

Medium-Chain Fatty Acids but Not L-Carnitine Accelerate the Kinetics of [¹⁴C]Triacylglycerol Utilization by Colostrum-Deprived Newborn Pigs¹

Kinam N. Heo,² Xi Lin, In K. Han*³ and Jack Odle⁴

Department of Animal Science, North Carolina State University, Raleigh, NC 27695-7621 and *Department of Animal Science and Technology, Seoul National University, Suwon 441-744, South Korea

ABSTRACT The effect of L-carnitine on in vivo fatty acid utilization was determined using colostrum-deprived newborn piglets fed emulsified triglycerides (TG) composed of [1-¹⁴C]octanoate (tri-8:0) or [1-¹⁴C]octadecanoate (tri-18:1). A soy protein-based liquid diet devoid of L-carnitine was fed piglets for 1 d to allow development of fatty acid-metabolizing enzymes and intestinal fat digestion and absorption before assessment of in vivo fat utilization. The radiolabeled TG were fed in isoenergetic amounts (97.7 kJ/kg^{0.75}), with or without L-carnitine (1 mmol/kg^{0.75}) as 30% (v/v) emulsions, using polyoxyethylene sorbitan monooleate as an emulsifier. Expired CO₂ was quantified and specific radioactivity (Bq/μmol) was determined at 20-min intervals over 24 h. The rate (mmol ATP·kg^{-0.75}·min⁻¹) and extent (mol ATP/kg^{0.75}) of TG oxidative utilization (i.e., composite of digestion, absorption and oxidation) were calculated from the kinetics of ¹⁴CO₂ expiration. The maximal rate and extent of tri-8:0 oxidation were three and fourfold greater than those of tri-18:1, respectively (*P* < 0.001), and tri-18:1 delayed the time to reach 10 and 50% of maximal oxidation rate by 1.2 and 1.9 h (*P* < 0.01, respectively), regardless of supplemental carnitine. Collectively, these findings quantify the accelerated oxidation of medium-chain vs. long-chain triglycerides, but fail to support a need for supplemental carnitine to maximize fat oxidation in colostrum-deprived piglets. *J. Nutr.* 132: 1989–1994, 2002.

KEY WORDS: • pigs • neonate • carnitine • triglycerides • fatty acid utilization

The central role of fat metabolism during the early neonatal period of all mammals is unequivocal (1). The predominant fuel sources provided to the fetus, in utero, are glucose, lactate and amino acids (2). In contrast, at birth, the metabolism of the newborn must rapidly adjust to accommodate the use of fat as a primary fuel because up to 70% of milk energy is contributed by fat (1). This nutritional/biochemical adaptation is paramount to the survival of the newborn, whose metabolic rate increases two- to threefold at birth. Indeed, nutrient composition of newborn pigs reveals negligible body fat (< 2%) (3) and little stored glycogen (4), indicating that immediate and efficient exogenous energy sources are needed during this critical life stage. Research using preparations of emulsified medium-chain triglycerides (MCT⁵) (3) has shown that neonatal piglets can digest and absorb MCT, and oxidize the medium-chain fatty acids (5), but oral doses of nonemulsified LCT are not efficiently absorbed and utilized by neonatal piglets (6).

The established biochemical role of carnitine is in the transport of long-chain fatty acids across the inner mitochondrial membrane (7). Carnitine is a cosubstrate of carnitine palmitoyltransferase I (CPT-I), and the carnitine status could plausibly have an impact on the use of LCT as a metabolic fuel (8–10). Furthermore, some investigators have shown the stimulatory effects of carnitine on octanoate oxidation by rat skeletal muscle (11), whereas others have suggested that oxidation of medium-chain fatty acids (C6 to C12) should be carnitine independent because they are presumably activated to their CoA thioesters within the mitochondrial matrix (12). In addition, carnitine and carnitine acetyltransferase provide a mechanism to modulate the acetyl-CoA to free CoA ratio to further oxidation by regenerating free CoA during fasting, high fat feeding and exercise (13).

Clinical concerns regarding the management of hypoglycemic patients and premature neonates led to the use of MCT and/or carnitine to enhance energetic supply during parenteral nutrition (14–18). However, other investigators have questioned the clinical benefits of MCT (19–22). Likewise, interest in production agriculture resulted from the desire to decrease mortality—asccribed to inadequate energy supply—of low-birth-weight piglets (23). Even though several in vitro and in vivo studies with neonatal pigs have shown increases in fatty acid oxidation upon carnitine supplementation (15,16,24,25), studies have yet to be conducted to confirm these effects under more practical conditions that may be extended for use in medical and agricultural sciences.

¹ Supported in part by the North Carolina Agriculture Research Service, Raleigh, NC; the Ministry of Agriculture & Forestry-SGRP/HTDP in Korea; and the U.S. Department of Agriculture National Research Initiative (grant no. 98-35206-6645).

² Present address: Macrogen, Inc., Seoul 110-061, South Korea.

³ Present address: Korean Academy of Science and Technology, Seoul 135-703, South Korea.

⁴ To whom correspondence should be addressed.

E-mail: jack_odle@ncsu.edu.

⁵ Abbreviations used: CPT-I, carnitine palmitoyltransferase I; LCT, long-chain triglycerides; MCT, medium-chain triglycerides; TG, triglycerides.

Specifically, experiments herein examined how fatty acid chain length and supplemental L-carnitine would affect the maximal rate and extent of oxidation of enteral [$1\text{-}^{14}\text{C}$]triglycerides (TG) by 1-d-old colostrum-deprived pigs. It is the first study to directly evaluate carnitine effects on in vivo fatty acid oxidation of piglets fed TG of various fatty acid chain lengths (medium vs. long). The data will show that fatty acid chain length considerably affects the kinetics of [$1\text{-}^{14}\text{C}$]TG utilization of newborn pigs, but that carnitine supplementation produced no detectable effect.

MATERIALS AND METHODS

Animals and diet. All animal procedures were approved by the Institutional Animal Care and Use Committee of North Carolina State University. Colostrum-deprived newborn pigs were obtained from the Lake Wheeler Field Laboratory of North Carolina State University. A total of 20 piglets (1.51 ± 0.04 kg, mean \pm SEM) were removed from the sow at birth, before suckling, in five replicates. A soy protein-based liquid diet⁶ devoid of L-carnitine was orogastrically gavaged to piglets for 13 h to allow time for early-postnatal development of fat digestive and metabolic capacity (5). Water was added to reconstitute the powder to 120 g/L. Negligible amounts of total carnitine were detected ($0.2 \mu\text{mol/L}$ of liquid diet) using the enzymatic radioisotope method (26), as modified by Heo et al. (9). Piglets were fed 1.6 times their basal metabolizable energy requirement ($8.2 \text{ kJ}\cdot\text{kg}^{-0.75}\cdot\text{h}^{-1}$) every h (0 to 5 h) and every 2 h (7 to 13 h), based on Kempen and Odle (16). Food was withheld for 6 h before initiation of the in vivo fatty acid oxidation study.

Triglyceride preparations. Radiolabeled trioctanoylglycerol (tri-8:0) was synthesized from glycerol and [$1\text{-}^{14}\text{C}$]octanoate (27), using *p*-toluenesulfonic acid (Sigma Chemical, St. Louis, MO) as catalyst. Reactants were refluxed at 135°C under N_2 for 72 h, and residual unreacted glycerol and fatty acids were extracted with an alkaline/ethanolic wash. Radiochemical purity of tri-8:0 was examined by high performance liquid chromatography (HPLC) (28) using a Millennium 2010 Chromatography Manager system (Waters Associates, Milford, MA) connected to a model A265 Radiomatic β -flow detector (Packard Instruments, Meriden, CT). The HPLC effluent (1 mL/min) was mixed with Bio-Safe II scintillation cocktail (3 mL/min ; Research Products International, Mount Prospect, IL) immediately prior to entry into the counting chamber of the β -flow detector. Purity of the synthesized tri-8:0 was $> 99\%$ as judged from the radio-HPLC chromatograms. [$1\text{-}^{14}\text{C}$]Trioctadecanoylglycerol (tri-18:1) was obtained from American Radiolabeled Chemicals (St. Louis, MO). Specific radioactivity of the triacylglycerols was adjusted to 35.9 kBq/mmol for tri-8:0 and 81.1 kBq/mmol for tri-18:1 using nonradioactive triacylglycerols (Sigma Chemical). Fresh oil-in-water emulsions (30% lipid, v/v) of each triacylglycerol were prepared (29) using 20 g Tween 80/L as the emulsifying agent. Because L-carnitine uptake by intestinal epithelial cells involves a carrier-mediated system that is Na^+ dependent, 9 g NaCl/L was used in preparing the emulsions (30).

Infusion and sampling. After piglets were gastrically infused with isoenergetic amounts of radiolabeled tri-8:0 ($6.5 \text{ mmol/kg}^{0.75}$) or tri-18:1 ($2.94 \text{ mmol/kg}^{0.75}$) with or without L-carnitine ($1 \text{ mmol/kg}^{0.75}$; Sigma Chemical), marking time zero of the experiment, they

were subsequently positioned into one of four respiration chambers designed for continuous collection of expired CO_2 (16). Each chamber measured 8.4 L in volume. Piglets rapidly acclimated to the chambers and were quietly resting within 10 min. The temperature of the chambers was maintained at 35°C for the duration of the 24-h experiment, and air flow through each chamber was maintained at 2.0 L/min . Total expired $^{14}\text{CO}_2$ was collected in 1.8 mol NaOH/L (16) over continuous 20-min periods throughout the first 12 h. During the second 12 h, CO_2 collection was reduced to one 20-min sampling each hour. The amount and specific radioactivity of expired CO_2 were determined after precipitation as BaCO_3 (31) and used to calculate the rate and extent of triacylglycerol oxidative utilization (i.e., composite of digestion, absorption and oxidation) throughout the 24-h experiment. The tri-8:0 dosage ($6.5 \text{ mmol/kg}^{0.75}$) was based on Odle et al. (28), as the maximal, nontoxic oral gavage. Similarly, the carnitine dosage ($1.0 \text{ mmol/kg}^{0.75}$) was selected based on a previous study (32), which determined the relationship between oral carnitine dose, plasma carnitine fractions and renal thresholds. The plasma-free carnitine (throughout 20-h postdosing) of piglets gavaged with $0.5 \text{ mmol/kg}^{0.75}$ of carnitine and $6.5 \text{ mmol/kg}^{0.75}$ of tri-8:0 was threefold higher than that of piglets not receiving supplemental carnitine (35.6 vs. $8.3 \mu\text{mol/L}$), but was less than the renal threshold (46.4 vs. $2.0 \mu\text{mol/L}$). Based on this, we reasoned that a dosage of $1.0 \text{ mmol/kg}^{0.75}$ would provide ample carnitine in the current study. At the end of each trial, piglets were killed by sodium pentobarbital overdose (200 mg/kg , i.v.).

Calculations and statistics. Piglets were allotted to one of four treatments according to a 2×2 factorial, randomized complete block design with five replicates (33): 1) tri-8:0, 2) tri-8:0 with L-carnitine, 3) tri-18:1, 4) tri-18:1 with L-carnitine. Oxidation data were analyzed using the general linear models (GLM) procedure of SAS (SAS Institute, Cary, NC) with an additional split-plot in time (33). One piglet from the tri-8:0 with L-carnitine group was excluded from the data because it died after 2.5 h into the experiment.

The transfer quotient, defined as the fraction of CO_2 -carbon derived from TG was estimated by dividing the specific radioactivity ($\text{Bq}/\mu\text{mol}$) of expired CO_2 at each sampling time by the specific radioactivity of the infused TG ($\text{Bq}/\mu\text{mol}$ of TG), with the assumption that the $1\text{-}^{14}\text{C}$ reflected the fate of all carbon in the TG molecule. The amount of CO_2 expired ($\mu\text{mol/min}$) at each sampling time was multiplied by the respective transfer quotient to estimate the rate of conversion of the TG to CO_2 , which was scaled to the metabolic body size ($\text{kg}^{0.75}$) of each respective pig ($\mu\text{mol MCT}\cdot\text{kg}^{-0.75}\cdot\text{min}^{-1}$). To correct for differences in molar energy content between tri-8:0 and tri-18:1, data were subsequently expressed in terms of ATP yield ($\text{mmol ATP}\cdot\text{kg}^{-0.75}\cdot\text{min}^{-1}$), assuming that 205 and 454 mol of ATP were derived from the complete oxidation of tri-8:0 and tri-18:1, respectively. The isotopic flux rates represented the composite rates of TG digestion, absorption and oxidation to CO_2 and were generally termed "oxidative utilization." Cumulative oxidative utilization curves were established by summation of TG utilization over time, which was subsequently divided by energy intake and expressed as percentage. A four-parameter logistic equation (34) was fitted to the data for each piglet using the NLIN procedure of SAS. The overall extent of oxidative utilization was estimated by extrapolation of the logistic curves to $t = \infty$. The times and magnitude of peak TG utilization, along with the extrapolated extent of oxidation, also were analyzed as a randomized complete block design with a 2×2 factorial arrangement of treatments (fatty acid chain length \times L-carnitine), using the GLM procedure of SAS. Significant differences were accepted at $P < 0.05$.

RESULTS

The expiration rate of CO_2 (Fig. 1A) was not affected by treatment ($P > 0.2$), but decreased by 22% over 24 h. The specific radioactivity of expired CO_2 from the pigs fed tri-8:0 peaked at $\sim 3 \text{ h}$, remained elevated through roughly 6 h and declined gradually over 24 h (Fig. 1B). In contrast to the tri-8:0, the specific radioactivity of the expired CO_2 from pigs fed tri-18:1 peaked near 5 h and declined more gradually. Effects of fatty acid chain length and an interaction between

⁶ Each kilogram of diet (dry matter basis) contained 338 g isolated soy protein, 148.1 g soybean oil, 341.1 g lactose, 50 g sucrose, 4.1 g L-lysine, 0.7 g L-histidine, 0.7 g L-leucine, 1.7 g L-valine, 2.8 g DL-methionine, 0.3 g L-tryptophan, 1.6 g L-threonine, 10 g xanthan gum, 13.2 g CaCO_3 , 43.3 g dicalcium phosphate, 5 g mineral mixture, 1.3 g vitamin mixture, 10 g lecithin, 8.3 g NaCl, 0.5 g MgSO_4 , 4.5 g K_2HPO_4 . The calculated nutrient composition of diet per kilogram was 16.14 MJ metabolizable energy, 300.9 g protein, 150.1 g fat, 343.8 g lactose, 15 g Ca, 10.3 g P, 3.5 g Na, 5.1 g Cl, 3.0 g K, 0.4 g Mg, 23.2 g arginine, 4.0 g cysteine, 8.3 g histidine, 14.4 g isoleucine, 23.1 g leucine, 21.1 g lysine, 6.2 g methionine, 14.7 g phenylalanine, 12.3 g threonine, 4.0 g tryptophan, 10.5 g tyrosine, 15.9 g valine, 14.4 mg retinyl acetate, 0.21 mg cholecalciferol, 46.8 mg α -tocopherol, 326.9 mg vitamin C, 10.6 mg riboflavin, 42 mg niacin, 0.08 mg biotin, 6.5 mg pyridoxine, 38.1 mg pantothenic acid, 0.056 mg vitamin B-12, 27 mg Mn, 146 mg Fe, 129 mg Zn, 14 mg Cu, 2 mg I, 1 mg Co, 0.35 mg Se.

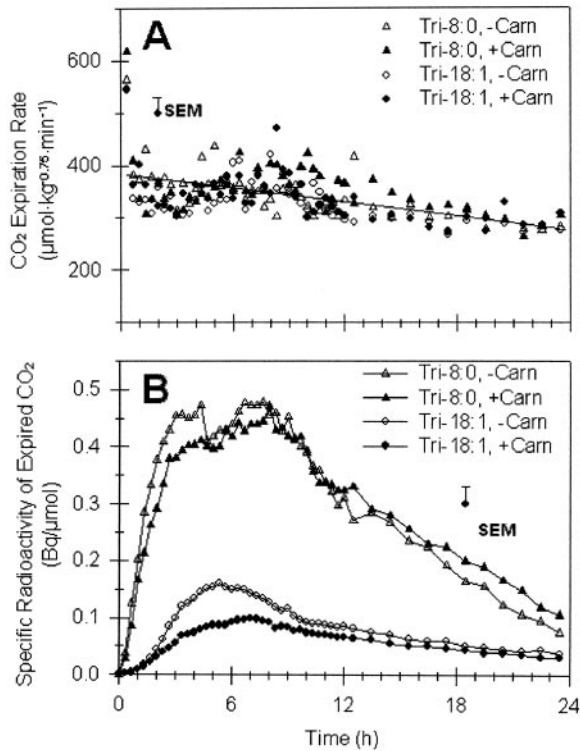


FIGURE 1 Expiration rate (A) and specific radioactivity (B) of expired CO_2 by neonatal piglets over 24 h after feeding $6.5 \text{ mmol/kg}^{0.75}$ of $[1\text{-}^{14}\text{C}]\text{tri-8:0}$ (35.9 kBq/mmol) or $2.94 \text{ mmol/kg}^{0.75}$ of $[1\text{-}^{14}\text{C}]\text{tri-18:1}$ (81.1 kBq/mmol) with or without $1 \text{ mmol/kg}^{0.75}$ of L-carnitine as a 30% (v/v) oil-in-water emulsion. Error bars represent pooled SEM for four or five observations per point. Effects on CO_2 expiration rate: linear decline over time ($P < 0.001$), unaffected by treatment ($P > 0.1$). Effects on CO_2 -specific radioactivity: fatty acid chain length effect ($P < 0.001$), fatty acid chain length \times time interaction ($P < 0.001$).

fatty acid chain length and time were observed for the entire duration after feeding ($P < 0.001$). However, carnitine supplementation was without effect ($P > 0.1$).

The data in Figure 1 were used to compute TG oxidative utilization rates (i.e., composite of digestion, absorption and oxidation), as shown in Figure 2, to provide a more effective assessment of fatty acid chain length and carnitine effects. Figure 2A illustrates the time course of TG oxidative utilization ($\text{mmol ATP}\cdot\text{kg}^{-0.75}\cdot\text{min}^{-1}$), which showed treatment effects similar to those in Figure 1B (i.e., fatty acid chain length effect and a fatty acid chain length \times time interaction, $P < 0.001$). To further compare the time course of TG oxidative utilization rates, the maximal utilization rate and observed times for corresponding rates (10, 50 and 100% of maximal utilization rate) were determined for each piglet (Tables 1 and 2). Because peak oxidative utilization rates rose at slightly different times among piglets within a treatment, these data differ from those of Figure 2A, which were averaged within the sampling time. The magnitude of maximal oxidative utilization rate was threefold higher for tri-8:0 than that for tri-18:1 ($P < 0.001$, Table 1). The time of maximal oxidative utilization was $6.8 \pm 0.9 \text{ h}$ after feeding and was not affected by fatty acid chain length or carnitine ($P > 0.4$, Table 2). On the other hand, the time of the peak in oxidative utilization of tri-8:0 preceded by 2.2 h that of tri-18:1 ($2.6 \pm 0.4 \text{ h}$ vs. $4.8 \pm 0.3 \text{ h}$, $P < 0.001$), which was similar to data in Figure 1B. Likewise, increased chain length delayed the time to reach 10 and 50% of maximal utilization rate by 1.2

and 1.9 h ($P < 0.001$ and $P < 0.01$, respectively). Based on these kinetic data, tri-8:0 was oxidized at a rate greater than 50% of the maximal oxidative utilization rate from 2 to 10 h after feeding. However, 50% of the maximal oxidative utilization rate was reached at 4 h and was maintained for only 6.5 h in tri-18:1-fed piglets. In contrast to the time course before the maximal rate was reached, the times to descend to 50 and 25% of the maximal utilization rate were not affected by fatty acid chain length ($P > 0.05$), and the slopes of utilization observed during the declining phase were more moderate than those observed previously (28).

To measure the overall extent of TG oxidative utilization, the areas under the utilization curves in Figure 2A were estimated by summation of the data and fitting logistic curves (Fig. 2B). The extent of TG oxidative utilization was fourfold greater for tri-8:0 than that for tri-18:1 ($P < 0.001$), but was not affected by carnitine ($P = 0.9$). When expressed as the percentage of energy intake, tri-8:0 and tri-18:1 were utilized 70 and 14.6%, respectively.

DISCUSSION

Orogastric vs. parenteral nutrition. Despite the substantial biochemical basis for formulas containing MCT, recent research has yet to confirm the putative advantages of MCT within total parenteral nutrition regimens used in clinical studies (20–22). Compared to LCT emulsions, MCT/LCT (50/50%) emulsions did not increase lipid oxidation in criti-

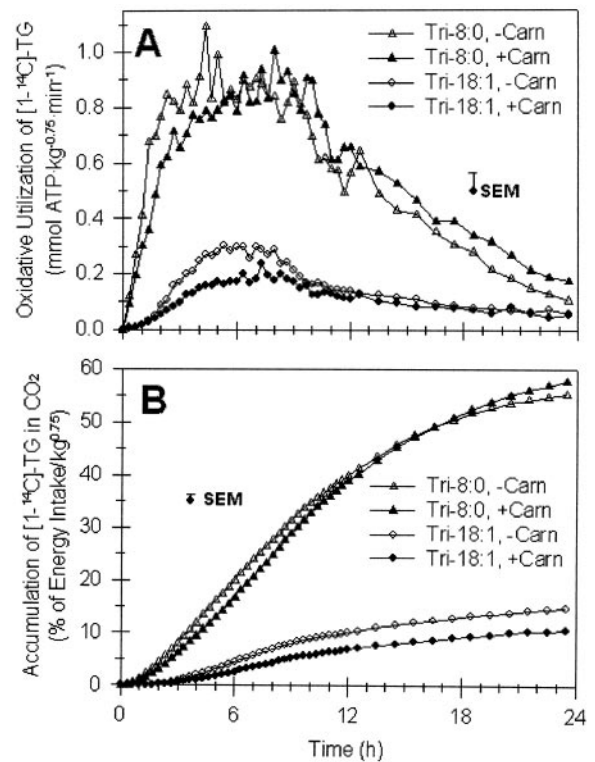


FIGURE 2 Instantaneous (A) and accumulative (B) oxidative utilization (i.e., digestion, absorption and oxidation) of $[1\text{-}^{14}\text{C}]\text{tri-8:0}$ (35.9 kBq/mmol) or $[1\text{-}^{14}\text{C}]\text{tri-18:1}$ (81.1 kBq/mmol) fed to neonatal piglets (6.5 and $2.94 \text{ mmol/kg}^{0.75}$, respectively) with or without $1 \text{ mmol/kg}^{0.75}$ of L-carnitine as a 30% (v/v) oil-in-water emulsion. Error bars represent pooled SEM for four or five observations per point. Fatty acid chain length effect (A and B, $P < 0.001$); fatty acid chain length \times time interaction (A and B, $P < 0.001$).

TABLE 1

Effects of fatty acid chain length and L-carnitine (Carn) on the rate and extent of emulsified triglyceride utilization by 1-d-old piglets^{1,2}

	Tri-8:0		Tri-18:1		SEM
	Carn (-)	Carn (+)	Carn (-)	Carn (+)	
Piglets, <i>n</i>	5	4	5	5	
Body weight, <i>kg</i>	1.48	1.49	1.46	1.46	0.05
Triglyceride (TG) intake					
<i>mmol TG/kg^{0.75}</i>	6.44	6.41	2.93	2.94	0.03
<i>mmol ATP/kg^{0.75}</i>	1.32	1.31	1.33	1.35	0.01
Maximal utilization rate, <i>mmol ATP·kg^{-0.75}·min⁻¹</i> ³	1.45	1.18	0.37	0.29	0.11
Extent of utilization (at <i>t</i> = ∞)					
<i>mol ATP/kg^{0.75}</i> ³	0.91	0.97	0.23	0.16	0.04
% of intake ³	68.4	73.0	16.9	12.3	3.2

¹ Values are means; SEM represent pooled SEM from ANOVA, *n* = 4–5 as indicated.

² After colostrum-deprived piglets were fed carnitine-deprived soy milk for 19 h, they were given trioctanoylglycerol (tri-8:0) or trioctadecanoylglycerol (tri-18:1) with or without 1 mmol/kg^{0.75} of L-carnitine by gavage in a 30% (v/v) oil-in-water emulsion. Utilization refers to the composite of digestion, absorption and oxidation to CO₂ after feeding triglycerides.

³ Fatty acid chain length effect (*P* < 0.001).

cally ill adult patients (21). In addition, high lipid formula (i.e., nonprotein energy as 70% lipid/30% glucose) did not significantly increase lipid oxidation in vivo. In stark contrast our results (Table 2, Fig. 2) showed faster and larger in vivo oxidative utilization of MCT than of LCT by orogastric feeding. Even though it is not excluded that the combinations of MCT and other nutrients (LCT, glucose and amino acids) in clinical studies may dilute the theoretical benefits of MCT usage, other possible reasons may account for these divergent results. First, efficiency of digestion, absorption and oxidation of LCT are much less developed in early postnatal piglets (6). Second, a low rate of ketogenesis is observed in pigs, unlike other animals (35), because of limited expression of mitochondrial HMG-CoA synthesis (36,37). In addition, other enzyme regulatory sites such as HMG-CoA synthase (or acetyl-CoA carboxylase and carnitine palmitoyltransferase I) may be a cause for an impaired rate of hepatic ketogenesis in pigs.

To our knowledge, this study is also the first to show

accurate utilization kinetics of dietary LCT at the resolution of 20-min intervals. Although Chiang et al. (6) conducted a similar study, investigating the time course of nonemulsified MCT and LCT utilization, they encountered some difficulties in comparing the kinetics of MCT vs. LCT utilization. Using continuous radioisotopic infusion techniques, supplemental L-carnitine increased the rate of oxidation of medium-chain fatty acids to CO₂ (16). On the other hand, similar intravenous infusion techniques to examine carnitine effects on long-chain fatty acid metabolism in vivo was not applied because of the insolubility of long-chain fatty acids without the use of a suitable binding protein (e.g., albumin). In addition, this technique is more difficult to apply directly to medicine and production agriculture because fatty acids are rarely fed or infused. For these reasons, we developed a gastric feeding protocol to study fatty acid chain length and carnitine effects in a more practical setting. However, the current study was somewhat different from the methodology of both Chiang et

TABLE 2

Effects of fatty acid chain length and L-carnitine (Carn) on the time course of emulsified triglyceride utilization by 1-d-old piglets^{1,2}

	Tri-C8:0		Tri-C18:1		SEM
	Carn (-)	Carn (+)	Carn (-)	Carn (+)	
Piglets, <i>n</i>	5	4	5	5	
Time of corresponding rate, <i>h</i>					
10% of maximal rate ^{3,a}	0.53	0.58	1.80	1.73	0.22
50% of maximal rate ^{3,b}	1.80	2.17	3.53	4.20	0.51
Maximal rate	7.00	7.42	6.60	6.33	0.93
50% of maximal rate ⁴	10.67	13.63	9.70	11.50	1.31
25% of maximal rate ⁴	17.50	20.50	19.50	19.90	1.54
Time of peak oxidation ^{5,a}	2.70	2.96	4.50	5.07	0.46

¹ Values are means; SEM represent pooled SEM from ANOVA, *n* = 4–5 as indicated. ^{a,b} Fatty acid chain length effect (*P* < 0.001 and *P* < 0.01, respectively).

² After colostrum-deprived piglets were fed carnitine-deprived soy milk for 19 h, they were given trioctanoylglycerol (tri-8:0) or trioctadecanoylglycerol (tri-18:1) with or without 1 mmol/kg^{0.75} of L-carnitine by gavage in a 30% (v/v) oil-in-water emulsion. Utilization refers to the composite of digestion, absorption and oxidation to CO₂ after feeding triglycerides.

³ Before the time of maximal rate.

⁴ After the time of maximal rate.

⁵ The first peak followed by decline after feeding was considered as the representative peak to describe the kinetics of in vivo oxidation.

al. (6) and Odle et al. (28). Instead of 1-d-old piglets suckling sow's colostrum (28), colostrum-deprived 1-d-old piglets were used to remove the maternal carnitine supply through milk. Because milk consumption affects enzymes of fat digestion and absorption (38,39), piglets were fed a soy-based liquid diet devoid of carnitine for 1 d to develop these enzymes (i.e., gastric and pancreatic lipase, etc.), unlike unsuckled 0-d piglets used by others (6). Specifically, LCT utilization may be significantly affected by these enzymes, and the lack of developed enzymes may limit long-chain fatty acid utilization compared to that of MCT (5). Furthermore, to minimize any developmental variation in diet intake among piglets, orogastric feeding scaled to metabolic body size was used. Although piglets were fed a soy-based liquid diet for 13 h (10 times), it cannot be excluded that the artificial diet was limited in some factors (compared with sow's colostrum) that are essential for intestinal enzymes to develop (40). If so, this could result in delayed time of maximal oxidative utilization, and decreased slope of utilization during the declining phase (c.f. Table 2, Fig. 2).

Effect of fatty acid chain length on estimated energetics.

The ATP turnover in the piglets (estimated from the rate of CO₂ production) was used to convert the rate and extent of TG utilization to the estimates of energetic contributions (41). The maximal rate and the average over 24 h of energy expenditure were 3.8 and 2.7 mmol ATP·kg^{-0.75}·min⁻¹, respectively. During peak utilization, tri-8:0 and tri-18:1 were oxidized at a rate to meet 35% (1.32/3.8) and 9% (0.33/3.8) of piglets' energy expenditure, respectively. The oxidative utilization (Table 1) of tri-8:0 could supply energy sufficient for 5.8 h (e.g., 940 mmol ATP/kg^{0.75} ÷ 2.7 mmol ATP·kg^{-0.75}·min⁻¹ ÷ 60 min/h); however, tri-18:1 could sustain the piglet for only 1.2 h (e.g., 195 ÷ 2.7 ÷ 60).

Carnitine effects. This study is the first to evaluate directly the effect of carnitine on in vivo fatty acid oxidation by neonatal pigs fed TG composed of various fatty acid chain lengths (medium vs. long). Because of the well-described metabolic roles of carnitine in fatty acid metabolism (7), we expected supplemental carnitine to increase dietary MCT and LCT oxidation in colostrum-deprived newborn pigs, even though the mechanism of stimulation might be different for each fatty acid. Indeed, we showed previously that supplemental carnitine could enhance the oxidation of intravenously infused octanoate, and that effects were elevated as infusion rates increased from 35 to 100% of piglet energy expenditure (15,16). We reasoned these effects to be related to carnitine's ability to buffer the acyl-CoA to free-CoA ratio. Failure to observe similar effects in the present study may stem from the overall lower rates of fatty acid oxidation when compared with the intravenous/infusion studies. In contrast, for long-chain fatty acids, we expected that the reduced carnitine status of colostrum-deprived piglets (42) might impair fat oxidation, compared with carnitine-supplemented littermates, by limiting entry into mitochondria by carnitine palmitoyltransferase I. Indeed, in slightly older pigs (8,9) we were able to detect alterations in nutrient partitioning with carnitine supplementation, consistent with this hypothesis. In the present study, inherently high animal-to-animal variation in development and digestion capacity of LCT may have precluded our detection of possible carnitine effects. The ranges of accumulative oxidation (% of energy intake) were 43.1–64.9% and 4.3–21.3% for tri-8:0 and tri-18:1, respectively; the CV after statistically removing variation attributable to replication for tri-18:1 was ninefold greater than that of tri-8:0 (57% vs. 6%). This may be not surprising, considering that digestion, absorption and metabolism of LCT include more complex and en-

zyme-regulated steps than those of MCT (5,43). It is possible that diverse ontogeny of digestion and absorption in piglets resulted from the liquid diet feeding as well as genetic differences. Although liquid diet feeding increased the capacity of fatty acid enzymes and intestinal fat absorption for LCT, it may not ameliorate relative variations (i.e., CV) after 1 d.

In summary, based on results from this study, L-carnitine did not significantly increase in vivo fatty acid oxidative utilization using safe doses of MCT (< 6.5 mmol/kg^{0.75}) over the short term. However, it cannot be excluded that L-carnitine may alleviate acyl intoxication when neonatal animals receive MCT long term (15). Collectively, this study shows that fatty acid chain length (medium vs. long) has a profound effect on the kinetics of oral [1-¹⁴C]TG utilization by newborn piglets. The maximal rate of tri-8:0 oxidative utilization (i.e., composite of digestion, absorption and oxidation) and the extent (Table 1) of utilization were three- and fourfold greater than that of tri-18:1, respectively, and tri-18:1 delayed the time to reach the 10 and 50% of maximal utilization rate by 1.2 and 1.9 h (Table 2), respectively, regardless of carnitine supplementation.

ACKNOWLEDGMENT

The authors thank Ralph House for his expert technical assistance with this experiment.

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