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Maternal Dietary L-Carnitine Supplementation Influences Fetal Carnitine Status and Stimulates Carnitine Palmitoyltransferase and Pyruvate Dehydrogenase Complex Activities in Swine[1,2]

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Abstract

Effects of increasing maternal L-carnitine on carnitine status and energy metabolism in the fetus were evaluated by feeding pregnant swine a corn-soybean–based diet containing either 0 or 50 mg/kg added L-carnitine ($n = 10$/treatment) during the first 70 d of gestation. Carnitine, carnitine palmitoyltransferase (CPT), and pyruvate dehydrogenase complex (PDHC) activities were analyzed in tissues collected from fetuses on d 55 and 70. Maternal L-carnitine supplementation increased both fetal free and long-chain carnitine concentrations by 45% in liver and free carnitine by 31% in heart tissues but did not affect kidney tissue. Elevations in free and acylcarnitines increased with gestational age from 55 to 70 d in liver but not in heart and kidney. The increased carnitine concentrations resulted in a 45% increase in PDHC activity in heart and liver on d 70 of gestation but did not affect kidney and liver on d 55 of gestation. The increases in carnitine concentrations were accompanied by a 70% increase in hepatic CPT activity in 70-d-old fetuses, but activities in heart and kidney were unaffected. The Michaelis constant (K_m) of CPT for carnitine in fetal tissues was not influenced by carnitine supplementation ($P > 0.1$). Notably, the concentrations of carnitine measured on d 70 were only 25–40% of the K_m values in liver, 60–70% in heart, and 30–40% in kidney ($P < 0.001$). We conclude that carnitine ingestion during pregnancy increases fetal carnitine concentrations and stimulates heart PDHC and liver CPT activity without altering carnitine K_m. J. Nutr. 138: 2356–2362, 2008.

Introduction

Carnitine functions primarily in the transfer of long-chain fatty acids into the mitochondrial matrix for fatty acid β-oxidation. In addition, carnitine has an important role in other intermediary metabolism, such as transfer of the peroxisomal β-oxidation product, acetyl-CoA, to the mitochondria (1), maintaining a favorable acetyl-CoA:CoA ratio and storage of energy in the form of acetylcarnitine (2). Recently, the role of carnitine in fetal-placental growth and energy metabolism has received much attention (3,4), because studies have shown that carnitine is critical in fetal growth (5,6), fetal maturation (7), and differential gene regulation of various metabolic pathways (8,9). Studies also have confirmed that the hypoglycemia associated with intrauterine growth retardation in infants (10), the mitochondrial-based bioenergetic decline with age, and hypoxia-ischemia in the newborn (11–13) are associated with decreased plasma free carnitine concentration and impaired fatty acid oxidation.

Carnitine carbon is derived metabolically from lysine and methionine in animals (14). The primary biosynthetic site is the liver, although butyrobetaine hydroxylase, the key enzyme catalyzing the final step in carnitine biosynthesis, is found in liver, kidney, and brain (2). The fetus is incapable of endogenous synthesis of carnitine due to the lack of this enzyme (15). Recently, butyrobetaine hydroxylase activity has been detected in fetal-placental tissues (16), but the activity is low. Thus, carnitine concentrations in fetal tissues are greatly dependent on maternal carnitine status and the rate of placental transfer (15,17). Carnitine deficiency develops among very low birth weight infants who do not receive exogenous carnitine supplementation (18). Therefore, adequate supply of exogenous carnitine may be important for fetal growth and organ development, especially for fetuses having potential genetic defects related to long-chain fatty acid oxidative metabolism.

Supplementation with L-carnitine may improve fetal energy metabolism by increasing carnitine level, subsequently altering the acetyl-CoA:CoA ratio, metabolic pathways, and energy supply. Carnitine was shown to modulate the expression and activity of placental short-chain 3-hydroxyacyl CoA dehydrogenase, one of the mitochondrial fatty acid oxidation enzymes

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(4). Dietary supplementation with L-carnitine highly stimulated carnitine palmitoyltransferase I (CPT I)[6] and CPT II transcription rates and significantly increased CPT I activity in liver (19,20) in young and adult animals. Furthermore, L-carnitine supplementation stimulated CPT I and subsequently accelerated palmitate oxidation in newborn piglets during cardiac development and hypoxia (21). In addition to stimulating CPT activity and increasing fatty acid oxidation, supplementation with L-carnitine was shown to stimulate pyruvate dehydrogenase complex (PDHC) activity in isolated human skeletal muscle mitochondria (22,23) and increase glucose oxidation in the fatty acid-perfused isolated working heart (24). We hypothesized that maternal dietary L-carnitine supplementation would increase carnitine content in fetal tissues and stimulate the development of the CPT system and/or the PDHC system. Correspondingly, increases in oxidative capacity could improve mitochondrial bioenergetics and aid development of fetuses at risk for prematurity and intrauterine growth retardation. We have examined this hypothesis by testing the effects of maternal supplementation with carnitine on carnitine status and CPT and PDHC activities in liver, kidney, and heart tissues from 55- and 70-d-old pig fetuses, the stage at which fetal weight and protein accretion are accelerated (25).

Materials and Methods

Animals and experimental design. Pregnant, first-parity swine (average body weight 137.7 kg; $n = 59$) were randomly divided into 2 groups and fed corn-soybean–based diets (1.75 kg/d) and received either with 0 or 0.64 mg/kg body weight supplemental L-carnitine during gestation as previously described (26). The pigs consumed diets and water ad libitum. On d 55 and 70, the pigs were killed by electrical stunning followed by exsanguination. A mid-lateral incision was made to gain access to the abdominal cavity. The ovarian pedicles and uterine stump, at the level of the cranial cervix, were cut and the uterus removed. Once the uterus was removed, fetuses were collected from the right uterine horn under aseptic conditions. Fetal weights averaged 73.5 ± 3.4 g at d 55 and 211.1 ± 11.8 g at d 70 of gestation, which are similar weights to those observed by Wise and Christenson (27). Liver, kidney, and heart from each individual fetus were removed and immediately snap-frozen in liquid nitrogen. The frozen samples were stored in −80°C until analysis. The experiment was conducted at the Kansas State University Swine Teaching and Research Center. All animal procedures were reviewed and approved by the Kansas State University Animal Care and Use Committee.

Carnitine and acylcarnitine analysis. Tissue samples were homogenized (PRO 200 homogenizer, Pro Scientific) in perchloric acid, and free carnitine and acylcarnitines were extracted as described by Lin and Odle (28). Carnitine and acylcarnitine concentrations were determined using an enzymatic radioisotope method (29,30).

CPT analysis. Tissue samples were homogenized in a buffer (pH = 7.2) containing mannitol (220 mmol/L), sucrose (70 mmol/L), HEPES (2 mmol/L), and EDTA (0.1 mmol/L) at 4°C using a hand-held glass homogenizer. The homogenization was conducted with a ratio of tissue and buffer of 1:4 (g:mL). After homogenizing, CPT activity was analyzed in the whole homogenate, with carnitine concentration incremented up to 5 mmol/L (28). To evaluate the 2 distinct acyltransferases, CPT I and CPT II, activities were analyzed with or without 50 μmol/L malonyl-CoA in the presence of 1 mmol/L carnitine. Homogenate protein concentration was determined by use of the biuret method (31). CPT activity was determined as palmitoylcarnitine production rate, expressed as nmol/(h·mg protein), after correction for time-0 blanks. The apparent kinetic constants (V_{max} and carnitine-K_m) were calculated using the iterative NLIN procedure of SAS according to the Michaelis-Menten equation, $V_i = V_{max}(s)/[K_m + (s)]$, where V_i = initial velocity, V_{max} = maximal velocity, K_m = Michaelis constant, and s = substrate (carnitine) concentration.

PDHC analysis. Tissue samples were homogenized and protein was determined as described above. PDHC-activated activity was measured in tissue homogenates using a modified method described by Uziel et al. (23). Briefly, 50-μL aliquots of homogenates (~5 mg of wet tissue) were incubated in a medium containing 1 mmol/L CoA, 2.5 mmol/L NAD, 0.3 mmol/L thiamin pyrophosphate, 10 mmol/L β-mercaptoethethanol, 30 mmol/L HEPES, 10 mmol/L potassium phosphate, 10 mmol/L $MgCl_2$, and 1 mmol/L $CaCl_2$ in a 25-mL Erlenmeyer flask in a final volume of 1 mL at 37°C. The reaction was initiated by adding 1 μmol [1-^{14}C]-pyruvate (7.4 MBq/mmol) after a 4-min preincubation. Each flask was sealed with a rubber cap from which a small tube was suspended containing 0.6 mL of ethanolamine. The reaction was terminated by injecting 0.5 mL of 10% $HClO_4$ after 10 min. The $^{14}CO_2$ produced from the reaction was trapped in the center tubes and radioactivity was measured by liquid scintillation counting (32).

Chemicals. L-[*N*-methyl-^{3}H]-carnitine (2.22 MBq/mmol), [1-^{14}C]-acetyl-CoA (148 MBq/mmol), and [1-^{14}C]-pyruvate (703 MBq/mmol) were purchased from American Radiolabeled Chemicals. Palmitoyl-CoA, acetyl-CoA, CoASH, NAD, carnitine acetyltransferase (CAT; EC 2.3.1.7), L-carnitine, and other chemicals were purchased from Sigma-Aldrich.

Statistics. Data from calculations using the NLIN procedure of SAS and from chemical analyses were subjected to ANOVA based on a 2 × 2 factorial design (dietary carnitine × fetal age) using the general linear models procedure of SAS. Tissue differences were also evaluated by multivariate ANOVA based on a completely randomized design using the general linear models procedure. Statistical differences between K_m values and tissue total carnitine were analyzed using the TTEST procedure of SAS with a paired Student's *t* test. Values were expressed as least square means ± SEM and differences were considered significant when $P < 0.05$.

Results

Fetal and organ weights did not differ between the 2 dietary groups ($P > 0.05$; data not shown). Maternal supplementation with dietary L-carnitine during early pregnancy significantly increased fetal free carnitine and long-chain acylcarnitine concentrations in liver and free carnitine concentration in heart, but did not affect the kidney tissues of fetuses (**Table 1**). Fetal free carnitine concentrations increased by 45% in liver and 32% in heart of fetuses from the dams supplemented with carnitine. The influence of maternal carnitine supplementation on fetal hepatic carnitine moieties increased with gestational age (carnitine × age; $P < 0.05$). Age did not affect carnitine status in fetal heart and kidney tissues. The effects of maternal carnitine supplementation and gestational age on total carnitine status were similar to the effects on free and acylcarnitines.

Carnitine distributions varied among tissues regardless of supplementation with dietary L-carnitine or gestational age, with total carnitine concentration in heart being 50% higher than that in liver and 2 times higher than that in kidney of the fetuses (Table 1).

Supplementation with dietary L-carnitine to dams had no effects on specific activity of CPT (malonyl-CoA inhibited and uninhibited) in kidney and heart tissue and its kinetic constants in any tissue from 55- or 70-d-old fetuses but was associated with a significant increase in CPT activity (**Fig. 1**), with a 60%

[6] Abbreviations used: CAT, carnitine acetyltransferase; CPT, carnitine palmitoyltransferase; K_m, Michaelis constant; PDH, pyruvate dehydrogenase; PDHC, pyruvate dehydrogenase complex.

TABLE 1 Effects of maternal carnitine supplementation (± carnitine) on carnitine concentrations in swine fetal tissues on 55 and 70 d of gestation[1]

	55 d		70 d			Source		
Tissues	−Carnitine	+Carnitine	−Carnitine	+Carnitine	SEM	Age	Treat	Age × treat
Liver			*nmol/g wet tissue*					
Free carnitine	110.8[a]	140.9[a]	140.5[a]	222.3[b]	10.9	<0.0001	<0.0001	0.027
Short-chain acylcarnitine	4.7	7.9	14.3	13.7	2.3	0.0038	0.58	0.43
Long-chain acylcarnitine	19.9[a]	22.5[a]	17.2[a]	31.0[b]	2.6	0.28	0.005	0.04
Total carnitine[c]	135.4[a]	171.3[a]	172.0[a]	267.0[b]	13.3	<0.0001	<0.0001	0.038
Kidney								
Free carnitine	73.7	78.7	76.6	90.1	7.7	0.36	0.24	0.59
Short-chain acylcarnitine	7.3	4.9	4.0	4.9	1.2	0.19	0.56	0.19
Long-chain acylcarnitine	8.3	11.2	8.8	9.8	2.0	0.82	0.35	0.67
Total carnitine[d]	89.3	94.8	89.4	104.8	9.8	0.61	0.30	0.62
Heart								
Free carnitine	191.0[a]	282.0[ab]	221.7[ab]	260.4[b]	28.2	0.87	0.033	0.37
Short-chain acylcarnitine	9.9	12.8	10.0	8.6	2.5	0.42	0.77	0.41
Long-chain acylcarnitine	23.3	38.5	37.0	38.2	6.3	0.20	0.28	0.20
Total carnitine[e]	224.2[a]	333.2[b]	268.6[ab]	307.2[ab]	32.6	0.78	0.035	0.29

[1] Values are least squares means, $n = 6$ fetal tissue samples from 6 gilts. Means in a row without a common letter (a or b) differ, $P < 0.05$. [c,d,e] Mean total carnitine concentrations differed among liver (186.4 ± 9.3 nmol/g), kidney (94.6 ± 9.3 nmol/g), and heart (283.3 ± 9.3 nmol/g), $P < 0.0001$.

increase in inhibited and 80% increase in uninhibited CPT activity in liver tissue from 70-d-old fetuses (**Table 2**). Maternal dietary L-carnitine and gestational age did not affect the malonyl-CoA inhibited:uninhibited enzyme activity ratio ($P = 0.83$), but the percentage of inhibited enzyme activity was 2.2-fold higher in heart and in kidney (53.2%) than in liver (16.4%). Neither the CPT activity nor carnitine-K_m changed in liver or kidney tissues with increasing gestational age; however, the K_m in heart tissue increased by 63% ($P < 0.04$) as fetuses aged from 55 (0.32 mmol) to 70 d (0.52 mmol) of age. Similar to the carnitine status, CPT activity and kinetic constants differed among the various fetal tissues. The apparent malonyl-CoA–inhibited activity in kidney was 1 times higher than in heart and 4 times higher than in liver. The uninhibited activity in kidney was similar to the activity in liver but was 1.8 times higher than in heart (Table 2). The K_m for carnitine obtained from liver (0.54 mmol) was 80% higher than that from kidney (0.3 mmol) and 30% higher than that from heart (0.42 mmol).

The values of K_m for carnitine were significantly higher than free carnitine concentrations in liver and kidney from 55-d-old fetuses and in all of the measured tissues from 70-d-old fetuses with or without maternal dietary carnitine supplementation (**Fig. 2**).

Supplementation of dietary L-carnitine to dams increased PDHC activity (**Fig. 3**) in fetal heart by 52% ($P < 0.01$). Supplementation also increased PDHC activity in liver by 37% ($P < 0.03$) in 70-d-old fetuses, but did not affect PDHC activity in kidney ($P = 0.63$). The enzyme activity decreased in heart tissue but increased in kidney tissue with gestational age. The enzyme activity in the kidney was greater than in liver and heart.

Discussion

Effects on carnitine status. Previous studies (5,33,34) demonstrate the placenta-fetal interrelationship in carnitine metabolism during pregnancy and suggest that supplementation of carnitine during pregnancy may be beneficial to both mother and fetus. However, the effect of maternal carnitine supplementation on fetal tissue carnitine status deserves evaluation, given the importance of placental transfer (7). Our results show that dietary supplementation of L-carnitine to pregnant dams significantly increased carnitine concentrations in fetal liver, heart, and muscle tissues (35), demonstrating that the placenta is capable of increasing the transport of carnitine to the fetus in the early stages of pregnancy. Results from earlier studies also showed that carnitine placental transfer in humans occurred primarily in the middle stage (33) or the last trimester of pregnancy (36). Our data are consistent with these observations in humans, suggesting that carnitine placental transfer in the porcine species occurs at the same stage of pregnancy in humans. Total tissue carnitine concentration (liver, heart, and kidney) measured at 55 and 70 d (corresponding to the middle or late-middle stage of human gestation) increased by 30% in fetuses from dams given carnitine supplementation. The patterns of carnitine distribution in fetal tissues also are similar to human neonates (34). Therefore, the data further support the pig as an

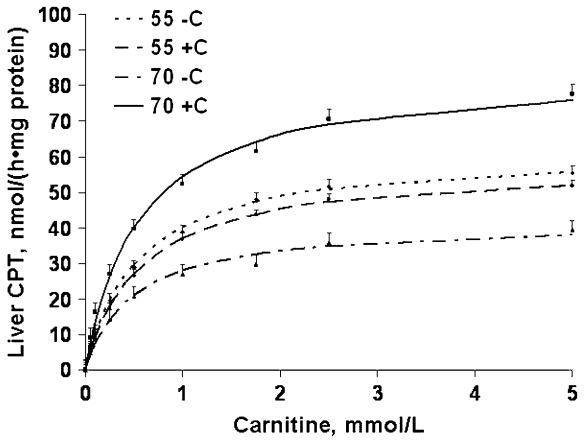

FIGURE 1 Hepatic CPT activity and kinetic response to increasing carnitine concentrations in fetal piglets from pregnant dams fed control (−C) or L-carnitine–supplemented diets (+C) for 55 or 70 d of gestation. Values are expressed as least square means ± SEM, $n = 8$.

TABLE 2 Effects of maternal carnitine supplementation (± carnitine) on CPT activity in the presence or absence of malonyl-CoA (50 μmol/L) in swine fetal tissues on d 55 or 70 of gestation[1]

	d 55		d 70		Source			
Tissues	−Carnitine	+Carnitine	−Carnitine	+Carnitine	SEM	Age	Treat	Age × treat
Liver	*CPT activity (nmol/g wet tissue)*							
Inhibited[2,c]	6.78[ab]	5.56[a]	5.00[a]	8.04[b]	0.76	0.24	0.64	0.009
Uninhibited[f]	32.05[a]	31.49[a]	24.83[a]	44.69[b]	4.13	0.47	0.025	0.018
% of inhibited[h]	17.16	15.31	16.69	15.74	1.17	0.83	0.27	0.62
Kidney								
Inhibited[d]	32.09	33.97	30.96	32.54	4.28	0.77	0.68	0.97
Uninhibited[f]	26.43	28.90	29.92	33.16	3.75	0.31	0.44	0.92
% of inhibited[i]	54.82	52.97	49.39	49.51	1.65	0.0002	0.20	0.28
Heart								
Inhibited[e]	14.14	15.54	16.03	16.55	2.36	0.56	0.70	0.86
Uninhibited[g]	11.14	12.8	12.30	13.43	2.13	0.93	0.27	0.55
% of inhibited[i]	55.51	50.98	57.11	55.20	1.47	0.0029	0.0013	0.14

[1] Values are least squares means, $n = 6$ fetal tissue samples from 6 gilts. Means in a row with superscripts without a common letter differ (a or b), $P < 0.05$. [c,d,e] Mean inhibited activity in kidney [32.38 ± 1.29 nmol/(h·mg protein)] and liver (6.35 ± 0.93) exceed that of heart (15.57 ± 1.11), $P < 0.0001$. [f,g] Mean uninhibited activity in kidney [29.60 ± 2.30 nmol/(h·mg protein)] and liver (33.27 ± 1.61) exceed that of heart (12.95 ± 1.94), $P < 0.0001$. [h,i] Mean percent inhibited activity in heart (54.71 ± 0.69%) and kidney (51.67 ± 0.81) exceeded that of liver (16.37 ± 0.38), $P < 0.0001$.

[2] Inhibited activities were measured in the presence of 50 umol/L malonyl-CoA.

appropriate animal model for studies concerning perinatal carnitine metabolism.

Supplementation with maternal dietary L-carnitine increases placenta carnitine transfer and fetal tissue carnitine deposition, but the uptake of carnitine varies greatly among tissues. Carnitine concentrations were higher in heart than in liver and kidney. Supplementation with L-carnitine significantly increased carnitine uptake in fetal liver and heart but did not affect kidney. Moreover, the uptake of carnitine was associated with increasing gestational age in liver but not in heart and kidney. These differences may reflect the differences in carnitine transporter expression and/or affinity of the transporters for carnitine in fetal tissues, which may be related to the metabolic functions of the organs. In addition, oxidative metabolism is greater in heart than in liver and kidney of fetuses; thus, the carnitine concentrations may reflect the difference in vital functions, such as the rate of oxidative metabolism for the production of energy in the tissues during fetal life (7).

Effects on CPT activity. L-Carnitine is an essential cofactor of CPT I, a key enzyme for long-chain fatty acid oxidation. Increasing carnitine concentration via dietary supplementation stimulated the transcription and activity of CPT in the liver of old and adult rats (20) as well as the enzyme activity in the liver of young pigs (19). Similar stimulation also occurred after the addition of carnitine to tissue incubation medium of hearts from newborn pigs (21). In this study, we examined the effects of supplementation with L-carnitine to the maternal diet on CPT activity and kinetics in fetal tissues during the middle or late-middle stages of gestation. The data revealed that the apparent CPT activity increased by 100% in liver of 70-d-old fetuses from dams supplemented with L-carnitine and the increase was associated with a substantial increase in tissue carnitine concentration. In contrast with liver, maternal carnitine supplementation did not alter CPT activity or carnitine concentration in the kidney. This suggests an association between CPT activity and tissue carnitine concentration. The stimulation of CPT activity by high levels of carnitine in liver might be due to increased mRNA for both CPT IA, the predominant isoform in liver and kidney tissues, and CPT II as observed in the liver of old rats fed dietary L-carnitine (20). Indeed, both malonyl-CoA inhibited and uninhibited enzyme activities increased (Table 2) in the fetus from carnitine-fed gilts. Besides kidney, however, neither malonyl-CoA inhibited or uninhibited CPT activities increased in heart tissue even though carnitine concentrations increased significantly in the tissue. Because both CPT IA and CPT IB isoforms are expressed in heart tissue, the diminished response of cardiac CPT activity to increased carnitine concentration might be partially caused by the different gene encoding CPT IB. In contrast to CPT IA, the B isoform is not regulated by PPARα and has no thyroid hormone responsive element, whereas L-carnitine stimulates gene expression of CPT IA, which does have thyroid hormone responsive element in its promoter (37). Thus, the influence of carnitine on CPT activity in heart could be attenuated by the presence of the carnitine-insensitive gene, CPT IB. In addition, the poor response of CPT activity to carnitine also might be due to reduced mRNA abundance of the CPT IA isoform in heart tissue, which accounts for 25% of the total activity during perinatal development (38) and is regulated differentially by hormones and diet (39). Therefore, the stimulatory effect of carnitine on CPT activity depends greatly on carnitine concentrations and the isotypes of CPT I in the tissues. The results support previous research in which liver is deemed most responsive to carnitine supplementation (20).

Supplementation with L-carnitine did not affect K_m of CPT for carnitine in any measured tissues, but the values in liver were higher than those in isolated mitochondria from liver and muscle (40). In the term fetus, CPT II is expressed during fetal development (41) and the K_m value for carnitine is higher for CPT II than for CPT I when they are expressed separately in yeast (42). Moreover, the malonyl-CoA–uninhibited enzyme activity was 4 times higher than malonyl-CoA–inhibited enzyme activity. Thus, a great portion of the K_m values measured in homogenates should reflect the presence of CPT II. In addition, the K_m value was lower in kidney than in liver. This might be explained by the potential differences in mitochondrial content, oxidative enzymes, and CPT expression in the 2 tissues as suggested by Doh et al. (43). We also noticed that the K_m in heart

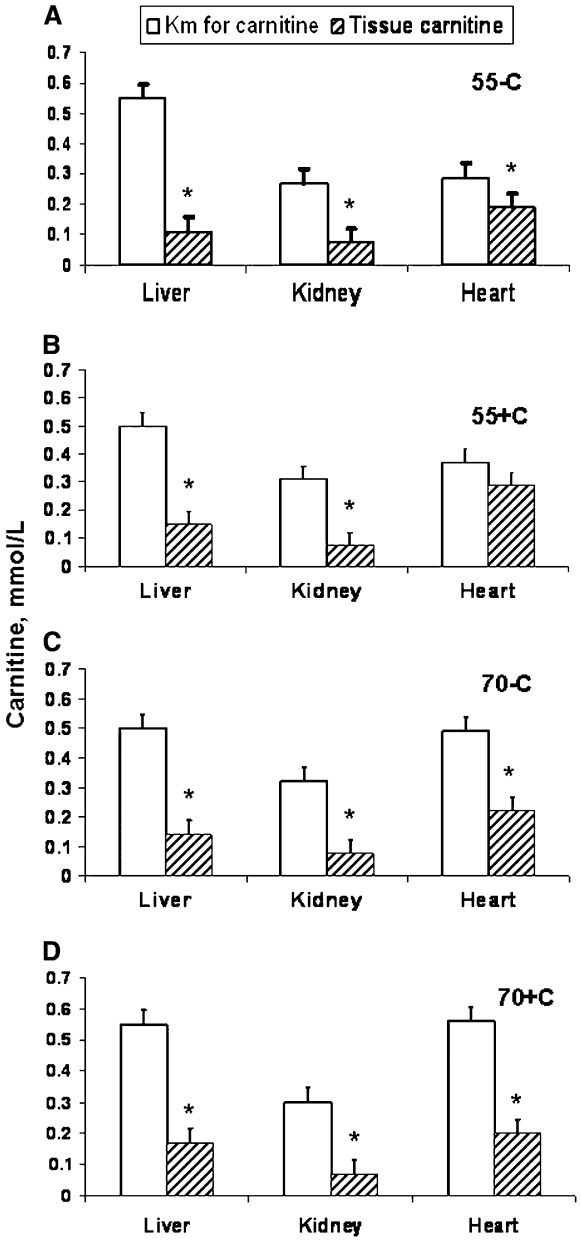

FIGURE 2 Comparison of the CPT K_m for carnitine and tissue carnitine concentrations in fetal pig tissues from pregnant dams fed control (−C) or L-carnitine–supplemented diets (+C) for 55 or 70 d of gestation. Tissue carnitine concentrations were corrected using tissue dry weight:wet weight ratios (52). Values are least square means ± SEM, $n = 8$. *Different from K_m in that tissue, $P < 0.05$.

was lower than liver on d 55 of gestation but increased to equal the liver value by 70 d. Because both CPT IA and CPT IB are expressed in heart tissue, the increased K_m may reflect a change in the 2 isoforms during fetal development. The 2 isotypes of CPT I have different kinetic properties and the carnitine K_m values measured in rat and human are 15 times higher for CPT IB than for CPT IA (44). During the neonatal period, CPT IA gradually decreases, whereas CPT IB increases and remains highly expressed after weaning (45). Therefore, the increase in carnitine the K_m value in heart may coincide with the increased proportion of CPT IB present in early fetal development. In addition to the differences among tissues, all of the tissue free carnitine concentrations were lower than the in vitro-measured K_m values for carnitine for both 55- and 70-d-old fetus regardless of carnitine supplementations (Fig. 2), suggesting carnitine may be a limiting substrate for CPT during the mid-gestational period.

Effects on PDHC activity. Because increasing carnitine stimulates PDHC activity by reducing the acetyl-CoA:CoA ratio in mitochondria via exporting the acetyl groups out of the mitochondria matrix, the role of carnitine in carbohydrate oxidation and the potential effect on fatty acid oxidation has been emphasized recently (46). In our experiment, increased carnitine concentrations increased PDHC activity in heart ($P < 0.03$) and liver at 70 d of gestation ($P < 0.04$). These results are consistent with observations in muscle of endurance athletes (22). During pregnancy, carbohydrates, especially glucose and lactate, are major sources of metabolic energy. Elevated glucose and lactate oxidation may increase acetyl-CoA accumulation within mitochondria when the production rate exceeds the rate of combustion in the tricarboxylic acid cycle. In adult animals, the increased acetyl-CoA level in mitochondria will inhibit PDHC activity by activating pyruvate dehydrogenase (PDH) kinase and inhibiting CPT I activity by increasing synthesis of malonyl-CoA. Additional carnitine allows the transfer of the extra acetyl-CoA to acetylcarnitine, thereby reducing the acetyl-CoA:CoA ratio. The intramitochondrial acetyl-CoA:CoA ratio is primarily involved in the regulation of PDHC activity (46). Thus, increasing carnitine can stimulate PDHC activity in fetal heart and liver by forming acetylcarnitine. The acetylcarnitine is then transported out of mitochondria and converted back to acetyl-CoA by CAT in the cytosol. Similar results were observed in a working rat heart model (24). Because CAT activity in heart is increased by carnitine supplement (47), the transfer of acetyl-CoA by CAT might reduce the concentration of acetyl-CoA sufficient for stimulation of PDHC in heart and liver on d 70 by inactivating PDH kinase. In addition, the increased PDHC activity also may be due to increased enzyme protein expression and/or a change of protein expression in their regulatory enzymes (PDH kinase and PDH phosphatase), which need to be further investigated.

Lactate concentration is extremely high and reaches 10 mmol/L in fetal circulation (48). A high lactate oxidation rate has been observed in the fetal heart following the high lactate concentration in the circulation and is consistent with a high oxidative capacity measured by oxygen consumption. It has

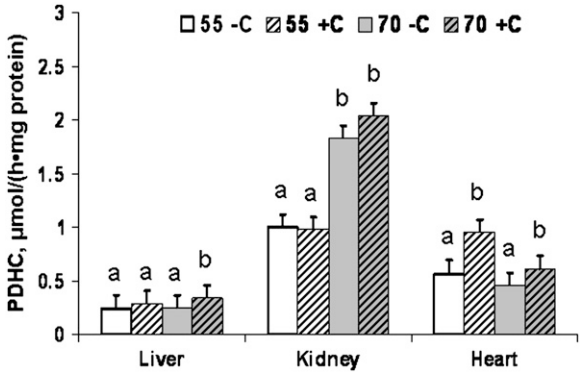

FIGURE 3 PDHC-activated activity in fetal pig tissues from pregnant dams fed control (−C) or L-carnitine–supplemented diets (+C) for 55 or 70 d of gestation. Values are least square means ± SEM, $n = 6$. Means for a tissue without a common letter differ, $P < 0.05$.

been hypothesized in adult heart that increased glucose oxidation and decreased fatty acid oxidation following L-carnitine treatment (at a given workload and constant need for ATP) is secondary to increased PDHC activity (47). Therefore, a high response of PDHC to supplementation with maternal carnitine in the fetal heart, with a potential low fatty acid oxidation rate, will increase lactate and glucose oxidation as observed in previous studies (24,49). The increase may significantly improve efficiency of energy utilization and the mechanical functions related to the heart in the fetus.

In summary, our study demonstrates that fetal pigs have a remarkably high activity of CPT with a high K_m value for carnitine in liver, heart, and kidney tissues. Supplementation with maternal L-carnitine significantly increases carnitine concentrations in fetal liver and heart, but the increase does not affect the K_m of CPT for carnitine. Carnitine concentrations measured in 55- and 70-d-old fetal tissues from the dams with or without supplementation with L-carnitine are significantly lower than K_m values for the carnitine, suggesting that carnitine might be a limited substrate for the enzyme. Maternal supplementation with L-carnitine stimulates CPT activity in fetal liver and PDHC activity in fetal heart and liver at d 70 of gestation. The stimulation may increase fatty acid and glucose oxidation and improve fetal energy metabolism, which are critical to embryonic and fetal development. Subsequently, the improvements afforded by providing a sufficient amount of carnitine may increase birth weight and postnatal growth rate, as observed previously in the newborn (50,51).

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