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# The corrected blood urea nitrogen predicts the developmental quotient of extremely low-birth-weight infants at the corrected age of 36 months

Ken Nagaya[a, *], Shinya Tanaka[b], Hiroyuki Kitajima[b], Masanori Fujimura[b]

[a]*Department of Pediatrics, Asahikawa Medical College, ZIP 078-8510, 2-1-1-1 Midorigaoka-higashi, Asahikawa, Hokkaido, Japan*

[b]*Department of Neonatal Medicine, Osaka Medical Center for Maternal and Child Health, Osaka, Japan*

Short title: BUN predicts cognitive outcome ~~of~~in preterm infants **[Note: Please check the change.]**

Corresponding author: Ken Nagaya

Tel.: +0166-65-2111; fax: +0166-68-2489.

*E-mail address:* nagaya5p@asahikawa-med.ac.jp (K. Nagaya).

**Abstract**

*Background*: Currently, there are no nutritional indices to predict the cognitive function in extremely low-birth-weight (ELBW) infants ~~that predict cognitive function~~.

*Objective*: To assess the neonatal blood urea nitrogen (BUN) values ~~of~~in ELBW infants according to their cognitive function at the corrected age of 36 months.

*Methods*: This was a retrospective study that assessed the neonatal factors affecting the developmental outcome in two groups "developmental quotient (DQ) ≥ 80" and "DQ < 80"; the groups were divided based on ~~developmental quotient (~~the DQ~~)~~ at the corrected age of 36 months. Between 1996 and 1999, ~~a total of~~ 178 ELBW infants born at ~~<~~ 28 weeks of gestation were admitted to our neonatal intensive care unit (NICU)~~.~~; of these,~~There were~~ 32 died~~deaths~~. ~~and only 37 o~~Of the surviving 146 infants, 37 infants without any exclusion criteria (~~to~~that would affect the cognitive function and BUN)~~,~~ ~~—~~ except the nutritional factor~~),~~ were assessed. Area under the curve (AUC) of corrected BUN (CBUN: BUN × 0.5/serum ~~-~~ creatinine) from 28 to 84 days of life was used as an index of protein intake.

*Results*: No significant differences were observed between the two groups ~~for~~with regard to the gestational age, birth weight, Z score of birth weight, and sex. However, ~~in comparison with~~compared to 15 infants with DQ < 80, ~~and the~~ 22 infants with DQ ≥ 80 had significantly shorter duration of artificial ventilation and $O_2$ supplementation, a higher Apgar score at 5 min, and a higher AUC of CBUN. On ~~a~~ multiple regression analysis, DQ ≥ 80 was observed to be significantly correlated with the AUC of CBUN (Odd's ratio~~OR~~: 1.03, 95% confidence interval:~~CI of~~ 1.002 ~~to~~ 1.06).

*Conclusion*: The CBUN level would provide an estimate of adequate protein intake and ~~improve~~ the subsequent development of an ELBW infant. **[Note: Please check the change.]**

*Keywords*: Preterm infant; Nutrition; Blood urea nitrogen; Protein fortification; Cognitive development

## 1. Introduction

Human milk is recommended for the management of extremely low-birth-weight (ELBW) infants [1,2]. However, it needs to be supplemented with proteins and other nutrients, because by itself, ithuman milk cannot meet the high nutrient requirements of the ELBW infants [3]. Human milk is usually fortified based on the nutritional recommendations such as those from the American Academy of Pediatrics (AAP) [4], or the European Pediatric Society of Gastroenterology and Nutrition (EPSGN) [5]. Compared to infants born at term, ELBW infants tend to have much higher nutritional requirements than those of term infants due to their poor nutrient store, rapid growth, severity of illnesses, and physiological immaturity [6,7]. It is well known that infants suffering from chronic lung diseases display poor weight gain as a resultbecause of inadequate nutrient intake [8]. These infants tend to have poor nutritional intakes due to fluid restrictions that are with respectimposed due to their respiratory status. Furthermore, the nutrient content of human milk is not constant. A gradual reduction in the concentrations of the key components occurs during the first 2 months of lactation [9]. Therefore, a fixed level of human milk fortification may be inadequate for ELBW infants because they haveof their variable nutritional demands. As recently advocated by Polberger et al. [10], individualized supplementation is recommended.; Hhowever, this has not yet been popularized. Moro et al. [11] have proposed a method of adjusting the amountlevel of human milk fortification based on corrected blood urea nitrogen (CBUN) levels. Since this monitoring method considers the infant's metabolic response in relation to protein intake, Thisit may allow enable optimal nutritional supplementation forin ELBW infants because this monitoring method considers the infant's metabolic response in relation to protein intake. InAt our neonatal intensive care unit (NICU), the human milk fortification method was not individualized according to the method, as described by Moro et al. The two types of fortification methods used were not adjusted based on the CBUN value.; As a resulthence, the observed CBUN values varied. The purpose of this retrospective study was to evaluate whether athe CBUN levels predicted the developmental outcome in ELBW infants at 36 months of post-conceptual age (PCA) for ELBW infants.

## 2. Materials and methods

Between 1996 and 1999, 178 ELBW infants born at <28 weeks of gestation were admitted to the neonatal intensive care unitNICU of the Osaka Medical Center

for Maternal and Child Health. Of these, 32 infants died ~~in~~during the neonatal period. In this study, ~~W~~we excluded infants with all neonatal factors, ~~except for the~~other than the nutritional factor, ~~in this study, which~~because these factors could influence the cognitive ~~function~~ and renal functions. Therefore, the exclusion criteria were ~~included~~ death, major congenital anomalies, intraventricular hemorrhage (grade 3–4), meningitis, congenital hydrocephalus, cerebral infarction, administration of prostag~~r~~landin E1 (PGE1) inhibitors, intestinal perforation, and renal failure. A total of 79 infants were followed up; ~~.~~ of these, 42 ~~of 79~~ infants ~~who followed up~~ were either not assessed for the developmental quotient (DQ) or not traceable at 36 months of PCA. The DQ was assessed ~~O~~only for the remaining 37 ~~of 79~~ eligible infants ~~had a developmental quotient performed~~ at 36 months of PCA (Fig. 1).

Two clinical psychologists in our hospital assessed the ~~developmental quotient (~~DQ~~)~~ ~~by~~using the revised Kyoto Scale of Psychological Development [19] at ~~about~~approximately 36 months of PCA (range, ~~from~~ 32 ~~to~~ –40 months ~~of PCA~~). This examination has been standardized and is widely used in Japan [20]. It has been modified from the Weschsler Intelligence Scale for Children Revised (WISC-R) [20], and it assesses all aspects of an infant's performance. The developmental performance of ~~the~~an infant~~s~~ is expressed as the developmental age for each behavioral area (postural-motor, cognitive-adaptive, and language-social areas) and all other areas. The DQ is obtained by dividing the estimated ~~the~~ developmental age by the chronological age and then multiplying the quotient by 100. The infants were divided into two groups ~~by~~based on their DQs at 36 months of PCA (DQ ≥ 80 ~~or~~and DQ ~~not~~< 80). At our center, DQ ≥ 80 is defined as a value showing typical~~ly~~ develop~~ment~~ing in an infant [19]. ~~in our center.~~ **[Note: Please check the change.]** ~~And~~Further, the clinical characteristics of the infants were compared between the two groups.

The CBUN level was calculated ~~by~~using Moro's formula (BUN × 0.5/serum-creatinine level). ~~CBUN~~It was ~~checked~~determined at least once a week, and the area under the curve (AUC) of CBUN (mmol × day/L) **[Note: See Editor's Note #1.]** between 28 and 84 days of life ~~(mmol × day/L)~~ was calculated. The BUN values usually correlate with the protein intake after 4 weeks of life [12–14]. However, the rise in the BUN level does not accurately reflect the protein load in premature infants during the first ~~four~~4 weeks of life because the urea cycle at this age is not as developed as ~~the one~~that in term infants [15]. Therefore, although nutrition is extremely important during the first ~~four~~4 weeks of life [16–18], ~~it~~the BUN level **[Note:**

**Please check the change.]** cannot be used as an index of protein intake. The AUC of CBUN was calculated using ImageJ® software (ver. 1.32, NIH, Bethesda, Maryland, USA) after plotting one CBUN value every ~~one~~ week; the CBUN value was obtained between 28 and 84 days of life. These values were plotted using Excel® software (Microsoft Corporation, USA) to evaluate the AUC of CBUN accurately.

The calorie and protein contents in human milk were estimated to be 0.69 kcal/ml and 1.3 g/dl, respectively. These values correspond to those observed in the milk at mid-lactation in Japanese women [21]. ~~We did not consider the distinction of~~Differences between the milk of an infant's~~own~~ mother~~'s milk~~ and donor human milk were not considered.

The study was approved by the local institutional review board, and an informed parental consent was obtained prior to the study.

*2.1. Feeding strategy ~~in~~at our NICU*

Table 1 summarizes the data of the nutritional ~~content~~supplements used in the human milk fortification method used ~~in~~at our NICU. We ~~A~~add~~ing~~ either 3 g or 5 g of the fortifier, HMS-1® (Morinaga Milk Industry Co. Ltd., Japan) (protein 0.26 g/~~G~~g of fortifier, **[Note: Please check the change.]** energy 3.37 kcal/g), ~~of either 3 g or 5 g~~ to 100 ml of human milk (HM) to achieve a target protein content of 3–4 g/kg/day. But there must also be a target calorie intake range that is not merely 120 kcal/kg/day **[Note: See Editor's Note #2.]** [4,5]. The infants were ~~feeding with~~fed HM + 3 g/dl HMS-1® (3H) fortification ~~(human milk + 3 g/dl HMS-1®)~~ when the amount of enteral feed~~ing~~ was ~~more than~~ ≥150 ml/kg/day. The infants were fed HM + 5 g/dl HMS-1® (5H) fortification ~~(human milk + 5 g/dl HMS-1®) was provided the infants~~ when the amount of enteral feed~~ing did not exceed~~ was <150 ml/kg/day; less quantity of feed was due to the~~ir~~ infants' condition. **[Note: Please check the change.]** When the calorie intake was less than ~~a~~the target calorie intake, we further supplemented the milk with medium-chain triglyceride oil (approximately 2 ml/kg/day). When ~~the~~ mother's milk ~~became~~was insufficient, we used donor milk ~~was used for~~during the first month and ~~after that time~~ preterm formula later (Neomilk PM®, Bean Stalk Snow Co. Ltd., Japan) ~~in~~for feeding the ELBW infants;~~,~~ these were used because ELBW infants fed on formula milk are at a risk of developing~~of a risk for~~ necrotizing enterocolitis ~~in the ELBW infants fed formula milk~~ **[Note: Please check the change.]** [22]. We adjust the concentration of the preterm formula ~~in~~as 16% and 18%~~.~~; ~~The~~ 16% concentration is equivalent to HM + 3H fortification and ~~the~~ 18% concentration is

equivalent to HM + 5H fortification.

*2.2. Statistical analysis*

Data were retrospectively analyzed. The statistical analyses included the χx$^2$ test, Mann-Whitney U test, and a multiple logistic regression analysis. In all cases, StatView software (ver. 5.0, SAS institute Inc., USA) was applied.

**3. Results**

Table 2 lists the detailed characteristics of infants included inas the study population. No significant differences were observed between the two groups forwith regard to the gestational age, birth weight, birth length, head circumference at birth, Apgar score at 1 min, sex, and human--milk feeding ratio (HMFR, defined as intake of human milk/intake of [(human milk + formula milk)] during the first 2 months). In comparison withCompared to the "DQ < 80" group, the "DQ ≥ 80" group displayed a higher Apgar score at 5 min, a shorter duration of artificial ventilation and $O_2$ supplementation, and a higher AUC of CBUN between 28 and 84 days of life. Table 3 shows the results of the multiple regression analysis of the overall DQ scores above≥ 80 points at 36 months of PCA. Only the AUC of CBUN between 28 and 84 days of life influenced the overall DQ score at 36 months of PCA, aAfter adjustingment for the gestational age, Z score of birth weight, sex, Apgar score at 5 min, and duration of ventilation days, we observed that only the AUC of CBUN between 28 and 84 days of life influenced the overall DQ score at 36 months of PCA.

Figure 2 illustrates the mean calorie and protein intakes calculated every 2 weeks in both the "DQ ≥ 80" and "DQ < 80" groups. With the exception of protein intake between 2 and 4 weeks of life, no significant differences were observed between the groups with regard to the protein and calorie intakes. **[Note: Please check the change.]**

Figure 3 shows the average CBUN and serum creatinine levels estimated every 2 weeks after birth in the two groups, thatwhich were divided based on the overall DQ score at 36 months of PCA. Although the average serum creatinine level did not differ, the CBUN level in the "DQ ≥ 80" group was greater than that in the "DQ < 80" group, except for the level during the first 2 weeks of life.

There were no significant differences between infants with DQ ≥ 80 and DQ < 80 inwith regard to infants' growth at 36 months of PCA between infants with DQ≥80 and DQ<80 (weight (kg): 12.0 ± 1.4 and 11.6 ± 1.5, length (cm): 92.0 ± 3.8 and 89.4 ±

3.4, and head circumference (cm): 49.1 ± 2.0 and 48.2 ± 2.2, respectively).

## 4. Discussion

There are no indices to predict the optimal protein intake ~~of~~for ELBW infants. We could not clarify~~reveal clearly~~ whether ~~that~~the CBUN value ~~of~~in ELBW infants used for estimating **[Note: Please check the change.]** the protein intake could predict their ~~later~~ cognitive function later in life. On ~~On a m~~multiple regression analysis, we observed that~~,~~ DQ ≥ 80 ~~was~~ significantly correlated with the AUC of CBUN. ~~It~~This may suggest that a high CBUN value reflects adequate protein intake in ELBW infants. However, ~~it is not clear that it has~~its clinical relevance is unclear because ~~of an~~the Odd's ratio (OR) was 1.03 with 95% confidence interval (CI) of 1.002~~ to ~~1.06.

Only 37 of the 146 survivors (25.3%) ~~have been~~were ~~estimated~~included in this study. ~~42 o~~Of the 79 infants who were followed up, 42 were either not assessed for the DQ or not traceable at 36 months of PCA. ~~34 o~~Of the 42 infants, the ~~were assessed~~ DQ was assessed in 34 infants after 36 months of PCA (from 4 to 9 years ~~of age~~)~~.~~ and ~~8~~eight ~~of 42~~ infants moved to other areas or were not traceable. Furthermore, we ~~thought~~believed that except the nutritional factor, other factors that would~~to~~ affect BUN, although slightly, ~~a little~~ should be excluded~~excluding nutritional factor should be not accepted~~. At our center, ~~Because~~ infants ~~who had~~with patent ductus arteriosus (PDA) were treated with PGE1 inhibitor whenever ~~as much as~~ possible, ~~in our center,~~ and they were often ~~administered~~administrated low ~~-~~dose PGE1 inhibitor ~~by about~~at approximately 1 month of life. Therefore, infants who were ~~administered~~administrated PGE1 inhibitors between 28 and 84 days of life were excluded from this study ~~which period was between day 28 and day 84~~. **[Note: Please check the change.]** ~~52 infants o~~Of the 146 survivors, 52 infants were ~~administered~~administrated PGE1 inhibitors for ~~patent ductus arteriosus (~~PDA~~)~~, ~~that~~which was one of the exclusion criteria in this study.

As shown in Table 4, Moro et al. reported a method for adjusting the level of protein fortification that involved the addition of proteins and was dependent on the CBUN level. The CBUN level was corrected based on the normal serum creatinine level because the low glomerular filtration rate observed in preterm infants leads to the elevation of BUN and is independent of the protein intake. The CBUN level was calculated ~~by~~using the formula BUN×0.5/serum ~~-~~ creatinine, where 0.5 is the normal serum creatinine concentration. Moro et al. concluded that this method was safe and

it ensured adequate nutrient intake and growth. However, the developmental outcome in this fortification program was not evaluated. Although the human milk fortification method used at our NICU ~~of human milk~~ was fixed~~in our NICU was fixed~~, a variation in the CBUN values was observed because of the infants' conditions. Maturation of metabolism and severity of illness may lead to considerable variation in the CBUN values. Therefore, the present retrospective study tested whether the CBUN values ~~could~~can be used to predict the developmental and anthropometric outcomes. Although the CBUN level was not used to predict the outcome ~~for~~of the anthropometric parameter in our study, ~~it~~the results ~~may~~ **[Note: Please check the change.]** suggest that a high CBUN value reflects adequate protein intake in ELBW infants.~~–~~

Renal function, fluid shift, or catabolism can affect the BUN level. Therefore, infants with renal diseases were excluded from the present study. Furthermore, no significant difference was observed in the serum creatinine levels between the two groups, and the CBUN level was corrected based on the serum creatinine levels. Therefore, renal factors hardly affected the CBUN values ~~were hardly affected by renal factors~~. Since no differences were observed between the two groups ~~in~~with regard to the amount of protein and calorie intakes (Fig.~~ure~~ 3) and weight gain (data not shown) ~~between the two groups~~, it was ~~felt~~considered that fluid shift and catabolism did not significantly affect the BUN values.

The results showed that infants in the "DQ < 80" ~~infants~~group had a significantly lower Apgar score at 5 min and ~~a~~ longer durations of artificial ventilation and $O_2$ supplementation. This indicates that these infants ~~in this group~~ might be ~~sicker~~more ill than those in the other group, and the severity of illness ~~in~~during the neonatal period may affect the developmental outcome later in life. However, the AUC of CBUN in infants in the "DQ ≥ 80" ~~infants~~group between 28 and 84 days of life was higher than those in infants in the "DQ < 80" ~~infants~~group. The fixed fortification method used in this study might have led~~lead~~ to inadequate protein intake in infants in the "DQ < 80" ~~infants~~group, as indicated by their low CBUN values. Some studies ~~in~~on critically ill adults and children showed that they not only have higher nutritional ~~needs~~requirements but also have a decreased capacity to maximize the use of different substrates [23]. Compared to healthy children, ~~C~~critically ill children were recommended a high protein intake based on a higher protein turnover in this population ~~as compared to healthy children~~ [24]. On multiple regression analysis, only the AUC of CBUN between 28 and 84 days of life was related to the DQ at 36

months of PCA, whereas severity of illness was not significantly related to the DQ. The energy expenditure ~~of~~in infants was not analyzed. However, infants in the “CBUN < 80” ~~infants~~group might have required more nutrients due to their illness.

The actual individual protein intake could not be determined because the protein content of human milk was not analyzed. In this study, the protein and calorie contents in ~~the~~ mother’s milk and donor milk was found to be the same as that observed at mid-lactation in Japanese women [21] (calories and protein values in human milk are estimated ~~at~~to be 0.69 **[Note: Please check the change.]** kcal/dl and 1.3 g/dl, respectively). Since the nutrient content of human milk is not always constant [25], the difference between the actual and calculated protein and calorie intakes could not be calculated. Moreover, a fixed level of human milk fortification may be inadequate for ELBW infants because they have variable nutritional demands ~~as~~based on the~~ir~~ severity of their illness and physiological immaturity. It was suggested that ~~it~~variable nutritional demands might have been ~~was~~ the reason ~~that~~for the differences ~~was made~~ in the CBUN values in this study, although nutritional fortification was the same in both the groups ~~in this study~~. Cooke and Embleton suggested that the ~~degree~~level of fortification that is required~~needed~~ to sustain adequate growth might vary daily~~from day to day,~~; ~~so~~therefore, preterm infants fed on current fortification regimens show less growth ~~less well~~ than those fed on a preterm infant formula [26]. However, ~~it is unlikely that the~~ routinely measuring the individual nutrient ~~needs~~requirements and the content of human milk ~~are routinely measured~~ at bedside appears unlikely because of the effort and cost involved. Adjusting ~~the~~ human milk fortification based on the CBUN values, as suggested by Moro et al., may rectify this problem.

Based on our small sample size and with limitations in the study design, we conclude that a low CBUN value is detrimental for ~~a~~the developmental outcome of an ELBW infant. However, we would argue that a low **[Note: Please check the change.]** CBUN value reflects inadequate rather than an excessive dietary protein intake as suggested by the systematic review of the Cochrane library [27] and Lucas et al. [3]. ELBW infants are prone to suffer from malnutrition due to their rapid growth and ~~the~~ risk of illness, and it is important to evaluate the nutritional state with reference to the physiological parameters. Adjustment of the protein intake based on the CBUN value, and not a fixed protein intake, may provide a method of human milk fortification that meets the infant's nutritional requirements. Since our study was retrospective in nature, ~~P~~prospective studies that would estimate the correlation

between the CBUN level ~~in~~during the neonatal period and the cognitive function in later life ~~need~~should ~~to~~ be conducted. ~~because our study was retrospective in nature.~~

## References


[1] Morley R, Cole TJ, Powell R, Lucas A. Mother''s choice to provide breast milk and developmental outcome. Arch Dis Child 1988; 63:1382–5.

[2] Lucas A, Morley R, Cole TJ, Lister G, Leeson-Payne C. Breast milk and subsequent intelligence quotient in children born preterm. Lancet 1992; 339:261–4.

[3] Lucas A, Fewtrell MS, Morley R, Lucas PJ, Baker BA, Lister G, et al. Randomized outcome trial of human milk fortification and developmental outcome in preterm infants. Am J Clin Nutr 1996; 64:142–51.

[4] Nutrition and feeding of preterm infants. Committee on Nutrition of the Preterm Infant, European Society of Paediatrics Gastroenterology and Nutrition. Acta Pediatr Scand Suppl 1987; 336:1–14.

[5] American Academy of Pediatrics Committee on Nutrition: nutritional needs of low-birth-weight infants. Pediatrics 1985; 75:976–86.

[6] Moro GE, Minoli I. Fortification of human milk. In: Ziegler EE, Lucas A, and Moro GE, editors. Nutrition of the Very Low Birth Weight Infant. Nestle Nutrition Workshop Series, Paediatric Programme. Vol. 43. Pennsylvania: Lippincott Williams & Wilkins; 1999. pp. 85–8.

[7] Satish C K, Sabine I. Protein metabolism in the extremely low-birth-weight infant. Clin perinatol 2000; 27:23–38.

[8] Ryan S. Nutrition in neonatal chronic lung disease. Eur J Pediatr 1998; 157 (suppl 1): S19–22.

[9] Lonnerdal B, Forsum E, Gebre-Medhin M, Hambraeus L. Breast milk composition in Ethiopian and Swedish mothers. II. Lactose, nitrogen, and protein contents. Am J Clin Nutr 1976; 29:1134–41.

[10] Polberger S, Raiha NCR, Juvonen P, Moro GE, Minoli I, Warm A. Individualized protein fortification of human milk for preterm infants: comparison of ultrafiltrated human milk protein and a bovine whey fortifier. J Pediatr Gastroenterol Nutr 1999; 29:332–8.

[11] Moro GE, Minoli I, Ostrom M, Jacobs JR, Picone TA, Raiha NCR, et al. Fortification of human milk: evaluation of a novel fortification scheme and of a new fortifier. J Pediatr Gastroenterol Nutr 1995; 20:162–72.

[12] Polberger SKT, Axelsson IA, Raiha NCE. Growth of very low birth weight infants on varying amounts of human milk protein. Pediatr Res 1989; 25:414–9.

[13] Polberger SKT, Axelsson IA, Raiha NCE. Urinary and serum urea as indicators

of protein metabolism in very low birth weight infants fed varying human milk protein intakes. Acta Paediatr Scand 1990; 79:737–42.

[14] Putet G, Rigo J, Salle B, Senterre J. Supplementation of pooled human milk with casein hydrolysate: energy and nitrogen balance and weight gain composition in very low birth weight infants. Pediatr Res 1987; 21:458–61.

[15] Boehm G, Muller DM, Raiha NCR. Evidence for functional immaturity of the ornithine-urea cycle in very-low-birth-weight infants. Biol Neonate 1988; 54:121–5.

[16] Georgieff MK, Hoffman JS, Pereira GR, Bernbaum J, Hoffman-Williamson M. Effect of neonatal caloric deprivation on head growth and 1-year developmental status in preterm infants. J Pediatr 1985; 107:581–7.

[17] Georgieff MK, Mills MM, Lindeke L, Iverson S, Johnson DE, Thompson TR. Changes in nutritional management and outcome of very-low-birth-weight infants. Am J Dis Child 1989; 143:82–5.

[18] Morris BH, Miller-Loncar CL, Landry SH, Smith KE, Swank PR, Denson SE. Feeding, medical factors, and developmental outcome in premature infants. Clin Pediatr (Phila). 1999; 38:451–7.

[19] Matsushita Y, Shimazu M, Ikuzawa M, et al. Kyoto Scale of Psychological Development. Kyoto: Nakanishiya, 1985.

[20] Matsuishi T, Ishibashi S, Kamiya Y, Yamashita Y, Fukuda S, et al. Early intervention for very-low-birth-weight infants. Brain Dev 1998; 20:18–21.

[21] Idota T, Sakurai T, Ishiiyama Y, Murakami Y, Kubota J, et al. The latest survey for composition of human milk obtained Japanease mothers. Part I. The contents of gross components and minerals. JJPGN [Note: See Editor's Note #3.] 1991; 5:145–158.

[22] McGuire W, Anthony MY. Donor human milk versus formula for preventing necrotising enterocolitis in preterm infants: systematic review. Arch Dis Child 2003; 88:F11–F14.

[23] Coss-Bu JA, Kish WJ, Walding D, Stein F, Smith EOB, Jefferson LS. Energy metabolism, nitrogen balance, and substrate utilization in critically ill children. Am J Clin Nutr 2001; 74:664–9.

[24] Curley MA, Castllo L. Nutrition and shock in pediatric patients. New Horiz 1998; 6:212–5.

[25] Hamosh M. Enzymes in human milk. In: Jensen RG, editor. Handbook of Milk Composition. San Diego: Academic Press; 1995. pp. 388–436.

[26] RJ Cooke, ND Embleton. Feeding issues in preterm infants. Arch Dis Child 2000; 83:F215—F218.

[27] Kuschel CA, Harding JE. Protein supplementation of human milk to promote growth in preterm infants. The Cochrane Library 1999 Issue 2, Update Software, Oxford, 2003.

**Table 1 Variation in the nutritional content ~~with~~using the milk fortification protocol used ~~in~~at our NICU [Note: Please check the change.]**

| | Feed~~ing~~ intake | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 130 ml/kg/day | | | | | 150 ml/kg/day | | | | | |
| | HM | HM + 3H | HM + 5H | 16% PM | 18% PM | HM | HM + 3H | HM + 5H | 16% PM | 18% PM | HMS-1™ (/g of HMS-1™) |
| Protein (g/kg/day) | 1.7 | 2.7 | 3.4 | 3.2 | 3.6 | 2.0 | 3.1 | 3.9 | 3.6 | 4.1 | 0.26 g |
| Fat (g) | 4.8 | 4.8 | 4.8 | 4.1 | 4.6 | 5.6 | 5.6 | 5.6 | 4.7 | 5.3 | 0 g |
| Carbohydrate (g/kg/day) | 10.0 | 12.2 | 13.7 | 12.5 | 14.0 | 11.6 | 14.1 | 15.8 | 14.4 | 16.2 | 0.56 g |
| Calories (kcal/kg/day) | 89.7 | 102.9 | 111.6 | 98.8 | 111.2 | 103.5 | 118.7 | 128.8 | 114.0 | 128.3 | 3.37 kcal |

HM: human milk
HMS-1®: human milk fortifier used in Japan (Morinaga Milk Industry Co. Ltd., Japan)
HM + 3H: fortified human milk + 3 g/dl HMS-1®
HM + 5H: fortified human milk + 5 g/dl HMS-1®
16% PM: standard concentration of Neomilk PM® (Bean Stalk Snow Co. Ltd., Japan)

**Table 2 Characteristics of the study population**

| | DQ ≥ 80 (n = 22) | DQ < 80 (n = 15) | *p* |
|---|---|---|---|
| Gestational age (weeks) | 25.9 ± 1.3 | 25.4 ± 0.9 | NS |
| Birth weight (g) | 739.5 ± 127.6 | 724.7 ± 155.6 | NS |
| Z score of birth weight | −0.7 ± 0.6 | −0.5 ± 0.8 | NS |
| Birth length (cm) | 32.3 ± 2.9 | 31.3 ± 1.9 | NS |
| Z score of birth length | −1.0 ± 1.0 | −0.8 ± 0.9 | NS |
| Birth head circumference (cm) | 23.1 ± 1.4 | 22.8 ± 2.0 | NS |
| Z score of birth head circumference | −0.7 ± 0.5 | −0.3 ± 1.1 | NS |
| Apgar score at 1 min | 1–8 (median, 5) | 1–8 (median, 3) | NS |
| Apgar score at 5 min | 5–9 (median, 8) | 1–9 (median, 6) | <0.01 |
| Sex (No. of males) | 11 | 9 | NS |
| Duration of artificial ventilation (days) | 30.0 ± 24.0 | 50.3 ± 33.8 | <0.05 |
| Duration of $O_2$ supplementation (days) | 71.1 ± 56.9 | 127.6 ± 112.6 | <0.01 |
| *AUC of CBUN (mmol*day/l) | 285.2 ± 113.5 | 206.2 ± 80.3 | <0.05 |
| **Average CBUN (mmol/l) | 4.5 ± 1.7 | 3.3 ± 1.2 | 0.05 |
| ***Human milk feeding ratio (HMFR) (2 months of life) (%) | 80.3 ± 31.3 | 66.2 ± 26.9 | 0.14 |

*Area under the curve of CBUN between day 28 and 84 days of life
** Average CBUN level between day 28 and 84 days of life
***Intake of human milk/intake of (human milk + formula)

NS: not significant

**Table 3 Logistic multiple regression analysis for an overall DQ ~~above~~> 80 points at 36 months of PCA**

| | OR | 95% CI | *p* |
|---|---|---|---|
| Gestational age (weeks) | 0.71 | 0.20–2.53 | 0.60 |
| Z score of birth weight | 0.40 | 0.07–2.41 | 0.32 |
| Sex (male) | 0.20 | 0.03–1.63 | 0.13 |
| Apgar score (5 min) | 2.00 | 0.89–4.28 | 0.10 |
| Duration of ventilation (days) | 0.99 | 0.95–1.03 | 0.50 |
| *AUC of CBUN (mmol*day/L) | 1.03 | 1.002–1.06 | <0.05 |

*~~–~~AUC of CBUN between 28 and 84 ~~days~~ days of life (n = 37, $R^2$ = 0.41)
OR: Odd's ratio
CI: confidence interval

**Table 4 Moro's protein fortification method and its equivalents ~~for~~in our NICU method [Note: Please check the change.]**

Modified from Moro et al. [11]

| Fortification level | CBUN (mmol/dl) | Added protein (g/dl) | *Total protein intake (g/kg/day) |
|---|---|---|---|
| +3 | <1.2 | 1.20 | 3.75 |
| +2 | 1.2–2.2 | 1.05 | 3.54 |
| +1 | 2.3–3.4 | 0.93 | 3.35 |
| 0 | 3.5–4.5 | 0.79 | 3.14 |
| –1 | 4.6–5.6 | 0.65 | 2.93 |
| –2 | 5.6–6.8 | 0.52 | 2.73 |
| –3 | >6.8 | 0.38 | 2.52 |

*Amount of enteral feeding = 150 ml/kg/day

Figure 1 Derivation of groups followed up at the PCA of 36 months

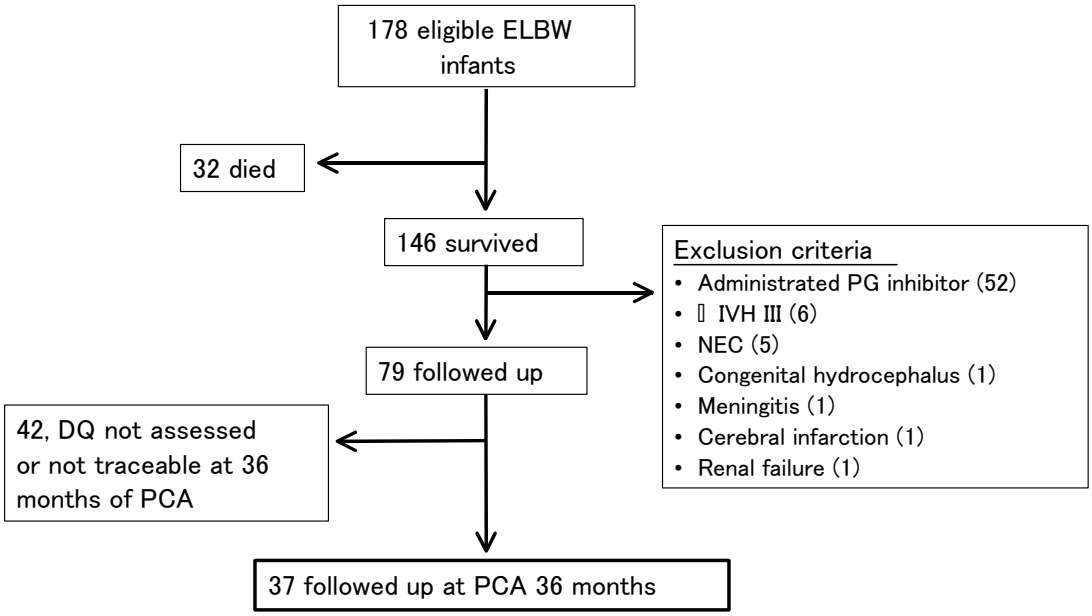
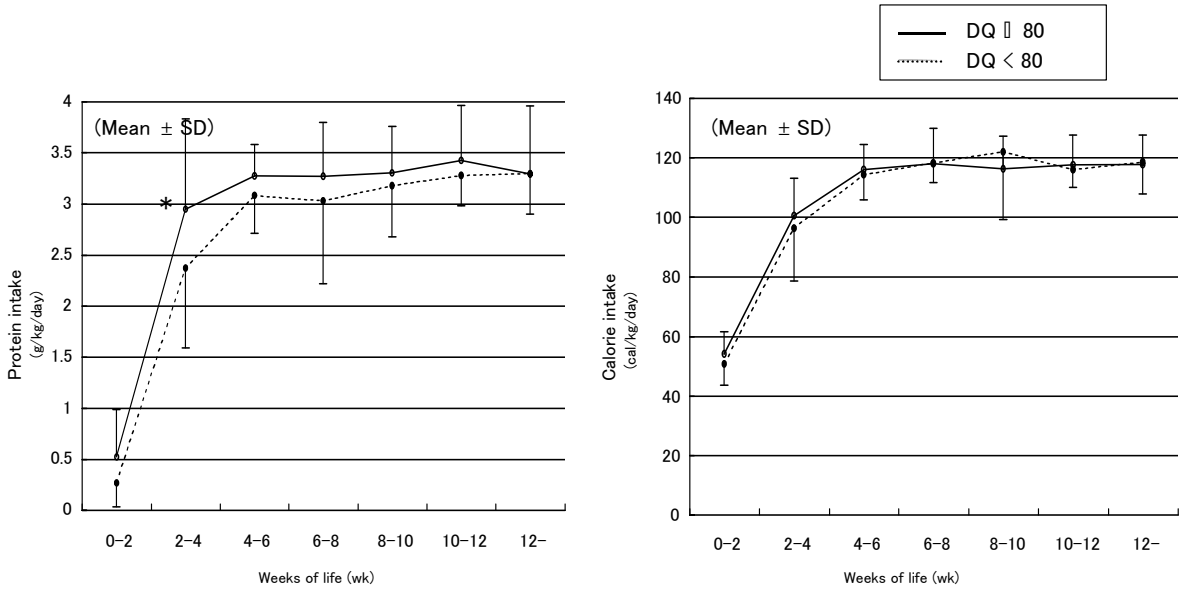


Figure 2

Comparison between DQ ≧ 80 and DQ < 80 groups for calculated calorie and protein intake

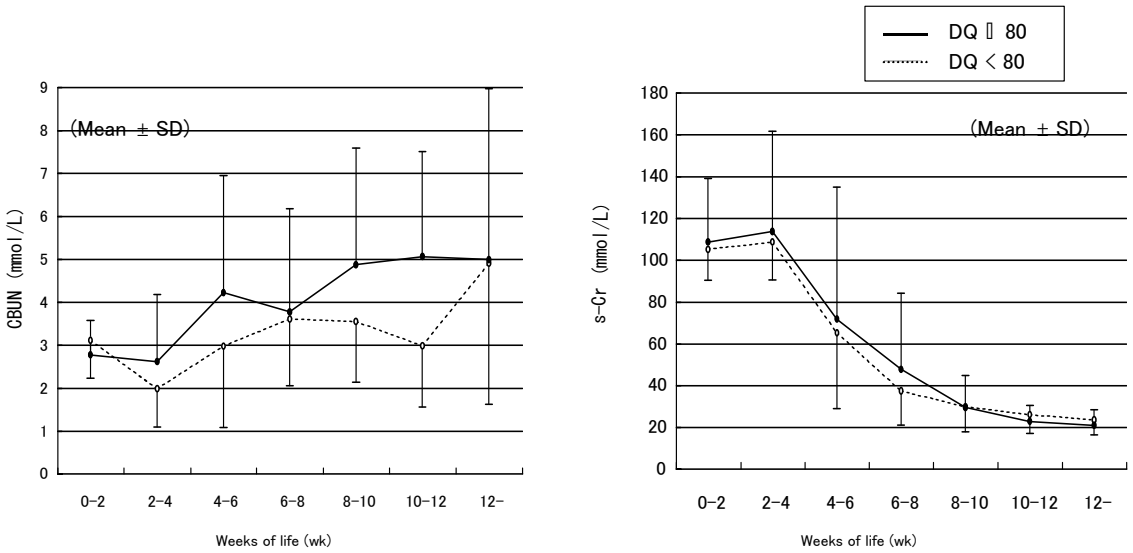



Bars : standard deviation

* $p < 0.05$

Figure 3

Comparison of average CBUN and serum creatinine levels between the DQ  80 and DQ < 80 groups




Bars : standard deviation