

# Ontogeny of Carnitine Palmitoyltransferase I Activity, Carnitine- $K_m$ , and mRNA Abundance in Pigs throughout Growth and Development<sup>1,2</sup>

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## Abstract

Carnitine palmitoyltransferase (CPT) I catalyzes an important regulatory step in lipid metabolism; however, no studies, to our knowledge, have evaluated the molecular and kinetic [maximal velocity and Michaelis constant ( $K_m$ )] for carnitine ontogeny of CPT I and prevailing tissue concentrations of carnitine in pigs. To this end, hepatic and skeletal muscle tissues were examined at various ages: birth; 24 h; 1, 3, 5, and 8 wk of age; and adult. Hepatic and skeletal muscle CPT I specific activities were low at birth and increased 100 and 70%, respectively, during the first week of life ( $P < 0.05$ ). Skeletal muscle transcript amounts were 2.7-fold greater ( $P < 0.001$ ) in 24-h-old pigs relative to newborns, whereas hepatic CPT I mRNA remained constant at each age studied. The apparent  $K_m$  for carnitine decreased 48% ( $P < 0.05$ ) during the initial 3 wk of life in liver and decreased 40% ( $P < 0.05$ ) during the first week of life in skeletal muscle. Plasma and liver free carnitine concentrations increased 95 and 62%, respectively, within 24 h after birth ( $P < 0.05$ ) and hepatic carnitine concentrations remained constant through 5 wk of age. Consequently, hepatic carnitine concentrations were 20–80% greater ( $P < 0.05$ ) than the  $K_m$  for carnitine during the suckling period. Skeletal muscle carnitine met or exceeded the apparent  $K_m$  for carnitine at each stage of development. Collectively, these findings suggest that postnatal increases in CPT I activity during the suckling period are accompanied by increased tissue carnitine; however, the lack of hepatic CPT I mRNA induction and low activity reported in both tissues prior to 1 wk of age may limit postnatal lipid utilization during the piglet's transition to extra-uterine life. *J. Nutr.* 137: 898–903, 2007.

## Introduction

Increased circulating fatty acids during postnatal development must be met with increased cellular  $\beta$ -oxidative capacity to ensure an adequate supply of energy to tissues for growth and development. Long-chain fatty acids supplied by the dam's milk are an essential energy source for several extra-hepatic tissues. In addition, they are indispensable to the liver where their metabolism corresponds with enhanced ketogenesis. Indeed, transient hyperketonemia is characteristic of the suckling neonate (1). The pig, however, does not display this postnatal hyperketonemia and the capacity of hepatic fatty acid utilization by this species has been questioned (2,3).

Because primary control of fatty acid oxidation is vested in the carnitine palmitoyltransferase (CPT)<sup>8</sup> system, most notably

CPT I (4), it is central to understanding the perinatal adaptive responses essential to postnatal fuel utilization. Mechanisms of regulation of postnatal fatty acid oxidation involve changes in maximal CPT activity, dependent on tissue carnitine availability. Increases in hepatic carnitine and CPT activity have been noted in pigs during the first 24 h of life, coincident with increased rates of  $\beta$ -oxidation (5). However, emergence of postnatal oxidation may be ascribed to mitochondrial maturation (6), not necessarily an increased reliance on fat as an energy source. Indeed, oxidative capacity is maximal at 24 h of age, with postnatal increases in hepatic CPT activity occurring in both the fed and food-deprived state (5). Recent characterization of liver and skeletal muscle CPT I expressed in *Pichia pastoris* (7,8) suggests that pig-specific CPT I isotypes are kinetically distinct from rat and human isotypes, which may contribute to species differences in postnatal lipid metabolism.

There are no publications, to our knowledge, characterizing postnatal changes in molecular or kinetic parameters of CPT in the liver and skeletal muscle of various aged pigs. Postnatal patterns of CPT that reflect dietary fat vs. carbohydrate energy sources have been established for the rat (9,10), canine (11), and feline (12) and support the role of CPT in postnatal fatty acid utilization. However, observations for pig-specific CPT I that

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<sup>2</sup> Supplemental Table 1 is available with the online posting of this paper at [jn.nutrition.org](http://jn.nutrition.org).

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<sup>8</sup> Abbreviations used: CPT, carnitine palmitoyltransferase;  $K_m$ , Michaelis constant;  $V_{max}$ , maximal velocity.

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appear to deviate from established models of fatty acid regulation in neonates (7,8,13) cautions extrapolation of observations between the species in ascertaining postnatal mechanisms of lipid handling by the latter. The aim of this study was to investigate changes in CPT I gene expression and kinetics during different stages of development that coincide with changes in dietary energy supply. The physiological relevance of kinetic changes was further established by assessing prevailing tissue carnitine concentrations. Due to the role of CPT I in fuel utilization, the fundamental knowledge gained from these findings is imperative to understanding piglet lipid metabolism, which deviates from that established in rodent models.

## Materials and Methods

**Animals and diets.** Procedures for this study were approved by the Institutional Animal Care and Use Committee of North Carolina State University. Male and female cross-bred pigs were obtained from the North Carolina State University Swine Facility at birth, 24 h, 1, 3, 5, and 8 wk of age weighing 1.2 kg ( $\pm$  0.23), 1.52 kg ( $\pm$  0.18), 3.2 kg ( $\pm$  0.24), 5.45 kg ( $\pm$  1.17), 14.12 kg ( $\pm$  2.60), and 19.57 kg ( $\pm$  5.32), respectively. Adult females were selected at a mean age of 7 mo ( $n = 5$  per each age group). Piglets selected at birth were sampled prior to suckling and additional pigs sampled were in the fed state. Three-week-old pigs removed from the sow represented the end of the suckling period. Colostrum or milk was manually expressed from the dams of suckling pigs studied and diet samples were collected for each age of pig postweaning. Diet samples ( $n = 5$  for each age group) were pooled prior to analysis. Diets for lactating dams, 8-wk and adult animals were corn-soybean meal diets manufactured by Southern States Cooperative. Lactating diets contained 14% protein, 6.6% fat, 0.85% lysine, 0.85% Ca (min), and 0.70% P; 8-wk-old pigs' diets contained 15.5% protein, 3.15% fat, 0.78% lysine, 0.67% Ca (min), and 0.56% P; and adult pigs' diets contained 14% protein, 4.15% fat, 0.63% lysine, 0.90% Ca (min), and 0.70% P. Diets fed to 5-wk-old pigs were manufactured by Renaissance Nutrition and contained 21.0% protein, 5.0% fat, 1.45% lysine, 0.6% Ca (min), and 0.7% P. Guaranteed analysis was provided by the diet manufacturer and all diets were formulated to meet or exceed requirements for vitamins and minerals.

**Tissue sampling.** Pigs were killed via American Veterinary Medical Association-approved electrocution. Liver was excised immediately and weighed. Portions of liver and semitendinosus muscle were collected and chilled on ice for enzyme analysis of fresh tissue. Duplicate tissue samples were frozen in liquid N and stored at  $-80^{\circ}\text{C}$  for determination of mRNA abundance and tissue L-carnitine concentrations.

**Measurement of L-carnitine.** Liver and skeletal muscle tissue (200 mg) were prepared using the procedure described by Bhuiyan et al. (14). Free carnitine was extracted from plasma (200  $\mu\text{L}$ ), as described by Lin and Odle (11). Dietary carnitine was extracted from pooled samples following the procedures outlined for tissues with the following modifications: 200  $\mu\text{L}$  of colostrum or milk was used in place of tissue, and for dry diet samples, 200  $\mu\text{L}$  of water was added to 100 mg of diet prior to homogenization (PowGen Homogenizer; Fisher Scientific) in 400  $\mu\text{L}$  of ice-cold 1 mol/L  $\text{HClO}_4$ . Extracted free carnitine was assayed by the enzymatic radioisotope method described by McGarry and Foster (15) and formed acetyl-carnitine was determined by liquid scintillation spectrometry (LS-6500 IC; Beckman Instruments) following elution of acetyl-carnitine from an anionic ( $\text{Cl}^-$ ) column. Tissue carnitine concentrations were corrected using wet/dry weight ratio for comparison of tissue carnitine against CPT I Michaelis constant ( $K_m$ ) for carnitine, as described previously (11).

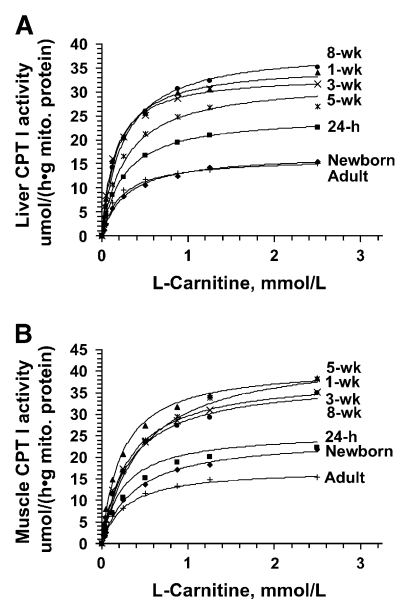
**CPT I activity.** Liver and skeletal muscle mitochondria were used to assess CPT I activity, as previously described (16). We isolated liver mitochondria by differential centrifugation, as described by Mersmann and et al. (6). Muscle mitochondria were isolated following the procedure of Power and Newshome (17) using isolation medium described

by Saggerson and Carpenter (18). We determined mitochondrial protein by the biuret method (19). Mitochondrial integrity was assessed following ADP-stimulated respiratory rates using succinate as the substrate (20). Mitochondria with respiratory rates exceeding 3 were used for determination of CPT activity. The assay measured the rate of formation of palmitoylcarnitine from palmitoyl-CoA and carnitine. Activity of CPT I was determined over a range of carnitine concentrations from 0 to 2.5 mmol/L, whereas palmitoyl-CoA was maintained at 80  $\mu\text{mol/L}$ . The CPT I activities were expressed as  $\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$  mitochondrial protein) and we generated Michaelis-Menten kinetic curves (Fig. 1) for determination of CPT I  $K_m$  for carnitine.

**CPT I mRNA analysis.** Total RNA was extracted from tissues using the guanidine isothiocyanate and phenol-based TRI Reagent solution (Sigma-Aldrich) and treated with TurboDNase (Ambion) according to the manufacturer's instructions for removal of genomic DNA. The RNA was recovered following phenol:chloroform extraction and reverse transcribed with oligo(dT) primers using Omniscript RT kit according to the manufacturer's instructions (Qiagen). Real-time quantitative PCR was performed using the DNA Engine Opticon (MJ Research), as described previously (21). We designed primers from pig-specific sequences available through GenBank and purchased from Sigma Genosys (Supplemental Table 1). Tissue-specific isoforms of CPT I were analyzed in their respective tissues. We normalized transcript amounts against the reference gene hypoxanthine phosphoribosyltransferase, which showed constitutive expression across developmental stages (data not shown) and expressed as fold of newborn according to the method of Pfaffl (22). Standard errors were determined according to Marino et al. (23).

**Reagents.** Methyl- $^3\text{H}$  carnitine (2.5 GBq/mol) and  $^3\text{H}$ -acetyl-CoA (148 MBq/mmol) were purchased from American Radiolabeled Chemicals. Palmitoyl-CoA, acetyl-CoA, acetyltransferase, and other chemicals were purchased from Sigma Chemicals. Ion-exchange resin (AG  $1 \times 8$ , 100–200,  $\text{Cl}^-$  form) was purchased from Bio-Rad Laboratories.

**Statistical analysis.** CPT I activity data were fit to Michaelis-Menten kinetic curves using the nonlinear model of SAS for determination of maximal velocity ( $V_{\text{max}}$ ) and  $K_m$  according to the equation:  $V_f = V_{\text{max}}[S]/(K_m + [S])$ . Kinetic parameters ( $V_{\text{max}}$  and  $K_m$ ) and tissue carnitine concentrations were subjected to 2-way ANOVA appropriate for a completely randomized design with age and tissue as independent variables.



**Figure 1** Ontogeny of CPT I-specific activity and Michaelis-Menten kinetic curves in liver (A) and skeletal muscle (B) of pigs. Values are means,  $n = 2-5$ . Carnitine was increased from 0 to 2.5 mmol/L. Maximal activities were obtained by 1 wk postnatal in liver (A) and skeletal muscle (B).

Protected least significant difference tests were performed for post hoc comparison of means. The age × tissue interaction also was modeled and reported when significant. We performed all computations using the general linear model procedure of SAS. To determine significance of gene expression data, we calculated relative expression ratios (22) and SEM (23). The mean ratio determined for each stage of development was compared with the ratio determined for the newborn using Student's *t* test with unequal variances. Data are reported as fold of newborn within liver and skeletal muscle tissues. Values in the text are means and significant difference was  $P < 0.05$ .

## Results

**Carnitine concentrations.** Free carnitine concentrations (nmol/g wet tissue) increased 95% in plasma during the first 24 h of life ( $P < 0.05$ ), concomitant with a marked increase in hepatic carnitine ( $P < 0.05$ ; Table 1). Whereas plasma carnitine decreased to values determined for the newborn by 1 wk of age, hepatic carnitine remained constant until 5 wk of age when concentrations were 35.5% of the value determined for the 3-wk-old pig ( $P < 0.01$ ). Subsequently, no further changes were measured for hepatic free carnitine, which remained low in the adult. Free carnitine determined in skeletal muscle remained constant from birth through adulthood (Table 1). Maximum dietary carnitine corresponded with birth and gradually decreased as lactation progressed. Diets fed postweaning corresponded to the lowest dietary carnitine concentrations (Table 1).

**CPT I kinetics.** Activity of CPT I was low in the newborn piglet at birth and 24 h of age and increased in liver and skeletal muscle (100 and 70%, respectively) between birth and 1 wk of age ( $P < 0.05$ ; Table 2). From 1 to 8 wk of age, CPT I activity remained constant in liver and skeletal muscle tissues, whereas adult values were similar to those determined for the newborn in both tissues (Table 2). Tissue differences in CPT I activity were not detected across the ages studied. Corresponding  $K_m$  estimates decreased 47% by 3 wk of life in liver and decreased 40% by 1 wk of life in skeletal muscle ( $P < 0.05$ ; Table 2). A transient increase (90%) in hepatic CPT I apparent  $K_m$  occurred during wk 3 and wk 5 ( $P < 0.05$ ), with  $K_m$  values returning to preweaning estimates by 8 wk postnatal age (Table 2). Early postweaning  $K_m$  values at 5 wk decreased 22 and 39% in liver and skeletal muscle, respectively, by adulthood ( $P < 0.05$ ). A comparison between liver and skeletal muscles CPT I apparent

**TABLE 2** Age-related changes in hepatic and skeletal muscle CPT I kinetic parameters ( $V_{max}$  and  $K_m$ ) in pigs<sup>1</sup>

Age	Liver		Skeletal muscle	
	$V_{max}, \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{g}^{-1} \text{ mitochondrial protein}^2$			
Newborn <sup>3</sup>	17.0 ± 5.5 <sup>b</sup>		24.6 ± 4.8 <sup>bc</sup>	
24 h	25.1 ± 5.5 <sup>b</sup>		25.8 ± 4.8 <sup>bc</sup>	
1 wk	35.6 ± 5.5 <sup>a</sup>		41.2 ± 4.8 <sup>a</sup>	
3 wk	33.5 ± 5.5 <sup>a</sup>		38.9 ± 4.8 <sup>ab</sup>	
5 wk	32.3 ± 5.5 <sup>a</sup>		44.4 ± 4.8 <sup>a</sup>	
8 wk	39.0 ± 5.5 <sup>a</sup>		38.0 ± 4.8 <sup>ab</sup>	
Adult	16.2 ± 5.5 <sup>b</sup>		17.3 ± 5.4 <sup>c</sup>	
	Apparent $K_m$ for L-carnitine, $\mu\text{mol/L}$			
Newborn	278 ± 29 <sup>ab</sup>		381 ± 52 <sup>ab</sup>	
24 h	254 ± 29 <sup>bc</sup>		245 ± 52 <sup>bc</sup>	
1 wk	179 ± 29 <sup>c</sup>		228 ± 52 <sup>c</sup>	
3 wk	147 ± 29 <sup>c</sup>		314 ± 52 <sup>abc*</sup>	
5 wk	280 ± 29 <sup>a</sup>		460 ± 52 <sup>a*</sup>	
8 wk	244 ± 29 <sup>bc</sup>		326 ± 52 <sup>abc</sup>	
Adult	218 ± 29 <sup>bc</sup>		280 ± 58 <sup>bc</sup>	

<sup>1</sup> Values are means ± SEM,  $n = 4-5$ . Means for a variable in a column without a common letter differ,  $P < 0.05$ . \*Different from liver,  $P < 0.05$ .

<sup>2</sup> Hepatic and skeletal muscle mitochondria with ADP stimulated respiratory rates less than 3 were excluded from analysis.

<sup>3</sup> Newborn pigs were selected prior to suckling and all other pigs were in the fed state.

$K_m$  for carnitine revealed similarities between the tissues except during wk 3 and wk 5 when the apparent  $K_m$  of CPT I for carnitine in skeletal muscle were greater (113 and 64%, respectively) than values determined for liver ( $P < 0.05$ ).

**CPT I relative transcript amounts.** We did not detect changes in hepatic CPT I transcript amounts throughout development (Fig. 2A). In contrast, relative transcript amounts of CPT I in skeletal muscle were 2.7-, 9.4-, and 10-fold greater in 24-h-, 1-, and 3-wk-old pigs, respectively, than amounts determined at

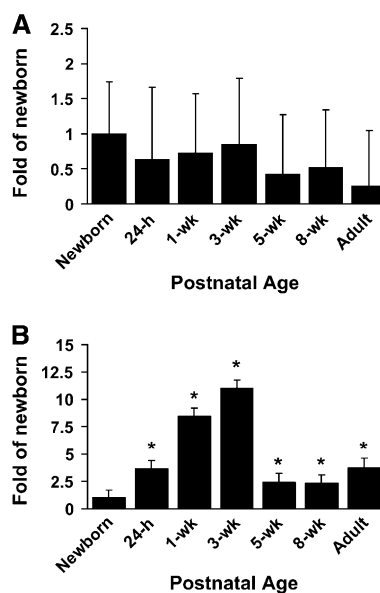
**TABLE 1** Age-related changes in free carnitine concentrations in plasma, liver, and skeletal muscle of pigs<sup>1</sup>

Age	Plasma	Free carnitine, nmol/g wet tissue			Diet <sup>2</sup>
		Liver	Skeletal muscle		
Newborn <sup>3</sup>	62 ± 17 <sup>b</sup>	189 ± 33 <sup>bc</sup>	397 ± 19	492	
24 h	121 ± 17 <sup>a</sup>	311 ± 33 <sup>a</sup>	396 ± 19	466	
1 wk	83 ± 17 <sup>b</sup>	329 ± 33 <sup>a</sup>	408 ± 19	334	
3 wk	73 ± 17 <sup>b</sup>	277 ± 33 <sup>ab</sup>	386 ± 19	250	
5 wk	84 ± 17 <sup>b</sup>	104 ± 33 <sup>c</sup>	415 ± 19	61	
8 wk	75 ± 17 <sup>b</sup>	183 ± 33 <sup>bc</sup>	369 ± 19	57	
Adult	86 ± 19 <sup>b</sup>	130 ± 37 <sup>c</sup>	390 ± 19	61	

<sup>1</sup> Plasma and tissue values are means ± SEM,  $n = 4-5$ . Means for a variable in a column without a common letter differ,  $P < 0.05$ .

<sup>2</sup> Diet samples,  $n = 5$ , were pooled prior to analysis. Carnitine was extracted from colostrum or milk samples for newborn, 24-h-, 1-, and 3-wk-old pigs. All other pigs were fed standard corn-soybean meal diets.

<sup>3</sup> Newborn pigs were selected prior to suckling and all other pigs were in the fed state.



**Figure 2** Relative transcript amounts of hepatic (A) and skeletal muscle (B) CPT I in newborn to adult pigs. Data are reported as fold of newborn pig and were normalized to hypoxanthine phosphoribosyltransferase. Bars are means ± SEM,  $n = 4-5$ . \*Different from newborn,  $P < 0.01$ .

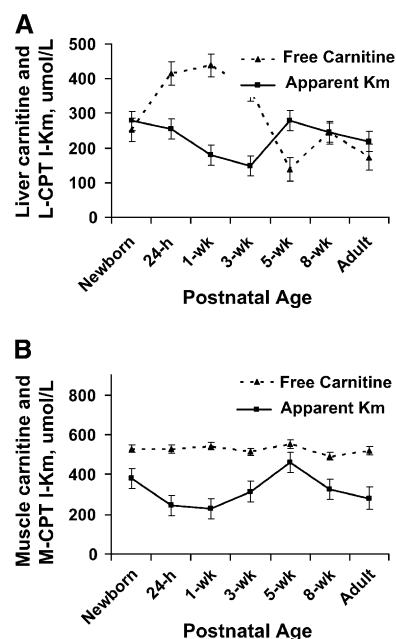
birth ( $P < 0.01$ ). Postweaning, CPT I mRNA abundance increased 140% at 5 and 8 wk of age and 270% in the adult relative to the newborn ( $P < 0.05$ ; Fig. 2B).

## Discussion

As part of an ongoing effort to elucidate the role of CPT I in the perinatal adaptive response of pigs, a molecular and enzymatic characterization of CPT I in the liver and skeletal muscle at different stages of postnatal piglet development was conducted. To our knowledge, this is the first study to evaluate the ontogeny of CPT I in pigs.

The developmental regulation that has been characterized most prominently in suckling rats (9,10) and more recently in canines (11) and felines (12) is a salient feature of CPT I. Changes in CPT I activity and maintenance of tissue carnitine in support of CPT I  $K_m$  for carnitine is an important aspect of regulation. Postnatal increases in tissue carnitine coincide with enhanced lipid utilization and are attributed to changes in dietary carnitine. Indeed, failure to suckle results in diminished tissue carnitine in neonatal rabbits (24) and rats (25) and maximal hepatic carnitine concentrations measured at birth gradually decrease with age following trends in dietary carnitine (11,26). This study suggests that initial increases in plasma and hepatic carnitine were reliant on dietary sources, whereas hepatic carnitine in pigs remained constant despite decreasing milk carnitine in late lactation and may be indicative of endogenous synthesis. Conversely, skeletal muscle carnitine was independent of dietary carnitine at all ages studied. At birth, skeletal muscle was the predominant tissue reserve for carnitine and may reflect increased capacity for carnitine uptake as well as increased retention of free carnitine. Indeed, metabolic compartmentalization of dietary carnitine differs among tissues, perhaps with carnitine deposition in skeletal muscle taking priority (27). However, the substantial increases in skeletal muscle carnitine that occur with age in humans (28) as well as felines (12) were not detected in pigs. The developmental profile determined for skeletal muscle carnitine was ascribed to postnatal hypertrophy of this tissue and its demand for carnitine to ensure adequate fatty acid oxidation for growth (11,12). The absence of developmental increases in skeletal muscle carnitine for pigs suggests that the tissue's biological demand for carnitine is met throughout development and further increases are not warranted.

To assess the metabolic demand for carnitine, we compared prevailing tissue concentrations to CPT I  $K_m$  for carnitine. In skeletal muscle, the  $K_m$  for carnitine was exceeded by tissue carnitine at all stages of development (Fig. 3B). Similarly, hepatic carnitine concentrations were greater than the apparent  $K_m$  for carnitine during the suckling period (Fig. 3A), inferring that CPT I was not limited by carnitine availability during early development. However, postweaning, the apparent  $K_m$  increased coincident with decreasing hepatic carnitine. Reciprocal shifts in CPT I  $K_m$  and tissue carnitine would ensure optimal CPT I activity as demanded by fatty acids prevailing as the energy source during the suckling period. Conversely, as the suckling-weaning transition is characterized by a transition from a diet rich in milk lipids to a carbohydrate rich energy source, the increase in CPT I  $K_m$  and decrease in hepatic carnitine may be integral to fuel-sensing regulation of CPT I. The mechanisms involved in the observed changes in CPT I  $K_m$  are unknown. In rodents, it was suggested that N-terminal motifs are responsible for determining carnitine affinities and malonyl-CoA sensitiv-



**Figure 3** CPT I  $K_m$  for carnitine and prevailing free carnitine concentrations in the liver (A) and skeletal muscle (B) of postnatal pigs. Data are means  $\pm$  SEM,  $n = 2-5$ . Tissue carnitine concentrations were corrected using wet weight/dry weight ratio (11). (A) Free carnitine concentrations in the liver exceeded the  $K_m$  during the suckling period and were 63% below the apparent  $K_m$  postweaning. (B) Skeletal muscle carnitine met or exceeded the  $K_m$  for carnitine at each stage of development.

ities of skeletal muscle and hepatic CPT I isotypes, respectively, through interactions with the enzyme's catalytic domain (29). Although not studied in pigs, similar N-terminal domains might account for carnitine affinity of the liver isotype in light of reports that the pig-specific liver isotype displays malonyl-CoA sensitivities similar to the rat muscle isotypes (7). Studies are required to verify, but it is tempting to speculate that postnatal changes in membrane fluidity may direct hepatic CPT I affinity for carnitine by altering domain interactions. Practical extrapolation of these transient postweaning changes suggests that supplemental dietary carnitine may be justified during this period.

In agreement with postnatal changes in tissue carnitine and CPT I  $K_m$  for carnitine, maximal rates of hepatic CPT I activity were reached by 1 wk of age. Similar observations were noted for skeletal muscle, which increased 70% ( $P < 0.05$ ) during wk 1 of life, in agreement with a previous study (13). Accordingly, our study suggests that postnatal maturation of the carnitine system is complete within 1 wk of life for maximal  $\beta$ -oxidative capacities. However, it should be noted that isolation of mitochondria was expected to provide a malonyl-CoA free system for assessing CPT I activity. Because malonyl-CoA is a potent inhibitor of CPT I in pigs (7), the importance of this regulatory mechanism and its role in oxidative capacity cannot be excluded. Furthermore, as liver glycogen is depleted within 48 h of birth (30) and fatty acids become the primary fuel source for the developing neonate, survival of the pig hinges on timely upregulation of the carnitine system. The lack of an immediate postnatal increase in CPT I activity noted during this study may interfere with the neonatal pig's ability to meet its energy needs, contributing to postnatal mortality in this species.

The emergence of the CPT system is coupled to profound nutritional and hormonal changes that occur in the immediate postnatal period (31). The increase in circulating fatty acids that

accompanies the transition to extra-uterine life is integral to postnatal lipid metabolism in several species. Studies in rodents (9), canines (10), and felines (12) infer that hepatic CPT activity is responsive to changes in dietary fat content. The current findings suggest that nutritional status is not the primary modifier of CPT I activity in pigs, as evidenced by the lack of an immediate postnatal increase as well as maintenance of liver and skeletal muscle CPT I activity following the transition from a lipid- to carbohydrate-rich diet that occurs at weaning (Table 2). Instead, modifications of pancreatic hormones that accompany increased circulating fatty acids may underlie postnatal CPT I profiles observed in pigs. The increased plasma glucagon and decreased insulin that prevail during the suckling period of the rodent (1) appear to exert long-term effects on CPT I activity by maintaining a reduced insulin/glucagon ratio that coincides with the early postnatal rise in CPT I activity (9). In suckling newborn pigs, postnatal changes in plasma glucagon and insulin promote an increase in the insulin/glucagon ratio during the first 48 h of life (30) and may antagonize early postnatal increases in CPT I activity.

Changes in CPT I activity may be coupled to the induction of the gene encoding for this enzyme (9,10). Although rapid accumulation of skeletal muscle CPT mRNA occurred after birth, postnatal increases in hepatic CPT I mRNA were not detected. Although the effects of dietary fatty acids on CPT I activity are controversial, marked increases in CPT I mRNA are stimulated by long-chain fatty acids (4). In skeletal muscle, transcriptional regulation of CPT I by fatty acids is mediated by peroxisome proliferator activated receptor  $\alpha$  (32). However, transcriptional activation of hepatic CPT I by fatty acids occurs independent of peroxisome proliferator activated receptor  $\alpha$  (33) and studies in rat hepatocytes indicate that fatty acid-induced accumulation of CPT I transcripts is antagonized by insulin (34). Accordingly, although fatty acids may induce CPT I transcription in skeletal muscle, postnatal induction of hepatic CPT I mRNA may be under hormonal control. Interestingly, changes in liver and skeletal muscle CPT I activity were independent of postnatal changes in gene expression. Although CPT I protein was not assessed in this study, Thumelin et al. (9) reported a developmental pattern in the concentrations of immunoreactive CPT I proteins that was closely related to the pattern established for mRNA transcript amounts. Increases in hepatic CPT I activity independent of transcriptional changes noted in our study may be indicative of posttranslational modifications. Recently, covalent modification of CPT I through phosphorylation was proposed to increase enzyme activity (35). Furthermore, although ADP-stimulated respiration rate of isolated mitochondria exceeded 3 when using succinate as the substrate, potential CPT II contamination cannot be discounted. However, although CPT II could result in an overestimation of the kinetic parameters established for CPT I, it is unlikely that contamination would support the developmental profile established in both liver and skeletal muscle. In fact, hepatic CPT II remains constant at whatever stage of development studied (9).

To our knowledge, this work provides the first developmental characterization of CPT I in pigs. Although hepatic and skeletal muscle CPT I-specific activities reach maximal rates within 1 wk of life and are supported by adequate carnitine concentrations, evidence that nutritional status is not the primary modifier of developmental shifts in CPT I for the pig is presented. As CPT I is integral to lipid utilization, the fundamental information contained herein is essential to understanding fatty acid metabolism in a species of agricultural and biomedical importance.

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