Executive Summary of the Workshop "Nutritional Challenges in the High Risk Infant"

Rosemary D. Higgins, MD[1], Sherin Devaskar, MD[2], William W. Hay, Jr, MD[3], Richard A. Ehrenkranz, MD[4], Frank R. Greer, MD[5], Kathleen Kennedy, MD, MPH[6], Paula Meier, RN, DNSc[7], LuAnn Papile, MD[8], and Michael P. Sherman, MD[9]

Pediatricians caring for preterm infants at the beginning of the 20th century faced the daunting challenge of providing nutritional support for their patients, who were surviving at increasing numbers because of the introduction of neonatal incubators. In a few decades, several key innovations followed. These included tube feeding, human milk banking systems, infant formulas, and, in the late 1970s through the 1980s, continuous intravenous nutrition.

Neonatal experts at the beginning of the 21st century are facing challenges similar to those faced by their professional colleagues a century ago. Recent advances in perinatal and neonatal care have increased survival rates of even the smallest and earliest born infants.[1] In the last 10 to 15 years, improvement in neonatal morbidity and mortality rates has plateaued.[2,3] A major concern, and part of the impetus for the workshop on "Nutritional Challenges in the High Risk Infant," is that this flattening in neonatal morbidity and mortality rates might be caused by inadequate or suboptimal (or both) nutrition of these extremely low birth weight, extremely preterm, and immature infants. Furthermore, newborn infants surviving a variety of critical care conditions appear to require focused attention to optimize nutritional support.

There is growing evidence, for example, that the "routine" intravenous nutritional mixtures, human milk, or currently available infant formulas (even those designed for preterm infants) may not be appropriate for infants recovering from major surgical conditions, especially those involving the gastrointestinal system. Similarly, late preterm, low birth weight newborn infants, and infants with congenital anomalies may need different nutritional requirements than growing healthy term infants. Thus, the healthcare team is challenged to develop strategies to meet high-risk infants' demands on the basis of stage of development, earlier and current growth disorders, underlying disease process, and stages of recovery from serious medical and surgical conditions.

Because >90% of preterm infants survive through their first birthday, there is an ever-increasing fraction of the population of preterm infants who will survive to adulthood. It is now clearly appreciated that abnormal perinatal nutritional conditions link directly to and may actually cause adult-onset illnesses.[4-6] There also is a growing realization that technical advances in providing intensive care have outpaced advances in perinatal nutritional research. Thus, there is a considerable urgency to refocus attention on much needed research in the field of neonatal nutritional support.

Now more than ever, we are facing new challenges for providing optimal nutritional support to the sickest and the smallest of newborn infants in our neonatal intensive care units (NICUs). The challenges are no longer limited to offering calories and proteins to promote weight gain. The challenges are more fundamental. There is new need to define what constitutes optimal postnatal growth, to find means of optimizing not only growth but also maturation (eg, the immune system, the brain, the skeletal muscle system, and bone), and to provide improved and firmer foundations for nutrition of the sick newborn infant that will better promote growth to healthy children and adults.

To address these wide-ranging issues, one needs to explore knowledge gaps and refocus a perinatal nutritional research agenda. Therefore, the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development held a workshop in 2009 to explore knowledge gaps and refocus interest on a perinatal nutritional research agenda. This review provides a brief summary of the discussions and is designed for researchers who deal with neonatal nutrition to consider for development of future investigations.

Fetal Nutrition and Long-Term Implications

Healthy embryonic development is a prerequisite for healthy fetal growth and development. A variety of maternal and fetal conditions and genetic and environmental factors also influence embryonic and fetal development. Normal fetal growth is affected by maternal diet and nutrition. The specific components of maternal nutrition and intrauterine nutritional milieu that are needed to promote healthy fetal growth still need to be delineated.

IUGR	Intrauterine growth restriction
NICU	Neonatal intensive care unit
TPN	Total parenteral nutrition

From the [1]Pregnancy and Perinatology Branch, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, Bethesda, MD; [2]Department of Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA; [3]Department of Pediatrics, University of Colorado School of Medicine, Aurora, CO; [4]Department of Pediatrics, Yale University School of Medicine, New Haven, CT; [5]Department of Pediatrics, University of Wisconsin, Madison, WI; [6]Division of Neonatal-Perinatal Medicine, University of Texas at Houston Medical School, Houston, TX; [7]Departments of Women, Children and Family Nursing, and Pediatrics, Rush University Medical Center, Chicago, IL; [8]Department of Pediatrics, Baylor College of Medicine, Houston, TX; and [9]Department of Child Health, University of Missouri at Columbia, Columbia, MO

This workshop was co-funded by the Office of Rare Disease and National Institutes of Health. The authors declare no conflicts of interest.

0022-3476/$ - see front matter. 10.1016/j.jpeds.2011.12.024

Most studies related to fetal nutrition have assessed changes in fetal growth rates. Although there are postnatal growth curves specific for African-American and Caucasian ethnic groups and for male and female infants, such standards are not widely used in clinical settings and do not reflect current demographic distributions. Moreover, the fetal growth curves currently in use have not been updated to reflect the contemporary population of pregnant women that has a higher proportion who are overweight or obese.

Meticulous assessment of fetal growth and growth rates can provide insights to fetal nutritional status. Normal growth curves have been developed for the fetus.[7] In a prenatal ultrasound scanning study, Doubilet et al[8] reported that singleton fetuses subsequently born preterm were, as fetuses, smaller and grew at slower rates than their gestational age-matched control subjects who went on to be born at term gestation, even in the absence of identifiable causes for growth restriction. This lag in fetal growth in infants who are born preterm occurs a few weeks before their birth. This may indicate that decline in fetal growth rate might be a signal of fetal malnutrition or illness, leading to preterm birth.

Normal fetal growth also requires adequate amounts of amniotic fluid and fetal swallowing activities. Although amniotic fluid composition, nutrients, and turnover rates vary across gestational ages, nutritional value of the amniotic fluid for the fetus has not been well investigated. Adequate placental health is critical for the volume and composition of the amniotic fluid. Birth before 29 weeks gestation hinders the development of intestinal secretory functions, absorptive characteristics, and motility. Amniotic fluid has growth factors, hormones, cytokines, and nutrients that stimulate the growth and maturation of the gastrointestinal tract during late gestation.[9] Fetal swallowing after 29 weeks of gestation is important in this process. Preterm birth and upper gastrointestinal anomalies disrupt this phenomenon. This critical fetal mechanism for intestinal maturation and its contribution to nutrition of the fetus during the third trimester requires additional investigation.

What are the optimal growth rates for the fetus and preterm infants? The impact of maternal overweight and underweight status and obesity on growth rates is not well described. Sex-, race-, and ethnicity-specific growth rates are needed to define normal and abnormal fetal and neonatal growth. The role of oxygen as a nutrient deserves further exploration. The impact of oxygen delivery on embryonic development, cell differentiation, growth, and development is not well understood. Intrauterine growth restriction (IUGR) can be caused by a single cause or multifactorial causes. Disorders in the uterus, placenta, and fetus all can be contributing factors. Advanced maternal diabetes with vascular disease and hypertension also results in IUGR. The role of each of these contributing factors in producing IUGR needs further investigation. The impact of changes in gestational age-specific growth rates and body composition at critical periods of development are not well described. Fetal programming as a result of such changes in growth rate and body composition likely play a major role in future development of health and disease and need further investigation. Ideal growth of the brain needs to be considered when studying somatic growth of the fetus. A healthy balance needs to be struck between optimal somatic growth and promoting normal fetal brain development and growth. Specific tools to assess fetal brain growth and determine its optimal development are needed. Genetic determinants of normal and abnormal growth including race, ethnicity, and sex need further investigation. Pregnancy complications that can significantly affect fetal growth and timing of these complications are likely to be gestational age specific for the outcomes for the fetus. The rate of fetal growth and conditions such as fetal growth "insufficiency" are likely to be sex- and gestational age-dependent and are in need of investigation.

There are critical periods when specific insults uniquely affect fetal development. Improvements in growth or insults to growth also depend on the duration of these events. Gestational age is shorter when the pregnant mother is undernourished, leading to the question: Could optimal nutrition of the pregnant mother prevent prematurity? Related research should focus on the particular nutritional factors, including macronutrients and micronutrients, which contribute to optimal pregnancy outcome. Coordinated studies in both mother and fetus should emphasize the role of the placenta in transferring nutrients to the fetus when changes in the diet of the pregnant mother are made. Such studies should include research on macronutrients and micronutrients, with more attention to the interaction of these major classes of nutrients. For abnormal fetal growth, life cycle studies should address longitudinal growth in time and generations. Development of model systems is desirable to study perturbations in growth, particularly with attention to gestational age.

Current Nutritional Practices: The Preterm Infant

Growth after birth can be investigated in several areas: short-term growth outcomes immediately after birth, matched versus mismatched growth between in utero and ex utero periods for preterm infants, and the effect of exogenous nutrition (enteral and parenteral) on physiological function.

Just after birth, there is an early window of nutritional management for which observational and interventional studies could potentially provide important information. Protein, fat, and carbohydrate intake and metabolism, energy expenditure, and temperature balance are all elements of an optimal nutritional environment. Practice strategies for parenteral nutrition are in flux, with increasing use of higher amino acid infusion rates earlier after birth, but there is no long-term assessment of this change in practice. Assessment of safety, efficacy, and appropriateness of both enteral and parenteral nutrition during this early postnatal period are needed for evidenced-based practice.

Gut microbiota and gut development are integral to developing strategies for optimizing both enteral and parenteral nutrition. Gut development and the effect of feeding are likely gestational age and postnatal age dependent, and both of these factors should be considered in study designs of optimizing nutrition after birth of preterm infants.

Further studies are needed to assess the long-term effects on growth, development, and particularly cognitive outcomes of the timing of initiation, initial volume, and rate of advancing feedings in the preterm infant. Optimal nutritional management is likely to depend on earlier fetal growth, gestational age at birth, and postnatal age. Total parenteral nutrition (TPN) has been lifesaving, but comes at a cost to infants that includes delayed gut growth, major changes in the gut microbiota, and metabolic complications. Early provision of amino acids has been associated with improved growth at hospital discharge and less suboptimal head growth at 18 months of age.[10] Modification of TPN to minimize or eliminate hepatic toxicities deserves further investigation, because cholestasis remains a major problem in infants on long-term TPN.

There also are many unanswered questions related to the use of human milk in preterm infants, including the timing of colostrum administration, volume of feedings, and supplements added to human milk. Although many physicians have noted that human milk feedings are considered the best type of nutrition for all infants, including those born preterm, many problems remain with its use in these infants, not the least of which is the need to optimize supplements.

Human milk is now known to contain probiotic bacteria in addition to lactoferrin, oligosaccharides, and lactose that promote their growth.[11-15] There is evidence that lactobacilli upregulate the epidermal growth factor receptor, thus increasing the effectiveness of human milk epidermal growth factor in promoting intestinal growth and maturation. Dvorak[16,17] and other investigators[18,19] have shown the mitigating effects of feeding epidermal growth factor on necrotizing enterocolitis in the neonatal rat model. Lactoferrin administration has been studied in the laboratory and is in need of clinical research.[20-22] One recent study with lactoferrin shows promise for prevention of late-onset sepsis.[23]

Several studies have been conducted to assess the effect of probiotic agents on necrotizing enterocolitis, sepsis, and death.[24-28] A recent meta-analysis from Australia concluded that probiotic supplements have significant benefits in reducing death and disease.[29] The meta-analysis concludes that additional trials are unnecessary when a suitable product is available.[29] An accompanying commentary[30] to the meta-analysis points out that no product with assured quality is available in the United States.

Feeding strategies during the transition from parenteral to enteral intake are understudied. Supplementation of milk during the transition from hospital discharge to home and continued nutritional management during infancy need rigorous exploration, particularly in light of recent evidence demonstrating potential effect of changes in growth rate and body composition during this critical period on later development of problems of overweight, obesity,[31] insulin resistance, and diabetes mellitus.

Poorly studied potential long-term effects of suboptimal nutrition include alterations of the renal, cardiovascular, metabolic, and neurological systems. Rapid catch-up growth, although positive in the short-term for weight gain, may have many long-term ramifications. Is a moderate growth rate better? Does this help provide the required balance in regulated somatic growth without compromise to cognition? Prevention of growth failure in preterm infants may be better than trying to treat growth failure after it is established. Thus, long-term investigations assessing the association between aberrant fetal and postnatal growth and later glucose intolerance, hypertension, and renal abnormalities are essential.

Brain maturation, growth, development, and function are important outcome measurements for assessing the nutrition of preterm infants. Such studies also should include the effect of the NICU environment and procedures and the effect of somatic growth and movement on long-term neurodevelopmental outcomes.[32] Nutritional strategies after brain injury may be important determinants of long-term outcomes, including cognitive development, sensory outcomes, and motor outcomes. This may be particularly important in population subgroups of infants with bronchopulmonary dysplasia, gastrointestinal disease, and cardiac disease. Bone growth, composition, and health also are understudied. Although most preterm infants regain bone mineralization later in infancy, the effect of the period of poor bone mineralization during the hospitalization of preterm infants on adult bone health remains uncertain and understudied.

Current nutritional practice and intervention include the many areas for investigation. Extremely low birth weight, preterm infants face significant challenges, including optimizing parenteral nutrition; initiation, advancement, and method of feeding; fortification and supplementation of feedings; and use of banked human milk when mother's milk is not available. Research barriers in this area include equipoise with current practices that are considered routine clinical practice in individual nurseries. Feeding practices, once established, may be difficult to change. The research agenda for nutrition in the preterm infant includes standardization of nutrition practices that optimize outcomes in these infants. Examples would include various aspects of intravenous nutrition, including protein, glucose/carbohydrates, and fats, and additional nutrients that are essential for growth. The parenteral intake of amino acids suggested for extremely preterm infants is approximately 3.0 to 4.0 g/kg/day. Concern remains for azotemia and metabolic acidosis with higher amino acid intakes during parenteral nutrition.[33] More information about adverse effects of high amino acid intake is needed. Evidence indicates concern for protein load and higher blood urea nitrogen levels in the first week of life.[34] Proinflammatory effects and toxicity of parenteral nutrition components for hepatic injury is an area for investigation of mechanisms causing injury, healing and repair, and prevention of toxicity. Failure of infants to feed or to be able to increase their feeding volume is an area that is markedly understudied. The goals for nutrition should be optimum growth and development. Most infants leaving the NICU after preterm birth have postnatal growth failure.[35]

Postnatal growth failure urgently needs research to define preventive strategies or interventions for optimizing outcomes in these infants. Optimal health includes the programming effects of early nutrition on short- and long-term health problems—late onset sepsis, necrotizing enterocolitis, re-hospitalizations after NICU stay, and early origins of adult-onset diseases. The true requirements of vitamins, trace elements and minerals for optimal growth and development of preterm infants are unknown.

Human Milk Feeding for Preterm Infants

Human milk functions as a source of nutrition and as a protective agent for the human newborn. The protective properties, which include immunomodulatory, anti-inflammatory, anti-oxidant, and intestinal growth-promoting and epigenetic functions reduce the risk of short- and long-term morbidities in extremely preterm infants.[36]

Human milk confers long-term benefit for infants in a dose-response relationship, with higher lifetime doses of human milk reducing the risk of atopy and increasing cognitive function. This same dose-response relationship was demonstrated in extremely preterm infants, with higher doses of human milk reducing the rates of re-hospitalization and increasing Bayley scores at 18 and 30 months of age, corrected for prematurity.[37,38] Special populations, including infants with surgical disease and infants who are neurologically impaired, may also benefit from human milk. The benefits of donor milk may not be the same as the benefits of the mother's own milk.

Human milk varies widely within and between mothers for volume, composition, and caloric density. Colostrum, the milk that is produced in the first days after birth, contains high concentrations of high-molecular weight protective proteins that cross into the milk via the open paracellular pathways that close with the onset of lactogenesis II. Animal studies suggest that colostrum has its greatest effect on protection, growth, and maturation of the gastrointestinal tract when received by the infant as "first feedings." Subsequently, mature human milk varies markedly in calories, primarily as a function of variability in the lipid content. Human milk lipid is not homogenized and clings to collection, storage, and feeding containers, further reducing the content that is delivered to the extremely preterm infant when these devices are needed.

Donor human milk has been used for preterm infants when mother's milk is not available in sufficient quantity. Donor human milk is available from commercial human milk banks, where it has collected from screened donors, pooled, and pasteurized. Some, but not all, human milk banks perform compositional analyses and provide this information on the individual milk containers. As a result of pasteurization, many protective components are either destroyed or their bioavailability is markedly reduced. A potential advantage of donor human milk is that it does not contain bovine protein, which may be important for extremely preterm infants and those with surgical conditions.

For human milk feedings to be successful, evidence-based education that is specific to the infant in the NICU and family should be provided to the entire NICU team, including physicians, nurses, and dietitians. This well-informed team should be encouraged to provide consistent counseling about optimizing lactation to provide as much human milk as feasible for preterm infants. The team should be knowledgeable about lactation technologies that optimize human milk feedings for infants in the NICU, including the creamatocrit and other milk analysis techniques, test-weights to measure milk intake, and the use of evidence-based breast pump technology.

Specific areas for consideration of research include: (1) Standardize definitions for human milk feeding including colostrum, human milk, foremilk, hind milk, and mature milk and for the amount of human milk (eg, dose) that is fed during the NICU hospitalization; (2) Large randomized studies comparing donor human milk with formula for preterm infants to determine whether banked human milk offers advantage compared with commercially available formula—studies about nutritional supplements of human milk for preterm infants are needed; (3) Mechanisms of human milk protection, specifically for neonatal immunity for preterm infants; (4) Optimum promotion of breast feeding and human milk use; and (5) Standardization of commercial and private milk donors to ensure safety first in the use of donor milk. There is need for standardization of the milk testing to inform care providers about the composition (eg, macro- and micro-nutrients).

Neonatal Nutrition Niche Areas

There are many areas of neonatal nutrition in need of additional investigation.

There is a growth promoting effect of amniotic fluid. Further investigation on potential use of amniotic fluid for postnatal growth may provide increased knowledge for clinical application. Probiotics have been studied in >2000 infants.[24-28] Meta-analysis confirms beneficial effects of probiotics, but points out the need for a product with well-documented and reliable composition and with established safety.[29] Studies reported to date have not demonstrated bacteremia with lactobacilli or bifidobacteria. Questions remain about the optimal probiotic(s), consistency of product, ideal target population, and timing of initiation and duration of treatment. The need for a product of insured quality is a major gap.[30] The importance of feeding probiotic bacteria goes beyond the concept of preventing necrotizing enterocolitis. Probiotic bacteria may enhance growth in infants who are born prematurely, but more research is needed because controversy exists about their effectiveness.[39,40] Prebiotics are gaining attention to promote optimal nutrition. The exact role(s) of prebiotics in the neonatal population needs further investigation. Dietary supplements, including trace elements and vitamins, may hold promise for neonatal nutrition. Transitional nutrition after birth to determine the optimal method, initiation, timing, quality, and quantity of feeding are important areas of study, particularly for the preterm infant. This needs to be tailored to the gestational age and medical status of the infant. Nutritional monitoring of high-risk infants to optimize outcomes including growth and neurodevelopment is needed.

In summary, nutritional management is a major challenge for the developing fetus and newborn. Very preterm and ill infants face many nutritional challenges. Optimal nutrition for growth and development to yield the best outcomes for infants are desired. Neonatal nutrition and its impact on overall health throughout the life course must be examined to make progress in this area of medicine. ■

Submitted for publication Oct 18, 2011; accepted Dec 12, 2011.

Reprint requests: Rosemary D. Higgins, MD, Pregnancy and Perinatology Branch, Center for Developmental Biology and Perinatal Medicine, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, 6100 Executive Blvd, Room 4B03, MSC 7510, Bethesda, MD 20892. E-mail: higginsr@mail.nih.gov

References

1. Stoelhorst GM, Rijken M, Martens SE, Brand R, de Ouden AL, Wit JM, et al. Leiden follow up project on prematurity. Changes in neonatology: comparison of two cohorts of very preterm infants (gestational age, 32 weeks): the project on preterm and small for gestational age infants 1983 and the Leiden follow-up project on prematurity 1996-1997. Pediatrics 2005;115:396-405.
2. Fanaroff AA, Stoll BJ, Wright LL, Carlo WA, Ehrenkranz RA, Stark AR, et al., NICHD Neonatal Research Network. Trends in neonatal morbidity and mortality for very low birth weight infants. Am J Obstet Gynecol 2007;196:147.e1-8.
3. Tommiska V, Heinonen K, Lehtonen L, Renlund M, Saarela T, Tammela O, et al. No improvement in outcome of nationwide extremely low birth weight infant populations between 1996-1997 and 1999-2000. Pediatrics 2007;119:158-60.
4. Devaskar SU, Calkins K. Developmental origins of adult health and disease in neonatal-perinatal medicine: diseases of the fetus and infant. In: Martin RJ, Fanaroff AA, Walsh MC, eds. 9th ed. St. Louis: Elsevier Mosby; 2011. p. 229-41.
5. Joss-Moore LA, Lane RH. The developmental origins of adult disease. Curr Opin Pediatr 2009;21:230-4.
6. Simmons RA. Developmental origins of adult disease. Pediatr Clin North Am. 2009;56:449-66.
7. Doubilet PM, Benson CB, Nadel AS, Ringer SA. Improved birth weight table for neonates developed from early gestations dated by early ultrasonography. J Ultrasound Med 1997;16:241-9.
8. Doubilet PM, Benson CB, Wilkins-Haug L, Ringer S. Fetuses subsequently born premature are smaller than gestational age-matched fetuses not born premature. J Ultrasound Med 2003;22:359-63.
9. Underwood MA, Sherman MP. Nutritional characteristics of amniotic fluid. NeoRev 2006;7:e310-6.
10. Poindexter BB, Langer JC, Dusick AM, Ehrenkranz RA, National Institute of Child Health and Human Development Neonatal Research Network. Early provision of parenteral amino acids in extremely low birth weight infants: relation to growth and neurodevelopmental outcome. J Pediatr 2006;148:300-5.
11. Martín R, Langa S, Reviriego C, Jiménez E, Marín ML, Xaus J, et al. Human milk is a source of lactic acid bacteria for the infant gut. J. Pediatr 2003;143:754-8.
12. Martín R, Olivares M, Marín ML, Fernández L, Xaus J, Rodríguez JM. Probiotic potential of 3 lactobacilli strains isolated from breast milk. J Hum Lact 2005;21:8-17.
13. Díaz-Ropero MP, Martín R, Sierra S, Lara-Villoslada F, Rodríguez JM, Xaus J, et al. Two Lactobacillus strains, isolated from breast milk, differently modulate the immune response. J Appl Microbiol 2007;102:337-43.
14. Martín R, Jiménez E, Heilig H, Fernández L, Marín ML, Zoetendal EG, et al. Isolation of bifidobacteria from breast milk and assessment of the bifidobacterial population by PCR-denaturing gradient gel electrophoresis and quantitative real-time PCR. Appl Environ Microbiol 2009;75:965-9.
15. Abrahamsson TR, Sinkiewicz G, Jakobsson T, Fredrikson M, Björkstén B. Probiotic lactobacilli in breast milk and infant stool in relation to oral intake during the first year of life. J Pediatr Gastroenterol Nutr 2009;49:349-54.
16. Dvorak B, Halpern MD, Holubec H, Williams CS, McWilliam DL, Dominguez JA, et al. Epidermal growth factor reduces the development of necrotizing enterocolitis in a neonatal rat model. Am J Physiol Gastrointest Liver Physiol 2002;282:G156-64.
17. Dvorak B, Khailova L, Clark JA, Hosseini DM, Arganbright KM, Reynolds CA, et al. Comparison of epidermal growth factor and heparin-binding epidermal growth factor-like growth factor for prevention of experimental necrotizing enterocolitis. J Pediatr Gastroenterol Nutr 2008;47:11-8.
18. Feng J, El-Assal ON, Besner GE. Heparin-binding epidermal growth factor-like growth factor decreases the incidence of necrotizing enterocolitis in neonatal rats. J Pediatr Surg 2006;41:144-9.
19. Radulescu A, Zorko NA, Yu X, Besner GE. Preclinical neonatal rat studies of heparin-binding EGF-like growth factor in protection of the intestines from necrotizing enterocolitis. Pediatr Res 2009;65:437-42.
20. Edde L, Hipolito RB, Hwang FF, Headon DR, Shalwitz RA, Sherman MP. Lactoferrin protects neonatal rats from gut-related systemic infection. Am J Physiol Gastrointest Liver Physiol 2001;281: G1140-50.
21. Sherman MP. New concepts of microbial translocation in the neonatal intestine: mechanisms and prevention. Clin Perinatol 2010;37:565-79.
22. Legrand D, Pierce A, Elass E, Carpentier M, Mariller C, Mazurier J. Lactoferrin structure and functions. Adv Exp Med Biol. 2008;606:163-94.
23. Manzoni P, Rinaldi M, Cattani S, Pugni L, Romeo MG, Messner H, et al., Italian Task Force for the Study and Prevention of Neonatal Fungal Infections, Italian Society of Neonatology. Bovine lactoferrin supplementation for prevention of late-onset sepsis in very low-birth-weight neonates: a randomized trial. JAMA 2009;302:1421-8.
24. Hoyos AB. Reduced incidence of necrotizing enterocolitis associated with enteral administration of *Lactobacillus acidophilus* and *Bifidobacterium infantis* to neonates in an intensive care unit. Int J Infect Dis 1999;3: 197-202.
25. Dani C, Biadaioli R, Bertini G, Martelli E, Rubaltelli FF. Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm infants. A prospective double-blind study. Biol Neonate 2002;82:103-8.
26. Bin-Nun A, Bromiker R, Wilschanski M, Kaplan M, Rudensky B, Caplan M, et al. Oral probiotics prevent necrotizing enterocolitis in very low birth weight neonates. J Pediatr 2005;147:192-6.
27. Lin HC, Hsu CH, Chen HL, Chung MY, Hsu JF, Lien RI, et al. Oral probiotics prevent necrotizing enterocolitis in very low birth weight preterm infants: a multicenter, randomized, controlled trial. Pediatrics 2008;122: 693-700.
28. Lin HC, Su BH, Chen AC, Lin TW, Tsai CH, Yeh TF, et al. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. Pediatrics 2005;115:1-4.
29. Deshpande G, Rao S, Patole S, Bulsara M. Updated meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates. Pediatrics 2010;125:921-30.
30. Soll RF. Probiotics: are we ready for routine use? Pediatrics 2010;125: 1071-2.
31. Bartok CJ, Ventura AK. Mechanisms underlying the association between breastfeeding and obesity. Int J Pediatr Obes 2009;4:196-204.
32. Ehrenkranz RA, Dusick AM, Vohr BR, Wright LL, Wrage LA, Poole WK. Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. Pediatrics 2006;117:1253-61.
33. Premji SS, Fenton TR, Sauve RS. Higher versus lower protein intake in formula-fed low birth weight infants. Cochrane Database of Systematic Reviews 2006;CD003959.

34. Balakrishnan M, Tucker R, Stephens BE, Bliss JM. Blood urea nitrogen and serum bicarbonate in extremely low birth weight infants receiving higher protein intake in the first week after birth. J Perinatol 2011;31: 535-9.
35. Dusick AM, Poindexter BB, Ehrenkranz RA, Lemons JA. Growth failure in the preterm infant: can we catch up? Semin Perinatol 2003;27:302-10.
36. Meier PP, Engstrom JL, Patel AL, Jegier BJ, Bruns NE. Improving the use of human milk during and after the NICU stay. Clin Perinatol 2010;37: 217-45.
37. Vohr BR, Poindexter BB, Dusick AM, McKinley LT, Wright LL, Langer JC, et al., NICHD Neonatal Research Network. Beneficial effects of breast milk in the neonatal intensive care unit on the developmental outcome of extremely low birth weight infants at 18 months of age. Pediatrics 2006;118:e115-23.
38. Vohr BR, Poindexter BB, Dusick AM, McKinley LT, Higgins RD, Langer JC, et al., National Institute of Child Health and Human Development National Research Network. Persistent beneficial effects of breast milk ingested in the neonatal intensive care unit on outcomes of extremely low birth weight infants at 30 months of age. Pediatrics 2007;120:e953-9.
39. Underwood MA, Salzman NH, Bennett SH, Barman M, Mills DA, Marcobal A, et al. A randomized placebo-controlled comparison of 2 prebiotic/probiotic combinations in preterm infants: impact on weight gain, intestinal microbiota, and fecal short-chain fatty acids. J Pediatr Gastroenterol Nutr 2009;48:216-25.
40. Chou IC, Kuo HT, Chang JS, Wu SF, Chiu HY, Su BH, et al. Lack of effects of oral probiotics on growth and neurodevelopmental outcomes in preterm very low birth weight infants. J Pediatr 2010;156:393-6.