC caused a greater decrease than did MC (treatment $\times$ time, $P = 0.01$). On d 10, the rate of palmitate esterification did not differ among the control, LC, and MC treatments, but remained lower for HC. Total

palmitate metabolism was decreased by the HC treatment compared with MC ($P = 0.01$) regardless of day relative to calving (treatment $\times$ time, $P = 0.19$). In vitro palmitate conversion to ASP and EP did not differ among treatments on d 28 after calving.

The HC treatment resulted in a greater proportion of palmitate converted to CO_2 than did MC (5.2 vs. 3.8%; $P = 0.05$). A clear pattern was evident for the proportion of palmitate converted to ASP and EP on d 2 and 10; the LC treatment resulted in metabolism similar to that of the control, whereas MC and HC increased the proportion of palmitate oxidized to ASP (treatment $\times$ time, $P = 0.01$) and decreased the proportion converted to EP (treatment $\times$ time, $P = 0.01$). Rates of total palmitate β -oxidation ($CO_2 + ASP$) postpartum for the control, LC, MC, and HC (10.7 , 12.1 , 18.0 , and $17.9 \pm 1.1\%$ of total palmitate metabolism, respectively) followed a pattern similar to that of ASP.

Compared with midlactation cows (Andersen et al., 2002; Carlson et al., 2006), in vitro palmitate β -oxidation by liver slices was greater in early lactation, consistent with hepatic adaptations favoring LCFA β -oxidation (Dann and Drackley, 2005; Loor et al., 2005). In the present study, however, hepatic LCFA β -oxidation for the control was insufficient to prevent hepatic TG accumulation, likely because of inadequate liver carnitine availability. Carnitine has been shown to stimulate hepatic LCFA β -oxidation in liver from several species, including cows (Jesse et al., 1986; Drackley et al., 1991b; Carlson et al., 2006), pigs (Owen et al., 2001a), and Atlantic salmon (Ji et al., 1996). Despite vastly different carnitine intakes, all carnitine treatments promoted conversion of palmitate to ASP on d 2 after calving. In pigs, only a very small amount of carnitine was needed to increase hepatic palmitate β -oxidation (50 mg/kg of diet; Owen et al., 2001a). When adjusted for BW, this amount of supplementation (1.22 mg/kg of BW; Owen et al., 2001a) was much less than that for the LC treatment (9.0 mg/kg of BW). The degree of ruminal carnitine degradation is unknown; therefore, we can neither predict how much carnitine was available for intestinal absorption nor determine optimal amounts of supplementation.

Despite similar liver carnitine concentrations, liver slices from HC cows consistently converted less palmitate to esterified products than did liver slices from MC cows. When conversion rates were expressed as a percentage of total palmitate metabolism, however, the partitioning of palmitate to ASP and EP was equivalent between MC and HC. This discrepancy was due to decreased total palmitate metabolism in the liver from HC cows. In cows fasted for 7 d, a marked increase in plasma NEFA was associated with numerically lower total palmitate utilization (Drackley et al., 1991b). In-

Table 7. Least squares means for concentration and yield of milk components during the first 8 wk of lactation for multiparous Holstein cows fed different amounts of L-carnitine from d -14 relative to calving until d 21 of lactation

Variable	Treatment ¹				SEM	P-value			Treatment × time
						Treatment contrast ²			
	Control	LC	MC	HC		1	2	3	
Fat									
%	4.02	3.98	4.39	4.59	0.18	0.12	0.02	0.42	0.11
kg/d	1.53	1.44	1.60	1.50	0.10	0.92	0.35	0.48	0.12
Protein									
%	2.86	2.92	2.83	3.00	0.07	0.43	0.94	0.09	0.52
kg/d	1.09	1.06	1.04	0.98	0.05	0.21	0.39	0.38	0.15
Lactose ³									
%	4.80	4.76	4.83	4.76	0.04	0.64	0.40	0.20	0.16
kg/d	1.88	1.75	1.78	1.53	0.11	0.04	0.40	0.08	0.01
TS									
%	12.6	12.6	12.9	13.2	0.2	0.12	0.04	0.35	0.24
kg/d	4.83	4.57	4.76	4.34	0.23	0.28	0.94	0.20	0.09
Urea N, mg/dL	12.7	12.6	14.2	12.7	1.1	0.68	0.47	0.30	0.07
3.5% FCM, ³ kg/d	41.5	39.2	42.2	38.8	2.3	0.55	0.64	0.30	0.14

¹Control, 0 g/d of L-carnitine; LC = low carnitine, 6 g/d; MC = medium carnitine, 50 g/d; HC = high carnitine, 100 g/d.

²Contrast 1 = control vs. LC + MC + HC; contrast 2 = LC vs. MC + HC; contrast 3 = MC vs. HC.

³Data from cows treated for clinical mastitis were omitted from analysis [control (n = 13), LC (n = 10), MC (n = 11), and HC (n = 6)].

⁴3.5% FCM = (0.4255 × milk yield) + (16.425 × milk fat yield).

Table 8. Least squares means for concentrations of total lipid, triacylglycerol (TG), and glycogen in liver from multiparous Holstein cows fed different amounts of L-carnitine from d -14 relative to calving until d 21 of lactation

Variable ¹	Treatment ²				SEM	P-value			Treatment × time
						Treatment contrast ³			
	Control	LC	MC	HC		1	2	3	
Total lipid, % wet weight						<0.01	0.30	0.55	<0.01
–21 d (covariate)	3.94				0.07				
2 d	7.50 ^a	6.20 ^{ab}	5.03 ^{bc}	4.29 ^c	0.68				
10 d	9.52 ^a	6.07 ^b	5.13 ^b	5.32 ^b	0.67				
28 d	6.87 ^a	4.73 ^b	5.33 ^{ab}	4.41 ^b	0.66				
TG, % wet weight						<0.01	0.27	0.65	0.01
–21 d (covariate)	0.25				0.02				
2 d	3.91 ^a	2.60 ^{ab}	1.32 ^b	0.64 ^b	0.74				
10 d	6.03 ^a	2.60 ^b	1.60 ^b	1.71 ^b	0.73				
28 d	3.45 ^a	1.20 ^b	1.50 ^b	0.84 ^b	0.72				
Glycogen, % wet weight						0.01	0.78	0.46	0.46
–21 d (covariate)	5.41				0.25				
2 d	1.39	1.77	2.00	2.24	0.32				
10 d	1.64	2.67	2.04	2.52	0.32				
28 d	2.80	3.31	3.59	3.53	0.32				
TG:glycogen						<0.01	0.54	0.74	0.02
–21 d (covariate)	0.06				0.01				
2 d	4.16 ^a	2.31 ^{ab}	1.43 ^b	1.43 ^b	0.88				
10 d	5.99 ^a	1.08 ^b	0.97 ^b	1.22 ^b	0.87				
28 d	1.48	0.42	0.44	0.31	0.84				

^{a-c}Means within a row with unlike superscripts differ ($P \leq 0.05$).

¹Data for d -21 were pooled because cows were not on treatment; postpartum data were covariately adjusted for differences in pretreatment values.

²Control, 0 g/d of L-carnitine; LC = low carnitine, 6 g/d; MC = medium carnitine, 50 g/d; HC = high carnitine, 100 g/d.

³Contrast 1 = control vs. LC + MC + HC; contrast 2 = LC vs. MC + HC; contrast 3 = MC vs. HC.

Table 9. Least squares means for conversion of [1-¹⁴C]palmitate to CO₂, acid-soluble products, and esterified products by liver slices from multiparous Holstein cows fed different amounts of L-carnitine from d -14 relative to calving until d 21 of lactation

Variable	Treatment ¹				SEM	P-value			Treatment × time
						Treatment contrast ²			
	Control	LC	MC	HC		1	2	3	
Conversion of [1- ¹⁴ C]palmitate	nmol/(g of wet tissue × h)								
CO ₂						0.97	0.55	0.17	0.79
2 d	13.8	15.7	14.1	15.2	3.1				
10 d	19.2	17.8	17.9	22.7	3.2				
28 d	20.3	16.3	16.2	23.3	3.2				
Acid-soluble products						0.01	0.01	0.01	0.01
2 d	34.8 ^c	54.9 ^b	83.6 ^a	74.5 ^a	7.4				
10 d	28.2 ^c	40.0 ^{bc}	78.3 ^a	49.1 ^b	7.4				
28 d	19.5	22.4	32.3	19.0	7.4				
Esterified products						0.01	0.01	0.02	0.01
2 d	420.8 ^a	398.4 ^a	355.6 ^b	286.7 ^c	19.6				
10 d	400.9 ^a	405.9 ^a	355.5 ^a	297.2 ^b	20.5				
28 d	360.7	376.0	366.8	347.1	20.5				
Total metabolism ³						0.22	0.05	0.01	0.19
2 d	472.1	467.3	453.6	375.0	20.5				
10 d	450.6	462.1	453.2	367.9	21.4				
28 d	403.5	413.5	415.6	390.1	21.4				
Conversion of [1- ¹⁴ C]palmitate	% of total palmitate metabolism								
CO ₂						0.94	0.21	0.05	0.63
2 d	3.06	3.41	3.27	4.06	0.69				
10 d	4.26	3.68	4.03	5.77	0.72				
28 d	5.23	3.97	3.94	5.88	0.72				
Acid-soluble products						<0.01	0.01	0.32	0.01
2 d	8.04 ^b	11.83 ^b	18.47 ^a	19.62 ^a	1.60				
10 d	6.40 ^b	8.49 ^b	16.59 ^a	13.58 ^a	1.67				
28 d	5.24	4.98	7.62	4.77	1.67				
Esterified products						<0.01	<0.01	0.98	0.01
2 d	88.90 ^a	84.77 ^a	78.27 ^b	76.32 ^b	1.60				
10 d	89.34 ^a	87.84 ^a	79.43 ^b	80.55 ^b	1.68				
28 d	89.53	91.06	88.44	89.36	1.68				

^{a-c}Means with unlike superscripts within the same row differ (treatment × time; $P \leq 0.05$).

¹Control, 0 g/d of L-carnitine; LC = low carnitine, 6 g/d; MC = medium carnitine, 50 g/d; HC = high carnitine, 100 g/d.

²Contrast 1 = control vs. LC + MC + HC; contrast 2 = LC vs. MC + HC; contrast 3 = MC vs. HC.

³Total palmitate metabolism = (CO₂ + acid-soluble products + esterified products).

teractions of complete palmitate β -oxidation and DMI may have affected in vitro palmitate utilization in our study.

As indicated by the increased proportion of palmitate converted to CO₂, the HC treatment may have increased hepatic ATP generation to the extent that DMI was inhibited. Under the conditions of the in vitro incubations, increased CO₂ would arise from partitioning of acetyl-CoA (from β -oxidation of palmitate) toward the tricarboxylic acid cycle rather than conversion to ketone bodies as a means to generate ATP. Evidence in rodent models suggests that hepatic ATP production from substrate oxidation is involved in food intake regulation (Friedman, 1998). Several studies in rodents have shown that inhibition of hepatic LCFA β -oxidation (Leonhardt and Langhans, 2004) or a reduction in hepatic glucose utilization (Friedman, 1998) stimulates food intake by decreasing hepatic ATP concentrations.

On the other hand, increasing hepatic oxidation of carbohydrates or LCFA might decrease food intake, and this mechanism may extend to ruminants (Allen et al., 2005). Propionate infusions decrease DMI in ruminants, most likely because of enhanced hepatic propionate oxidation (Allen et al., 2005); however, data regarding the effects of increased hepatic LCFA β -oxidation on food intake are lacking (Leonhardt and Langhans, 2004). We speculate from our data that ATP production may have been higher in liver from HC cows; however, these results are confounded somewhat by lower rates of total palmitate metabolism for the HC treatment.

Ala Metabolism. Carnitine supplementation increased Ala conversion to glucose (Table 10) compared with the control ($P = 0.01$) regardless of day relative to calving (treatment × time, $P = 0.61$) or amount of carnitine supplemented. On d 2 postpartum, liver slices from

Table 10. Least squares means for conversion of [1-¹⁴C]L-alanine to CO₂ and glucose by liver slices from multiparous Holstein cows fed different amounts of L-carnitine from d -14 relative to calving until d 21 of lactation

Conversion of [1- ¹⁴ C]L-alanine	Treatment ¹				SEM	P-value			Treatment × time
						Treatment contrast ²			
	Control	LC	MC	HC		1	2	3	
— μmol/(g of wet tissue × h) —									
Glucose						0.01	0.59	0.55	0.61
2 d	0.67	0.80	1.02	0.80	0.15				
10 d	0.67	0.93	0.94	1.14	0.15				
28 d	0.64	1.02	0.86	1.11	0.15				
CO ₂						0.08	0.49	0.22	0.06
2 d	0.28 ^a	0.28 ^a	0.32 ^a	0.19 ^b	0.04				
10 d	0.25	0.29	0.29	0.33	0.03				
28 d	0.18 ^b	0.21 ^{ab}	0.28 ^a	0.26 ^a	0.04				

^{a,b}Means with unlike superscripts within the same row differ (treatment × time; $P \leq 0.05$).

¹Control, 0 g/d of L-carnitine; LC = low carnitine, 6 g/d; MC = medium carnitine, 50 g/d; HC = high carnitine, 100 g/d.

²Contrast 1 = control vs. LC + MC + HC; contrast 2 = LC vs. MC + HC; contrast 3 = MC vs. HC.

HC cows tended to convert less Ala to CO₂, no differences were apparent on d 10, and the control treatment tended to have lower rates of conversion than MC and HC on d 28 (treatment × time, $P = 0.06$). Increased rates of in vitro hepatic conversion of Ala to glucose suggest that carnitine-supplemented cows derived relatively more glucose from hepatic gluconeogenesis than from glycogenolysis, which may have contributed to maintenance of hepatic glycogen concentrations (Table 8).

Previously, abomasal carnitine infusion did not alter in vitro gluconeogenesis from Ala in midlactation cows (Carlson et al., 2006). In the present study, dietary carnitine supplementation may have stimulated the flux of glucogenic intermediates through PC. The activity and mRNA expression of PC increases around parturition (Greenfield et al., 2000; Loores et al., 2006), concomitant with the increased contribution of lactate and Ala to hepatic gluconeogenesis during the periparturient period compared with other stages of lactation (Reynolds et al., 2003). Carnitine-stimulated hepatic β -oxidation of LCFA was accompanied by increased PC activity and greater gluconeogenesis from lactate in liver of nonruminants (Ji et al., 1996; Owen et al., 2001a). In sheep, hepatic CPT-I inhibition resulted in depressed gluconeogenesis (Chow and Jesse, 1992), suggesting that glucose production from Ala and lactate is modulated by the rate of LCFA β -oxidation in ruminant liver. Increasing palmitate β -oxidation would increase the intramitochondrial concentration of acetyl-CoA, which would stimulate the flux of lactate and Ala through PC rather than pyruvate dehydrogenase (PDH). Despite functioning similarly in ruminant liver and in nonruminant liver and increasing the conversion of Ala to glu-

cose, the effects of carnitine supplementation did not confer a measurable advantage in cow performance under the conditions of this study.

Conversion of Ala to CO₂ by liver slices was lower than previously reported for cows during the periparturient period (Overton, 1998). Abomasal carnitine infusion into midlactation cows increased Ala conversion to CO₂ in liver slices from ad libitum-fed cows but not in feed-restricted cows (Carlson et al., 2006). Carnitine supplementation might have affected CO₂ production by diverting some Ala through PDH in addition to stimulating flux through PC, which could result in increased Ala conversion to both glucose and CO₂. Despite the fact that products of LCFA β -oxidation inhibit PDH activity, addition of palmitate to rat hepatocyte suspensions increased the flux of pyruvate through both PC and PDH, but this effect was abolished by CPT-I inhibition (Agius and Alberti, 1985). The rate of pyruvate flux through PDH can be accelerated by increased ketogenesis in rat liver (Zweibel et al., 1982), as a result of stimulated exchange of pyruvate for ketone bodies (Halestrap, 1978). Previously, addition of Ala, lactate, or pyruvate to incubation media potentially accelerated palmitate β -oxidation to ASP, whereas acetoacetate addition inhibited ketogenesis (Drackley et al., 1991a). Considering that the vast majority of radiolabeled Ala is converted to pyruvate or lactate in vitro (Knapp et al., 1992), greater rates of LCFA β -oxidation to ASP might promote exchange of mitochondrial acetoacetate for cytosolic pyruvate, thus allowing flux of pyruvate through either PC or PDH and a net increase in gluconeogenesis. Determining to what compounds Ala is converted under conditions of carnitine-stimulated LCFA

Table 11. Least squares means for concentrations of plasma and serum components for multiparous Holstein cows fed different amounts of L-carnitine from d -14 relative to calving until d 21 of lactation

Variable	Treatment ¹				SEM	P-value			Treatment × time
						Treatment contrast ²			
	Control	LC	MC	HC		1	2	3	
NEFA, μEq/L									
Prepartum ³	181	154	149	172	24	0.36	0.82	0.45	0.72
Postpartum ³	413	317	373	374	45	0.22	0.30	0.99	0.77
BHBA, μmol/L									
Prepartum	443	488	433	477	26	0.38	0.29	0.19	0.60
Postpartum	673	605	756	826	84	0.53	0.07	0.54	0.01
Cholesterol, mg/dL									
Prepartum	65.2	65.1	63.4	60.3	2.4	0.36	0.26	0.33	0.68
Postpartum	102.7	98.5	104.7	89.8	3.7	0.18	0.78	0.01	0.12
Glucose, mg/dL									
Prepartum	67.5	64.6	66.6	65.7	1.1	0.12	0.26	0.58	0.37
Postpartum	57.4	57.5	58.8	60.2	1.3	0.33	0.21	0.46	0.64
Insulin, μIU/mL									
Prepartum	5.14	5.86	6.58	6.98	0.76	0.12	0.32	0.70	0.69
Postpartum	1.96	2.44	3.03	2.91	0.36	0.03	0.23	0.81	0.56
Urea N, mg/dL									
Prepartum	11.0	11.7	10.0	10.2	0.5	0.54	0.01	0.81	0.25
Postpartum	12.1	12.1	10.8	10.4	0.6	0.08	0.03	0.53	0.64
Total protein, g/dL									
Prepartum	7.55	7.52	7.58	7.71	0.14	0.71	0.46	0.48	0.11
Postpartum	7.73	7.74	8.12	7.99	0.16	0.18	0.10	0.55	0.51

¹Control, 0 g/d of L-carnitine; LC = low carnitine, 6 g/d; MC = medium carnitine, 50 g/d; HC = high carnitine, 100 g/d.

²Contrast 1 = control vs. LC + MC + HC; contrast 2 = LC vs. MC + HC; contrast 3 = MC vs. HC.

³Prepartum = d -21 to calving; postpartum = calving to d 56.

β -oxidation would help clarify Ala flux (Knapp et al., 1992).

Blood Metabolites and Hormones

Prepartum concentrations of NEFA, BHBA, cholesterol, glucose, insulin, and total protein in plasma were not altered by dietary carnitine supplementation (Table 11). Prepartum urea N concentrations were lower for MC and HC than for LC ($P = 0.01$).

Carnitine supplementation had a more pronounced effect on postpartum blood components (Table 11). Postpartum plasma BHBA tended to be higher for MC and HC treatments than for the LC treatment ($P = 0.07$) despite similar serum NEFA concentrations (Figure 4, panel A); however, treatment differences were evident only during the first 2 wk of lactation (treatment $\times$ time, $P = 0.01$; Figure 4, panel B). Increased BHBA coupled with similar NEFA concentrations for MC and HC cows may have contributed to the greater milk fat contents for those cows (Table 7).

Carnitine-stimulated hepatic palmitate β -oxidation was accompanied by higher plasma BHBA in MC and HC cows without affecting the NEFA supply, which was similar to responses documented in midlactation cows infused with carnitine (Carlson et al., 2006). Our

results suggest that a greater amount of NEFA was converted to BHBA rather than being converted to TG.

Plasma cholesterol concentration was lower for HC than for MC ($P = 0.01$), which might be a result of lower DMI. Plasma glucose was not altered, but serum insulin concentrations were greater for carnitine-supplemented cows than for the control ($P = 0.03$), regardless of carnitine intake. Elevated blood insulin is associated with increased hepatic glycogen concentrations in dairy cows (Andersen et al., 2002; Hayirli et al., 2002), which agrees with our data. Enhanced hepatic ketogenesis may have affected insulin secretion. Infusion of BHBA increased blood insulin concentrations in sheep, likely as a means for autoregulation of hepatic ketogenesis (Heitmann and Fernandez, 1986). This concept may partially explain increased serum insulin in carnitine-supplemented cows, although increased *in vitro* ASP production was not reflected in greater plasma BHBA for LC cows. Enhanced hepatic gluconeogenic capacity in the carnitine-supplemented groups also might explain the increase in serum insulin because plasma glucose concentrations were numerically greater than in the control.

Prepartum differences in plasma urea N carried over to the postpartum period, such that MC and HC had lower urea N than LC ($P = 0.03$). Plasma total protein

Figure 4. Least squares means for concentrations of NEFA in serum (panel A) and BHBA in plasma (panel B) from multiparous Holstein cows fed different amounts of L-carnitine from d -14 relative to calving until d 21 of lactation. Treatments: control, 0 g/d of L-carnitine; LC = low carnitine, 6 g/d; MC = medium carnitine, 50 g/d; HC = high carnitine, 100 g/d. Orthogonal contrasts were used to test overall treatment effects: 1) control vs. LC + MC + HC; 2) LC vs. MC + HC; 3) MC vs. HC. (A) Prepartum: largest SEM = 61.9 μ Eq/L; all contrasts, $P > 0.45$; treatment $\times$ time, $P = 0.72$. Postpartum: largest SEM = 75.8 μ Eq/L; all contrasts, $P > 0.22$; treatment $\times$ time, $P = 0.99$. (B) Prepartum: largest SEM = 53.0 μ mol/L; all contrasts, $P > 0.19$; treatment $\times$ time, $P = 0.60$. Postpartum: largest SEM = 136.8 μ mol/L; contrast 1, $P = 0.53$; contrast 2, $P = 0.07$; contrast 3, $P = 0.54$; treatment $\times$ time, $P = 0.01$. Asterisks (*) indicate ($P \leq 0.05$): d 7, HC > control, LC, and MC; d 10, HC > control, LC, and MC; d 13, HC > control and LC; d 13, HC > MC.

tended to be higher for the MC and HC treatments than for LC ($P = 0.10$). Protein catabolism increases around parturition to support milk protein synthesis and hepatic gluconeogenesis (Overton, 1998; Bell et al., 2000; Reynolds et al., 2003). Carnitine allows tissues to utilize LCFA for energy while decreasing AA catabolism (Owen et al., 2001a), which has resulted in decreased urinary N excretion (Heo et al., 2000b) and greater rates of protein accretion in pigs (Heo et al., 2000b; Owen et al., 2001b). Further, Owen et al. (2001a) found that carnitine supplementation increased hepatic protein synthesis and AA concentrations in the liver and muscle of pigs. Our results indicate that carnitine supplementation positively affected N metabolism in periparturient dairy cows, although the mechanism is unclear.

Prepartum serum clinical chemistry profiles revealed that the LC treatment resulted in elevated concentrations of aspartate aminotransferase ($P = 0.04$) and GGT ($P = 0.01$) compared with those of MC and HC (Table 12), whereas all carnitine treatments tended to increase serum SDH relative to the control ($P = 0.07$). In samples obtained on d 7 after calving, carnitine supplementation tended to decrease serum albumin ($P = 0.09$). Cows on the LC treatment had lower alkaline phosphatase concentrations ($P = 0.05$) and tended to have higher GGT ($P = 0.10$) than did MC and HC. The HC treatment had increased concentrations of total bilirubin ($P = 0.04$) compared with MC. The biological significance of these changes is likely minimal because concentrations were within normal ranges, except for the low values obtained for SDH (Boyd, 1984).

Postpartum Health

The incidence of health disorders is presented in Table 13. Carnitine supplementation was associated with a numeric increase in the number of cows classified as ketonemic (plasma BHBA $\geq 1,400$ μ mol/L) for at least 1 d between d 1 and 10 after calving. Ketonemia was not accompanied by decreases in blood glucose concentration, indicating that cows were not in the metabolic state typical of subclinical ketosis. Increased β -oxidation, as reflected by increased conversion of palmitate to ASP, coupled with evidence for enhanced gluconeogenic capacity, resulted in a redistribution of metabolic fuels that does not represent a pathological state. Therefore, the ketonemia induced by carnitine does not seem to equate to increased disease risk.

Six cows assigned to the HC treatment were treated for clinical mastitis at some point during the first 8 wk of lactation, compared with 1 cow on each of the other 3 treatments. Whether this difference is linked causally to the greater amount of carnitine supplemented cannot be determined from our data. The number of cows assigned to each treatment was insufficient to accurately determine differences in health outcomes; thus, the information is presented for reference only.

CONCLUSIONS

Dietary carnitine supplementation decreased liver TG accumulation during the periparturient period as a result of enhanced capacity for hepatic LCFA β -oxidation. As little as 6 g/d of rumen-available L-carnitine lessened liver TG concentrations, but supplementation of 50 and 100 g/d had more potent effects on in vitro and in vivo lipid metabolism. Estimates of ruminal carnitine degradability may have been inaccurate for dairy cows during the periparturient period, because 100 g/d de-

Table 12. Least squares means for serum clinical chemistry profile at -7 d prior to calving and +7 d after calving for multiparous Holstein cows fed different amounts of L-carnitine from d -14 relative to calving until d 21 of lactation

Variable	Treatment ¹				SEM	P-value		
						Treatment contrast ²		
	Control	LC	MC	HC		1	2	3
Albumin, g/dL								
-7 d	3.36	3.20	3.33	3.17	0.08	0.15	0.65	0.16
+7 d	3.29	3.16	3.13	3.05	0.10	0.09	0.55	0.57
Alkaline phosphatase, U/L								
-7 d	44.4	36.2	42.0	43.8	4.2	0.40	0.20	0.76
+7 d	36.8	29.9	34.8	41.5	3.4	0.70	0.05	0.13
Alanine transaminase, U/L								
-7 d	15.6	15.3	15.3	13.9	1.4	0.61	0.70	0.45
+7 d	12.1	11.6	11.8	12.2	1.6	0.90	0.84	0.87
Aspartate aminotransferase, U/L								
-7 d	49.3	58.2	44.8	49.8	4.3	0.72	0.04	0.38
+7 d	80.0	82.4	85.9	102.3	13.4	0.46	0.47	0.35
γ -Glutamyltransferase, U/L								
-7 d	17.9	22.0	17.4	17.3	1.3	0.48	0.01	0.96
+7 d	20.7	25.3	19.8	20.3	2.6	0.67	0.10	0.88
Total bilirubin, mg/dL								
-7 d	0.29	0.29	0.27	0.28	0.03	0.76	0.51	0.81
+7 d	0.41	0.40	0.38	0.55	0.07	0.61	0.42	0.04
Sorbitol dehydrogenase, U/L								
-7 d	0.66	1.39	1.00	1.31	0.30	0.07	0.52	0.45
+7 d	2.13	2.36	1.26	2.90	0.70	0.95	0.74	0.08

¹Control, 0 g/d of L-carnitine; LC = low carnitine, 6 g/d; MC = medium carnitine, 50 g/d; HC = high carnitine, 100 g/d.

²Contrast 1 = control vs. LC + MC + HC; contrast 2 = LC vs. MC + HC; contrast 3 = MC vs. HC.

Table 13. Frequency of health disorders and twins for multiparous Holstein cows fed different amounts of L-carnitine from d -14 relative to calving until d 21 of lactation

Condition	Treatment ¹			
	Control	LC	MC	HC
Ketosis				
Ketonemia ²	2	6	5	8
Clinical ³	0	0	0	1
Displaced abomasum	0	1	1	3
Retained placenta	1	1	0	1
Metritis	1	1	1	1
Mastitis ⁴	1	1	1	6
Milk fever	0	0	0	0
Hoof disorders ⁵	2	2	1	2
Twins	0	2	0	1

¹Control, 0 g/d of L-carnitine; LC = low carnitine, 6 g/d; MC = medium carnitine, 50 g/d; HC = high carnitine, 100 g/d.

²Cow had plasma BHBA $\geq 1,400$ $\mu\text{mol/L}$ (15 mg/dL) at least 1 d during 1 to 10 DIM.

³Cow had ketonemia, showed signs of anorexia, ataxia, or abnormal behavior.

⁴Cow was treated with intramammary antibiotics.

⁵Includes abscess, pododermatitis circumscripta (ulcer), interdigital hyperplasia (corn), interdigital phlegmon (foot rot), digital dermatitis, and white line disease.

creased DMI and milk yield. Carnitine modulated lipid, carbohydrate, and N metabolism in periparturient cows, consistent with observations in nonruminants. This study clearly demonstrated that carnitine decreases liver TG accumulation. Additional studies with larger numbers of cows are required to assess whether application of L-carnitine for periparturient cows might confer longer term benefits in energy balance, BCS, health, and reproduction.

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