Determination of Carnitine Renal Threshold and Effect of Medium-Chain Triglycerides on Carnitine Profiles in Newborn Pigs

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ABSTRACT : Colostrum deprived, newborn pigs (N=12, 1.64±0.05 kg) were used to study the renal threshold of carnitine, and effects of emulsified medium-chain triglyceride (MCT, tri-8:0) feeding on kinetics of plasma carnitine and urinary carnitine excretion. An arterial catheter was inserted through an umbilical artery, and a bladder catheter was inserted via the urachus. Piglets were oro-gastrically gavaged with one of six carnitine levels (0, 60, 120, 180, 240, 480 μmol/kg $W^{0.75}$) with (+MCT) or without medium-chain triglycerides (-MCT) in 0.9% NaCl solution. Blood was sampled into heparinized tubes at 0, 1, 2, 4, 6, 8, 14, and 20 h after gavage, and urine was collected and pooled into 1 h or 2 h composite samples to determine free- and short-chain carnitine concentrations. Plasma from the 12 newborn piglets before gavage contained 10.6±1.2 μmol/L free carnitine and 7.2±0.6 μmol/L acid-soluble acyl carnitine. The renal threshold for carnitine was similar between the MCT and the +MCT group (42.6±13.1 and 46.4±2.0 μmol/L, respectively), but the correlation between plasma free carnitine and urinary excretion was altered. Plasma free carnitine linearly increased with increasing carnitine dosage (-MCT group, R^2=0.95, $p<0.001$; +MCT group, R^2=0.91, $p<0.001$), but was decreased by 50% when medium-chain triglycerides were fed. The peak in plasma free carnitine concentration was depressed by medium-chain triglycerides feeding also. Therefore, the plasma and urinary short-chain/free carnitine ratio of the +MCT group was increased by 100% and 40%, respectively ($p<0.01$). Feeding of medium-chain triglycerides may delay plasma carnitine elevation via altering the kinetics of absorption. Similarly, the plasma and urinary short-chain/free carnitine ratio were affected by interaction between medium-chain triglycerides and time ($p<0.01$). The present study suggests that an oral carnitine dose over 480 μmol/kg $W^{0.75}$ may be needed to reach the free carnitine renal threshold within a short period, especially when provided together with medium-chain triglyceride. (*Asian-Auts. J. Anim. Sci. 2001. Vol. 14, No. 2 : 237-242*)

Key Words : Carnitine, Renal Threshold, Medium-Chain Triglycerides, Newborn Pigs, Acyl Intoxication

INTRODUCTION

Medium-chain triglycerides (MCT) and L-carnitine have been researched in our laboratory, considering their potential roles as a supplemental energy source and as a fatty-acid-oxidation enhancer for the neonatal pigs, respectively (Kempen and Odle, 1993 and 1995; Odle, 1997 and 1998). Due to the improved efficiency of digestion, absorption, and oxidation of medium-chain triglycerides relative to long-chain triglycerides, numerous clinical studies have evaluated the efficacy of medium-chain triglycerides in formulas for premature and/or small-for-gestational-age infants (Odle, 1997). Similarly, the efficacy of medium-chain triglycerides as a supplemental energy source to improve survival of neonatal piglets has been investigated (Chiang et al., 1990; Kempen and Odle, 1993; Odle et al., 1994; Wieland et al., 1993a, b).

L-carnitine is an essential cofactor in the transport of activated long-chain fatty acids from the cytosol into the mitochondria matrix (McGarry and Brown, 1997), and carnitine and carnitine acetyltransferase provide a mechanism to modulate the acetyl-CoA to free CoA ratio during metabolic changes (i.e., fasting, high fat feeding, exercise). In humans, renal clearance of short-chain acyl carnitine esters is greater than that of free carnitine, even though acyl carnitine esters only comprise about 20-30% of total circulating carnitine. However, they comprise over 50% of total carnitine in urine (Lombard et al., 1989; Davis et al., 1990). In pigs, there is no comparable data for carnitine fractions in urine.

Some research has been conducted to find the threshold value as well as the corresponding dose of carnitine required to reach the threshold level in rodents and humans (Gross and Henderson, 1984; Engel et al., 1981). Recently, Penn et al. (1997) reported the renal carnitine threshold was between 15 and 35 μmol/L of plasma free carnitine in pigs reared by total parenteral nutrition over 11 to 14 d of age.

Specifically, the experiment herein determined the renal threshold and corresponding oral dose with a continuous blood and urinary collection, and a regression model in colostrum-deprived newborn pigs. Furthermore, we tested the hypothesis that high medium-chain triglyceride feeding can alter the time-course of plasma and urinary carnitine.

MATERIALS AND METHODS

Animals and experimental protocol

All animal procedures were approved by the

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Institutional Animal Care and Use Committee (IACUC) of North Carolina State University. Colostrum deprived, newborn pigs (N=12, 1.64±0.05 kg) were obtained from the Lake Wheeler Field Laboratory of North Carolina State University. Piglets were removed from the sow at birth, prior to suckling. An arterial catheter was inserted into piglets through the umbilical artery via a minor surgical procedure, and a bladder catheter was inserted via the urachus as described by Kempen and Odle (1995) using general isoflurane anesthesia (Anaquest Inc., Liberty Corner, NJ, USA).

After recovery from surgery (1 h), an oro-gastric catheter (12 french) was inserted into the pigs stomach through the esophagus. An infusion line was connected to the oral catheter of each pig and connected to an six-channel peristaltic infusion pump (model 7524, Cole Parmer Instrument, Chicago, IL, USA) which allowed a continuous delivery of saline from intravenous bags (Baxter Healthcare, Deerfield, IL, USA). To facilitate continuous urine collection, hypo-osmotic saline (0.45%) was used. Piglets were placed into the respiration chambers (Kempen and Odle, 1993) and were infused continuously with 0.45% saline (20 mL/h) via the oro-gastric catheter for 1 h to collect a baseline urine sample.

Trioctanoylglycerol (Karlshamns Lipid Specialties, Columbus, OH, USA) was mixed together with the L-carnitine (Sigma Chemical, St. Louis, MO, USA) in a 30% (vol/vol) emulsion using 2% (wt/vol) Tween 80 (polyoxy-ethylene sorbitan monooleate) as the emulsifying agent (Wieland et al., 1993a). Piglets were gavaged with one of six carnitine levels (0, 60, 120, 180, 240, 480 μmol/kg $W^{0.75}$) with or without 6.5 mmol/kg $W^{0.75}$ of trioctanoylglycerol in a 0.9% NaCl solution. The total time from birth until the start of oral gavage was less than 12 h. Carnitine infusion amounts were based upon preliminary studies (data not shown) and daily piglet consumption from sows milk (~120 μmol carnitine/d; Kerner et al., 1984). The trioctanoylglycerol amount was based on Wieland et al. (1993). Because L-carnitine uptake by intestinal epithelial cells involves a carrier-mediated system that is Na^+-dependent, 0.9% NaCl was in the solution (McCloud et al., 1996).

During the collection period, piglets slept most of time in the heated (32℃) respiratory chambers. The chambers were connected to an air inlet, and air was drawn through the chambers using a vacuum pump at a rate of 2L/min. The arterial catheter (inserted 20 cm) was connected to a blood sampling line (flushed with heparinized saline, 5×10^3 units/L). Blood was sampled into heparinized tubes at 0 (before the start of the gavage), 1, 2, 4, 6, 8, 14 and 20 h after the start of the gavage. Blood samples were centrifuged at 2,300×g for 25 min at 4℃ and plasma was stored at -70℃ before analysis. Saline (0.45%) was infused at a rate of 3.6 mL/h via oro-gastric catheter throughout the experiment. Urine was collected and pooled into 1-h or 2-h composites over the 21 h period of the experiment for analysis of free and short chain carnitine excretion. Urine samples were stored at -70℃ until analysis.

Carnitine analysis

[Methyl-^{3}H]carnitine and [1-^{14}C]acetyl-CoA were purchased from American Radiolabelled Chemicals, Inc. (St. Louis, MO, USA). Acetyl-CoA, carnitine acetyltransferase (EC 2.3.1.7) and other chemicals were obtained from Sigma Chemical (St. Louis, MO, USA). Scintillation fluid (Scintisafe) and ion-exchange resin (AG 1×8, 100-200, chloride form) were obtained from Fisher Scientific (Atlanta, GA, USA) and Bio-Rad Lab. (Richmond, CA, USA), respectively. Carnitine fractions were assayed by the enzymatic radioisotope method of McGarry and Foster (1976) modified as described by Heo et al. (2000).

Statistical analysis

The relationships between plasma carnitine fractions and urinary carnitine were analyzed (PROC NLIN; SAS, 1989) as a regression model (figure 1) as follows:

$Y = B_0X + B_1Max\ (X - i, 0)$

X = plasma carnitine concentration
Y = urinary carnitine concentration
i = the apparent threshold point of plasma carnitine
B_0 = the slope related to plasma carnitine concentration
B_1 = the slope related to renal threshold

Area under the response curve for plasma free carnitine on each carnitine dose was calculated using trapezoidal geometry for the time period 0 to 20 h after gavage, and correlations between carnitine doses and plasma free carnitine were evaluated by a linear regression (figure 2). Other data were analyzed as a completely randomized design with 2 treatments (six pigs per treatment) over time using the GLM procedure of SAS (1989). Significant relationships were accepted at $p<0.05$.

RESULTS

Plasma from 12 newborn piglets before gavage contained 17.7±1.3 μmol/L total acid-soluble carnitine. It consisted of 10.6±1.2 mol/L free carnitine and 7.2±0.6 μmol/L acid-soluble acyl carnitine.

Apparent renal threshold

The effect of emulsified medium-chain triglycerides on the relationship between plasma free carnitine and

urinary excretion, and renal threshold in colostrum-deprived newborn piglets is shown in figure 1. The best-fit two-broken-line equations for the apparent renal threshold were $Y=0.00058X+0.00194\max(X-42.6, 0)$, $R^2=0.78$ for the -MCT group, and $Y=0.00145X+0.03899\max(X-46.4, 0)$, $R^2=0.88$ for the +MCT group. Renal threshold was not changed by medium-chain triglycerides (-MCT group, $i=42.6\pm13.1$ μmol/L; +MCT group, $i=46.4\pm2.0$ μmol/L), but the correlation between plasma free carnitine and urinary excretion was different (figure 1). The renal threshold for short-chain carnitine was not detected because piglets excreted a wide range of short-chain carnitine regardless of plasma short-chain carnitine levels (data not shown).

Effect of carnitine level

Plasma free carnitine calculated from area under the response curve was linearly increased ($p<0.001$) up to 122.5 (-MCT group, $R^2=0.95$) and 35.6 μmol · h/L (+MCT group, $R^2=0.91$) by increasing carnitine dose (figure 2), and consequently the short-chain/free carnitine ratio fell down by 40% (data not shown).

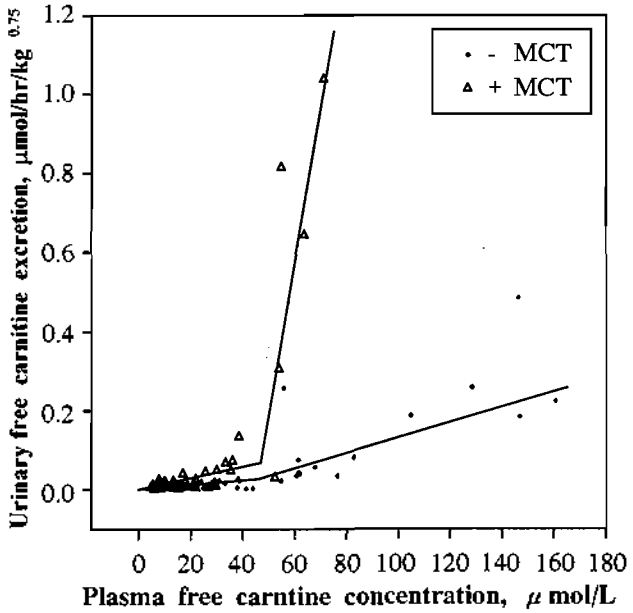

Figure 1. Effect of emulsified medium-chain triglycerides (MCT; tri-8:0) on the relationship between plasma free carnitine and urinary excretion, and renal threshold in colostrum-deprived newborn piglets. n=48 points per regression. The relationships between plasma carnitine fractions and urinary carnitine were analyzed as a regression model $[Y=B_0X+B_1\max(X-i, 0)]$ using the NLIN procedure of SAS (-MCT group, $i=42.6\pm13.1$ μmol/L; +MCT group, $i=46.4\pm2.0$ μmol/L). Abbreviations used: X, plasma carnitine concentration; Y, urinary carnitine concentration; i, the apparent threshold point of plasma carnitine; B_0, the slope related to plasma carnitine concentration before threshold; B_1, the slope related to plasma carnitine after threshold; MCT, without MCT; +MCT, 6.5 mmol/kg $W^{0.75}$ of MCT.

Effect of medium-chain triglycerides and interaction with time

Plasma free carnitine concentration was decreased by co-administration of medium-chain triglycerides (50%, $p<0.001$, figure 3). Therefore, plasma short-chain /free carnitine ratio was increased by 100% ($p<0.001$, figure 4) and its ratio in urine was increased by 40% ($p<0.01$, figure 5). Although plasma free carnitine was increased by carnitine dosage regardless of medium-chain triglycerides, the peak and response curve over time was depressed by feeding medium-chain triglycerides (figure 3). Plasma free carnitine concentration peaked at 6 h in piglets gavaged without medium-chain triglycerides and it was maintained for 20 h (figure 3). On the other hand, piglets fed medium-chain triglycerides showed a continuous increase without any peak for the entire period.

DISCUSSION

The primary aim of this study was to determine the renal threshold of carnitine fractions and the corresponding oral dose in colostrum-deprived newborn pigs. Dietary supplementation resulting in plasma

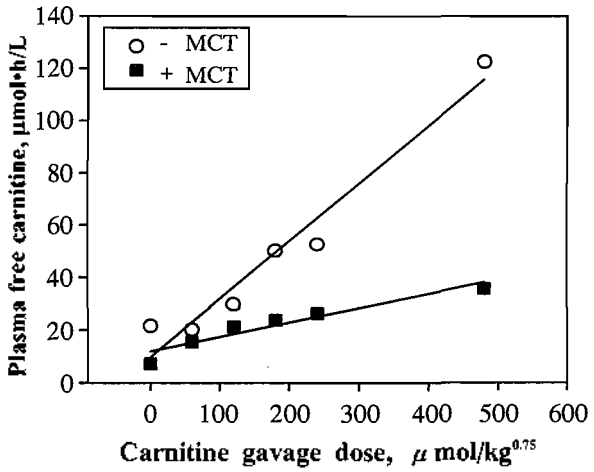

Figure 2. Effect of oro-gastric carnitine doses and emulsified medium-chain triglycerides (MCT; tri-8:0) on area under the curve for plasma free carnitine in colostrum-deprived newborn piglets measured during 20 h postgavage. $Y=9.62+0.22X$, $R^2=0.95$, $p<0.001$ for -MCT group and $Y=11.77+0.054X$, $R^2=0.91$, $p<0.001$ for +MCT group. Abbreviations used: X, carnitine gavage dose; Y, area under the curve for plasma free carnitine during 20 h; -MCT, without MCT; +MCT, 6.5 mmol/kg $W^{0.75}$ of MCT.

carnitine concentration above the renal threshold may not yield further increases in the body carnitine pool. Rather, excess carnitine will be excreted without reabsorption by the kidneys.

We were aware of inaccurate renal threshold estimates caused by variation in urinary collection and poor coordination with blood sampling. By using a continuous blood and urinary collection technique, we could determine more accurately the carnitine renal threshold. As shown in figures 3, 4, and 5, plasma free and short-chain carnitine concentrations changed quickly after gavage and correspondingly resulted in changed urinary excretion. Even though we used one pig per oral carnitine level (total six levels) in each MCT group, we could collect eight plasma carnitine concentrations and corresponding urinary excretion composites (total 48 observations) in each MCT group. We felt justified that each sampling of 1-h or 2-h urinary composite was the narrowest period to be combined with the corresponding plasma carnitine concentration simultaneously compared to any animal modeling. Unlike total parenteral nutrition which supplies adequate water at the same time, our study needed additional water supply for complete hydration. To solve this, saline (0.45%) was oro-gastrically infused throughout the experiment.

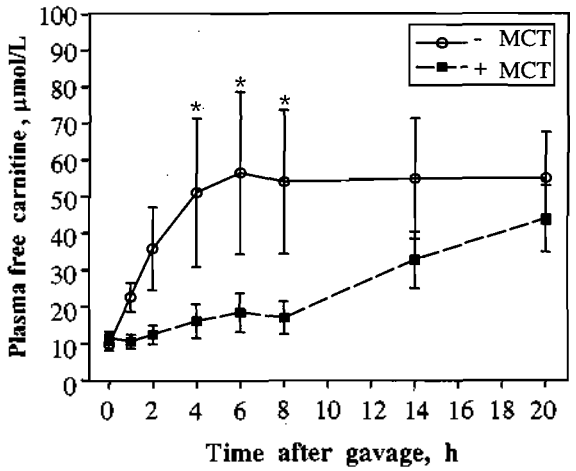

Figure 3. Effect of emulsified medium-chain triglycerides (MCT; tri-8:0) on kinetics of plasma free carnitine in colostrum-deprived newborn piglets. n=6. Error bar represents SEM. Effect of MCT ($p<0.001$). Effect of time ($p<0.02$). * MCT effect within each respective hour ($p<0.05$).

Secondary aim was to assess the interactive kinetics between medium-chain triglycerides feedingand carnitine metabolism. It was reported that medium-or long-chain triglycerides feeding increases short-chain /free carnitine ratio in plasma and urine (Seccombe et al., 1978; Cederblad, 1987; Stadler et al., 1993), but the responding profile over time have not been determined.

Apparent carnitine renal threshold

Dietary carnitine is absorbed from the gastrointestinal tract to a large degree in mammals (Gross and Henderson, 1984). Carnitine is mainly

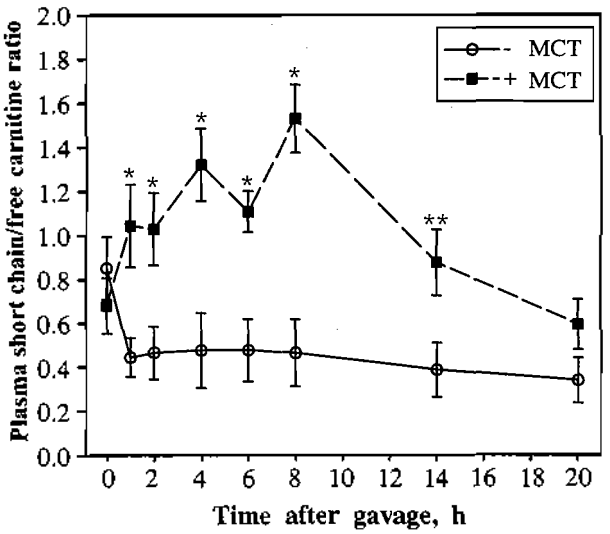

Figure 4. Effect of emulsified medium-chain triglycerides (MCT; tri-8:0) on kinetics of plasma short-chain/free carnitine ratio in colostrum-deprived newborn piglets. n=6. Error bar represents SEM. Effect of MCT ($p<0.001$). Effect of time ($p<0.02$). Effect of MCT time ($p<0.01$). MCT effect within each respective hour (* $p<0.01$ and ** $p<0.02$, respectively).

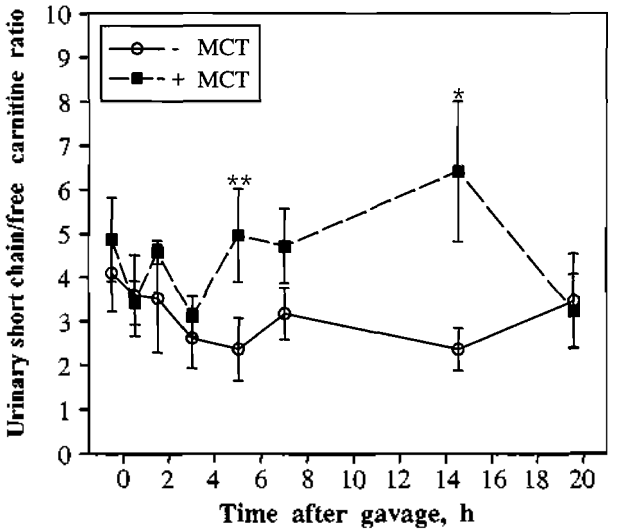

Figure 5. Effect of emulsified medium-chain triglycerides (MCT; tri-8:0) on kinetics of urinary short-chain/free carnitine ratio in colostrum-deprived newborn piglets. n=6. Error bar represents SEM. Effect of MCT ($p<0.01$). MCT effect within each respective hour (* $p<0.01$ and ** $p<0.05$, respectively).

absorbed by Na^+-dependent facilitated diffusion at low lumen concentrations, but via a passive process at high lumen concentrations (Rebouche, 1998; Shennan et al., 1998). Penn et al. (1997) reported that the apparent renal threshold for plasma free carnitine was between 15 and 35 μmol/L for young pigs, and the value obtained in the present study was higher than their result but less than that of adult animals (80 and 55 to 65 μmol/L for rats and humans, Gross and Henderson, 1984; Engel et al., 1981, respectively). The difference in threshold between neonatal pigs and adult humans may be due to not only species but also physiological status. Generally, renal reabsorption capability in neonates is less than in adults, but this generality has not been confirmed for carnitine to date. Rebouche et al. (1993) reported a low correlation between plasma free carnitine and urinary carnitine excretion that was due to variations in capacity of renal reabsorption among individuals. They proposed that diet composition, physiological and pathological factors (i.e., high fat, high protein, and pregnancy) also affect the rate of urinary carnitine excretion.

However, the failure to detect the renal threshold of short-chain carnitine is not surprising because the percentage of urinary acyl carnitine varied greatly, from 3 to 91% in human adults (Lombard et al., 1989). Furthermore, fasting or high fat intake increased the urinary short-chain carnitine excretion (Cederblad, 1987; Stadler et al., 1993). Urinary long-chain carnitine was not detected in our study, because it is not normally filtered by the kidney except during renal malfunction.

Dietary carnitine level and renal threshold

Because 240 μmol/kg $W^{0.75}$ of carnitine increased average plasma free carnitine concentration close to the measured renal threshold in the MCT group, excess oral carnitine over this value may not yield further increases in the body carnitine pool, but be excreted into urine primarily. On the other hand, plasma free carnitine concentration calculated from area under the curve did not reached renal threshold up to the 480 μmol carnitine/kg $W^{0.75}$ in the +MCT group. However, the blood sampling over time showed that plasma free carnitine concentration reached the renal threshold level at 1 and 4 h after gavage (-MCT and +MCT, respectively) with the 480 μmol/kg $W^{0.75}$ carnitine dose (data not shown).

Medium-chain triglycerides alter carnitine kinetics

Feeding of medium-chain triglycerides may delay plasma carnitine elevation due to the different kinetics from the intestinal enterocyte into the bloodstream or by delaying gastric emptying. A similar trend was reported by Gross and Savaiano (1993) in that fasting increased the uptake of carnitine into enterocytes of rats. Plasma short-chain/free ratio were significantly affected by the interaction between medium-chain triglycerides and time ($p<0.01$, figure 4), and the short-chain/free ratio in urine was increased by medium-chain triglycerides at 5 and 14 h after the gavage ($p<0.05$ and $p<0.01$, respectively). At 20 h after the gavage plasma short-chain/free ratio in the +MCT group dropped to the ratio seen in the -MCT group (figure 4). As a result, urinary short-chain/free ratio was recovered to the ratio before the gavage (figure 5). This reinstatement after 20 h fits the insignificant oxidative rate of the radiolabelled trioctanoylglycerol after 20 h in newborn pigs (Heo, 2000; Odle et al., 1994). Seccombe et al. (1978) also reported using rats that a diet rich in medium-chain triglycerides increased the ratio of acyl carnitine to free carnitine, acyl carnitine and β-hydroxybutyrate in serum. Even though it was expected that medium-chain triglycerides could increase plasma short-chain carnitine (mainly acetylcarnitine) via acetyltransferase activities and fatty acid oxidation in various tissues, plasma short-chain carnitine was not increased by medium-chain triglycerides (data not shown, $p>0.1$). Because the total carnitine concentration of the +MCT group was lower than the -MCT group, the absolute short-chain carnitine concentration was not increased but the relative ratio to total carnitine was increased via carnitine acyltransferase. It is also considered that the neonatal pigs could maintain a higher filtration rate of short-chain carnitine than that of free carnitine in kidneys to prevent acyl intoxication (Kempen and Odle, 1995) caused by an overload of medium-chain triglycerides (figure 5).

Congruently, not only plasma carnitine status but also the response curve over time was affected by medium-chain triglycerides as well as dietary L-carnitine intake. This alteration resulted in the change in urinary carnitine excretion. However, further studies are needed to confirm if kidney functions (i.e., efficiency of filtration and reabsorption) and efficiency of dietary carnitine absorption into blood stream (via enterocytes) from the intestinal lumen are affected by a diet rich in medium-chain triglycerides.

IMPLICATIONS

Medium-chain triglycerides and L-carnitine have the potential to serve as a supplemental energy source and as a fatty-acid-oxidation and enhancer to reduce the mortality for the neonatal pigs. The experiment herein was conducted to support their appropriate application. Oral carnitine doses over 480 μmol/kg $W^{0.75}$ may be needed to reach the renal threshold within a short period (e.g., in a hour), especially when provided together with medium-chain triglyceride. Even though medium-chain triglycerides are a promising supplemental

energy source of neonates, it can have detrimental effects causing nausea by feeding overdose. Previously our studies determined an optimal oral dose is 6.5 mmol/kg$^{0.75}$, and this current study suggests that the safe interval of medium-chain triglycerides would be around 20 h after feeding.

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