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Nutrition

Effect of L-carnitine and medium-chain triglyceride on plasma and urinary carnitine in newborn piglets

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Summary

Colostrum deprived, newborn pigs (N = 12, 1.64 ± 0.05 kg) were used to study the effects of oral L-carnitine and emulsified trioctanoylglycerol (TG) feeding on kinetics of plasma carnitine and urinary excretion. An arterial catheter was inserted through an umbilical artery, and a bladder catheter was inserted via the urachus. Plasma from the 12 newborn piglets before gavage contained 17.7 ± 1.3 μmol/L total acid-soluble carnitine. It consisted of 10.6 ± 1.2 μmol/L free carnitine and 7.2 ± 0.6 μmol/L acid-soluble acylcarnitine. The apparent renal threshold of plasma free carnitine was similar between - TG and + TG 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.

Introduction

L-carnitine is an essential cofactor in the transport of activated long-chain fatty acids from the cytosol into mitochondria matrix (McGarry & Brown, 1997), and is absorbed from the gastrointestinal tract to a large degree in mammals (Gross & Henderson, 1984). Because dietary or intravenous supplementation resulting in plasma carnitine concentration above the renal threshold may not yield further increases in the body carnitine pool, 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 & 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 to14 d of age. Specifically, the experiment herein determined the renal threshold and corresponding oral dose in colostrum-deprived newborn pigs by using oro-gastric, umbilical and bladder catheters which facilitate continuous blood and urine collection. Furthermore, to test the hypothesis that high medium-chain triglyceride feeding can alter kinetics of plasma and urinary carnitine, we fed various doses of L-carnitine with or without trioctanoylglycerol.

Material and Methods

All animal procedures were approved by the 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. 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 & Odle (1995) using general isoflurane anesthesia (Anaquest Inc., Liberty Corner, NJ). After recovery from the surgery (1 h), an oro-gastric catheter (12 french) was inserted into the pig's stomach through 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) which allowed a continuous delivery of saline from intravenous bags (Baxter Healthcare, Deerfield, IL). Piglets were placed into the respiration chambers (Kempen & Odle, 1993) followed by a continuous 0.45% saline infusion (20 mL/h) via oro-gastric catheter for 1 h to collect baseline urine sample. Trioctanoylglycerol was mixed together with the L-carnitine in a 30 % v/v emulsion using 2 % (w/v) Tween 80 (polyoxy-ethylene sorbitan monooleate) as the emulsifying agent (Wieland et al. 1993). Piglets were gavaged with one of six carnitine levels (0, 60, 120, 180, 240, 480 μmol/kg $^{0.75}$) with or without 6.5 mmol/kg $^{0.75}$ wt of trioctanoylglycerol in 0.9% NaCl solution. During the collection period, piglets slept most of the time in the heated (32 ^{o}C) respiratory chambers. 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. Saline (0.45%) was infused at a rate of 3.6 mL/h via oro-gastric catheter to maintain continuous urine sampling throughout the experiment. Urine was collected and pooled into 1-h or 2-h composites over the 21 h period of the experiment. Carnitine fractions were assayed by the enzymatic radioisotope method described by Bhuiyan et al. (1992). The relationships between plasma carnitine fractions and urinary carnitine were analyzed (PROC NLIN; SAS 1989) as a regression model (Figure 1.).

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

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

Linear and quadratic polynomials were used to evaluate correlations between carnitine doses and average plasma free carnitine. Significant relationships were accepted at $P < 0.05$.

Results and Discussion

Plasma carnitine status at birth. 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 acylcarnitine.

Apparent renal threshold. The effect of emulsified trioctanoylglycerol (TG) on the relationship between plasma free carnitine and urinary excretion, and renal threshold in colostrum-deprived newborn piglets is shown Figure 1. The best-fit equations for the apparent renal threshold were $Y = 0.00058X + 0.00194 \max(X - 42.6, 0)$, $R^2 = 0.78$ for the - TG group and $Y = 0.00145X + 0.03899\max(X - 46.4, 0)$, $R^2 = 0.88$ for the + TG group. Renal threshold was not changed by TG (- TG group, i = 42.6 ± 13.1 μmol/L; + TG group, i = 46.4 ± 2.0 μmol/L), but the correlation between plasma free carnitine and

urinary excretion was different (Fig. 1). However, the renal threshold for short-chain carnitine could not be determined because piglets excreted a wide range of short-chain carnitine regardless of plasma short-chain carnitine levels (data not shown). This result is not surprising because the percentage of urinary acylcarnitine 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).

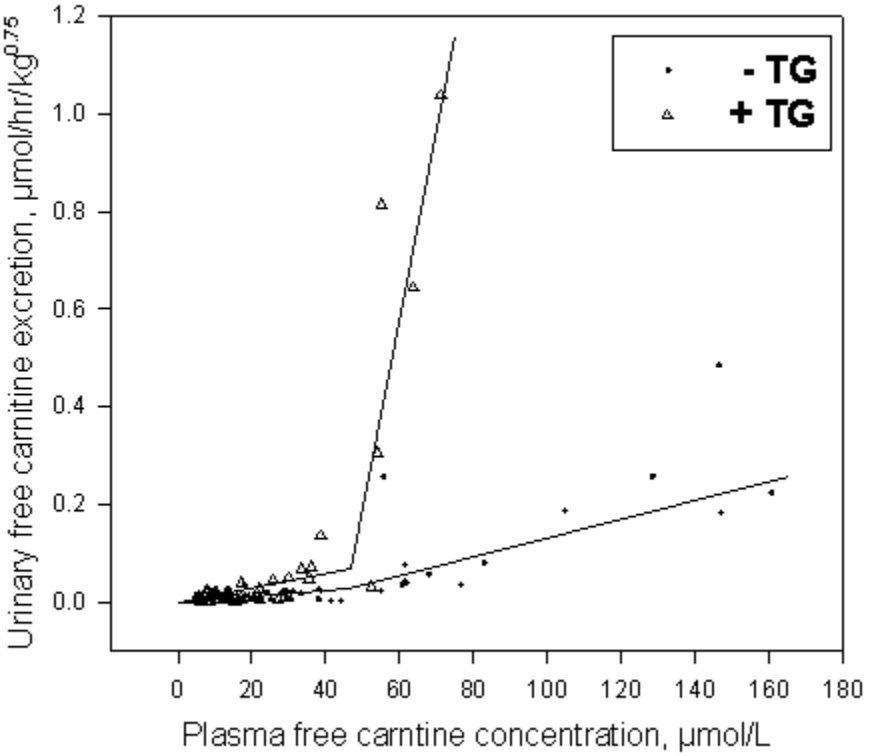

Figure 1. Effect of emulsified trioctanoylglycerol on the relationship between plasma free carnitine and urinary excretion, and renal threshold in colostrum-deprived newborn piglets. n = 24 points per regression. The relationships between plasma carnitine fractions and urinary carnitine were analyzed as a regression model ($Y = B_0X + B_1Max(X - i, 0)$) using the NLIN procedure of SAS (- TG group, i = 42.6 ± 13.1 µmol/L; + TG group, i = 46.4 ± 2.0 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; TG, trioctanoylglycerol.

Effect of carnitine level. Average plasma free carnitine was increased up to 120 :mol/L by increasing carnitine dose (- TG group, linear, $R^2 = 0.95$, $P < 0.001$; + TG group, linear, $R^2 = 0.91$, $P < 0.001$; Figure 2.), and consequently the short-chain/free carnitine ratio fell down by 40% (data not shown). Because 480 µmol/kg$^{0.75}$ of carnitine

increased average plasma free carnitine concentration close to the measured renal threshold, excess oral carnitine over this value may not yield further increases in the body carnitine pool, but be excreted into urine primarily. Congruently, plasma carnitine status was affected by TG as well as dietary L-carnitine intake. This alteration resulted in the change of urinary carnitine excretion and kinetics. 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 lumen may be affected by a diet rich in medium-chain triglycerides.

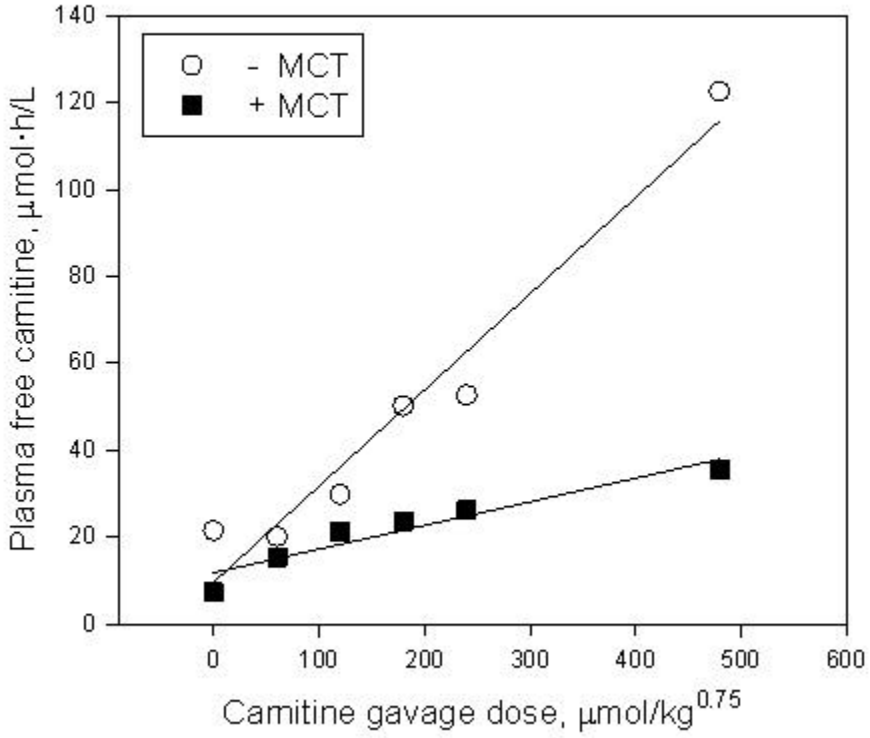

Figure 2. Effect of oro-gastric carnitine dose and emulsified trioctanoylglycerol on average plasma free carnitine in colostrum-deprived newborn piglets measured during 20 h postgavage. . Y = 9.62 + 0.22X, R^2 = 0.95, P <0.001 for - MCT group and Y = 11.77 + 0.054X, R^2 = 0.91, P <0.001 for + MCT group. Abbreviation used: X, carnitine gavage dose; Y, average plasma free carnitine of 8 measurements per point; TG, trioctanoylglycerol.

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