Available online at www.sciencedirect.com
ScienceDirect
Nutrition Research 30 (2010) 233–239
Nutrition Research
www.nrjournal.com

L-Carnitine–supplemented parenteral nutrition improves fat metabolism but fails to support compensatory growth in premature Korean infants

So-Hui Seong[a], Soo-Chul Cho[b], Yongsoon Park[c], Youn-Soo Cha[a,*]

[a]*Department of Food Science and Human Nutrition and Research Institute of Human Ecology, Chonbuk National University, Jeonju, Jeonbuk 561-756, South Korea*
[b]*Department of Pediatris, Chonbuk National University Medical School, Jeonju, Jeonbuk 561-712, South Korea*
[c]*Department of Food and Nutrition, Hanyang University, Seoul, 133-791, South Korea*

Received 12 March 2010; accepted 8 April 2010

Abstract

We have previously shown that pregnant Korean mothers often have especially poor carnitine status, which may be responsible for the suboptimal carnitine levels of newborn Korean infants. This study tested the hypothesis that carnitine obtained from premature infant formula alone is adequate in sustaining optimal lipid metabolism and growth in premature infants. Accordingly, we investigated the effects of parenteral carnitine supplementation on carnitine status, growth parameters, and lipid metabolism in premature infants by measuring serum lipid profiles, carnitine and β-hydroxybutyrate concentrations, and body weight, size, and length. Twenty-five low–birth weight Korean infants were randomly assigned to control (LCNS, n = 12) or L-carnitine–supplemented (10 mg/[kg d], LCS, n = 13) groups. On day 9, the triacylglycerol concentration was lower in the LCS group; but the high-density lipoprotein cholesterol concentration and free, acyl, and total carnitine and β-hydroxybutyrate were significantly increased compared with the LCNS group. The ratio of acyl carnitine to free carnitine was significantly lower on day 5 in the LCS compared with the LCNS group. Body weight, height, Apgar score (1 and 5 minute), head circumference, and chest circumference were recorded on day 0; and body weight was measured again on days 5 and 9. Infant formula intake was recorded every day. There was no significant difference in body weight or growth parameters between the groups from days 0 to 9.Therefore, we concluded that, in low–birth weight infants, the addition of 10 mg/(kg d) supplemental carnitine significantly improves lipid profiles and serum carnitine level but does not enhance growth.

Keywords: L-carnitine; Infant; Low birth weight; Triacylglycerol; 3-hydroxybutyrate; Carnitine; Growth

Abbreviations: β-HB, β-hydroxybutyrate; AIAC, long-chain acyl carnitine; ANOVA, analysis of variance; ASAC, acid-soluble, short- and medium-chain acyl carnitine; GA, gestational age; HDL, high-density lipoprotein; LBW, low birth weight; LCNS, non–carnitine-supplemented group; LCS, L-carnitine–supplemented group; NEC, nonesterified, free carnitine; PCA, perchloric acid; TC, total serum cholesterol; TCNE, total carnitine; TG, triacylglycerol; TPN, total parenteral nutrition.

1. Introduction

Birth initiates major metabolic challenges for the emerging neonate. After sudden deprivation of the continuous transplacental supply of glucose by alternating periods of feeding and fasting, the neonate must switch on the endogenous production of glucose until reliable nutrition intake becomes established. The neonate now has to meet its need for glucose through glycogenolysis and gluconeogenesis and, to a lesser extent, from lactate. As time passes, fat utilization becomes more important in meeting the

* Corresponding author. Tel.: +82 63 270 3822; fax: +82 63 270 3854.
E-mail address: cha8@chonbuk.ac.kr (Y.-S. Cha).

0271-5317/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.nutres.2010.04.004

energy needs and for maintaining constant body temperature. These metabolic differences are reflected by a change in the respiratory quotient from 0.9 to 1.0 to 0.7 during this period [1].

Low–birth weight (LBW) infants have high energy requirements and are dependent upon high fat intake to maintain adequate postnatal growth. Fat is transported in plasma as triglycerides, which are derived from either the diet or de novo lipogenesis [2]. Very LBW infants have low carnitine levels with impaired ketogenesis and are dependent upon parenteral nutrition for a prolonged period [3].

Carnitine (3-hydroxy-4-*N*-trimethylaminobutyric acid) is an important cofactor in β-oxidation, as it facilitates the transport of long-chain fatty acids into the inner mitochondrial matrix in the form of acyl carnitines [4]. L-Carnitine causes a remarkable decrease in the content of saturated fatty acids in the very low density lipoprotein + low-density lipoprotein fraction and increases this content in the high-density lipoprotein (HDL) particle. This response is consistent with an increase in fatty acid β-oxidation and/or an enhancement in lipoprotein metabolism [5]. Therefore, carnitine supplementation may be needed to support lipid metabolism in LBW infants because these infants have a greater need for energy met by endogenous and exogenous fat. L-Carnitine supplementation in infants receiving long-term total parenteral nutrition (TPN) enhances fatty acid oxidation, as evidenced by increased ketogenesis [6], demonstrating that the ability of premature infants to oxidize fatty acids is linked to carnitine status. Fatty acid oxidation after a fat challenge is improved if TPN includes L-carnitine [7]. The preterm infant is born with limited carnitine reserves. During TPN, plasma and tissue carnitine concentrations decrease, indicating that the rate of carnitine biosynthesis is inadequate because of a reduced capacity for liver and kidney carnitine biosynthesis; and thus, they are dependent upon exogenous sources of carnitine [8].

We previously reported that LBW infants in Korea have either carnitine deficiency or low bioavailability [9]. Although commonly available neonatal formulas contain L-carnitine, the adequacy of L-carnitine in these formulas has not been fully assessed. This study was designed to test the hypothesis that the L-carnitine in commercial neonatal formulas is adequate to support normal energy metabolism and growth in LBW infants who are supplemented with parenteral nutrition. If carnitine availability is insufficient to maintain normal energy metabolism, this may be reflected in poor growth, high triglycerides, and low ketogenesis (due to impaired fatty acid oxidation). Accordingly, weight gain, serum lipid profiles, serum β-hydroxybutyrate (β-HB), and serum carnitine concentrations were used to assess differences in growth and lipid metabolism in LBW infants with or without L-carnitine–supplemented parenteral formulas. This study provides valuable information on carnitine requirements for premature infants in a population with marginal carnitine status and may be applicable to premature infants in subsets of populations that normally maintain adequate carnitine status.

2. Methods and materials

The studies were performed on 25 LBW infants (13 boys and 12 girls). The infants were less than 1 week old, were born in the Chonbuk National University Hospital, and were patients in the pediatrics unit and neonatal intensive care unit. Gestational age (GA) was calculated from the first day of the mother's last menstrual period or, when dates were uncertain, by the method of Dubowitz et al [10]. The non-Korean neonates were excluded because of racial differences in birth weight distribution. Infants with severe malformations, metabolic diseases, perinatal asphyxia, or proven bacterial infection were also excluded from the study. At birth, the LBW infants had body weights of less than 2500 g. The LBW infants were divided into 2 groups: L-carnitine supplemented (LCS, n = 13) and non–L-carnitine-supplemented (LCNS, n = 12). This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Chonbuk National University Ethical Board of Clinical Experiments. Informed and written consent was obtained from all parents and attending physicians.

2.1. Carnitine supplementation

Infants were randomly assigned to receive their TPN diets (as shown in Table 1) with or without L-carnitine 10

Table 1
Nutrient composition of parenteral nutrition solutions

	Per 100 mL
Amino acids (%) (TrophAmine; Braun, Irvine, CA, USA)[a]	3
Dextrose (%)	10
Sodium (mEq)	4
Potassium (mEq)	2
Chloride (mEq)	4.6
Calcium (mEq)	3-4.5
Phosphorus (mEq)	1.4
Magnesium (mEq)	0.4
Zinc (μg)	500
Copper (μg)	60
Manganese (μg)	5
Selenium (μg)	3
Iodine (μg)	8
Iron (μg)	0.1
Vitamins (MVI Pediatric; Mayne Pharm, Paramus, NJ, USA) (mL)	2.5
Heparin (U)[b]	50
Fat emulsions (g/[kg d])[c]	1-3

[a] Maintains the serum amino acid pattern similar to breast-fed infants.
[b] Heparin accelerates the release of active lipoprotein lipase. This increases the release of free fatty acids from chylomicrons and very low density lipoproteins. Therefore, there is an increase in free fatty acid availability for β-oxidation.
[c] The fat emulsion from soybean oil that contains long-chain triglyceride and medium-chain triglyceride in 1:1 ratio.

mg/(kg d) (L-carn, Ildong Pharmaceutical Co Ltd, Seoul, Korea) beginning from 5 days after birth. The infants in this study were also fed with premature infant's formula (Maeil Dairy Industry Co Ltd, Pyeongtaek, Korea) containing 10 mg of carnitine per 100 g of formula as reported (per product labeling).

2.2. Outcome measures

Measurements of body weight, height, Apgar score (1 and 5 minutes), head circumference, and chest circumference were recorded on the first study day; and subsequent weights were recorded on days 5 and 9. Infant formula intake was recorded every day during the study period.

2.3. Sample collection

Blood samples were obtained by vein puncture on the day of birth, day 5 (before carnitine supplementation), and day 9. The collected blood samples were transferred into tubes, immediately chilled on ice, allowed to coagulate, and placed in a refrigerated centrifuge for 15 minutes at 3500 rpm to obtain the serum within an hour. The serum was immediately separated, coded, and stored at −80°C. All laboratory analyses were conducted in the Nutrigenomics and Assessment laboratory in the Department of Food Science and Human Nutrition, Chonbuk National University, Jeonju, Korea.

2.4. Serum lipids and β-HB assay

Total serum cholesterol (TC) and triacylglycerol (TG) were enzymatically measured using commercial kits (Asan Pharm Co, Seoul, Korea). The HDL cholesterol assay was carried out with a commercial kit based on phosphotungstic acid/$MgCl_2$ precipitation (Asan Pharm Co). Serum TC and HDL cholesterol [11] were assayed at 500 nm, TG [12] was assayed at 550 nm, and β-HB was assayed at 450 nm using a commercial kit (Biovision Research Products, Mountain View, CA). All assays were carried out spectrophotometrically using a UV spectrophotometer (UV-1601; Shimadzu, Australia).

2.5. Carnitine assay

Serum carnitine was assayed according to the modification of Sachan et al [13] of the radioisotope method of Cederblad and Lindstedt [14]. International isotope standards for each carnitine fraction were added to correct sample data. One hundred– to 200-μL serum samples were homogenized with 200 μL of 6% perchloric acid (PCA). After centrifugation at 1500*g* for 10 minutes, supernatant and pellet were separated. Nonesterified, free carnitine (NEC) was assayed in 150 μL of neutralized supernatant. Acid-soluble, short- and medium-chain acyl carnitine (ASAC) was assayed using 100 μL of the PCA supernatant after it was hydrolyzed with 1 N KOH at 37°C for 30 minutes followed by neutralization. Long-chain acyl carnitine (AIAC) was assayed using the original PCA pellet after it was washed with 6% PCA for 3 to 4 times to remove NEC and ASAC remnants, hydrolyzed with 0.5 N KOH, and incubated in a hot water bath for 60 minutes at 65°C. After cooling to room temperature, it was neutralized with a PCA-MOPS (3-[4-morpholino] propane sulfonic acid) solution (1 mol/L MOPS in 3% PCA) and centrifuged; then 100 μL of supernatant was assayed for acid-insoluble AIAC. Each carnitine fraction was assayed with the same reaction mixture (1 mol/L MOPS buffer, 0.1 mol/L potassium ethyleneglycotetraacetate, 0.1 mol/L sodium tetrathionate, 0.1 mmol/L [1-C14] acetyl–coenzyme A solution [Amersham, Little Chalfont, Buckinghamshire, England]). This was then added to the supernatant of each sample, and the reaction was started with 1 unit of carnitine acetyl transferase (Sigma Chemical Co, St Louis, MO) and carried on to completion by incubating at 37°C for 30 minutes. A 200-μL aliquot of reacted supernatant was passed through a column with ion exchange resin (AG 1x8, 200-400 mesh), which collects the unreacted [1-^{14}-C]acetyl carnitine; and the ^{14}C isotope was measured in a Beckman LS-3801 liquid scintillation counter (Beckman Instruments, Palo Alto, CA). Total carnitine (TCNE) was calculated as the sum of NEC, ASAC, and AIAC values.

2.6. Statistical analyses

A sample size of 10 infants in each group was determined to be sufficient to detect a clinically important difference of 6.5 mg/dL for TG, assuming a standard deviation of 8 mg/dL, using a 2-tailed *t* test of the difference between means, a power of 80%, and a significance level of 5%. The calculation was based on the assumption that the measurements of TCNE are normally distributed. This assumption of normal distribution was found in NEC (σ = 0.45 μmol/dL, δ = 0.56 μmol/dL) and TCNE (σ = 1.97 μmol/dL, δ = 2.5 μmol/dL). This number was increased to 13 per group (for a total of 26) to allow for a predicted dropout rate of 20% from the treatment group. At the end stage of the study period, one subject from the LCS group withdrew from continuing the experiment; and hence, the number of participants changed from 13 to 12.

All data were analyzed using the SPSS (Chicago, IL) 12.0.1 package. Student *t* test for unpaired samples was used to test for differences between the 2 groups. Comparisons of differences among the 3 periods were analyzed by analysis of variance (ANOVA) and Duncan multiple range tests. Results are presented as means ± SD. Differences were considered significant at $P \leq .05$.

3. Results

Between May 2006 and September 2007, 25 neonates completed the study, 8 males and 5 females in LCS and 5 males and 7 females in the LCNS group. General characteristics and growth parameters of the subjects are shown in Table 2. The supplemented and control groups did not differ

Table 2
Growth parameters of 25 preterm infants fed either a carnitine-supplemented (10 mg/[kg d]) or non–carnitine-supplemented parenteral nutrition formula

Measurement	LCS (n = 13)	LCNS (n = 12)
GA at birth (wk)	31.31 ± 2.39	33.00 ± 3.26
Apgar index (1 min)	6.09 ± 1.92	6.36 ± 1.03
Apgar index (5 min)	7.08 ± 1.98	7.36 ± 0.67
Height (cm)	40.06 ± 2.18	41.29 ± 2.34
Weight (cm)	1509 ± 241	1643 ± 228
Head circumference (cm)	29.28 ± 1.55	29.48 ± 1.45
Chest circumference (cm)	24.94 ± 1.69	25.29 ± 0.98

Values are means ± standard deviations.

significantly in GA, Apgar index, birth weight, height, head circumference, or chest circumference. Mean GA at birth of the subjects was 32.08 ± 2.89 weeks. Mean Apgar scores at 1 and 5 minutes were 6.23 ± 1.51 and 7.21 ± 1.50, respectively. Mean height and body weight immediately after birth were 40.63 ± 2.29 cm and 1570.42 ± 239.65 g. Mean head and chest circumferences were 29.37 ± 1.48 and 25.10 ± 1.39 cm, respectively. Infant formula intake for each study period is reported in Table 3. There were no statistically significant differences between the groups. Table 4 shows L-carnitine intakes by enteral feeding and L-carnitine supplementation by TPN. L-Carnitine intakes of LCNS and LCS from the premature infant's formula during the study period were 10.61 ± 7.51 and 11.42 ± 7.66 mg/(kg d), respectively. The neonates receiving carnitine-supplemented TPN received approximately 10 mg/(kg d) supplemental carnitine in addition to that provided by the enteral formula; and thus, TCNE intake levels were approximately 2 times higher in LCS than in LCNS. Efficacy of the L-carnitine supplementation on weight gain is shown in Table 5. In this study, there were no significant differences in body weight gain between LCNS and LCS from days 0 to 9.

Serum TG, TC, and HDL cholesterol concentrations on days 0 and 5, before L-carnitine supplementation, and on day 9 are shown in Table 5. The TG levels in both groups were significantly increased on day 9 compared with day 0 ($P \leq .05$). However, on day 9, infants receiving carnitine (group LCS) had a lower TG level than the non–carnitine-supplemented group (group LCNS). The TC concentrations increased significantly with time, but no significant differences were observed by carnitine supplementation.

Table 3
Daily intake of formula and kilojoules by preterm infants during study period

Variables	Intake (mL)		Intake (kJ)	
	LCS (n = 13)	LCNS (n = 12)	LCS (n = 13)	LCNS (n = 12)
1 d	0.00 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0
2 d	31.10 ± 43	46.5 ± 39	69.9 ± 60	71.0 ± 110
3 d	56.2 ± 67	63.1 ± 70	82.4 ± 165	149.0 ± 227
4 d	73.8 ± 65	81.6 ± 68	130.0 ± 181	220.0 ± 269
5 d	106.9 ± 84	108.0 ± 78	271.1 ± 238	302.0 ± 294
6 d	129.0 ± 89	141.9 ± 109	398.4 ± 235	435.0 ± 395
7 d	187.2 ± 108	172.8 ± 115	428.8 ± 312	471.0 ± 425
8 d	184.0 ± 122	199.1 ± 143	408.7 ± 360	507.0 ± 491
9 d	199.8 ± 125	192.1 ± 115	444.0 ± 376	407.0 ± 388

Values are means ± standard deviations. Enteral feeding by premature infant's formulas (Maeil Dairy).

Table 4
Carnitine intake by the preterm infants fed a carnitine-supplemented (10 mg/[kg d]) or non–carnitine-supplemented parenteral nutrition formula

Variables	LBW	
	LCS (n = 13)	LCNS (n = 12)
L-Carnitine intake (mg/d)	11.42 ± 7.66	10.61 ± 7.51
L-Carnitine supplement (mg/[kg d])	10	–
Total intake (mg/d)	23.39 ± 10.42	10.61 ± 7.51

Values are means ± standard deviations. L-Carnitine supplement by TPN.

Unlike TG and TC, serum HDL cholesterol concentrations in the LCS were significantly increased on day 9. However, no significant differences in serum HDL were observed during the study period in the LCNS group. The free and acyl carnitine concentrations in plasma for each group are presented in Fig. 1. There was a significant increase in the level in serum free and acyl carnitine in the LCS group on day 9 but not on day 0 or day 5 (before supplementation). Infants receiving carnitine supplement (LCS group) showed a higher concentration of NEC, AIAC, and TCNE levels in their plasma compared with the LCNS group. However, the LCNS had significantly higher ASAC on days 0 and 5; and on day 5, the LCNS had significantly higher TCNE than their LCS counterparts ($P \leq .05$).

The serum β-HB concentrations of each group are shown in Fig. 2. The concentration of β-HB increased on day 5 in LCNC and kept increasing with time in LCS, which resulted in significantly higher β-HB on day 9 in the carnitine-supplemented group.

4. Discussion

Carnitine, a quaternary trimethylamine present in both breast milk and infant formulas, plays a major role in the oxidation of long-chain fatty acids. Premature neonates are dependent on exogenous supplies of carnitine for the maintenance of serum carnitine levels. However, carnitine is not routinely provided in parenteral nutrition solutions; and consequently, non–carnitine-supplemented parenterally fed infants have very low tissue carnitine levels. Although neonates are provided with prolonged parenteral nutrition containing the essential precursors (lysine and methionine) for the synthesis of carnitine, serum carnitine levels continue to decrease or remain low with systemic carnitine deficiency until carnitine is supplied exogenously [3].

Carnitine deficiency may be a causative factor in the inability of premature babies to use parenteral lipid. In related studies, it has been suggested that fatty acid oxidation is impaired when tissue carnitine levels fall to less than 10% of normal. Therefore, relative carnitine deficiency may impair fatty acid oxidation, thus reducing the utilization of available energy and impairing growth.

Table 5
Changes in body weight and lipid profile between carnitine-supplemented and non–carnitine-supplemented groups

Parameter	LCS (n = 13)			LCNS (n = 12)		
	Day 0	Day 5	Day 9	Day 0	Day 5	Day 9
Body weight (g)	1587 ± 218.99	1479 ± 232.88	1535 ± 268.34	1600 ± 187.91	1537 ± 196.64	1533.33 ± 150
Triglyceride (mg/dL)	16.3 ± 6.38^{y}	28.7 ± 15.84^{x}	36.03 ± 7.62^{x}*	17.44 ± 9.76^{b}	30.68 ± 21.57^{ab}	49.65 ± 28.83^{a} *
TC (mg/dL)	63.43 ± 25.1^{y}	95.46 ± 23.48^{y}	160.86 ± 91.27^{x}	53.28 ± 24.35^{b}	85.2 ± 30.94^{a}	89.27 ± 32.39^{a}
HDL cholesterol (mg/dL)	7.07 ± 3.31^{y}	8.79 ± 4.03^{y}	12.9 ± 6.32^{x}	8.61 ± 3.95	9.94 ± 2.76	10.97 ± 4.28

The neonates were fed parenteral nutrition with carnitine (LCS) or without carnitine (LCNS). Their body weight, serum triglyceride, TC, and HDL cholesterol were measured on different days. The values are the means ± SD. Different letters indicate significantly different at $P < .05$ among the group.

* Significantly different between the groups on ninth day at $P < .05$ (Student t test).

Neonates who received parenterally administered carnitine had normal serum carnitine levels within 2 weeks [3,15]. In the present study, the basic developmental and metabolic characteristics were measured in LBW Korean infants to assess any specific changes in serum lipid profiles and in carnitine and β-HB concentrations as a consequence of carnitine supplementation.

The LCS had serum carnitine levels approximately 2-fold higher than those of the LCNS, which enhanced the TG utilization in the LCS. Despite this high level of carnitine, our study did not reveal any beneficial effects on growth, as measured by weight gain. In the review by Cairns and Stalker [15], only one study showed a significant effect on weight gain during the second week of supplementation; and this effect was noted only in infants who weighed 1001 to 1500 g [16]. This beneficial effect on growth was not sustained, and another study on carnitine supplementation by Whitfield et al [17] showed that routine carnitine supplementation (2 mg/[kg d]) in 33 preterm infants who weighed less than 1500 g had no demonstrable effect on growth.

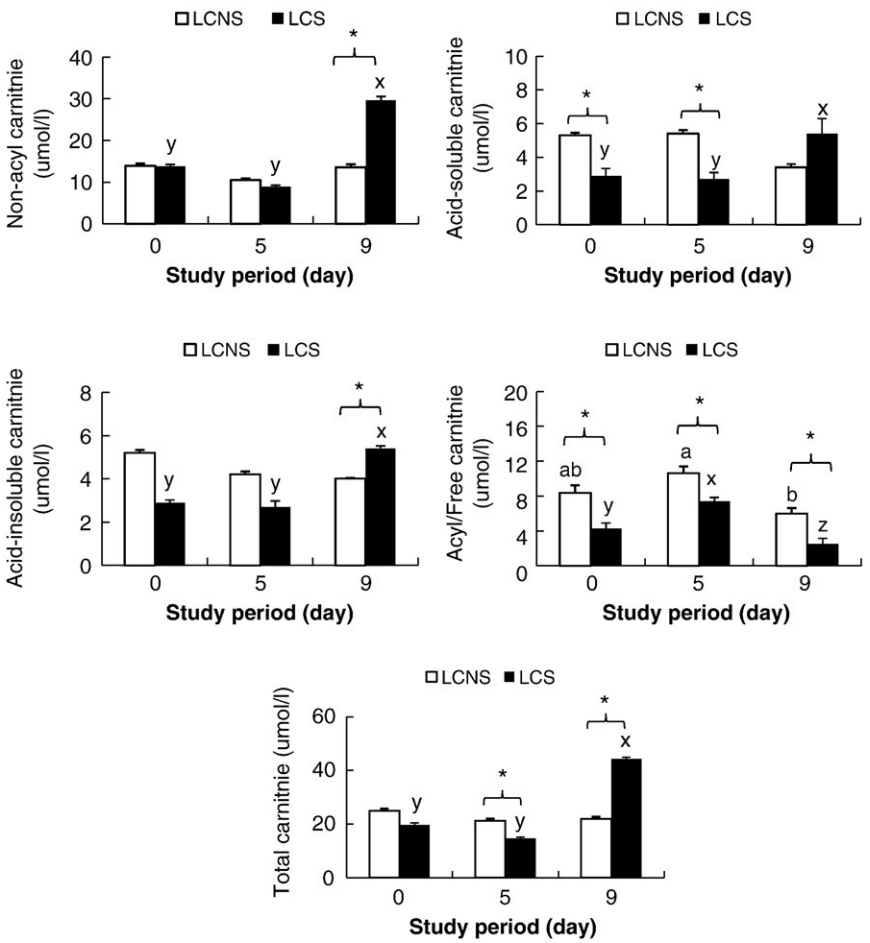

Fig. 1. Serum carnitine concentrations (in micromoles per deciliter). Values are means ± standard deviations. Values with different superscript letters indicate significant differences within treatment groups (LCS or LSNS) at different time points at $P < .05$ by ANOVA and Duncan multiple range test. *Significant differences between LCS and LCNS on day 9 by Student t test at $P < .05$. Acyl carnitine: NEC = (ASAC + AIAC)/NEC.

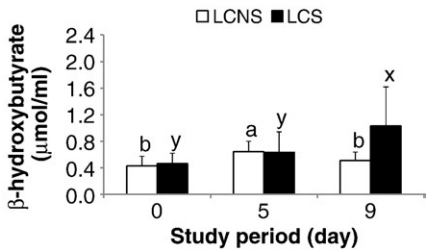

Fig. 2. Serum β-HB concentrations (in micromoles per milliliter). Values are means ± standard deviations. Those with different superscript letters indicate significant differences within treatment groups (LCS or LSNS) at different time points at $P < .05$ by ANOVA and Duncan multiple range test. *Significant differences between LCS and LCNS on day 9 by independent t test at $P < .05$.

The most recent placebo-controlled trial of carnitine supplementation (20 mg/[kg d]) used 29 preterm infants of 25 and 29 weeks GA and supplied carnitine through parenteral nutrition. During the study period of 8 weeks, the carnitine group of neonates regained their birth weight more rapidly than the placebo group of neonates (by day of life 11.8 ± 6 vs 16.9 ± 6.3, $P = .034$). In other words, carnitine supplementation at 20 mg/(kg d) had a positive effect on catch-up of growth [18]. However, this supplementation (10 mg/[kg d]) study for 9 days did not result in any significant effect on weight gain or increase in body size. The absence of any significant differences in growth and weight gain in our carnitine-supplemented group suggests that either the study period was too short and/or the supplemented level of carnitine was too low compared with the study by Crill et al [18].

A reduced clearance of TG after lipid infusion in neonates could be related to a deficiency in carnitine bioavailability [19]; it was an important observation that both LCNS and LCS had significantly increased TG on day 9, but TG level was lower in the carnitine-supplemented group than in the non–carnitine-supplemented group. The difference in the TG levels between the groups may be due to the fact that the carnitine supplementation increased the availability of carnitine in the LCS, which helped them in using the TG; and hence, the TG level dropped in the LCS. Prolonged carnitine administration has been reported to decrease serum TG levels in hyperlipoproteinemic individuals [20]. Orzali et al [21] also demonstrated that carnitine prevents the increase in circulating fatty acids and TG in fasting volunteers.

Carnitine is already known to enhance in vitro and in vivo triglyceride breakdown in neonatal adipose tissue [22]. On day 9, infants receiving carnitine (LCS) showed a lower TG level than the LCNS. Intravenous carnitine supplementation, along with parenterally administered nutrition, allows for more rapid growth and better fat utilization in very LBW infants [3]. Schmidt-Sommerfeld et al [23] evaluated 29 premature infants (15 receiving L-carnitine supplement 10 mg/[kg d] and 14 receiving no supplement), with results that shared some similarities to ours with respect to increases in serum carnitine and decreases in triglyceride in LCS as compared with the LCNS. Premature infants (<34 weeks GA), requiring TPN for 5 to 9 days, exhibited impaired fatty acid oxidation and ketogenesis related to nutritional carnitine deficiency.

Concentrations of TC were significantly increased in the current study in both groups, but the significant differences were not due to carnitine supplementation. This was similar to the results of Berkow et al [24] in which the plasma cholesterol and glucose levels cumulatively increased when TPN and Intralipid (Baxter Healthcare, Deerfield, IL, USA; 1, 2, and 3 g/[kg d] over 15 hours on days 1, 2, and 3, respectively) were administered. Comparing the study by Rubin et al [25], the addition of carnitine again did not seem to have any significant effect on TC. Serum HDL cholesterol concentrations in the LCS were significantly increased after carnitine supplementation. Studies have found that a treatment of 500 mg/d carnitine taken orally for 2 months reduces serum levels of TG and very low density lipoprotein cholesterol and increases HDL cholesterol, HDL2 cholesterol, and albumin in hemodialysis patients [26]. In a case study of a neonate with neonatal medium-chain acyl–coenzyme A dehydrogenase deficiency, administering L-carnitine (100 mg/[kg d]) for 3 weeks increased serum cholesterol (4.9 mmol/L) and maintained HDL cholesterol at the lower limit of normal (0.94 mmol/L) [27]. All these studies, along with our study, indicate that L-carnitine intake decreases triglycerides while increasing HDL levels.

Carnitine is essential for a developing fetus and is required for appropriate hepatic ketone synthesis. Neonates are able to use ketones as an energy source for their developing brains [28]. Carnitine synthesis in the neonate is limited by low levels of γ-butyrobetaine hydroxylase, and neonates are dependent on external sources of carnitine. Although carnitine is readily available in breast milk, extremely LBW infants are not usually able to tolerate an oral intake to get adequate nutrition [29]. Furthermore, we have previously shown that pregnant Korean mothers often have poor carnitine status [30], which may be the cause of our subsequent observation of suboptimal carnitine levels in newborn Korean infants [9].

Intravenous carnitine supplementation reportedly increased plasma carnitine levels and enhanced ketogenesis in premature infants maintained on a regimen of prolonged parenteral nutrition devoid of carnitine [3]. Furthermore, preterm infants have limited carnitine reserves [8] that may be bolstered by parenteral carnitine supplementation. In this study, carnitine administration tended to increase serum β-HB, which indicates enhanced ketogenesis due to increased β-oxidation in the LCS.

Infants in this study were fed premature infant formula that contained carnitine (10 mg/100 g). Under the conditions of this study, there was a rise in carnitine levels in preterm infants, even when not supplemented (LCNS). This was similar to the results of Whitfield et al [17] which suggested that the exogenous sources in the preterm formulas and/or endogenous synthesis are sufficient to maintain carnitine homeostasis. However, we found that the ratio of acyl to free carnitine was significantly lower in the LCS compared with the LCNS.

With regard to our hypothesis, the addition of carnitine as a nutritional supplement at a dose of 10 mg/(kg d) did not enhance growth in this group of Korean premature infants; but it did increase serum carnitine concentrations, which appeared to increase fatty acid oxidation, as evidenced by lower serum triglycerides and higher serum β-HB levels. Therefore, our findings suggest that continuous carnitine supplementation does not increase growth but does normalize or enhance lipid metabolism in premature infants in Korea. This not only may be an important observation for Korean infants who are at increased risk for carnitine deficiency compared with many other populations, but also may be important for subsets of mothers and infants who may be at risk of marginal carnitine status within populations that typically maintain adequate carnitine status.

Our study had some limitations. We were unable to directly measure fatty acid oxidation, although the indirect measurements provide convincing evidence of enhanced capacity for fatty acid oxidation. Furthermore, growth as measured by weight gain and other anthropometric measurements may be too narrow of a parameter to eliminate the possibility of improved development in the premature infants. Further investigations are required to establish the carnitine dosage level and duration of dosage administration for improving growth in preterm neonates and whether supplemental carnitine intake produces any adverse effects.

Acknowledgment

The present study was partially supported by the selection of research-oriented professor of Chonbuk National University in 2008.

References

[1] Ward Platt M, Deshpande S. Metabolic adaptation at birth. Semin Fetal Neonatal Med 2005;10:341-50.
[2] Garg M, Bassilian S, Bell C, Lee S, Lee WN. Hepatic de novo lipogenesis in stable low–birth-weight infants during exclusive breast milk feedings and during parenteral nutrition. J Parenter Enteral Nutr 2005;29:81-6.
[3] 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.
[4] Jo HS, Ko YH, Soh JR, Cha YS. Effects of aerobic exercise on carnitine concentration in rat's skeletal muscle. The Korean Journal of Exercise Nutrition 2004;8:235-41.
[5] Diaz Gómez MF, Urbina JA, López F, Hernández Rosales F. L-Carnitine–induced modulation of plasma fatty acids metabolism in hyperlipidemic rabbits. Rev Electron Biomed / Electron J Biomed 2006;1:33-41.
[6] 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.
[7] Schmidt-Sommerfeld E, Penn D. Carnitine and total parenteral nutrition of the neonate. BioL Neonate 1990;58:81-8.
[8] Crill CM, Storm MC, Christensen ML, Hankins CT, Bruce Jenkins M, Helms RA. Carnitine supplementation in premature neonates: effect on plasma and red blood cell total carnitine concentrations, nutrition parameters and morbidity. Clin Nutr 2006;25:886-96.
[9] Ahn EM, Cho SC, Lee M, Cha YS. Serum carnitine, triglyceride and cholesterol profiles in Korean neonates. Br. J. Nutr 2007;98:373-9.
[10] Dubowitz LMS, Dubowitz D, Goldberg C. Clinical assessment of gestational age in the newborn infant. J Pediatr 1970;77:1-10.
[11] Finely PR, Schifman RB, Williams RJ, Luchti DA. Cholesterol in high density lipoprotein: use of $Mg2^{+}$/dextran sulfate in its measurement. Clin Chem 1978;24:931-3.
[12] Wahlefeld AW. Triglycerides determination after enzymatic hydrolysis. In: Bergmeyer HU, editor. Methods of enzymatic analysis. 2nd English ed. New York: Academic Press; 1974. p. 18-31.
[13] Sachan DS, Rhew TH, Ruark RA. Ameliorating effects of carnitine and its precursors on alcohol induced fatty liver. Am J Clin Nutr 1984; 39:738-44.
[14] Cederblad G, Lindstedt S. A method for the determination of carnitine in the picomole range. Clin Chem Acta 1972;37:235-43.
[15] Cairns PA, Stalker DJ. Carnitine supplementation of parenterally fed neonates. Cochrane Database Syst Rev 2000;4:CD000950.
[16] 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.
[17] 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.
[18] Crill CM, Christensen ML, Storm MC, Helms RA. Relative bioavailability of carnitine supplementation in premature neonates. JPEN J Parenter Enteral Nut 2006;30:421-5.
[19] Editorial. The importance of carnitine in the perinatal period. Nutr Rev 1980;38:310-2.
[20] Maebashi M, Kawamura N, Sato M, Imamura A, Yoshinaga K. Lipid-lowering effect of carnitine in patients with type-IV hyperlipoproteinaemia. Lancet 1978;2:805-7.
[21] Orzali A, Donzelli F, Enzi G, Rubaltelli FF. Effect of carnitine on lipid metabolism in the newborn. I. Carnitine supplementation during total parenteral nutrition in the first 48 hours of life. Biol Neonate 1983;43: 186-90.
[22] Novak M, Penn-Walker D, Hahn P, Monkus EF. Effect of carnitine on lipolysis in subcutaneous adipose tissue of newborns. Biol Neonate 1974;25:85-94.
[23] 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.
[24] Berkow SE, Spear ML, Stahl GE, Gutman A, Polin RA, Pereira GR, et al. Total parenteral nutrition with Intralipid in premature infants receiving TPN with heparin: effect on plasma lipolytic enzymes, lipids, and glucose. J Pediatr Gastroenterol Nutr 1987;6:581-8.
[25] Rubin M, Naor N, Sirota L, Moser A, Pakula R, Harell D, et al. Are bilirubin and plasma lipid profiles of premature infants dependent on the lipid emulsion infused. J Pediatr Gastroenterol Nutr 1995;21(1):25-30.
[26] Argani H, Rahbaninoubar M, Ghorbanihagjo A, Golmohammadi Z, Rashtchizadeh N. Effect of L-carnitine on the serum lipoproteins and HDL-C subclasses in hemodialysis patients. Nephron Clin Pract 2005; 10:c174-9.
[27] Catzeflis C, Bachmann C, Hale DE, Coates PM, Wiesmann U, Colombo JP, et al. Early diagnosis and treatment of neonatal medium-chain acyl-CoA dehydrogenase deficiency: report of two siblings. Eur J Pediatr 1990;149:577-81.
[28] Medina JM, Tabernero A, Tovar JA, Martin-Barrientos J. Metabolic fuel utilization and pyruvate oxidation during the postnatal period. J Inherit Metab Dis 1996;19:432-42.
[29] O'Donnell J, 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.
[30] Cho SW, Cha YS. Pregnancy increases urinary loss of carnitine and reduces plasma carnitine in Korean women. Br J Nutr 2005;93:685-91.