Kinetics of Carnitine Palmitoyltransferase-I Are Altered by Dietary Variables and Suggest a Metabolic Need for Supplemental Carnitine in Young Pigs

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 x 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 ± 44 μmol/L. In contrast, the K_m for carnitine in liver was increased from 164 to 216 ± 20 μmol/L by dietary L-carnitine supplementation (P < 0.01) and from 169 to 211 ± 20 μmol/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 μmol/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)* 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 in young pigs (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, Pégourié 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 (Düée 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 protein and carnitine 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 diets* 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

* Nutrient composition of diets (per kilogram): low protein diets contained 136 g protein, 69.1 g fat, 8.6 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-tocopheryl acetate, 22.1; menadione, 2.6; riboflavin, 5.8; niacin, 28; choline, 308; biotin, 0.08; pyridoxine, 1.45, folic acid, 1.13; pantothenic acid, 22; vitamin B-12, 0.029; Mn, 64; Fe, 104; Zn, 141; Cu, 25; I, 1.6; Se, 0.3; carboxamid, 55. See Heo et al. (2000) for the ingredient composition of each 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 [L-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 CI 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 mmol/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 × 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 (Vmax and Km 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 × 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 (Vmax and Km for carnitine) from pigs fed the four experimental diets (low and high protein, each with or without 500 mg/kg l-carnitine) are summarized in Table 1. The Km for carnitine in liver was increased by l-carnitine (32%, P < 0.01) and high protein feeding (25%, P < 0.05). The Vmax in liver and muscle was not affected by dietary l-carnitine or protein level. The Km for carnitine in muscle was 2.5 times that of liver (0.48 vs. 0.19 mmol/L), and the Vmax 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 1**

<table>
<thead>
<tr>
<th></th>
<th>Low protein level</th>
<th>High protein level</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-carnitine, mg/kg diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K_m for carnitine, μmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>163 ± 16</td>
<td>110 ± 18</td>
<td>7.0</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>206 ± 21</td>
<td>165 ± 19</td>
<td>8.0</td>
</tr>
<tr>
<td>V_max, nmol/min mg mitochondrial protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.14 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>0.16 ± 0.02</td>
<td>0.14 ± 0.02</td>
<td>0.04</td>
</tr>
</tbody>
</table>

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).

**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 ± 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 μmol/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 μmol/L), and V_max (per mitochondrial protein) in muscle was < half that in liver (0.54 vs. 1.22 nmol/min).
Skeletal muscle carnitine 
Liver carnitine alteration in the K  
the pig model. To our knowledge, it also is the first to show  
trast with results from other mammalian species indicating  
constrained by carnitine availability. However, they con- 
carnitine to ensure that the activity of CPT-I in vivo is not 

L-carnitine (80 and 760
shortening of liver and muscle were compared with the corre- 
partitioning. Toward this aim, the free carnitine concen- 
terminate whether the in vitro kinetics of CPT-I would 

K for carnitine with tissue carnitine concentrations in  
mol/L, respectively) were less  
K for carnitine of CPT-I by changes in 
m (160 and 460
Km and high malonyl-CoA sensi - 
Km for carnitine similar to that of other species (Friolet  
ratios of acetyl-CoA (via carnitine acetyltransferase) as a product 
recently, heart and adipose tissue possess both isoforms, and 
the isoform ratio changes with development and physio -
logic status (Brown et al. 1995 and 1997). Because the 
the liver is not a major site of lipogenesis in pigs (Pégorier 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 for and high malonyl-CoA sensi- 
We further postulate that increasing hepatic carnitine by supplementation may change the Km 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.

**DISCUSSION**

This study is the first to compare directly data regarding the CPT-I Km for carnitine with tissue carnitine concentrations in the pig model. To our knowledge, it also is the first to show alteration in the Km 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 Km for carnitine (Fig. 2). The free carnitine concentrations in liver and muscle of the group without l-carnitine (80 and 760 μmol/L, respectively) were less than or near the respective Km (160 and 460 μmol/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 Km of CPT-I by 5–10 times (Friolet et al. 1994, McGarry and Brown 1997). Interest-ingly, heart and adipose tissue possess both isoforms, and the isoform ratio changes with development and physio -logic status (Brown et al. 1995 and 1997). Because the 

**TABLE 2**

<table>
<thead>
<tr>
<th>L-carnitine, mg/kg diet</th>
<th>Low protein level</th>
<th>High protein level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>500</td>
</tr>
<tr>
<td>nmol/g wet tissue (% of total carnitine)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Liver carnitine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freea</td>
<td>73.7 (91.4)</td>
<td>181.4 (82.9)</td>
</tr>
<tr>
<td>Short chaina</td>
<td>2.2 (2.8)</td>
<td>26.0 (11.9)</td>
</tr>
<tr>
<td>Long chaina</td>
<td>4.7 (5.9)</td>
<td>11.0 (5.2)</td>
</tr>
<tr>
<td>Totala</td>
<td>80.7</td>
<td>218.4</td>
</tr>
<tr>
<td><strong>Skeletal muscle carnitine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freea</td>
<td>759.8 (76.9)</td>
<td>1348.5 (72.3)</td>
</tr>
<tr>
<td>Short chaina</td>
<td>193.7 (19.3)</td>
<td>446.5 (24.2)</td>
</tr>
<tr>
<td>Long chainb</td>
<td>37.1 (3.8)</td>
<td>63.8 (3.5)</td>
</tr>
<tr>
<td>Totala</td>
<td>990.6</td>
<td>1857.9</td>
</tr>
</tbody>
</table>

1 Values are means, n = 5/treatment. Pigs were fed treatment diets for 10 d. 
ab L-Carnitine effect (P < 0.001, P < 0.01, respectively).
and free carnitine concentration was increased to the CPT-I Km.

Km (464 mol/L) were half the value of the Km (163 mol/L) that was 70% higher than the Km (464 mol/L) in liver with dietary supplementation. (β) Skeletal muscle of the Carn(-) group showed a free carnitine concentration (770 mol/L) that was 70% higher than the Km (464 mol/L) and was 1.7-fold greater than the Km of the Carn(+).

LITERATURE CITED


