Serum carnitine, triglyceride and cholesterol profiles in Korean neonates

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This study evaluated carnitine and lipid status of fifty Korean newborns. Each subject was assigned to two groups: one according to body weight at birth and the other according to gestational age. Serum total, HDL- and LDL-cholesterol were significantly lower and triacylglycerols were significantly higher, by 14 %, in the low birth weight infant (LBWI, 1310–2490 g) group compared with the normal birth weight infant (NBWI, 2570–4420 g) group. Neither birth weight nor gestational age affected serum total carnitine concentrations. However, serum ASAC (acid-soluble acylcarnitine) concentrations were 43 % higher (P<0.001) in the LBWI group compared with the NBWI, and approximately twice as high (P<0.05) in the 28–32 gestational age group compared with the other gestational age groups. NEC (non-esterified acyl carnitine) fractions were significantly higher in the NBWI and 28–32 week groups (P<0.001 and P<0.05); consequently serum acyl/NEC carnitine ratios were four times higher in the LBWI group compared with the NBWI group and 2–3 times higher in the 25–32 week age group compared with the more advanced gestational age groups. Urinary carnitine excretion, including the NEC fraction and total carnitine, was significantly higher (P<0.001) for LBWI than for NBWI. By gestational age, NEC excretion of the 28–32 week group was significantly (P<0.05) higher than that of the other two groups, but total carnitine excretion was not different among the groups. This study demonstrated that Korean immature and preterm newborns have higher serum triacylglycerol concentrations but lower carnitine status than NBWI. Therefore, the lower carnitine status and moderately higher triacylglycerols may suggest that LBWI in Korea might be at risk for poor carnitine status and decreased capacity to utilise fatty acids for energy.

Carnitine: Triglyceride: Cholesterol: Low birth weight infant

The growth and development of adipose tissue in late gestation is primarily in preparation for life after birth (Stephenson et al. 2001). A major source of energy for the fetus is glucose (Warshaw & Curry, 1980), but immediately after birth NEFA are mobilised from adipose tissue stores (Bieber et al. 1973). A rapid increase in the activity of carnitine palmitoyltransferase I and II, and a rise in the capacity to oxidise fatty acids are seen in liver and heart, reflecting prompt adaptation to lipid as a preferred metabolic fuel for the newborn (Roschinger et al. 2000).

Carnitine (L-3-hydroxy-4-N,N,N-trimethylaminobutyrate) is an essential metabolic mediator, which has a number of indispensable roles in intermediary metabolism (Vaz & Wanders, 2002). It has important roles in fatty acid metabolism, as well as in carbohydrate oxidation in cardiac myocytes, including facilitating β-oxidation by transporting activated fatty acids into the mitochondrial matrix (Kelly, 1998), and the enhancement of the metabolic flux in the tricarboxylic acid cycle by sparing free coenzyme A (CoA) (Neeley & Morgan, 1974).

In humans, the carnitine supply is derived in part from food and in part by endogenous synthesis from lysine and methionine (Koumantakis et al. 2003). Exogenous L-carnitine is used clinically for the treatment of carnitine deficiency disorders and a range of other conditions (Evans & Fornasini, 2003).

During the first hours after birth, the mitochondria, particularly in the heart, use fatty acids as the major source of energy. However, if fatty acid oxidation is impaired, increased use of glucose can result in hypoglycaemia which may cause cardiomyopathy (Guertl et al. 2000). Carnitine can, therefore, be regarded as essential for long-chain fatty acid metabolism and neonatal growth and development. Newborns, and especially premature newborns, have a very limited capacity for carnitine biosynthesis (Borum & Bennett, 1986). Furthermore, renal tubular reabsorption of carnitine is less efficient and correlates with gestational age (GA) in neonates (Zamora et al. 1995), although it is still in a near normal range. The primary determinant of carnitine status in neonates appears to be carnitine stores at birth resulting from the transfer of maternal carnitine to the fetus (Koumantakis et al. 1997), and carnitine in the mother’s milk at birth must be sufficient to maintain carnitine status until biosynthesis is developed after a few weeks. In a previous study of neonatal

Abbreviations: AIAC, acid-insoluble acyl carnitine; ASAC, acid-soluble acylcarnitine; GA, gestational age; LBWI, low birth weight infant; NBWI, normal birth weight infant; NEC, non-esterified acyl carnitine; TCNE, total carnitine.

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carnitine status (Meyburg et al. 2002), an immature baby was reported to be slower than normal neonates in the recovery of carnitine levels at each stage of development.

On the basis of this, carnitine is being added as an essential nutrient to total parenteral nutrition and to the diets of immature and normal neonates in advanced nations of Western Europe (Schmidt-Sommerfeld et al. 1983). Few investigators, however, have evaluated the carnitine status of immature newborns in their particular domestic region or in populations in which the traditional diets are low in carnitine.

Korean diets are typically lower in carnitine than Western diets (Lee et al. 2002), but there have been few studies of carnitine status or dietary intake in the Korean population. In our previous study (Cho & Chu, 2005), we found that low carnitine intake could result in marginal carnitine status of Korean mothers, which could in turn exacerbate the impaired carnitine status of premature infants. Therefore, this study investigated the effects of low birth weight and early GA on parameters of lipid metabolism, including serum lipid profiles and serum and urinary carnitine, in Korean newborn infants.

Materials and methods

Subjects and experimental design

This study was performed on fifty newborns, thirty-two boys and eighteen girls less than 1 week old, born in the obstetrics units and neonatal intensive care unit (NICU) at Chonbuk National University Hospital in Jeonju, Korea. Non-Korean newborns having different birth weight distributions were excluded due to racial differences. Infants with severe malformations, metabolic diseases, prenatal asphyxia or proven bacterial infection were also excluded from the study. Each newborn was categorised both by body weight and by GA immediately after birth. There were two groups according to body weight. The low birth weight infant (LBWI) group was composed of twenty-five under 2500 g body weight newborns, and the normal birth weight infant (NBWI) group had twenty-five over 2500 g body weight newborns.

A sample size of twenty in each group was determined to be sufficient to detect a clinically important difference of 15 μmol/l in serum total carnitine between LBWI and NBWI, the primary outcome, assuming a standard deviation of 17 μmol/l (as estimated from previous data) using a two-tailed t test of the difference between means, a power of 80 % and a significance level of 5 %. The number of subjects was increased to twenty-five to allow for the unexpected possibility of subjects dropping out or being eliminated from the study. Power tests were also conducted to ensure adequacy of secondary outcomes of serum non-esterified acyl carnitine (NEC) and triacylglycerols, and urinary total carnitine.

The infants were also divided into three groups by GA: 28–32 week group, 33–36 week group and 37–40 week group. All infants reached the final trimester of a normal full-term delivery (28–40 weeks) by WHO’s method of classification (World Health Organization, 1972) and were divided into groups according to a modified Yerushalmy’s classification (Yerushalmy, 1972). Sample collection and general data records were obtained from September 2003 to October 2004.

Growth parameters were documented at birth, on the day of study entry and 7 d after study entry. This study was approved by the Chonbuk National University Ethic Board of Clinical Experiments, and informed consent was obtained from the parents and attending physicians.

Blood and urine sample collections

Samples were collected from each infant at birth and at 7 d of age. Blood samples were obtained by venepuncture, transferred into tubes, immediately allowed to coagulate on ice and centrifuged in a refrigerated centrifuge for 15 min at 3500 r.p.m. to obtain serum within an hour. In addition, urine samples from each subject were collected into plastic containers containing toluene as a preservative. The serum was coded and immediately separated and frozen, as were untreated urine samples, at −80°C until analysis.

Serum lipids and urinary creatinine assay

Serum triacylglycerols, HDL-cholesterol and total cholesterol were estimated by an enzymatic colorimetric method using a commercial assay kit (Asan Pharm. Co., Seoul Korea). LDL-cholesterol was calculated using the equation of Friedwald et al. (1972) (LDL cholesterol = total cholesterol − HDL cholesterol − triacylglycerols/5). Urinary creatinine levels were assayed by the Jaffe method using a commercial kit (Asan Pharm. Co., Seoul, Korea).

Carnitine assay

Carnitines in serum and urine were assayed using the radiometric method of Cederblad & Lindstedt (1972) as modified by Sachan et al. (1984). Internal isotope standards for each carnitine fraction were added to correct sample data. The coefficient of variation was 2.44–3.43 % within and between assay. A 100 μl sample was hydrolysed with 200 μl of 0.5 M-KOH and then centrifuged at 1500 g for 10 min. Acid-soluble acyl carnitine (ASAC) and NEC were determined in the supernatant, and acid-insoluble acyl carnitine (AIAC) was determined in the pellet. A 150 μl aliquot of the supernatant was neutralized with 1 M-KHCO3 for measurement of NEC. In the ASAC assay, 100 μl of supernatant was hydrolysed with 0.5 M-KOH and neutralized with PCA/MOPS-II solution. The pellet containing AIAC was washed with 0.6 M-PCA and drained. It was then hydrolysed in 0.5 M-KOH for 60 min in a waterbath at 60°C, and neutralised with PCA/MOPS-II. The reaction mixture (1 M-MOPS buffer, 0.1 M-potassium ethyleneglycoltetra-acetate, 0.1 M-sodium tetrathionate and 0.1 mg.[1-14C]acetyl-CoA solution (Amer sham, Little Chalfont, Buckinghamshire, UK) was then added, diluted with water to 100 μl, and incubated with carnitine acetyl transferase (Sigma Chemical Co., St Louis, MO, USA) in a water bath at 37°C for 30 min. At the end of the incubation time, 200 μl of incubation mixture was transferred onto an ion exchange mini-column. After the sample had been absorbed, eluted with two 500 μl portions of water and then further diluted with water, it was then transferred to a 20 ml scintillation vial and counted with liquid scintillation fluid. The radioactivity of each sample was determined in a Beckman LS-3801 liquid scintillation counter (Beckman Instruments, Palo Alto, CA, USA).
Statistical analysis

Data were analysed using the SPSS 12.0.1 package. Student’s *t* test was used for testing significance of differences between the two groups of unpaired samples according to birth weights. Significant differences among the three groups according to GA were analysed by ANOVA and Duncan’s multiple range test. The level of significance for all the tests was set at \( P < 0.05 \). Pearson’s correlation coefficient and regression coefficient were used to determine correlations between continuous variables.

Results

General characteristics of subjects

General characteristics and growth parameters of the subjects are shown in Table 1. Mean GA at birth was 255.79 (sd 21.32)d. Mean Apgar scores at 1 and 5 min were 7.45 (sd 1.48) and 8.69 (sd 1.69), respectively. Newborns with birth weights under 2500 g (LBWI) had significantly lower Apgar indexes at 1 and 5 min, and lower GA at birth than those in the over 2500 g birth weight group (NBWI). Mean height and body weight immediately after birth were 46.21 (sd 3.86) cm and 2650 (sd 766.01) g. Mean head and chest circumferences were 32.76 (sd 2.28) and 30.00 (sd 3.63) cm, respectively. All growth parameters in this study were significantly higher in the NBWI group than in the LBWI group.

Table 1 shows growth parameters in the subjects based upon GA. Apgar scores at 1 and 5 min were not different between the 33–36 week and 37–40 week GA newborns. However, the score for the 28–32 week GA babies was significantly lower than for the 37–40 week group. Newborns with a GA of 28–32 weeks had lower Apgar scores, heights, weights, and head and chest circumferences than the others.

Serum lipid concentrations

Triacylglycerols were significantly higher in the LBWI group than in the NBWI group, but total cholesterol, HDL-cholesterol and LDL-cholesterol concentrations were significantly higher in the NBWI group than in the LBWI group (Table 2).

Lipid concentrations according to GA groups are shown in Table 2. Triacylglycerol concentrations in the 37–40 week group were significantly lower than in the 33–36 week group. Total cholesterol, HDL-cholesterol and LDL-cholesterol concentrations were significantly higher in the 37–40 week group than in the earlier GA groups. There were no differences in lipid profiles between the 28–32 week and 33–36 week groups.

Serum carnitine concentrations

Serum carnitine concentrations are shown in Table 2. NEC concentrations were significantly higher in the NBWI group than in the LBWI group. On the one hand, ASAC concentrations were significantly higher in the LBWI group than in the NBWI group and there were no differences in AIAC and total carnitine (TCNE) concentrations. On the other hand, the acylcarnitine/NEC ratio was significantly higher in the LBWI group than in the NBWI group. The NEC/TCNE ratio was significantly higher in the NBWI group than in the LBWI group.
Correlations between variables and parameters

Serum triacylglycerol concentrations after birth were negatively correlated with birth weight ($r = -0.655$, $P < 0.05$) and gestational age ($r = -0.516$, $P < 0.05$). Serum NEC concentrations positively correlated with body weight ($r = 0.582$, $P < 0.05$) and GA ($r = 0.571$, $P < 0.05$). In contrast, serum ASAC were negatively correlated with body weight ($r = -0.409$, $P < 0.05$). Urinary NEC excretion was negatively correlated with body weight ($r = -0.545$, $P < 0.05$)

Discussion

It is important to view this study in terms of the newborn’s energy metabolic adaptation. The small size and the correspondingly high surface-to-volume ratios make newborns especially prone to heat loss. Prior to birth, the mother maintains the body heat of the infant, but after birth the infant must do so. However, it adapts to this risk by elevating its metabolic rate (Singer, 1998). Likewise, immature (preterm and low birth weight) infants must also make the extrauterine adaptation after birth. However, for them it is a greater challenge than for an infant born at full term and normal birth weight, because the hormones and enzymes controlling intermediary metabolism are not fully developed in preterm neonates (Plides, 1986).

During delivery, plasma concentrations of adrenaline, noradrenaline and glucagon increase rapidly, whereas the insulin concentration declines. The effect of the hormonal changes is to mobilise stored glycogen and fatty acids (Sinha & Donn, 2006). During the first hour after birth, there is usually a brisk ketogenic response to low blood glucose levels (Rooy & Hawdon, 2002). Fasting ketosis develops over hours in children, increasing gradually after postprandial nutrients are utilised and tissue glycogen is progressively depleted (Bonnefont et al., 1990). Compared with mature newborns, immature newborns have low ketone body concentrations which vary little during the first postnatal week (Hawdon et al., 1992). During this ‘brisk ketogenic response’...
of neonates, fatty acids must be used as an energy source as well as to maintain the fluidity, permeability and conformation of membranes, and as precursors of important bioactive compounds such as the prostacyclins, prostaglandins, thromboxanes and leukotrienes (Haggarty, 2002).

Low birth weight is a marker for nutrient insufficiency at particular stages of gestation, and seems to be associated with persisting changes in cholesterol metabolism (Barker, 1997). In a previous study, triacylglycerols were higher in newborns with intrauterine growth restriction, compared with normally developed newborns (Molina et al. 2000).

Kaser et al. (2001) reported that neonates who were small for gestational age had significantly higher triacylglycerol levels than those of appropriate size or large for gestational age. The results in this study are similar to the previous data (Skinner et al. 1983; Molina et al. 2000). Triacylglycerols in the LBWI group were significantly higher than in the NBWI group, and in the 33–36 week group were significantly higher than in the 37–40 week group. Considering the low ketogenic capacity of immature babies (Hawdon, 1999), high triacylglycerols in this situation seem to be related to an increased hypoglycaemic risk of immature babies (Embleton & Ward Platt, 2003).

A previous study of serum total, HDL- and LDL-cholesterol levels in newborns revealed no significant differences according to the degree of their maturation. There were no differences in total, HDL- and LDL-cholesterol between intrauterine growth-retarded and normally developed newborns (Molina et al. 2000). Skinner et al. (1983) reported that there were no correlations between the composition or concentration of any cord blood lipoprotein fractions and birth weight or gestation. Kaser et al. (2001) reported that birth weight was not the sole determinant of lipoprotein levels in neonates. However, in this study, total, HDL- and LDL-cholesterol levels were significantly higher in the NBWI group than in the LBWI group. Total, HDL- and LDL-cholesterol levels were all significantly elevated in the 37–40 week group compared with the below-36 week groups. The different result in this study, compared with the previous study, seems to be caused by the different GA, birth body weight, sex and standard of ‘immaturity’ in the subjects.

Carnitine is present in biological materials in both the non-esterified form (NEC) and as acylcarnitine (Swell & Bohles, 1995). NEC typically accounts for around 80% of total carnitine, and serum concentrations lower than 20 µmol/l are considered a marker of ‘carnitine deficiency’ (Winter et al. 1990).

In this study, mean NEC concentrations of NBWI were similar to those reported by other investigators (Campoy et al. 1998), but the LBWI group infants had serum NEC concentrations of 13.08 (SD 7.40) and 13.08 (SD 7.76), respectively. Therefore, this study indicates that Korean low birth weight or short GA newborns have ‘carnitine deficiency’. A low NEC/TCNE ratio has also been considered to be indicative of ‘carnitine deficiency’. A normal NEC/TCNE ratio noted in neonates is thought to reflect the increased production of acyl-CoA derivatives, mainly acetyl-CoA produced by normally enhanced fatty acid oxidation in the newborn period (Warshaw & Curry, 1980; Girard et al. 1992). The value for the LBWI group was significantly lower than for the NBWI group, and those for the 33–36 and 37–40 week groups were higher than that for the 28–32 week group. However, if we use an NEC/TCNE ratio below 0.70 to define carnitine deficiency, then all the subjects in this study were ‘carnitine deficient’ and LBWI and the 28–32 week newborns had significantly greater deficiency.

In serum, AIAC and ASAC are considered very good markers of the activation of fatty acids (Aggett et al. 1991). Therefore, the intramitochondrial relationship between acyl-CoA and free CoA is reflected by the extramitochondrial acylcarnitine to NEC ratio, acyl-CoA/CoA = acylcarnitine/NEC; a high acylcarnitine/NEC is ‘a marker of carnitine insufficiency’ (Schmidt-Sommerfield et al. 1983; Winter et al. 1990). Since 1982, values > 0.40 are considered abnormal and indicate a low availability of NEC to the cells, thus ‘carnitine insufficiency’ (Bohles et al. 1994). In this study, the acyl carnitine/NEC ratios of all groups were > 0.40, but were especially higher in the LBWI group compared with the NBWI group. By GA group, the 28–32 week group had significantly higher acylcarnitine fractions than the 33–36 week and 37–40 week groups. It seems that the higher acylcarnitine/NEC ratios in this study are chiefly caused by increased ASAC levels, especially in immature newborns. Thus it seems that a newborn infant, especially an immature one, is born with limited carnitine reserves. The high acylcarnitine/NEC ratio noted in neonates is thought to reflect the increased production of acyl-CoA derivatives, mainly acetyl-CoA produced by normally enhanced fatty acid oxidation in the newborn period (Warshaw & Curry, 1980; Girard et al. 1992; Arenas et al. 1998). The association of low birth weight with higher TCNE values may be related to decreased tissue carnitine uptake (Chace et al. 2003). In the current study, however, there were no differences in TCNE concentrations by birth weight or by GA.

The selective excretion of acylcarnitine provides a route for eliminating accumulating intermediates in metabolic disorders (Siliprandi, 1986). Excessive loss of these specific acylcarnitines in the urine results in loss of free carnitine and increases the probability of secondary carnitine deficiency (Pons & de Vivo, 1995). Urinary excretion of NEC and TCNE fractions was significantly higher in the LBWI group than in the NBWI group. By GA, urinary NEC excretion in the 28–32 week neonates was significantly higher than in the 33–36 week group. By weight group, ASAC excretion was not statistically different between groups, but AIAC excretion was significantly higher in the LBWI group.

In previous studies, immature newborns have been shown to have higher plasma and red blood cell carnitine concentrations at birth than full-term neonates (Borum, 1995). However, in this study, there were no differences in TCNE values between mature and immature newborns, but carnitine fractions were different and markers of ‘deficiency’ and ‘insufficiency’ significantly greater for the low birth weight and early gestational groups.

All infants have carnitine stores, well below adult levels, at birth, but preterm infants have much lower stores than do full-term infants (Shenai & Borum, 1984). We have previously found that pregnant Korean women have marginal carnitine.
status due to relatively low dietary intake and increased urinary excretion of carnitine (Cho & Cha, 2005). Low dietary intakes of folate, protein and energy may also contribute to the marginal carnitine status of Korean women of childbearing age (Cha et al. 1998; Lee et al. 2005). This study was based on the premise that compromised carnitine status in Korean mothers might exacerbate low carnitine stores in preterm Korean infants. The high acyl/free serum carnitine in Korean preterm infants suggests that Korean preterm infants may indeed have lower carnitine stores than their counterparts in Western countries. Marginal or insufficient carnitine status is not unique to Korean preterm infants. Muscle tissue begins to accumulate carnitine stores at about the same time as fat stores begin to accumulate, in preparation for the utilisation of lipids as a major energy source after birth (Reichman, 1981; Shenai & Borum, 1984), and the degree of accumulation is largely a factor of gestational age. The source of carnitine for the fetus is exclusively from the blood supply from the mother, and the transfer of carnitine from the mother is a major determinant of carnitine status of a neonate (Koumantakis et al. 1997). When an infant is born prematurely, there is no further supply of carnitine from the mother and the carnitine biosynthesis enzymes are not yet fully active (Melegh et al. 1996). Consequently, preterm infants have little of their own fat stores to use for energy and less capacity to utilise exogenous fat for energy than do full-term infants. Preterm infants have abnormal circulating lipid-derived metabolites, and little capacity for producing ketone bodies, an important early energy source for the brain. For those reasons and others, an expert panel established by the Life Sciences Research Office (LSRO) and the American Society for Nutritional Sciences recommended to the United States Food and Drug Administration that a minimum of 2.0 mg/100 kcal and a maximum of 5·9 mg/100 kcal of carnitine be added to carnitine-free preterm infant formulas (American Society of Nutritional Sciences & Life Sciences Research Office, 2002). These amounts were based on the amounts of carnitine supplied from mother’s milk and the amount at which an increased fractional excretion of carnitine is seen in preterm infants. The 5·9 mg/100 kcal dose has a history of safe use and it was believed to be well within a safe range by the expert panel. Nevertheless, the conclusions of the expert panel have not been universally accepted due to the lack of a demonstratable effect of carnitine on growth and development in preterm infants (Shortland et al. 1998; Whitfield et al. 2003). Currently, reasonable and informed scientists can reach disparate conclusions about the need for including carnitine in carnitine-free parenteral and enteral infant formulas. However, if such a need does exist, it is reasonable to conclude that Korean preterm infants need carnitine at least as much as, and probably more than, infants from Western countries.

In summary, this study revealed that the immature newborn group had higher serum triacylglycerol concentrations than the mature newborn group, and that there was no difference in TCNE levels between mature and immature newborns. However, the carnitine fractional differences in this study suggest that Korean low birth weight and preterm newborns suffer from compromised carnitine status and abnormal carnitine availability. Therefore, in populations with low carnitine intake, as in Korea, the carnitine status of premature and low birth weight infants may be less than optimal. Therefore, these infants need to be carefully evaluated to ensure that their lipid metabolism is unimpaired by poor carnitine status.

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