Clinical Nutrition (2006) **25**, 886–896

ELSEVIER

Clinical Nutrition

http://intl.elsevierhealth.com/journals/clnu

ORIGINAL ARTICLE

Carnitine supplementation in premature neonates: Effect on plasma and red blood cell total carnitine concentrations, nutrition parameters and morbidity

Catherine M. Crill[a,b,c,d,*], Michael C. Storm[a,c], Michael L. Christensen[a,b,c,d], Charles T. Hankins[e,1], M. Bruce Jenkins[b,d], Richard A. Helms[a,b,c,d]

[a]*Department of Pharmacy, The University of Tennessee Health Science Center, Memphis, TN, USA*
[b]*Department of Pediatrics, The University of Tennessee Health Science Center, Memphis, TN, USA*
[c]*The Center for Pediatric Pharmacokinetics and Therapeutics, The University of Tennessee Health Science Center, Memphis, TN, USA*
[d]*Methodist Le Bonheur Healthcare, Memphis, TN, USA*
[e]*Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA*

Received 2 September 2005; accepted 2 May 2006

KEYWORDS
Carnitine supplementation;
Premature neonates;
Neonatal morbidity;
Nutrition

Summary
Background & aims: Carnitine may be considered conditionally essential in the neonatal population. The purpose of this study was to evaluate the effects of long-term carnitine supplementation on total carnitine status and morbidity in premature neonates.
Methods: In this prospective, randomized, placebo-controlled, double-blinded study, premature neonates received carnitine supplementation (20 mg/kg/day) or placebo. Plasma (nmol/ml) and red blood cell (RBC) (nmol/mg hemoglobin) total carnitine concentrations, 24-h nitrogen excretion, intake and weight, and respiratory, gastroesophageal, and infectious morbidity were assessed.
Results: Twenty-nine neonates (13 placebo, 16 carnitine; 27±2 weeks gestation; 976±259 g birthweight) were studied for up to 8 weeks. Plasma total carnitine concentrations exceeded the reference range in the carnitine group (weeks 1–8); however, concentrations did not reach reference range until week 4 in the placebo group. RBC total carnitine concentrations increased, but remained below reference

*Corresponding author. Department of Pharmacy, The University of Tennessee Health Science Center, 26 South Dunlap, Suite 210, Memphis, TN 38163, USA. Tel.: +1 901 448 6045; fax: +1 901 448 6064.
E-mail address: ccrill@utmem.edu (C.M. Crill).
[1]Dr. Hankins was a neonatologist with Methodist Le Bonheur Healthcare in Memphis, TN when the project was conducted.

0261-5614/$ - see front matter © 2006 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.
doi:10.1016/j.clnu.2006.05.002

range in both the carnitine (weeks 1–6) and placebo (weeks 1–8) groups. Carnitine group neonates regained their birthweight more rapidly than placebo group neonates (day of life 11.8 ± 6 vs. 16.9 ± 6.3, $P = 0.034$). In addition, percent periodic breathing calculated from cardiopulmonary trend monitor data (weeks 1–8) was lower in the carnitine group (0.4 ± 0.9 vs. 1.4 ± 1.9, $P = 0.014$). There was no difference with respect to other markers of respiratory, gastroesophageal and infectious morbidity or nitrogen balance.

Conclusions: Carnitine supplementation at 20 mg/kg/day results in increased plasma and RBC total carnitine concentrations, has a positive effect on catch-up growth, and may improve periodic breathing in premature neonates.

© 2006 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.

Introduction

Carnitine, a nutrient normally synthesized from methionine and lysine in the liver and kidney, has been gaining increasing attention as a therapeutic agent since the mid-1960s. Carnitine transports long-chain fatty acids (LCFA) across the mitochondrial membrane where they undergo beta-oxidation to produce energy. Carnitine deficiency decreases LCFA availability for oxidation, thereby resulting in LCFA accumulation in the cytosol, and decreased ketone and energy production. Other carnitine functions include the maintenance of adequate free coenzyme-A required for various metabolic pathways, the protection of cells against toxic accumulation of acyl-coenzyme-A compounds by shuttling acyl groups out of the mitochondria, and the storage and transport of energy.

Although carnitine is considered a nonessential nutrient in adults, it may be conditionally essential in the neonatal population. Neonates have a reduced ability to synthesize carnitine, primarily due to a diminished amount of protein-bound trimethyllysine and decreased activity of the enzyme gamma butyrobetaine hydroxylase, which is necessary for the conversion of gamma butyrobetaine to carnitine. In addition, due to decreased tissues stores and since placental transfer of carnitine occurs during the third trimester, premature neonates are at particular risk of developing carnitine deficiency.[1–4] Since premature neonates and infants may not adequately synthesize endogenous carnitine, they must rely on exogenous intake to prevent deficiency.

In various cow's milk infant formulas, carnitine concentrations have been found to be comparable to or higher than that found in human milk.[5] Soy-based formulas are now supplemented with carnitine as a result of documented deficiency in infants receiving all soy diets.[6] Premature human milk and modified protein enteral formulas may not contain adequate carnitine concentrations for the premature neonate.

Parenteral nutrition (PN) does not contain carnitine unless it is extemporaneously added. Routine PN without carnitine supplementation in term and premature infants results in decreased carnitine plasma concentrations.[7–10] The benefits of carnitine supplementation in PN and enteral feedings on neonatal nutrition markers, primarily enhanced fatty acid oxidation and clearance, improved lipid tolerance, and increased nitrogen balance, are well documented.[11–18] In addition, carnitine supplementation has been found to be important for weight gain in the neonatal population.[17,18] While studies have evaluated carnitine status using plasma total, free, and acyl carnitine concentrations, measurement of red blood cell (RBC) total carnitine concentrations is also important in that erythrocytes may be a critical vehicle for delivery of carnitine to tissues.[19]

Carnitine deficiency may have important effects on the premature neonate. Ketone production provides a critical energy source for the developing brain and nervous system. Similarly, skeletal and cardiac muscles primarily rely on LCFA breakdown for energy needs. In addition, decreased energy production secondary to impaired beta-oxidation can impact any number of metabolic processes in the body. A case report describing an infant with low total carnitine concentrations and gastrointestinal dysmotility suggests that carnitine may be important in intracellular energy production in smooth muscle as well.[20] Similarities exist between complications that are common in premature neonates and those that develop from carnitine deficiency syndromes occurring outside the neonatal period, specifically respiratory and gastrointestinal dysfunction, hypotonia, hypoglycemia, failure to thrive, and increased infection risk.[20–22] We hypothesized that many of the complications seen with prematurity may be actually secondary to

carnitine deficiency or insufficiency. The primary objective of this study was to evaluate the effect of long-term carnitine supplementation on total carnitine status and morbidity in premature neonates.

Patients and methods

This was a prospective, randomized, placebo-controlled, double-blinded study enrolling neonates from two level-three neonatal intensive care units (NICU) in Memphis, TN. The institutional review boards of The University of Tennessee Health Science Center and Methodist LeBonheur Healthcare approved this study. Informed parental consent was obtained prior to study enrollment. Neonates were eligible for enrollment if they were $\leqslant$4 days of age, $\leqslant$32 weeks gestation, had a birthweight of $\leqslant$1500 g, and initially required PN support. Neonates were excluded from the study if they had inborn errors of metabolism, chromosomal abnormality, end-stage liver or renal disease, were receiving peritoneal dialysis or hemofiltration, or had grade IV intraventricular hemorrhage. The research protocol stipulated that a subject be replaced in the randomization schedule by another patient if within 14 days of enrollment any of the following occurred: death, diagnosis of grade IV intraventricular hemorrhage, discharge from the NICU, or parent/legal guardian withdrawal from the study. Neonates were studied for up to 8 weeks.

Randomization into carnitine or placebo groups was conducted based on a list of random numbers; randomization occurred simultaneously at both centers. The subjects in the carnitine group received PN with 130 mg/l of intravenous (IV) L-carnitine (Carnitor®, Sigma Tau Pharmaceuticals, Gaithersburg, MD) added to the solution, so that an infusion rate of 150 ml/kg would provide 20 mg/kg/day. The addition of L-carnitine to the PN solutions was done at the time of PN preparation in the pharmacy at each of the hospitals.

Once the neonates began the transition to enteral feedings, oral syringes containing either sterile water for the neonates in the placebo group or oral L-carnitine (Carnitor®, Sigma Tau Pharmaceuticals, Gaithersburg, MD) 10 mg/ml for the neonates in the carnitine group were compounded under clean conditions in the pharmacy at LeBonheur Children's Medical Center and transported to the NICUs under refrigerated conditions. NICU nursing staff added the contents of one syringe to each 90 ml feeding of either Pregestimil®, Nutramigen®, or Enfamil Premature® (Mead Johnson Nutritionals, Evansville, Indiana) or expressed human milk. The commercial products contained a reported 1.35 mg of carnitine per 100 ml of formula (per product labeling), and the carnitine content of expressed human milk was estimated at 1.05 mg/100 ml.[5] Subjects in the carnitine group had carnitine added to their feeds so that an enteral feeding rate of 180 ml/kg would provide 20 mg/kg/day of carnitine. Total carnitine intake was calculated based on reported content in enteral diet in addition to the amount supplemented in parenteral and enteral formulas. Any carnitine contribution from the administration of packed RBCs was also calculated.

All investigators, nursing and technical staff, as well as patients and their families were blinded to treatment group. Study points were baseline and weeks 1, 2, 4, 6, and 8. All laboratory analysis was conducted in the Center for Pediatric Pharmacokinetics and Therapeutics laboratory at The University of Tennessee Health Science Center, Memphis, TN.

Blood for plasma and RBC total carnitine concentrations was drawn at all study points. Samples were centrifuged under refrigeration within 1 h of collection, the buffy coat was removed, and the plasma was collected and stored at $-70\,^{\circ}$C until analysis. The RBC portion was washed three times with cold normal saline and centrifuged, and the supernatant was discarded after each spin. The packed RBC sample was then stored at $-70\,^{\circ}$C until analysis. Carnitine concentrations were analyzed in duplicate via a radio-enzymatic assay.[23] The reference plasma total carnitine concentration was 31.1–60.5 nmol/ml and RBC total carnitine concentration was $\geqslant$0.2 nmol/mg hemoglobin.[19,24,25]

Twenty-four-hour nitrogen excretion and balance determination was conducted at all study points. The protocol was initially designed to conduct 24-h bag collections of urine ($n = 4$ patients). Early into study enrollment and due to the problems associated with urine bag collections in premature neonates, we revised the protocol to collect diapers for 24 h ($n = 25$ patients). We conducted analysis of nitrogen recovery from the types of diapers utilized in each NICU and determined recovery to be approximately 98.0%. The diapers, which contained both urine and stool, were stored in a refrigerated cooler at bedside over the 24-h collection period. Each diaper was stored in a separate plastic bag at $-20\,^{\circ}$C until analysis. Diapers were combined and placed in 4 l sealed bottles and 500 ml of citrate buffer (2 mmol/l, pH 5.0) per diaper was added to each container. The containers were periodically agitated to ensure nitrogen extraction and were allowed to sit at room temperature for 24 h. A fixed sample was then

taken from each container, filtered through a 0.2 μ filter and analyzed for nitrogen content on a chemiluminescent analyzer (Antek Instruments, Houston, TX) by a standard methodology.[26,27] The total nitrogen content of each sample was then added together for nitrogen balance calculations according to a previously published method.[28]

Weight, intake, output, and clinical laboratory were recorded daily. Infectious morbidity was evaluated by positive blood cultures $\geqslant$7 days of life (defined as late onset infection). Respiratory morbidity was evaluated by number of ventilator days, apneic and bradycardic events that were noted in the nursing notes, use of methlyxanthines, and results of polysomnography testing within the 8-week study period. Polysomnography provided continuous multichannel recordings (respiratory airflow, heart rate, oxygen saturation, sleep states, esophageal pH) of infants during sleep and wakefulness and were utilized to determine periodic breathing during sleep and the need for outpatient apnea monitoring in selected patients who were clinically in need of this diagnostic study. In addition, cardiopulmonary trend monitor (Athena, Simonsen and Weel, Denmark) data, apneic episodes, periods of less than 90% desaturation, and bradycardia were recorded over a 15 h period for all patients that were off ventilatory support at each study period. The percent periodic breathing was calculated based on the number and length of apneic events that occurred within each evaluation period. For example, in a neonate experiencing 84 events of 5 s duration, 26 events of 10 s duration, 2 events of 15 s duration and 1 event of 20 s duration during the 15 h evaluation period, the percent periodic breathing would be 1.35% based on the equation as follows: [84(5)+26(10)+2(15)+1(20)/54,000 $\times$ 100]. Gastroesophageal morbidity was evaluated by feeding tolerance, incidence of residuals or emesis, the need for motility agents, and pH probe study results if the infant underwent polysomnography testing during the study.

A power analysis, using a two-sided test with a significance level of 0.05 and a β of 0.2 determined that a sample size of 30 would be sufficient to detect a 50% reduction in respiratory parameters (apneic episodes and periodic breathing) and incidence of reflux between groups, assuming 40% variability in data. This sample size was also sufficient to determine a significant decrease in the incidence of infection based on estimates that 55–60% of neonates had at least one episode of late-onset, culture positive sepsis. Statistical analysis of data between groups was conducted with Student *t*-test (reported as mean $\pm$ standard deviation) for continuous data and χ^2 or Fisher exact tests for categorical data. Repeated measures analysis of variance with multiple comparison testing was used to determine differences between groups across the study periods. Kruskal–Wallis analysis of variance on ranks was utilized for parametric data when normality failed and is reported as median and 25% and 75% percentiles. Data with *P* values $\leqslant$0.05 are considered significantly different.

Results

Thirty-two neonates were enrolled in the study between August 1997 and May 1999. Two neonates (one in the placebo group and one in the carnitine group) died within 14 days of study enrollment and therefore were not included in data analysis. Another neonate enrolled to the placebo group did not meet eligibility criteria (birthweight of 1773 g) and was therefore a protocol violation and not included in data analysis. Twenty-nine neonates remained for analysis (13 placebo, 16 carnitine). Patient characteristics (Table 1) were similar between the two groups.

Intakes at each study period are reported in Table 2. There was no statistically significant difference between groups with respect to protein and IV lipid intake and percent enteral calories at any study period. Placebo group neonates received greater nonprotein calories at weeks 4 and 6 and greater fluid at week 6 compared to carnitine group neonates. These differences can be attributed to one neonate in the carnitine group who was fed parenterally and was being treated for candidemia during these study periods. Limited caloric intake in this patient was due to the lack of central venous access and antifungal administration during this period. If this patient's data are removed from analysis, there would be no difference between groups with respect to nonprotein calories at weeks 4 and 6, as well as fluid intake at week 6.

The plasma total carnitine concentrations by study period for each group are reported in Fig. 1. Concentrations were below reference range at baseline for both the placebo and carnitine groups. Concentrations were significantly increased from baseline to all other study points and exceeded the reference range from week 1 through the last study period in the carnitine group. In the placebo group, concentrations did not reach reference range until week 4. Statistically significant differences in concentrations between the groups occurred at weeks 1 and 2 study periods only. Statistically significant differences in the number of patients

Table 1 Demographic characteristics of study population.

	Placebo ($n = 13$)	Carnitine ($n = 16$)	P value
Gestation (weeks)*	27.2±2 (25–30)	27±2 (24–30)	0.80
< 25†	0	2	
25–29†	9	11	
⩾30†	4	3	
Birth weight (g)*	988±243 (608–1421)	966±279 (449–1476)	0.82
⩽750†	2	4	
751–1000†	6	6	
>1000†	5	6	
Apgar score			
1 min‡	6 (1–10)	3 (1–8)	0.11
5 min‡	8 (2–10)	7 (3–9)	0.06
PNA at enrollment (days)*	2.9±0.9 (1–4)	2.9±1 (1–4)	0.97
Male *n* (%)	7 (54)	4 (25)	0.14
Ethnicity *n* (%)			
African-American	6 (46)	4 (25)	0.27
White	7 (54)	12 (75)	0.27
Cesarean section *n* (%)	8 (62)	7 (45)	0.46

PNA = postnatal age.
*Data are mean±standard deviation (range).
†Data are number of patients.
‡Data are median (range).

with plasma total carnitine concentrations within or above reference range between the placebo and carnitine groups occurred at weeks 1 and 2 ($P < 0.001$) and week 4 ($P = 0.033$).

The RBC total carnitine concentrations by study period for each group are reported in Fig. 2. Both groups had concentrations greater than 0.2 nmol/mg hemoglobin at baseline. However, concentrations were below reference minimum for both groups between weeks 1 and 6 and also for the placebo group at week 8. Statistically significant within group differences occurred in the placebo group between baseline and week 2. Between group differences were not evident at any study point. Statistically significant differences in number of patients with RBC total carnitine concentrations at or above reference minimum between the placebo and carnitine groups occurred only at week 2 ($P = 0.037$).

Patient outcome data are reported in Table 3. Adverse events or side effects outside of expected complications that occur with prematurity were not seen in any of the neonates during the study. The requirement for packed red cell transfusion was similar between the groups and the majority of transfusions were given during the first 4 weeks of the study (74% in placebo group neonates and 83% in carnitine group neonates). The carnitine group neonates regained their birthweight more rapidly (approximately 5 days earlier) than the placebo group neonates. There was no difference in nitrogen balance between groups across all study periods.

All patients received artificial surfactant after delivery and theophylline during the study period. There was no difference between groups in the incidence of bronchopulmonary dysplasia, intraventricular hemorrhage, or necrotizing enterocolitis. There was also no difference between groups in ventilator use at enrollment, ventilator days, number of patients requiring reintubation, need for caffeine at discharge, and events of apnea and bradycardia recorded in the nurses' notes, or number of apneas >20 s from 15 h cardiopulmonary monitoring at each of the study periods. When total percent periodic breathing was evaluated for all patients off ventilatory support during the time of study intervention (i.e., between weeks 1 and 8), the carnitine group neonates had significantly lower percent periodic breathing compared to the placebo group neonates.

Eleven neonates (6 placebo, 5 carnitine) underwent polysomnography evaluation during the study period. Two patients were on methylxanthine therapy at the time of the study (1 placebo group neonate with 9% periodic breathing, 1 carnitine

Table 2 Nutrition and fluid intake by study period.

	Baseline (13 P/16 C)	Week 1 (13 P/16 C)	Week 2 (13 P/16 C)	Week 4 (13 P/15 C)	Week 6 (10 P/14 C)	Week 8 (9 P/12 C)
Fluid (ml/kg/day)						
Placebo	135.7±37.3	157.2±32.9	163.2±22.5	164.7±16.7	155.5±16*	155.9±27
Carnitine	157.9±49.8	163.2±36.9	161.7±22.9	156.6±29	141.2±17.5*	150.5±27.2
Nonprotein calories (kcal/kg/day)						
Placebo	57.5±19.8	82.2±14.9	101.2±25.3	125.6±13.5*	125.8±18.2*	121.6±24.9
Carnitine	63.5±22.5	88.7±25	104.7±28	112.3±19.6*	107.1±22.9*	114.6±31.1
% Enteral calories						
Placebo	10.4±15.9	35.5±35.6	53.5±39.9	84.5±37.5	80±42.2	83±35.7
Carnitine	10.6±16.4	34.6±36.1	60.3±40.1	71.7±39.2	83.9±35.1	84.7±35.8
Protein (g/kg/day)						
Placebo	1.2±0.7	3.2±0.8	3.5±1	3.5±0.8	3.4±0.5	3.2±1
Carnitine	1.6±1	3.7±0.8	3.6±1	3.5±0.8	3±0.6	3.3±0.6
Intravenous lipid (g/kg/day)						
Placebo	1.3±0.8 (10)	2.1±0.6 (10)	2.3±0.7 (7)	2.5±0 (2)	2.8±0.4 (2)	2.5 (1)
Carnitine	1.3±0.6 (14)	2.1±0.3 (14)	2.3±0.4 (9)	2.6±0.2 (5)	2.5±0 (2)	1.4 (1)
Carnitine (mg/kg/day)						
Placebo	0.1±0.3	0.5±0.5†	1±0.7†	1.7±0.8†	1.6±1†	1.7±0.8†
Carnitine	0.2±0.3	17.4±4†	19.4±3.1†	19.2±3.3†	18±2.6†	17.5±4.4†

Data are represented as mean±standard deviation.
Data represent both parenteral and enteral sources of fluid, calories, and protein.
Number of patients at each study point represented as number and P (placebo group) or C (carnitine group).
Number of patients at each study point for lipid dose noted in parentheses.
*$P \leqslant 0.05$ (placebo vs. carnitine).
†$P \leqslant 0.001$ (placebo vs. carnitine).

group neonate with 3.8% periodic breathing). There were no differences between groups with respect to percent periodic breathing (placebo group neonates 23.5±9.8 vs. carnitine group neonates 25.7±9.8; $P = 0.85$), apneas >20 s (3 placebo group neonates vs. 1 carnitine group neonate; $P = 0.55$), and incidence of gastroesophageal reflux (4 neonates in each group) during polysomnography. Additionally, there were no differences between groups with respect to the use of motility agents, gastric residuals, or need to stop enteral feedings. There was also no difference with respect to the incidence of late onset sepsis.

One set of multiple birth neonates (30 weeks gestation triplets) were randomized to different groups and underwent polysomnography evaluation during the study. One triplet (female, birthweight 1297 g), randomized to the carnitine group, had lower periodic breathing via polysomnography when compared to her siblings (female, birthweight 1257 g and male, birthweight 1273 g) randomized to the placebo group (3.7% compared to 29.5% and 24.1%, respectively). The carnitine group triplet went home off methylxanthine therapy while her siblings required caffeine at discharge.

Discussion

This study is the first to document both plasma and RBC total carnitine concentrations in a group of premature neonates over an extended period of supplementation. Neonates receiving 20 mg/kg/day carnitine greatly exceeded the plasma reference range during the study. Neonates in the placebo group did not reach plasma reference range until week 4, when neonates were taking in approximately 80% of nonprotein calories enterally. RBC total carnitine concentrations were above reference minimum in both groups at baseline and were similar to previous studies that have found higher RBC total carnitine concentrations, particularly in premature neonates, immediately after delivery.[19,25] RBC total carnitine concentrations fell in both groups and were below the reference

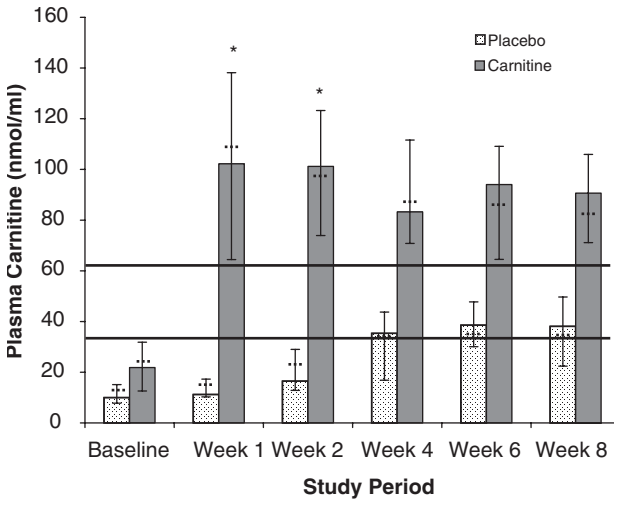

Figure 1 Plasma total carnitine concentrations by study period. Number of patients at each assessment are as follows: baseline (13 P/16 C), week 1 (13 P/15 C), week 2 (13 P/14 C), week 4 (13 P/14 C), week 6 (9 P/12 C), and week 8 (7 P/9 C). Data are represented as median with bars denoting 25th and 75th percentile range. The two horizontal lines delineate the reference plasma total carnitine concentration (31.1–60.5 nmol/ml).[24] Dashed lines represent mean data. Between group statistical significance denoted by asterisk ($P<0.05$). Within group differences noted between carnitine group baseline and carnitine group weeks 1, 2, 4, 6, and 8 ($P<0.05$).

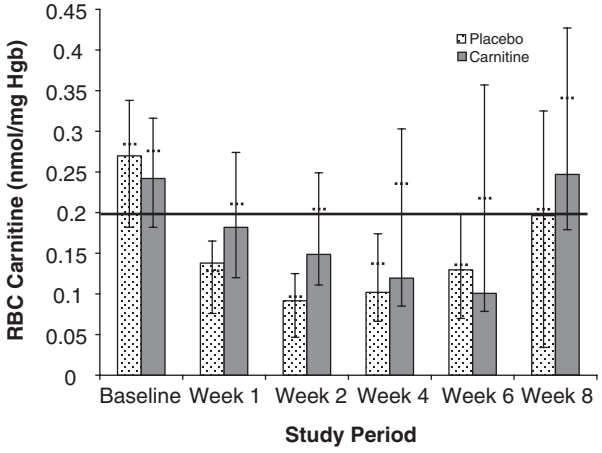

Figure 2 Red blood cell total carnitine concentrations by study period. Number of patients at each assessment are as follows: baseline (13 P/16 C), week 1 (13 P/15 C), week 2 (12 P/16 C), week 4 (13 P/14 C), week 6 (9 P/12 C), and week 8 (6 P/9 C). Data represented as median with bars denoting 25th and 75th percentile range. Expected concentrations in premature neonates should be $\geqslant 0.2$ nmol/mg hemoglobin (Hgb).[23,25] Dashed lines represent mean data. No between group statistical significance was determined. Within group difference noted between placebo group baseline and placebo group week 2 ($P<0.05$).

minimum for the majority of the study. Statistically significant differences between neonates with RBC total carnitine concentrations at or above reference minimum existed only during the early part of the study. The data suggest that carnitine supplementation may be important early in infancy and that once adequate enteral feedings are established, the amount of carnitine provided in enteral nutrition (approximately 2 mg/kg/day at week 4) is sufficient to achieve reference plasma carnitine status.

The failure to maintain RBC total carnitine concentrations despite achieving plasma concentrations above reference range clearly implies that RBC total carnitine concentrations are not equilibrated with plasma total carnitine concentrations. Plasma total carnitine concentrations may not be related to de novo carnitine synthesis and ''loading'' into new erythrocytes. If there were a relationship, we would have seen an effect since approximately one and a half to two half-lives for erythrocytes in premature neonates (lifespan of 60–90 days compared to 120 days in adults) occurred over the 8-week study. It is possible that, at least in the premature infant, a process that is not influenced by circulating plasma total carnitine concentrations predominantly governs total carnitine concentrations in erythrocytes. Therefore, it is unclear which is the preferred marker for evaluating carnitine status in premature infants.

The RBC carnitine concentration of donor blood has been reported as 0.16 ± 0.07 nmol/mg Hgb.[29] In the current study, packed RBC transfusions were similar between groups and the total carnitine contribution from all transfusions was negligible (less than 1% of the daily carnitine provision in the supplemented group). It is unlikely that the provision of packed RBCs during the course of the study had any effect on RBC total carnitine concentrations in the neonates.

The majority of the neonates in this study regained their birthweight over the first 3 weeks of life. Neonates who received carnitine supplementation regained their birthweight more rapidly than did placebo neonates (approximately 5 days earlier) and this effect occurred during the period when differences between plasma and RBC total carnitine concentrations were evident. Time to regain birthweight (and other indicators of fluid status) has been related to the prevalence of necrotizing enterocolitis in a longitudinal study of very low birthweight infants.[30] There was no difference between groups with respect to fluid, protein, or caloric intake during this period of time, suggesting that true weight gain occurred. Time to regain birthweight may be an indicator of catch up

Table 3 Clinical outcomes by study group.

	Placebo ($n = 13$)	Carnitine ($n = 16$)	P value
Patients on ventilator at enrollment	10	14	0.63
Initial days on ventilator	13.5±18.9	16.5±14.5	0.63
Patients requiring reintubation	3	8	0.25
Patients receiving PRBC transfusion	11	12	0.66
PRBC transfusions per patient	3.6±2.8	3.8±1.4	0.83
Total PRBC carnitine (mg/kg)*	0.18±0.1	0.17±0.03	0.91
BPD	7	10	0.13
IVH	2	3	1.00
NEC	3	2	0.63
Caffeine at discharge	11	13	1.00
GI motility agents	8	7	0.46
Gastric residuals	13	16	1.00
Need to stop enteral feedings	5	4	0.69
Late onset positive cultures	7	8	0.87
Birthweight regained			
Day of life	16.9±6.3	11.8±6	0.03
Study day	14±6.2	8.9±6.5	0.04
Study end weight	1.8±0.2	1.8±0.4	0.73
Change in weight (g)	806±186	870±317	0.52
Nitrogen balance (mg/kg/day)			
Baseline	172.8±141.8	181.7±157.8	0.88
Weeks 1–8	405.8±83.7	396.8±109.2	0.81
CP trend monitor % PB†			
Baseline (4 P/1 C)	1.3±1.7	1.6	—
Weeks 1–8 (29 P/32 C)	1.4±1.9	0.4±0.9	0.01

Data are mean±standard deviation.
Number of patients noted or represented as number and P (placebo) or C (carnitine).
BPD = bronchopulmonary dysplasia; CP = cardiopulmonary; GI = gastrointestinal; IVH = intraventricular hemorrhage; NEC = necrotizing enterocolitis; PB = periodic breathing; PRBC = packed red blood cell.
*Represents total carnitine intake (mg/kg) from PRBC transfusions across the study period.
†Percent periodic breathing (% PB): addition of number apneic events multiplied by duration of apneic events in seconds (i.e., 5, 10, 15, 20, 25, and 30 s) for each monitored period /total number of seconds evaluated (for 15 h recordings, denominator is 54,000 s).

growth throughout infancy and childhood. The successful achievement of catch-up growth in premature infants has been related to greater neurodevelopmental outcomes.[31,32] More recently, rapid early weight gain has been associated with markers of later cardiovascular disease (abnormal lipid profile, insulin and leptin resistance, increased blood pressure).[33]

Iafolla et al. reported a significant decrease in the incidence of apnea and greater wean from ventilatory support in carnitine supplemented premature neonates.[34] A systematic review of carnitine supplementation in apnea of prematurity later excluded this study after learning from one of its authors that the results were flawed.[35] The same investigators have also reported resolution of apnea and periodic breathing following carnitine supplementation in young siblings of an infant who died of sudden infant death syndrome.[21]

The use of an estimated percent periodic breathing from cardiopulmonary trend monitor recordings was used in the NICUs as a clinical tool for rapidly assessing apnea and respiratory morbidity. We found lower percent periodic breathing during the intervention period (from weeks 1 to 8) in the carnitine supplemented neonates. In addition, the rather large difference in periodic breathing during polysomnography between the carnitine supplemented triplet and her placebo group siblings lends further support to an effect of carnitine on respiratory morbidity.

One study correlated the incidence of coagulase-negative staphylococcal bacteremia in neonates with IV lipid administration.[36] Since carnitine supplementation in neonates enhances lipid utilization,[13,17] we hypothesized that this enhanced lipid utilization diverts exogenous, predominantly omega-6 fatty acids found in IV lipid preparations

from pro-inflammatory and immunoinhibitory eicosanoid production to energy production. Through this mechanism, we speculated that carnitine may have an effect on lowering the incidence of infections in the preterm neonatal population. In the current study, we found no effect of carnitine supplementation on the incidence of late onset sepsis.

Other studies have evaluated carnitine supplementation via both the parenteral and enteral route in premature neonates and have found no effect of carnitine on growth.[37–40] Shortland et al.[37] reported plasma total carnitine concentrations (160 nmol/ml on day 7 to 104 nmol/ml on day 28) similar to those of the current study. Two other studies, utilizing more aggressive dosing regimens, reported surprisingly high plasma total carnitine concentrations at 2 (up to 343.6 nmol/ml) and 4 (up to 250 nmol/ml) weeks.[38,39] Higher carnitine concentrations achieved in these studies may have negated any potential benefit of supplementation. An earlier study found increased fat and protein oxidation, decreased nitrogen balance, and less weight gain in neonates receiving 48 mg/kg/day L-carnitine within the first week of life. Plasma total carnitine concentrations were 197 ± 60 nmol/ml at 7 days; the investigators speculated that the negative effects may have been dose related.[41]

In addition to finding no effect of carnitine supplementation on growth, two of these studies, one by O'Donnell et al. and the other by Whitfield et al., also reported there was no effect on respiratory morbidity. The effect of carnitine in the current study on time to regain birthweight and periodic breathing, and the lack of an effect in two similar previous studies, may be explained by differences in study design. In addition to more aggressive dosing regimens and differences in carnitine assessment across the study period, one critical difference exists. One of the inclusion criteria in the O'Donnell study was that subjects be either nonintubated on nasal continuous positive airway pressure or intubated with a rate of less than 6 breaths/min. This study design likely excluded the more severely affected neonates. In fact, median ventilation time was 2 days for both groups.[38] In the current study, neonates remained on ventilatory support for a considerably greater period of time (13.5 ± 18.9 days, placebo group and 16.5 ± 14.5, carnitine group). The only statistical difference between groups in the O'Donnell study was the initial cardiorespirogram recording (recorded on day of life 4 and neonates were enrolled at 48 h or less).[38] By the next cardiorespirogram recording 4 days later, there was no difference. This may suggest that the carnitine group had greater respiratory compromise shortly after enrollment and before the effect of carnitine could be established.

Weaknesses of the current study include small sample size and methodology limitations, specifically with respect to the nitrogen analysis. When we conducted the diaper nitrogen recovery studies to validate this methodology, we did not incorporate a method to determine if we could elute nitrogen from stool in diapers. At 2 weeks into the study, neonates were taking approximately 50% of caloric intake from enteral diet and our diaper collections contained stool for the majority of the nitrogen analyses. The nitrogen recovery from the diapers included a filtering procedure and it is possible that the nitrogen in the stool was filtered out of the solution and we were actually measuring nitrogen losses from urine alone. While this defect in methodology would be expected to affect both groups similarly, variability between subjects in diaper collections (number of diapers per 24 h period and amount of stool per diaper) could have obscured a potential difference in nitrogen balance. Finally, a protocol violation in enrollment brought our final study subjects just under that supported by our power analysis. Though it's unlikely that one patient would have changed our results significantly, our results are clearly limited by the number of subjects reported in this small trial.

In summary, we found that long-term supplementation of carnitine at 20 mg/kg/day resulted in increased plasma and RBC total carnitine concentrations. Early carnitine supplementation in premature neonates has a positive result on time to regain birthweight and may improve periodic breathing in premature neonates. The results of the current study may be generalized to premature neonates initially receiving PN support. Whether there is any additional benefit of continued carnitine supplementation once neonates are primarily fed by the enteral route is uncertain at this time. Future studies evaluating the effect of carnitine supplementation on neonatal morbidity parameters should include an assessment of different dosing regimens and the effect of supplementation after enteral feedings have commenced.

Acknowledgements

Supported in part by the Le Bonheur Children's Medical Center Small Grants Program and Sigma Tau Pharmaceuticals. We thank the dedicated NICU nurses and pharmacy staffs at Methodist Le Bonheur

Healthcare, North and Germantown hospitals; Dianne Woodall, M.D. for assistance with patient recruitment; Jason West, Pharm.D. for study coordination, patient randomization, study drug distribution and nitrogen analysis; Bo Wang, M.D. and Lee Wright, Pharm.D. for assistance with analysis of plasma and red blood cell total carnitine concentrations; and Laura Plummer, Pharm.D. for assistance with study coordination, study drug distribution and nitrogen analysis.

References

1. Schmidt-Sommerfeld E, Penn D, Wolf H. The influence of maternal fat metabolism on fetal carnitine levels. *Early Hum Dev* 1981;**5**:233–42.
2. Schmidt-Sommerfeld E, Penn D, Sodha RJ, Progler M, Novak M, Schneider H. Transfer and metabolism of carnitine and carnitine esters in the in vitro perfused human placenta. *Pediatr Res* 1985;**19**:700–6.
3. Penn D, Schmidt-Sommerfeld E, Pascu F. Decreased tissue carnitine concentrations in newborn infants receiving total parenteral nutrition. *J Pediatr* 1981;**98**:976–8.
4. Shenai JP, Borum PR. Tissue carnitine reserves of newborn infants. *Pediatr Res* 1984;**18**:679–82.
5. Penn D, Dolderer M, Schmidt-Sommerfeld E. Carnitine concentrations in the milk of different species and infant formulas. *Biol Neonate* 1987;**52**:70–9.
6. Novak M, Monkus EF, Buch M, Lesmes H, Silverio J. The effect of a L-carnitine supplemented soybean formula on the plasma lipids of infants. *Acta Chir Scand Suppl* 1983;**517**: 149–55.
7. Schiff D, Chan G, Secombe D, Hahn P. Plasma carnitine levels during intravenous feeding of the neonate. *J Pediatr* 1979; **95**:1043–6.
8. Schmidt-Sommerfeld E, Wolf H. Carnitine deficiency in premature infants receiving total parenteral nutrition. *Early Hum Dev* 1980;**4**:23–34.
9. Schmidt-Sommerfeld E, Penn D, Wolf H. Carnitine blood concentrations and fat utilization in parenterally alimented premature newborn infants. *J Pediatr* 1982;**100**:260–4.
10. Christensen ML, Helms RA, Mauer EC, Storm MC. Plasma carnitine concentration and lipid metabolism in infants receiving parenteral nutrition. *J Pediatr* 1989;**115**:794–8.
11. Schmidt-Sommerfeld E, Penn D, Wolf H. Carnitine deficiency in premature infants receiving total parenteral nutrition: effect of L-carnitine supplementation. *J Pediatr* 1983;**102**: 931–5.
12. Orzali A, Donzelli F, Enzi G, Rubaltelli FF. Effect of carnitine on lipid metabolism in the newborn. *Biol Neonate* 1983;**43**: 186–90.
13. Helms RA, Whitington PF, Mauer EC, Catarau EM, Christensen ML, Borum PR. Enhanced lipid utilization in infants receiving oral L-carnitine during long term parenteral nutrition. *J Pediatr* 1986;**109**:984–8.
14. Melegh B, Kerner J, Sandor A, Vinceller M, Kispal G. Oral L-carnitine supplementation in low-birth-weight newborns: a study on neonates requiring combined parenteral and enteral nutrition. *Acta Paediatr Hung* 1986;**27**:253–8.
15. Melegh B, Kerner J, Sandor A, Vinceller M, Kispal G. Effects of oral L-carnitine supplementation in low-birth-weight premature infants maintained on human milk. *Biol Neonate* 1987;**51**:185–93.
16. Melegh B, Szucs L, Kerner J, Sandor A. Changes of plasma free amino acids and renal clearances of carnitine in premature neonates during L-carnitine-supplemented human milk feeding. *J Pediatr Gastroenterol Nutr* 1988;**7**: 424–9.
17. Helms RA, Mauer EC, Hay WW, Christensen ML, Storm MC. Effect of intravenous L-carnitine on growth parameters and fat metabolism during parenteral nutrition in neonates. *J Parenter Enteral Nutr* 1990;**14**:448–53.
18. Bonner CM, DeBrie KL, Hug G, Landrigan E, Taylor BJ. Effects of parenteral L-carnitine supplementation on fat metabolism and nutrition in premature neonates. *J Pediatr* 1995;**126**: 287–92.
19. Shenai JP, Borum PR, Mohan P, Donlevy SC. Carnitine status at birth of newborn infants of varying gestation. *Pediatr Res* 1983;**17**:579–82.
20. Weaver LT, Rosenthal SR, Gladstone W, Winter HS. Carnitine deficiency: a possible cause of gastrointestinal dysmotility. *Acta Paediatr* 1992;**81**:79–81.
21. Iafolla AK, Browning IB, Roe CR. Familial infantile apnea and immature beta oxidation. *Pediatr Pulmonol* 1995;**20**:167–71.
22. Winter SC, Szabo-Aczel S, Curry CJ, Hutchinson HT, Hogue R, Schug A. Plasma carnitine deficiency. Clinical observations in 51 pediatric patients. *Am J Dis Child* 1987;**141**:660–5.
23. Cederblad G, Lindstedt S. A method for determination of carnitine in the picomole range. *Clin Chim Acta* 1972;**37**: 235–43.
24. Patera A, Neylan MM, Borum PR. Plasma and red blood cell carnitine concentrations of healthy one week old infants receiving dietary carnitine. In: Borum PR, editor. *Clinical aspects of human carnitine deficiency*. Elmsford, NY: Pergamon Press; 1986. p. 46–7.
25. Borum PR. Carnitine in neonatal nutrition. *J Child Neurol* 1995;**10**:2S25–31.
26. Konstantinides FN, Boehm KA, Radmer WJ, et al. Pyrochemiluminescence: real time, cost-effective method for determining total urinary nitrogen in clinical nitrogen-balance studies. *Clin Chem* 1988;**34**:2518–20.
27. Boehm KA, Ross FA. Determination of total nitrogen in urine by pyrochemiluminescence: collaborative study. *JAOAC Int* 1995;**78**:301–6.
28. Van Goudoever JB, Wattimena JDL, Carnielli VP, Sulkers EJ, Degenhart HJ, Sauer JJ. Effect of dexamethasone on protein metabolism in infants with bronchopulmonary dysplasia. *J Pediatr* 1994;**124**:112–8.
29. Borum PR. Plasma carnitine compartment and red blood cell carnitine compartment of healthy adults. *Am J Clin Nutr* 1987;**46**:437–41.
30. Uauy RD, Fanaroff AA, Korones SB, Phillips EA, Phillips JB, Wright LL. Necrotizing enterocolitis in very low birth weight infants: biodemographic and clinical correlates. National Institute of Child Health and Human Development Neonatal Research Network. *J Pediatr* 1991;**119**:630–8.
31. Hack M, Merkatz IR, Gordon D, Jones PK, Fanaroff AA. The prognostic significance of postnatal growth in very low birth weight infants. *Am J Obstet Gynecol* 1982;**143**:693–9.
32. Latal-Hajnal B, Von Sibenthal K, Kovari H, Bucher HU, Largo RH. Postnatal growth in VLBW infants: significant association with neurodevelopmental outcome. *J Pediatr* 2003;**143**:163–70.
33. Singhal A, Lucas A. Early origins of cardiovascular disease: is there a unifying hypothesis? *Lancet* 2004;**363**:1642–5.
34. Iafolla AK, Roe CR. Carnitine deficiency in apnea of prematurity. *Pediatr Res* 1995;**2**:309A.
35. Kumar M, Kabra NS, Paes B. Role of carnitine supplementation in apnea of prematurity: a systematic review. *J Perinatol* 2004;**24**:158–63.

36. Freeman J, Goldmann DA, Smith NE, Sidebottom DG, Epstein MF, Patt R. Association of intravenous lipid emulsion and coagulase-negative staphylococcal bacteremia in neonatal intensive care units. *N Eng J Med* 1990;**323**: 301–8.
37. Shortland GJ, Walter JH, Stroud C, Fleming PJ, Speidel BD, Marlow N. Randomised controlled trial of L-carnitine as a nutritional supplement in preterm infants. *Arch Dis Child Fetal Neonatal Ed* 1998;**78**:F185–8.
38. O'Donnell, Finer NN, Rich W, Barshop BA, Barrington KJ. Role of L-carnitine in apnea of prematurity: a randomized, controlled trial. *Pediatrics* 2002;**109**:622–6.
39. Whitfield J, Smith T, Sollohub H, Sweetman L, Roe CR. Clinical effects of L-carnitine supplementation on apnea and growth in very low birth weight infants. *Pediatrics* 2003; **111**:477–82.
40. Pande S, Brio LP, Campbell DE, Gayle Y, Esteban-Cruciani NV. Lack of effect of L-carnitine supplementation on weight gain in very preterm infants. *J Perinatol* 2005;**25**:470–7.
41. Sulkers EJ, Lafeber HN, Degenhart HJ, Przyrembel H, Schlotzer E, Sauer PJJ. Effects of high carnitine supplementation on substrate utilization in low-birth-weight infants receiving total parenteral nutrition. *Am J Clin Nutr* 1990;**52**:889–94.

Available online at www.sciencedirect.com
ScienceDirect