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Kinetics of Carnitine Palmitoyltransferase-I Are Altered by Dietary Variables and Suggest a Metabolic Need for Supplemental Carnitine in Young Pigs^{1,2}

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ABSTRACT To examine the kinetics of carnitine palmitoyltransferase-I (CPT-I) and the influence of dietary variables, young pigs (18 kg, $n = 20$) were fed corn-soybean meal diets supplemented with 40 g soy oil/kg and containing either 136 or 180 g crude protein/kg and either 0 or 500 mg/kg L-carnitine (2 × 2 factorial design). Diets were offered for 10 d (85% of ad libitum); CPT-I activities in liver and skeletal muscle mitochondria were determined, and enzyme kinetic constants (V_{max} and K_m for carnitine) were estimated. Kinetics of CPT-I in muscle were not affected by diet ($P > 0.1$; carnitine $K_m = 480 \pm 44 \mu\text{mol/L}$). In contrast, the K_m for carnitine in liver was increased from 164 to $216 \pm 20 \mu\text{mol/L}$ by dietary L-carnitine supplementation ($P < 0.01$) and from 169 to $211 \pm 20 \mu\text{mol/L}$ by high protein feeding ($P < 0.05$). Dietary L-carnitine increased muscle and liver free carnitine concentrations by 72 and 158% over control concentrations (770 and 80 $\mu\text{mol/kg}$ wet muscle and liver, respectively). Because tissue carnitine concentrations were within the range of the respective K_m for both liver and muscle tissue, it is inferred that alteration of tissue carnitine concentrations via dietary supplementation could modulate CPT-I activity in young pigs. **J. Nutr.** 130: 2467-2470, 2000.

KEY WORDS: • pigs • carnitine • protein
• carnitine palmitoyltransferase-I • liver • muscle

Carnitine palmitoyltransferase-I (CPT-I; EC 2.3.1.21)⁴ is a major regulatory enzyme of lipid metabolism, required

for the transport of long-chain fatty acids across the inner mitochondria membrane; this transport is L-carnitine dependent [see McGarry and Brown (1997) for review]. Biosynthesis of carnitine in the liver and kidney appears sufficient (Rebouche and Seim 1998) to surpass the metabolic needs of mammalian adults in that tissue carnitine concentrations typically exceed the K_m of CPT-I for carnitine (Friolet et al. 1994, McGarry et al. 1983, Pearson and Tubbs 1967). Thus, carnitine is not considered an essential nutrient for healthy adults; however, this may not be true for young animals in which carnitine might be conditionally essential (Borum 1983). Indeed, using a young pig model, several researchers have shown increased fatty acid utilization upon carnitine supplementation (Heo et al. 2000, Kempen and Odle 1995, Penn et al. 1997, Wolfe et al. 1978). Extrapolation of findings from the piglet model to other species must be done cautiously because pigs show some peculiar idiosyncrasies related to lipid metabolism. In particular, low hepatic lipogenesis (Odle et al. 1995, Pégrier et al. 1983) is associated with low malonyl-CoA concentrations, but correspondingly, the sensitivity of hepatic CPT-I to malonyl-CoA inhibition is higher than that observed in other species (Duée et al. 1994). In addition, hepatic ketogenesis is markedly attenuated in pigs compared with other species (Adams and Odle 1993).

Because the kinetics of CPT-I have not been well described for young pigs, in this paper, we report enzyme activities in liver and skeletal muscle, with focused attention on the K_m for carnitine. Furthermore, because these data were collected from pigs in the course of a larger study (Heo et al. 2000) examining the influence of dietary variables on carnitine status, effects of dietary carnitine and protein levels also are assessed.

MATERIALS AND METHODS

Animals and diets. All animal procedures were approved by the IACUC of North Carolina State University. The pigs used in this research were part of a larger project examining effects of dietary carnitine and protein level on nutrient partitioning in young pigs during abrupt transition to a strict vegetarian diet (Heo et al. 2000). Pigs (18 kg, $n = 20$) were fed corn-soybean meal diets⁵ containing 136 or 180 g protein/kg with either 0, designated Carn(-), or 500 mg/kg added L-carnitine, designated Carn(+). Diets were formulated to contain 14.24 MJ metabolizable energy (ME) and 40 g supplemental soy oil/kg diet, and to exceed requirements for vitamins and

with 500 mg/kg L-carnitine; CPT-I, carnitine palmitoyltransferase-I; ME, metabolizable energy.

⁵ Nutrient composition of diets (per kilogram): low protein diets contained 136 g protein, 69.1 g fat, 8.0 g Ca, 7.0 g P, 9.0 g lysine, 3.0 g methionine, 5.9 g threonine; high protein diets contained 180 g protein, 66.3 g fat, 8.0 g Ca, 7.0 g P, 12.0 g lysine, 4.0 g methionine, 7.8 g threonine. Vitamin and mineral premixes provided the following (mg/kg diet): retinol, 2.2; cholecalciferol, 0.042; α -tocopherol, 22.1; menadione, 2.6; riboflavin, 5.8; niacin, 29; choline, 308; biotin, 0.08; pyridoxine, 1.45; folic acid, 1.13; D-pantothenic acid, 22; vitamin B-12, 0.029; Mn, 64; Fe, 104; Zn, 141; Cu, 25; I, 1.6; Se, 0.3; carbadox, 55. See Heo et al. (2000) for the ingredient composition of each diet.

¹ Presented in part at Experimental Biology 99, April 1999, Washington, DC [Heo, K. N., Lin X., Odle, J. & Han, I. K. (1999) Carnitine palmitoyltransferase I activity and carnitine concentration in liver and skeletal muscle of 20 kg pigs. FASEB J. 13: A908 (abs.)].

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⁴ Abbreviations used: Carn(-), diet without added L-carnitine; Carn(+), diet

minerals (NRC 1988). Detailed composition of each diet was reported previously (Heo et al. 2000). The low protein diet was marginally adequate in protein, containing 0.63 g lysine/MJ ME; the high protein diet contained 0.84 g lysine/MJ ME. Diets were offered at 85% of ad libitum for 10 d before collection of tissues for enzyme kinetic and metabolite assays.

Chemicals. L-Carnitine used for dietary supplementation was donated by Algroup Lonza (Fair Lawn, NJ). [Methyl-³H]carnitine and [1-¹⁴C]acetyl-CoA were purchased from American Radiolabeled Chemicals, (St. Louis, MO). Palmitoyl-CoA, acetyl-CoA, carnitine acetyltransferase (EC 2.3.1.7) and other chemicals were obtained from Sigma Chemical (St. Louis, MO). Scintillation fluid (Scintisafe) and ion-exchange resin (AG 1×8, 100–200, Cl form) were obtained from Fisher Scientific (Atlanta, GA) and Bio-Rad Laboratories (Richmond, CA), respectively.

Isolation of liver and muscle mitochondria. Pigs were killed by American Veterinary Medical Association–approved electrocution and tissues (liver and soleus muscle) were obtained immediately and chilled on ice. Liver mitochondria were isolated by differential centrifugation as described by Mersmann et al. (1972). Skeletal muscle mitochondria were prepared by the method of Power and Newsholme (1997) using isolation medium described by Saggerson and Carpenter (1981).

The integrity of the mitochondrial membranes was assessed by measuring respiratory control ratios as described by Aprille and Asimakis (1980), and mitochondrial protein was determined by the biuret method (Gornall et al. 1949), using bovine serum albumin as the standard.

Carnitine palmitoyl transferase-I activity analysis. The activity of CPT-I was determined over a range of carnitine concentrations from 0 to 3 mmol/L (and palmitoyl-CoA fixed at 80 μ mol/L). The assay (Bremer et al. 1985) measured the rate of formation of palmitoylcarnitine from palmitoyl-CoA and carnitine. The CPT-I activities of liver and muscle mitochondria were expressed as nmol palmitoylcarnitine produced/(min · mg mitochondrial protein). The assay was verified to be linear with time and proportional to the amount of tissue assayed (data not shown).

Carnitine analysis. All samples were prepared using the procedure outlined by Bhuiyan et al. (1992). Liver and muscle (~ 500 mg) tissues were homogenized in 1 mL of ice-cold 1 mol/L HClO₄ using a PowerGen Homogenizer (Model 700; Fisher Scientific; 6

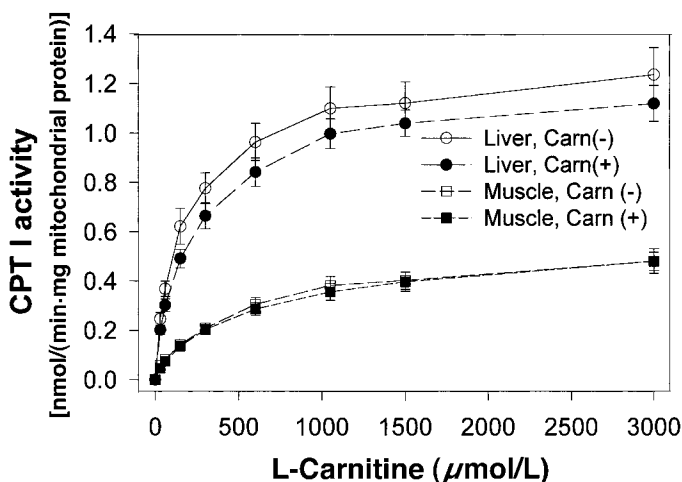


FIGURE 1 Main effect of carnitine on the kinetics of carnitine palmitoyltransferase-I (CPT-I) in liver and skeletal muscle mitochondria of young pigs. Values are means \pm SEM; $n = 10$. Carn(-) and Carn(+) refer to diets without and with 500 mg/kg L-carnitine, respectively. CPT-I K_m for carnitine without and with dietary L-carnitine were 163 ± 11 and 219 ± 21 μ mol/L in liver mitochondria, respectively ($P < 0.01$). The K_m for carnitine in muscle tissue was 1.5 times higher than that in liver (480 vs. 190 μ mol/L), and V_{max} (per mitochondrial protein) in muscle was less than half that in liver (0.54 vs. 1.22 nmol/min).

TABLE 1

Effects of dietary L-carnitine and protein level on kinetics of carnitine palmitoyltransferase-I and mitochondrial protein concentration of liver and muscle of 20-kg pigs¹

L-carnitine, mg/kg diet	Low protein level		High protein level		SEM
	0	500	0	500	
K_m for carnitine, μ mol/L					
Liver ^a	155	182	172	250	16
Skeletal muscle	419	490	510	498	44
V_{max} , nmol/(min · mg mitochondrial protein)					
Liver	1.184	1.106	1.336	1.262	0.098
Skeletal muscle	0.548	0.546	0.526	0.544	0.088

¹ Values are means $n = 5$ /treatment. Pigs were fed treatment diets for 10 d. Respiratory control ratios were 5.2 ± 0.2 in all hepatic mitochondria and 2.1 ± 0.2 in skeletal muscle mitochondria.

^a L-Carnitine effect ($P < 0.01$).

^b Protein effect ($P < 0.05$).

$\times 10$ s at 30,000 rpm). Three carnitine fractions (free, short-chain and long-chain esters) were assayed by the enzymatic radioisotope method of McGarry and Foster (1976), as modified by Bhuiyan et al. (1992).

Statistical analysis. Pig was used as the experimental unit. Michaelis-Menten kinetic constants of CPT-I (V_{max} and K_m for carnitine) for each pig were calculated using the iterative nonlinear procedure of SAS (1989). All data were analyzed as a randomized complete block (5 replicates) with a 2×2 factorial arrangement of treatments (L-carnitine \times protein level), employing the General Linear Models procedure of SAS (1989). Significant differences were accepted at $P < 0.05$.

RESULTS

Kinetics of CPT-I in the liver and skeletal muscle. Composite curves showing the kinetic response of CPT-I in liver and skeletal muscle to increasing carnitine are illustrated in **Figure 1**. Corresponding kinetic parameter estimates (V_{max} and K_m for carnitine) from pigs fed the four experimental diets (low and high protein, each with or without 500 mg/kg carnitine) are summarized in **Table 1**. The K_m for carnitine in liver was increased by L-carnitine (32%, $P < 0.01$) and high protein feeding (25%, $P < 0.05$). The V_{max} in liver and muscle was not affected by dietary L-carnitine or protein level. The K_m for carnitine in muscle was 2.5 times that of liver (0.48 vs. 0.19 mmol/L), and the V_{max} in muscle tissue was half of that in liver tissue [0.54 vs. 1.22 nmol/(min·mg mitochondrial protein)]. No interactions between L-carnitine and protein level were detected ($P > 0.10$).

Liver and skeletal muscle carnitine and acyl-carnitine concentrations. Free carnitine and short- and long-chain acyl-carnitine concentrations increased in liver (160, 690 and 140%, respectively) and skeletal muscle (70, 130 and 90%, respectively) with dietary L-carnitine supplementation ($P < 0.001$), but concentrations were not affected by high protein feeding (**Table 2**). The proportions of total tissue carnitine (shown parenthetically in **Table 2**) existing as short-chain esters in liver and skeletal muscle were increased by L-carnitine supplementation (200 and 30% respectively, $P < 0.01$), whereas the proportion of long-chain carnitine esters in liver decreased by 12% ($P < 0.001$), but did not change in skeletal muscle ($P > 0.10$).

TABLE 2

Effects of dietary L-carnitine and protein level on liver and muscle carnitine concentrations in 20-kg pigs¹

L-carnitine, mg/kg diet	Low protein level		High protein level		SEM
	0	500	0	500	
	<i>nmol/g wet tissue (% of total carnitine)</i>				
Liver carnitine					
Free ^a	73.7 (91.4)	181.4 (82.9)	86.0 (90.5)	231.3 (85.9)	12.8 (1.1)
Short chain ^a	2.2 (2.8)	26.0 (11.9)	4.2 (4.2)	24.8 (9.2)	3.0 (1.1)
Long chain ^a	4.7 (5.9)	11.0 (5.2)	4.9 (5.4)	12.2 (4.8)	1.1 (0.4)
Total ^a	80.7	218.4	95.1	268.3	14.6
Skeletal muscle carnitine					
Free ^a	759.8 (76.9)	1348.5 (72.3)	781.5 (78.4)	1296.1 (71.6)	73.9 (1.7)
Short chain ^a	193.7 (19.3)	445.6 (24.2)	194.7 (18.2)	442.9 (24.4)	32.7 (1.7)
Long chain ^b	37.1 (3.8)	63.8 (3.5)	33.4 (3.4)	73.1 (4.1)	8.0 (0.3)
Total ^a	990.6	1857.9	1009.6	1812.1	80.3

¹ Values are means, *n* = 5/treatment. Pigs were fed treatment diets for 10 d.^a L-Carnitine effect (*P* < 0.001, *P* < 0.01, respectively).

Short-chain acyl-carnitine concentration (per g wet tissue) and its relative composition were very low in liver compared with muscle [3 nmol (3%) vs. 194 nmol (19%)]; this difference between the tissues was not altered by carnitine supplementation [26 nmol (11%) vs. 445 nmol (24%)].

DISCUSSION

This study is the first to compare directly data regarding the CPT-I K_m for carnitine with tissue carnitine concentrations in the pig model. To our knowledge, it also is the first to show alteration in the K_m for carnitine of CPT-I by changes in dietary variables.

In typical swine husbandry, animals transition from a mixed-ingredient neonatal diet, formulated with various carnitine-containing animal products, to a strict vegetarian diet (i.e., corn-soy based) at ~7–8 wk of age. Therefore, pigs of this age were selected for study on the basis of the supposition that removal of dietary carnitine sources (animal products) might occur while pigs were not fully competent with respect to de novo carnitine biosynthesis. We reported previously (Heo et al. 2000) that when these pigs were supplemented with carnitine at 500 mg/kg, nitrogen balance and protein accretion were increased and carcass fat composition was reduced. In this study, we wanted to determine whether the in vitro kinetics of CPT-I would further corroborate the in vivo findings of altered nutrient partitioning. Toward this aim, the free carnitine concentrations in liver and muscle were compared with the corresponding K_m for carnitine (Fig. 2). The free carnitine concentrations in liver and muscle of the group without L-carnitine (80 and 760 μ mol/L, respectively) were less than or near the respective K_m (160 and 460 μ mol/L). These data imply that young pigs may require supplemental carnitine to ensure that the activity of CPT-I in vivo is not constrained by carnitine availability. However, they contrast with results from other mammalian species indicating that free carnitine concentration may surpass the K_m of CPT-I by 5–10 times (Friolet et al. 1994, McGarry et al. 1983, Pearson and Tubbs 1967).

The low hepatic carnitine content in the unsupplemented pigs compared with other species is consistent with observations reported for neonatal piglets (Kempen and Odle 1995, Kerner et al. 1984). Because liver and kidney

have a much faster carnitine turnover rate than muscle (Rebouche and Engel 1983), liver carnitine concentration of our control pigs (carnitine-deprived for 10 d) was reduced to the levels reported for colostrum-deprived newborn piglets. Especially the short-chain carnitine content in liver was very low, and the relative distribution of the three carnitine fractions in liver was atypical of that observed in rats and humans regardless of carnitine supplementation, whereas skeletal muscle showed relative carnitine composition similar to that of other species (Friolet et al. 1994, Pearson and Tubbs 1967). Carnitine concentrations and the proportions of short- and long-chain carnitine were increased by dietary carnitine in liver and muscle. The observed increase in concentrations of short-chain carnitine may result from increased production of acetyl-CoA (via carnitine acetyltransferase) as a product of β -oxidation of long-chain acyl-CoA, which also could have been accelerated by elevated tissue carnitine concentration.

Recent studies of the molecular biology of CPT-I have identified two isoforms, i.e., the L-form (liver), which possesses relatively low sensitivity to malonyl-CoA inhibition and has a low K_m for carnitine (~0.03 mmol/L), and the M-form (muscle), which shows very high sensitivity to malonyl-CoA and has a high K_m for carnitine (~0.50 mmol/L; McGarry and Brown 1997). Interestingly, heart and adipose tissue possess both isoforms, and the isoform ratio changes with development and physiologic status (Brown et al. 1995 and 1997). Because the liver is not a major site of lipogenesis in pigs (P egorier et al. 1983), unlike other mammalian species (e.g., rats or rabbits), we speculate that it may express some of the M-isoform (or perhaps a unique isoform), thus resulting in the higher observed K_m and high malonyl-CoA sensitivity. We further postulate that increasing hepatic carnitine by supplementation may change the K_m for carnitine via changing the ratio of isoforms (L and M). Furthermore, the finding that malonyl-CoA sensitivity of pig liver CPT-I (Lin and Odle 1995, Schmidt and Herpin 1998) is close to the average value reported for the L and M isoforms strengthens this notion. The pig gene for CPT-I will have to be cloned and characterized to resolve these issues definitively.

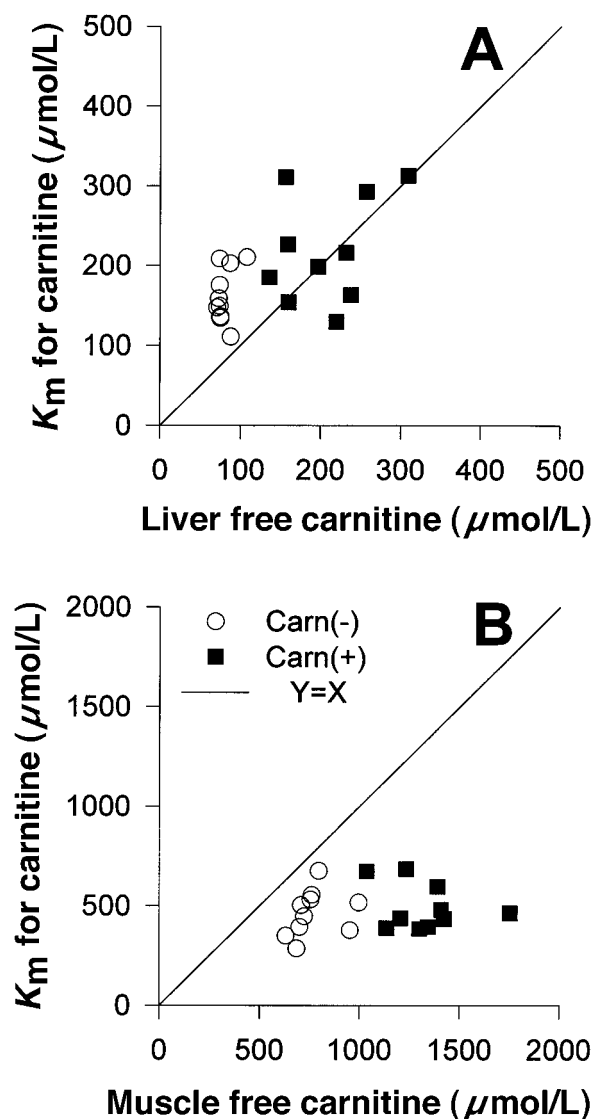


FIGURE 2 Carnitine palmitoyltransferase-I (CPT-I) K_m for carnitine against the measured liver free carnitine concentration (A) and skeletal muscle free carnitine concentration (B) in 20-kg pigs; $n = 10$ /group. Carn(-) and Carn(+) refer to diets without and with 500 mg/kg diet L-carnitine, respectively. (A) Free carnitine concentrations in liver of the Carn(-) group (80 $\mu\text{mol/L}$) were half the value of the K_m (163 $\mu\text{mol/L}$), and free carnitine concentration was increased to the CPT-I K_m in liver with dietary supplementation. (B) Skeletal muscle of the Carn(-) group showed a free carnitine concentration (770 $\mu\text{mol/L}$) that was 70% higher than the K_m (464 $\mu\text{mol/L}$) and was 1.7-fold greater than the K_m of the Carn(+) group.

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