Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Nutritional Biochemistry 16 (2005) 331–338

Journal of Nutritional Biochemistry

RESEARCH ARTICLES

Ontogeny and kinetics of carnitine palmitoyltransferase in liver and skeletal muscle of the domestic felid (*Felis domestica*)☆

Xi Lin[a], Ralph House[b], Jack Odle[a,b,*]

[a]*Department of Animal Science, North Carolina State University, Raleigh, NC 27695-7621, USA*
[b]*Functional Genomics Program, North Carolina State University, Raleigh, NC 27695-7621,USA*

Received 26 July 2004; received in revised form 12 November 2004; accepted 6 December 2004

Abstract

The ontogeny of carnitine palmitoyltransferase (CPT) was examined in liver and muscle throughout growth and development of the domestic felid. Homogenates from animals in six age categories (newborn, 24-h, 3-, 6- and 9-week-old, and adult) were examined. Hepatic CPT specific activity increased progressively from birth to 6 weeks and then declined slightly into adulthood, with maximal values for animals greater than 24 h of age [171 nmol/(min g wet tissue)] being 70% higher than for newborns [99 nmol/(min g wet tissue)] ($P < .05$). Specific activity in adults was similar to that in 6- and 9-week-old juveniles. Total hepatic CPT activity [nmol/(min liver)] increased linearly with age, but the activity expressed per kg body weight [nmol/(min kg BW)] declined after 3 weeks. In contrast, skeletal muscle CPT-specific activity remained unchanged from birth to 3 weeks and then increased significantly, with maximal values at 9 weeks being 90% greater than those for young animals (newborn to 3 weeks; $P < .05$), whereas specific activity in adults was 50% lower than that observed in 9-week-old animals ($P < .05$). Hepatic and muscle apparent K_m's for carnitine averaged 440 μM and did not vary with age. Hepatic carnitine concentrations remained relatively constant during development, but were lower in adult lactating females, whereas skeletal muscle concentrations increased markedly with age. Hepatic concentrations were 20–50% higher than apparent K_m's for carnitine in young and growing animals, but concentrations were similar to the apparent K_m at 6 weeks and significantly lower than the apparent K_m in adults. Carnitine concentrations in skeletal muscle were 37% lower than apparent K_m during the neonatal period, but significantly higher in cats >3 weeks of age. We conclude that postnatal increases in CPT activity support increased capacity for fatty acid oxidation in the developing felid and that dietary carnitine may be required to maximize enzyme activity.

Keywords: Fatty acid oxidation; Feline development; Lipid metabolism; Cats; CPT

1. Introduction

Fatty acid oxidation changes dramatically during growth and development of all mammals, especially during the transition from fetal to postnatal life and at weaning. Studies of omnivorous and herbivorous species such as rats [1], pigs [2] and rabbits [3] have suggested that fatty acid oxidation increases greatly in the first 24 h after birth. Elevated fatty acid oxidation persists throughout the suckling period and decreases quickly at weaning with transition from a high fat (milk) diet to a high carbohydrate diet [4,5]. It is believed that the changes in postnatal fatty acid oxidation are predicated on developmental regulation of the carnitine-dependent acyltransferase system in which carnitine palmitoyltransferase I (CPT I; Enzyme Commission No. 2.3.1.21) facilitates transport of long-chain fatty acids across the inner mitochondrial membrane [6] for subsequent beta oxidation.

In contrast, despite their natural selection of carnitine-rich diets, surprisingly few data are available regarding carnitine status and the CPT transport system in carnivorous species. Indeed, variations in kinetics (i.e., K_m and V_{max}) and capacity of CPT are likely of heightened importance for developing felids due to their sustained reliance on high-fat, high-protein diets. For example, the fat content of feline milk increases from 17% in early lactation to 30% in late lactation [7], and whereas dietary fat decreases after

☆ This work was funded by the North Carolina Agriculture Research Service.

* Corresponding author. Department of Animal Science, North Carolina State University, Box 7621, Raleigh, NC 27695-7621, USA. Tel.: +1 919 515 4050; fax: +1 919 515 7780.

E-mail address: jack_odle@ncsu.edu (J. Odle).

0955-2863/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2004.12.012

weaning, the fat content of commercial cat food remains relatively high (25%) compared with that consumed by other species. This suggests that a high fatty acid oxidative capacity must be developed after birth, and sustained capacity is required given the relatively high-fat diet of the carnivore postweaning. Furthermore, studies with adult cats have shown a strong ability to increase fatty acid oxidation in response to increasing dietary fat [8].

Carnitine, an essential cofactor for CPT, can be synthesized de novo from trimethyl-lysine (see Ref. [9]) or may be consumed in the diet. Plants are essentially devoid of carnitine, but animal products (especially meat) are rich in carnitine [9]. De novo synthesis is inadequate in human neonates, resulting in low plasma carnitine concentrations in premature infants [10]. Therefore, dietary carnitine supplementation is advocated for infants and, similarly, may be beneficial for domestic livestock species [11–13]. Studies examining systemic carnitine distribution have shown that concentrations vary widely depending on age [14], tissue [15], physiological status [16], species [17] and dietary carnitine content [18]. Carnitine concentrations in 10-week-old kittens and adult cats were similar to values observed in other animals [19,20]; however, a full ontogeny, especially during the neonatal period, has not been reported.

In the present study, we have analyzed the kinetics (V_{max} and K_m for carnitine) of CPT and carnitine concentrations in liver and skeletal muscle homogenates throughout the development of the domestic felid, spanning from the early postnatal period, through weaning, and into adulthood. The relationship between CPT K_m for carnitine and carnitine concentrations also was evaluated to gain insight into the metabolic need for carnitine vs. the supply via the diet and/or de novo biosynthesis.

2. Methods and materials

2.1. Chemicals

L-[*N*-methyl-^{3}H]-Carnitine (60 Ci/mmol) and [1-^{14}C]-acetyl-CoA (4 mCi/mmol) were purchased from American Radiolabeled Chemicals (St. Louis, MO). Palmitoyl-CoA, acetyl-CoA, carnitine acetyltransferase (Enzyme Commission No. 2.3.1.7), L-carnitine and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Sample preparation

Timed-pregnant, domestic short-haired cats were housed in standard cat cages designed to meet PHS and AAALAC housing criteria. Cats were fed commercial cat foods (The Iams, Dayton, OH) formulated to exceed published NRC nutrient requirements [21]. Food and water were offered ad libitum. Liver and skeletal muscle tissues were collected from newborn, 24-h, 3-, 6-, 9-week-old and adult cats (n=6–12 per age group). Fresh tissues were frozen in liquid nitrogen immediately after sampling and stored at −80°C until analysis.

2.3. Carnitine palmitoyltransferase analysis

Using a hand-driven ground-glass tissue grinder, tissues (1 g for liver and 0.3 g for muscle) were homogenized with four volumes of a medium containing 220 mM mannitol, 70 mM sucrose, 2 mM HEPES and 0.1 mM EDTA (pH 7.2 at 4°C). The whole tissue homogenates were used directly for CPT (Enzyme Commission No. 2.3.1.21) analysis. The enzyme analyses were conducted at 30°C as described by Bremer et al. [22], over a range of carnitine from 0 to 2.5 mM. Briefly, homogenates (50 μL) were preincubated for 3 min in 0.95 ml of a reaction medium containing 75 mM KCl, 50 mM mannitol, 25 mM HEPES, 0.2 mM EGTA, 2.0 mM KCN, 1% fatty acid free bovine serum albumin (BSA), 1 mM dithiothreitol and a saturating concentration (80 μM) of palmitoyl-CoA. The reaction was started by addition of L-[methyl-^{3}H]-carnitine (50 μL, 4.5 μCi/μmol) and terminated by addition of 6% $HClO_4$ (2 ml) after 6 min of incubation. The labeled palmitoyl-carnitine generated from the reaction was extracted using water-saturated butanol and quantified using liquid scintillation spectrometry (Model LS 6500, Beckman Instruments, Fullerton, CA), with correction for the time-zero control. The specific activity of CPT was expressed as nmol/(min g wet tissue). The apparent kinetic constants of CPT (V_{max} and carnitine-K_m) were calculated using the iterative NLIN procedure of SAS [23], according to the Michaelis–Menten equation,

$$V_i = V_{max}[s]/(K_m + [s]) \quad (1)$$

where V_i=initial velocity, V_{max}=maximal velocity, K_m=Michaelis constant and s=substrate concentration.

2.4. Carnitine analysis

Carnitine and carnitine esters were quantified by the enzymatic radioisotope method of McGary and Foster [24] with some modifications as described by Bhuiyan et al. [25]. Tissue-free carnitine and carnitine esters were extracted with perchloric acid as described by Lin and Odle [5]. The extracted supernatants were incubated in 0.5 ml of HEPES-EDTA buffer (pH 7.3) with 25.5 nmol [1-^{14}C]-acetyl-CoA (1 μCi/μmol), 2 μmol *N*-ethylmaleimide and 1 IU carnitine acetyltransferase at 25°C for 30 min. The reaction mixture was then loaded on a 5×50-mm column packed with AG 1×8 resin (100–200 mesh, chloride form; Bio-Rad, Richmond, CA). Acetyl-carnitine produced from the reaction was separated on the column and eluted with 4 ml of water. Radioactivity in the column eluate was measured via liquid scintillation spectrometry (Model LS 6500, Beckman Instruments). Short- and long-chain acyl-carnitines were quantified as described above after alkaline hydrolysis [5].

2.5. Statistics

All data from calculations using the NLIN procedure of SAS and from chemical analyses were subjected to analysis of variance based on a completely randomized design using

the general linear model (GLM) procedure of SAS (Statistical Analysis System, Cary, NC) [23]. Values were expressed as means±pooled SE, and differences were considered significant when $P < .05$.

3. Results

3.1. Carnitine palmitoyltransferase activity

The specific activity of CPT (assayed with saturating concentrations of carnitine and palmitoyl-CoA) measured in liver tissue increased with age from newborn to 6 weeks and decreased gradually after 6 weeks (Fig. 1, top panel). The maximal enzyme activity measured in cats ≥6 weeks of age was about twofold greater than that in newborns ($P < .05$; Table 1). No differences were detected among animals older than 24 h, indicating that the greatest increase occurred during the first day of life. In contrast, the specific activity of CPT measured in skeletal muscle tissue remained low during the neonatal period and started increasing from 3 to 9 weeks (Fig. 1, bottom panel). The maximal activity observed at 9 weeks of age was twofold higher than the activity in neonates (newborn and 24 h) or adults ($P < .05$; Table 1). There were no differences detected between neonates and adults. The maximal specific activities obtained from muscle [150–334 nmol/(min g wet tissue)] were 50–70% greater than that observed in liver [100–196 nmol/(min g wet tissue)]. The apparent kinetic parameter, K_m for carnitine, did not significantly change during development in either liver or skeletal muscle (Table 1), with values ranging from 372 to 534 μM. Total liver CPT activity increased linearly with age ($P < .0001$; Fig. 2) and

Table 1
Apparent kinetic constants (V_{max} and K_m for carnitine) of carnitine palmitoyltransferase in liver and skeletal muscle of developing cats[1]

Age	Liver	Muscle
V_{max}, nmol/(min g wet tissue)		
Newborn	100.0±22.1[a]	177.5±48.8[a]
24 h	139.8±22.1[a,b]	150.6±48.8[a]
3 weeks	154.6±26.1[a,b]	197.1±52.7[a,b]
6 weeks	196.0±23.8[b]	297.1±52.7[a,b]
9 weeks	195.4±23.8[b]	334.1±48.8[b]
Adult	178.8±23.8[b]	156.4±48.8[a]
Apparent K_m for carnitine, μM		
Newborn	431.4±59.3	457.1±82.8
24 h	527.5±59.3	534.3±82.8
3 weeks	428.0±70.2	441.7±89.5
6 weeks	435.0±64.1	371.7±89.5
9 weeks	437.7±64.1	431.4±82.8
Adult	438.3±64.1	424.3±82.8

[a,b] Values within a column lacking a common letter differ, $P < .05$.
[1] Values are means±SE ($n = 6–7$). V_{max} and K_m were derived from data in Fig. 1 using the iterative NLIN procedure of SAS [23].

was correlated strongly with liver weight ($r = .9$). Indeed, total liver CPT activity measured in adult cats was 3–20 times greater than that in juveniles. When scaled per body weight, hepatic CPT activity increased from birth to 3 weeks, but remained relatively stable through 9 weeks and then declined in adults.

3.2. Carnitine concentrations

Free carnitine concentration determined in liver tissue remained relatively constant during development from birth to 9 weeks (Table 2); however, the concentration from 3 and 9 weeks (438 nmol/g wet tissue) was on average 80% greater than that observed in adult cats (230 nmol/g wet tissue). There were no significant differences among the differently aged cats for short- and long-chain acylcarnitine in hepatic tissue. Thus, the concentrations of total hepatic carnitine did not change in kittens from birth to 9 weeks (426–582 nmol/g wet

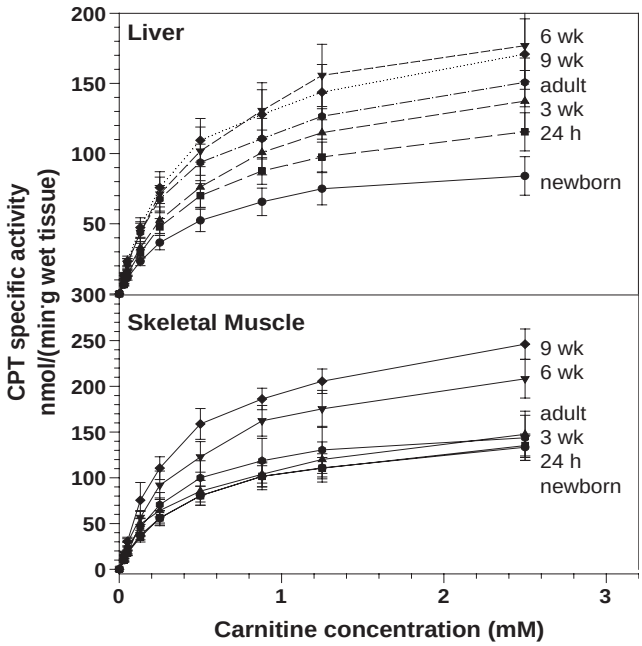

Fig. 1. Changes in carnitine palmitoyltransferase activity in liver (top panel) and skeletal muscle (bottom panel) during feline development and kinetic response to carnitine concentration. Values are means±SE, $n = 6–7$.

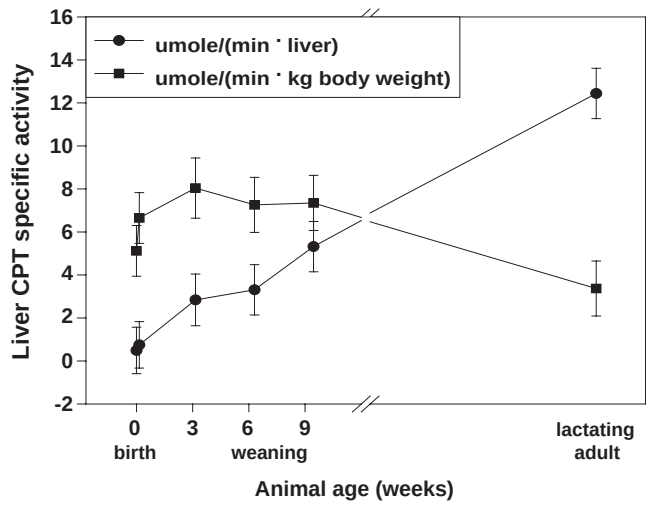

Fig. 2. Relationship between total hepatic CPT-specific activity and cat age. Values are means±SE, $n = 6–7$.

Table 2
Free and acyl-carnitine concentrations in liver and skeletal muscle of developing cats[1]

Age	*n*	Free carnitine	Short-chain acylcarnitine	Long-chain acylcarnitine	Total carnitine
Liver, nmol/g wet tissue					
Newborn	12	384.5±46.7[a,b]	78.3±20.2	60.2±8.0	523.0±53.3[a]
24 h	10	373.7±49.0[a,b]	79.5±21.2	49.9±8.4	503.1±55.9[a]
3 weeks	6	433.0±63.3[a]	24.2±27.4	31.4±10.8	477.8±72.1[a,b]
6 weeks	7	330.3±58.9[a,b]	54.8±25.3	44.7±10.0	425.5±66.8[a,b]
9 weeks	6	442.2±63.3[a]	85.2±27.4	54.9±10.8	582.3±72.1[a]
Adult	8	230.6±54.8[b]	31.0±23.7	37.3±9.4	297.8±62.5[b]
Muscle, nmol/g wet tissue					
Newborn	9	243.2±76.8[a]	60.7±72.6[a]	27.9±40.1[a]	331.9±109.5[a]
24 h	7	232.9±87.1[a]	106.5±82.3[a]	23.4±45.5[a]	363.0±124.2[a]
3 weeks	8	414.6±81.5[a]	193.8±77.0[a,b]	115.1±42.6[a,b]	723.5±116.1[b]
6 weeks	8	894.7±81.5[b]	78.2±77.0[a]	267.6±42.6[c]	1240.5±116.1[c]
9 weeks	8	865.6±81.5[b]	370.2±77.0[b]	201.1±42.6[b,c]	1436.9±116.1[c]
Adult	7	1237.9±87.1[c]	791.1±82.3[c]	201.4±45.5[b,c]	2230.4±124.2[d]

[a,b,c,d] Values within a column lacking a common letter differ, $P < .05$.
[1] Values are means±SE, n = number of observations.

tissue), but the concentrations were greater in neonates (newborn and 24 h) and 9-week-old animals than in adults (298 nmol/g wet tissue). There was no difference in the ratio of free/total carnitine (FC/TC) and acylcarnitine/free carnitine (AC/FC) in liver at any age (Table 3).

Concentrations of free and total carnitine in skeletal muscle tissue were low at birth, but increased markedly after 24 h (Table 2). By 6 weeks, the concentration of free carnitine reached 895 nmol/g wet tissue and was four times greater than the concentration in newborns ($P < .05$; Table 2). Even so, the concentration was 30% less in these young cats than in adults (1238 nmol/g wet tissue). The concentration of short-chain acylcarnitine increased progressively with age (from 60 in newborn to 790 in adult), except that the concentration observed at 6 weeks (78 nmol/g wet tissue) was similar to concentrations in the newborn. The concentration of short-chain acylcarnitine in adults was sevenfold greater than that in juveniles from birth to 6 weeks of age and two times greater than in 9-week-old animals. Concentrations of long-chain acylcarnitine also increased after birth, but remained constant after 6 weeks of age. The ratio of FC/TC remained relatively stable from birth to 9 weeks (0.63–0.76), but was 26% greater than the ratio in adults (0.54; Table 3). The ratio of AC/FC in adult cats was 2.5 times greater than in newborns, but no differences were observed among other ages. Compared with liver, FC and TC concentrations measured in skeletal muscle were lower during the first 24 h, but were significantly higher after 3 weeks.

Table 3
Concentration ratios of free/total carnitine (FC/TC) and acyl/free carnitine (AC/FC) in liver and skeletal muscle of developing cats[1]

Age	*n*	FC/TC	AC/FC
Liver			
Newborn	12	0.74±0.04	0.44±0.09
24 h	10	0.75±0.04	0.35±0.09
3 weeks	6	0.88±0.06	0.16±0.12
6 weeks	7	0.79±0.05	0.33±0.11
9 weeks	6	0.75±0.06	0.41±0.12
Adult	8	0.77±0.05	0.33±0.10
Muscle			
Newborn	9	0.76±0.06[a]	0.39±0.18[a]
24 h	7	0.65±0.07[a,b]	0.64±0.20[a,b]
3 weeks	8	0.62±0.06[a,b]	0.85±0.19[a,b]
6 weeks	8	0.74±0.06[a]	0.42±0.19[a,b]
9 weeks	8	0.63±0.06[a,b]	0.68±0.19[a,b]
Adult	7	0.54±0.07[b]	1.01±0.20[b]

[a,b] Values within a column lacking a common letter differ, $P < .05$.
[1] Values are means±SE, n = number of observations.

4. Discussion

To our knowledge, the activity and kinetic properties of CPT have not been determined in the developing domestic felid. The maximal liver CPT activity increased 40% in 24 h after birth, 55% in 3 weeks and 96% in 6 weeks. The increase was consistent with the CPT I activity observed in isolated mitochondria from other species such as rats [1], rabbits [3], pigs [2] and dogs [5]. Although the increase in CPT-specific activity was strongest early in development, the affinity of the enzyme for carnitine (K_m) did not change. Rodent studies have suggested that the postnatal increase in CPT I-specific activity may be due to induction of the gene encoding this enzyme [26]. Indeed, the concentration of the enzyme protein and CPT I mRNA increased sevenfold at birth and remained elevated during the suckling period. In contrast with CPT I, the specific activity and mRNA abundance of CPT II remained relatively constant. Furthermore, the elevation of CPT I mRNA and concomitant increase in specific activity were correlated with increased dietary fat content. The postnatal period represents a stage in which hormones and nutrients change dramatically. Milk fat content increases progressively throughout lactation in the cat [7]. Therefore, we speculate that the developmental increase in CPT-specific activity observed in cats may

likewise be due to induction of CPT I gene expression, stemming from an increase in dietary fat. However, differences in ontogenic profile of CPT were observed for cats compared with those of other species. In rodents [4] and dogs [5], CPT-specific activity decreased greatly during late suckling and at weaning, which was not observed for cats in the present study. The change in enzyme-specific activity at the suckling–weaning transition is likely triggered by the abrupt change in nutrient profile from a relatively high-fat milk diet to a lower-fat solid food. Transcription of CPT I remained high when rats were weaned to a high fat diet, whereas weaning to a high carbohydrate (low fat) diet resulted in reduced CPT I mRNA [26]. Because cats are true carnivores, dietary fat remains relatively high (and carbohydrate low), even after weaning [7]. Therefore, we consider that the maintenance of high CPT-specific activity in the felid after weaning (Fig. 2) is related to the sustained intake of dietary fat. Total hepatic CPT activity increased linearly with age due to the corresponding increase in liver weight with age. When the liver reached mature size, the total hepatic activity (scaled per body weight) decreased significantly, implying that the proportion of hepatic fatty acid oxidation declined in the adult cats compared with developing juveniles.

Compared with the maximal specific activity of liver CPT, muscle CPT was lower in the newborn, but increased significantly after 24 h. The increase remained until 9 weeks and was not affected by the suckling–weaning transition that occurred between 3 and 6 weeks. This is consistent with our previous report in dogs [5]. The muscle isoform of CPT I is distinct from the liver isoform [27], being about 60% homologous based on amino acid sequence [28]. Functionally, the muscle isoform has a higher K_m for carnitine and is more sensitive to malonyl-CoA inhibition, but the sensitivity is not altered by insulin and thyroid hormone [29]. In addition, the muscle isoform is less responsive to changes in nutritional status [30]. Dietary fatty acids during the suckling period did not affect the mRNA abundance of the enzyme in skeletal muscle, nor was it altered when rats were weaned onto low- or high-fat diets [29]. Therefore, the specific activity of CPT observed in cat (this study) and dog [5] skeletal muscle during postnatal development is in accord with the muscle isoform of CPT I gene expression reported in developing rats. This suggests that the regulation of muscle CPT I throughout development is different from the liver isoform. Also, the muscle mass of the cat expands quickly after 1 week of birth, which is congruent with the accumulation of muscle carnitine. Collectively, these observations indicate that the postnatal development of muscle CPT is associated with the physical growth of skeletal muscle and may be important in supplying energy (from fat oxidation) required to fuel growth.

Free carnitine and total carnitine concentrations in liver tissue remained relatively constant throughout the development of the felid. This profile contrasts that of developing rats [14] and dogs [5], but is very close to observations made in humans [15]. Because carnitine synthesis by neonates is limited due to low activity of butyrobetaine hydroxylase [10], tissue carnitine concentrations in the neonate are dependent on dietary carnitine (e.g., milk [31]). In rats, only 26% of the total carnitine originated from de novo synthesis from birth to 3 weeks, during which the contribution from milk carnitine increased greatly [18]. Thus, differences in liver carnitine concentration between felids and other species during early development may reflect carnitine concentration differences in the mother's milk as well as the development of butyrobetaine hydroxylase; however, neither milk carnitine nor butyrobetaine hydroxylase has been reported in cats. In addition, we observed that the carnitine concentration in liver from adult cats was only half that of developing juveniles. The adult feline hepatic carnitine concentrations were lower than the values reported by Jacobs et al. [19,20] and also lower than concentrations observed in adult dogs [5]. Milk carnitine may be derived primarily from maternal liver stores, because hepatic carnitine concentrations decrease dramatically during lactation [14]. We believe this to be the reason for the low hepatic concentrations in our adult samples, which were drawn from females at the end of lactation. The FC/TC and AC/FC ratios in plasma have been used for evaluating carnitine status, and values less than 0.7 for FC/TC and greater than 0.4 for AC/FC are considered to be abnormal [32]. Values for FC/TC and AC/FC ratios obtained from liver tissue in this study fell within this normal range.

Carnitine concentrations in cat skeletal muscle increased substantially with age. Similar results were reported in developing humans [15] and dogs [5], as skeletal muscle is the predominant body pool in all species, regardless of age [33], and is incapable of de novo carnitine synthesis. However, the concentration in skeletal muscle does not necessarily reflect plasma carnitine status, and there is no evidence indicating that skeletal muscle carnitine buffers plasma carnitine levels [19]. The large increase in skeletal muscle carnitine with age may be related to an increased capacity for carnitine uptake and retention, as well as the rapid hypertrophy of muscle tissue during growth. Skeletal muscle carnitine also has a longer turnover time compared with that in liver [9]. In addition, the AC/FC ratio in tissue is an index of the physiological state of metabolism, with a higher percentage of AC being correlated with higher fatty acid oxidative metabolism. The higher proportion of AC, especially for short-chain acylcarnitine obtained in neonatal and young cats, was consistent with the observations reported in earlier studies with cats [19] and dogs [5]. Therefore, the high percentage of AC may be due to a higher fatty acid oxidation rate occurring after birth, which has been observed also in rats, rabbits and pigs [34]. Furthermore, we noticed that the ratios of FC/TC in cats after 24-h were lower than 0.7, and the ratios of AC/TC were greater than 0.4. The latter was at least partially associated with the high percentage of AC measured in the

tissue. Because concentrations of carnitine and carnitine esters in plasma and muscle have no correlation [19], whether a low ratio of FC/TC or a high ratio of AC/FC in muscle is indicative of abnormal metabolism or carnitine insufficiency will require further investigation.

Carnitine status has been studied extensively in adult animals and developing human neonates. However, only in few cases [5,12,35] have tissue carnitine concentrations been compared with the CPT K_m for carnitine as a physiological basis for determining carnitine status/sufficiency. Depending on species and physiological status, tissue carnitine concentrations in adult animals can vary dramatically. Markedly different concentrations of carnitine might reflect the different requirements of CPT I for this substrate [36]. Further study of various tissues from adult animals of multiple species [35] found that tissue carnitine concentrations for any tissue studied were higher than the respective K_m of CPT I for carnitine, which was considerably higher than the concentration found necessary for half-maximal rates of overall fatty acid oxidation. The results demonstrated that the activity of CPT I was not limited by the carnitine availability of adult animals under normal physiological status. Recently, we evaluated the relationship between carnitine status and CPT-specific activity in liver and skeletal muscle during canine development, and found that CPT activity may be limited by carnitine availability around the time of weaning [5]. The present study used similar techniques to evaluate the relationship between carnitine K_m and carnitine status in cats. The results show that concentrations of FC were similar to or lower than the values of apparent K_m for carnitine, but the concentrations were significantly higher than the K_m for carnitine in liver from newborn, 3- and 9-week-old cats, and in muscle from 3 weeks to adulthood (Figs. 3 and 4). These findings are consistent with the results reported in other adult animal tissues by McGarry et al. [34,35], suggesting that young, growing cats are able to obtain enough carnitine to support

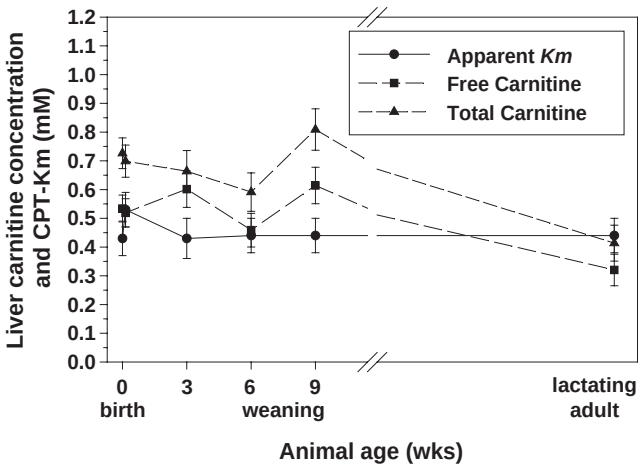

Fig. 3. Relationship between the apparent CPT K_m for carnitine and carnitine concentrations (free and total) in liver during growth and development of cats. Values are means±SE, $n=6–7$.

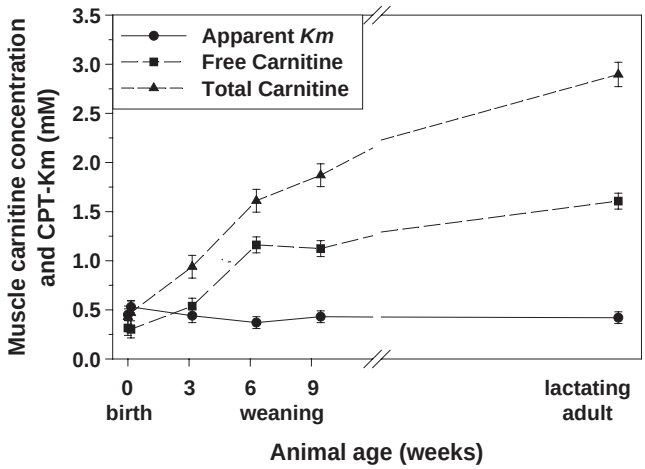

Fig. 4. Relationship between apparent CPT K_m for carnitine and carnitine concentrations (free and total) in skeletal muscle during growth and development of cats. Values are means±SE, $n=6–7$.

their requirement for the total CPT activity during development. However, we noticed that the concentration of FC and TC in liver tended to decrease at 6 weeks, and the value of FC was close to the value of apparent K_m for carnitine. Because milk is the primary source of carnitine during the neonatal period, the decrease might be due to a reduction of carnitine content in the milk near the end of lactation, which itself may be associated with the low level of hepatic carnitine observed in lactating adult. This might be indicative of a risk for carnitine insufficiency in liver during late lactation.

In addition, differences existed in liver and skeletal muscle tissue between neonatal cats and the adult female. First, in neonatal cats, the carnitine concentration was equal to (FC) or higher (TC) than the CPT K_m for carnitine in liver tissue, but the concentrations were significantly lower than the CPT K_m for carnitine in muscle. This phenomenon could be caused by low concentrations of carnitine in circulation and/or by a limited carnitine uptake capacity by skeletal muscle prior to birth. Furthermore, this might be related to the difference in fatty acid oxidative development between the two tissues (liver and muscle) because CPT-specific activity in skeletal muscle did not change greatly during the neonatal period. Second, in the adult female a reversed pattern was observed. The concentration of carnitine was lower than the CPT K_m in liver tissue, but was much higher than the CPT K_m in muscle tissue. This was probably due to carnitine transfer from liver to milk after birth, resulting in a depletion of hepatic carnitine. Apparently, the depletion could not be compensated for by carnitine in the muscle, despite carnitine accumulation in the muscle. This is supported by the conspicuous lack of correlation between muscle and plasma carnitine [19].

Beyond supplementation of carnitine in support of normal growth and development, recent studies on hepatic lipidosis in felids have shown some favorable response to supplemental carnitine [37–39]. The mechanisms underly-

ing this disorder likely involve increased fatty acid transport to the liver, increased triacylglycerol synthesis, decreased fatty acid oxidation and decreased release of very low density lipoprotein from the liver [40,41]. Insofar as carnitine is necessary for fat oxidation, its clinical efficacy in treating this important feline disorder will await further investigation.

In summary, the data reported in this study show that CPT-specific activity increased with age of the domestic felid. The developmental pattern of the enzyme showed only a minor difference between liver and muscle tissue. The CPT-specific activity peaked at 6 weeks in liver and 9 weeks in skeletal muscle, suggesting that the increase of fatty acid oxidative capacity occurring in skeletal muscle was delayed as compared to that in liver. The CPT K_m for carnitine remained relatively constant for both liver and skeletal muscle, although specific activity of the enzyme increased greatly in both tissues. Carnitine status in liver did not vary much during development, but the concentrations of FC and TC decreased after 6 weeks, and the FC was close to the CPT K_m for carnitine. This may be associated with a depletion of liver carnitine in late lactation in which the FC and TC concentration was about 60% lower than the CPT K_m for carnitine. The concentrations of FC and TC in skeletal muscle increased greatly with age and were significantly higher than the CPT K_m for carnitine after 3 weeks, but the proportion of acylcarnitine was especially high in the adults. Collectively, we infer that supplementation of carnitine to the maternal diet may benefit both the developing juveniles as well as the lactating mother.

Acknowledgments

We thank R. K. Buddington (Mississippi State University) for providing tissues for this study and M. Watts for her assistance with sample analysis.

References

[1] Augenfeld J, Fritz IB. Carnitine palmitoyltransferase activity and fatty acid oxidation by livers from fetal and neonatal rats. Can J Biochem 1970;48:288–94.

[2] Bieber LL, Markwell MAK, Blair M, Helmrath TA. Studies on the development of carnitine palmitoyltransferase and fatty acid oxidation in liver mitochondria of neonatal pigs. Biochim Biophys Acta 1973;326:145–54.

[3] Herbin C, Pegorier JP, Duee PH, Kohl C, Girard J. Regulation of fatty acid oxidation in isolated hepatocytes and liver mitochondria from newborn rabbits. Eur J Biochem 1987;165:201–7.

[4] Decaux JF, Ferre P, Robin D, Robin P, Girard J. Decreased hepatic fatty acid oxidation at weaning in the rat is not linked to a variation of malonyl-CoA concentration. J Biol Chem 1988;263:3284–9.

[5] Lin X, Odle J. Changes in kinetics of carnitine palmitoyltransferase in liver and skeletal muscle of dogs (*Canins familiaris*) throughout growth and development. J Nutr 2003;133:1113–9.

[6] McGarry JD, Woeltje KF, Kuwajima M, Foster DW. Regulation of ketogenesis and the renaissance of carnitine palmitoyltransferase. Diabetes Metab Rev 1989;5:271–84.

[7] Lepine AJ. Nutrition of the neonatal canine and feline. In: Reinhart GT, Carey DC, editors. Recent Advances in Canine and Feline Nutrition. Wilmington (Ohio): Orange Frazer Press; 1998. p. 249–56.

[8] Lester T, Czarnecki-Maulden G, Lewis D. Cats increase fatty acid oxidation when isocalorically fed meat-based diets with increasing fat content. Am J Physiol 1999;277:R878–86.

[9] Rebouche CJ, Paulson DJ. Carnitine metabolism and function in humans. Annu Rev Nutr 1986;6:41–66.

[10] Penn D, Schmidt-Sommerfeld E, Pascu F. Decreased tissue carnitine concentrations in newborn infants receiving total parenteral nutrition. J Pediatr 1981;98:976–8.

[11] Heo K, Odle J, Han IK, Cho W, Seo S, VanHeugten E, et al. Dietary L-carnitine improves nitrogen utilization in growing pigs fed low energy, fat-containing diets. J Nutr 2000;130:1809–14.

[12] Heo K, Lin X, Odle J, Han IK. Kinetics of carnitine palmitoyltransferase-I are altered by dietary variables and suggest a metabolic need for supplemental carnitine in young pigs. J Nutr 2000;130:2467–70.

[13] Heo KN, Lin X, Han IK, Odle J. Medium-chain fatty acids but not L-carnitine accelerate the kinetics of [^{14}C]triacylglycerol utilization by colostrum-deprived newborn pigs. J Nutr 2002;132:1989–94.

[14] Robles-Valdes C, McGarry JD, Foster DW. Maternal–fatal carnitine relationship and neonatal ketosis in the rat. J Biol Chem 1976;251:6007–12.

[15] Nakano C, Takashima S, Takeshita K. Carnitine concentration during the development of human tissues. Early Hum Dev 1989;19:21–7.

[16] Seccomb DW, Hahn P, Novak M. The effect of diet and development on blood levels of free and esterified carnitine in the rat. Biochim Biophys Acta 1978;448:562–77.

[17] Alhomida AS, Duhaiman AS, Al-Jafari AA, Junaid MA. Determination of L-carnitine, acylcarnitine and total carnitine levels in plasma and tissues of camel (*Camelus dromedarius*). Comp Biochem Physiol 1995;111B:441–5.

[18] Davis AT. Fractional contributions to total carnitine in the neonatal rat. J Nutr 1989;119:262–7.

[19] Jacobs G, Keene B, Cornelius LM, Rakich P. Plasma, tissue, and urine carnitine concentrations in healthy adult cats and kittens. Am Vet Res 1990;51:1345–8.

[20] Jacobs G, Cornelius L, Keene B, Rakich P. Comparison of plasma, liver, and skeletal muscle carnitine concentrations in cats with idiopathic hepatic lipidosis and in healthy cats. Am J Vet Res 1990;51:1349–51.

[21] NRC. Nutrient requirements of cats. Revised ed. Washington (DC): National Academy Press; 1986.

[22] Bremer J, Woldegiorgis G, Schalinske K, Shrago E. Carnitine palmitoyltransferase activation by palmitoyl-CoA and inactivation by malonyl-CoA. Biochem Biophys Acta 1985;833:9–16.

[23] SAS. SAS/STAT user's guide (version 6). 4th ed. Cary (NC): SAS Institute; 1989.

[24] McGarry JD, Foster DW. An improved and radioisotopic assay for the determination of free and esterified carnitine. J Lipid Res 1976;17:277–81.

[25] Bhuiyan AK, Jackson S, Turnbull DM, Aynsley-Green A, Leonard JV, Bartlett K. The measurement of carnitine and acyl-carnitines: Application to the investigation of patients with suspected inherited disorders of mitochondrial fatty acid oxidation. Clin Chim Acta 1992;207:185–204.

[26] Thumelin S, Esser V, Charvy D, Kolodziej M, Zammit VA, McGarry D, et al. Expression of liver carnitine palmitoyltransferase I and II genes during development in the rat. Biochem J 1994;300:583–7.

[27] Winder WW, Arogyasami J, Barton RJ, Elayan IM, Vehrs PR. Muscle malonyl-CoA decreases during exercise. J Appl Physiol 1989;67:2230–3.

[28] Morillas M, Gómez-Puertas P, Bentebibel A, Sellés E, Casala N, Valencia A, et al. Identification of conserved amino acid residues in rat

liver carnitine palmitoyltransferase I critical for malonyl-CoA inhibition. J Biol Chem 2003;278:9058–63.
[29] Cook GA, Edwards TL, Jansen MS, Bahouth SW, Wilcox HG, Park EA. Differential regulation of carnitine palmitoyltransferase-I gene isoforms (CPT-I α and CPT β) in the rat heart. J Mol Cell Cardiol 2001;33:317–29.
[30] Mynatt RL, Lappi MD, Cook GA. Myocardial carnitine palmitoyltransferase of the mitochondrial outer membrane is not altered by fasting. Biochim Biophys Acta 1992;1128:105–11.
[31] Takahashi M, Sawaguchi S. Lipid metabolism in parenterally alimented neonates: Carnitine blood concentrations and fat utilization. Indian J Pediatr 1983;50:161–8.
[32] Böhles H, Evangeliov A, Bervoets K, Eckert I, Sewell AC. Carnitine esters in metabolic disease. Eur J Pediatr 1994;153:s57–61.
[33] Shenai JP, Borum PR. Tissue carnitine reserves of newborn infants. Pediatr Res 1984;18:679–82.
[34] Girard J, Duee PH, Ferre P, Pegorier JP, Escriva F, Decaux JF. Fatty acid oxidation and ketogenesis during development. Reprod Nutr Dev 1985;25:303–19.
[35] McGarry JD, Mills SE, Long CS, Foster DW. Observations on the affinity for carnitine, and malonyl-CoA sensitivity, of carnitine palmitoyltransferase I in animal and human tissues. Demonstration of the presence of malonyl-CoA in non-hepatic tissues of the rat. Biochem J 1983;214:21–8.
[36] Long CS, Haller RG, Foster DW, McGarry JD. Kinetics of carnitine-dependent fatty acid oxidation: Implications for human carnitine deficiency. Neurology 1982;32:663–6.
[37] Blanchard G, Paragon BM, Miliat F, Lutton C. Dietary L-carnitine supplementation in obese cats alters carnitine metabolism and decreases ketosis during fasting and induced hepatic lipidosis. J Nutr 2002;132:204–10.
[38] Center SA, Harte J, Watrous D, Reynolds A, Watson TD, Markwell PJ, et al. The clinical and metabolic effects of rapid weight loss in obese pet cats and the influence of supplemental oral L-carnitine. J Vet Intern Med 2000;15:598–608.
[39] Ibrahim WH, Bailey N, Sunvold GD, Bruckner GG. Effects of carnitine and taurine on fatty acid metabolism and lipid accumulation in liver of cats during weight gain and weight loss. Am J Vet Res 2003;64:1265–77.
[40] Dimski DS. Feline hepatic lipidosis. Vet Med Surg (Small Anim) 1997;12:28–33.
[41] Ibrahim WH, Szabo J, Sunvold GD, Kelleher JK, Bruckner GG. Effect of dietary protein quality and fatty acid composition on plasma lipoprotein concentrations and hepatic triglyceride fatty acid synthesis in obese cats undergoing rapid weight loss. Am J Vet Res 2000;61:566–72.