

Symposium: Animal Models in Neonatal and Infant Nutrition Research

Use of the Colostrum-Deprived Piglet to Evaluate Parenteral Feeding Formulas¹

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ABSTRACT Several different neonatal and infant piglet models have been invaluable as animal models in nutrition research. A preterm colostrum-deprived piglet model has been developed that is delivered at the desired gestational age, is cared for using the standard of care provided preterm human neonates including procedures for nutritional support and can be studied during pathological conditions induced under controlled conditions. The absolute values for each nutrient requirement would not be expected to be identical for preterm piglets and preterm human neonates. However, the effect of different levels of gestational maturity and superimposed pathophysiologies on nutrient requirements should be similar in the piglet and human neonate. *J. Nutr.* 123: 391-394, 1993.

INDEXING KEY WORDS:

• total parenteral nutrition • neonate • piglet

The phrase human neonate refers to individuals with extraordinarily diverse characteristics. The field of neonatal clinical nutrition encompasses a wide spectrum of neonates from the healthy breast-fed term neonate to the critically ill extremely preterm neonate. Intrauterine growth and gestational age at parturition are important determinants of nutritional needs during the neonatal period. Dramatic improvements in neonatal critical care medicine have led to the survival of a new population of neonates that could not have survived two decades ago. Preterm neonates are now divided into low birth weight (LBW), very low birth weight (VLBW), and extremely low birth weight (ELBW) groups. Just as the nutritional requirements of the term neonate differ from those of the adult, the nutritional requirements of the term neonate differ from those of the ELBW neonate. The nutritional requirements of the three different groups of preterm neonates probably differ from each other.

The window of tolerance of nutrient intake is extremely narrow for these infants. The formulation

must meet the special nutrient requirements of the neonate with organ systems that are still developing and must not exceed the limited concentrations that these same premature organ systems can tolerate. At the present time, our knowledge concerning the limits of this window of tolerance of nutrient intake is sparse.

Superimposed upon organ immaturity, many neonates experience major medical complications. Organ support protocols range from increased oxygen level in the inhaled atmosphere to extracorporeal membrane oxygenation. Surgery can range from the relatively simple procedure to organ transplantation. Infection can range from the common bacterial infection that is antibiotic responsive to human immunodeficiency virus (HIV) infection that awaits a cure.

If the neonate cannot nipple feed, enteral nutrition is provided via a tube placed in the stomach or the small intestine. If the neonate cannot absorb human milk or whole protein formula, a more easily digested enteral formula or parenteral nutrition is used for nutritional support. Investigations in adult animals have shown that use of the parenteral route for administration of nutrients requires that some nutrients be supplied in the diet that are not required when nutrients are supplied via the enteral route. Thus, the nutrient requirements of the enterally fed ELBW neonate may differ from those of the parenterally fed ELBW neonate.

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Research techniques are too invasive to be performed in preterm human neonates

Gestational age, clinical status, concurrent medical therapies and route of administration of nutrition are recognized as critical components in designing the nutritional support of an individual neonate. Because blood and urine concentrations of nutrients are often not good indicators of tissue concentrations, tissue sampling is needed. Determination of nutrient requirements during metabolic stress is also important for optimal nutritional support of this population. However, minor invasive techniques cannot be performed in an extremely preterm human neonate with multiple metabolic stresses even though this is the type of neonate who has the greatest need for nutritional support tailored to his or her specific needs.

Because much of this research involves techniques that are too invasive to be performed in human neonates, a neonatal animal model is required that is similar in anatomy and physiology to the human neonate (Table 1). The animal model must permit evaluation of nutritional regimens at different gestational ages, during different pathological conditions, with different medical therapies and with different routes of administering nutritional formula. The piglet has been used for more than 25 years to study human infant formula products (1, 2). The milk-fed piglet continues to be a valuable animal model for studying aspects of digestion and absorption in milk-fed human infants (3).

Many of the critical questions concerning the effect of early nutrition on the development of the central nervous system can be addressed in the piglet. The pattern of brain growth before and after birth is similar in the piglet and the human (4). The piglet is used to refine many surgical procedures performed on neonates. The piglet model has proven useful in studying heart transplantation during the neonatal period (5). The model would also be an excellent one to investigate nutritional support of the surgical neonate.

Preterm neonates are often colostrum deprived

Preterm neonates who require parenteral nutritional support are usually neonates who have not ingested any colostrum. Colostrum is a source of such a large number of nutrients, growth factors and hormones that the nutrient needs of the colostrum-fed neonate may not be the same as that of the colostrum-deprived neonate. A recent report indicates that protein synthesis in intestines of suckling piglets during the early neonatal period is increased by colostrum feeding but that the high rate of protein accretion in colostrum-fed piglets may be attributable largely to the absorption and retention of colostrum proteins, especially immunoglobins (6). Hepatic gluconeogenesis in the neonatal piglet has been shown to be a function of colostrum intake (7). Colostrum intake, with its myriad metabolic effects, should be con-

TABLE 1

Characteristics of preterm neonatal animal model that must simulate those of the preterm human neonate

1. Size, anatomy and physiology
2. Pattern of nutrient accretion and organ development during gestation
3. Delivery at gestational ages corresponding to ELBW, VLBW, LBW and term¹
4. Pathophysiological conditions but induced under controlled conditions
5. Care using the standard of care of Neonatal Intensive Care Units
6. Control of colostrum intake or colostrum deprivation
7. Administration of enteral and/or parenteral nutrition using the procedures of Neonatal Intensive Care Units

¹ ELBW, extremely low birth weight; VLBW, very low birth weight; LBW, low birth weight.

trolled to simulate the colostrum intake of the type of human neonates being addressed.

Total parenteral nutrition (TPN) piglet models

Infant and pediatric piglets have proven to be excellent animal models for TPN. Six-week-old weaned piglets received amino acids, glucose and fat emulsion intravenously or intragastrically for 3 weeks (8). The total body weight gain was the same in both groups, but the intravenously fed piglets had reduced growth of the stomach, small bowel and pancreas. Female Landrace pigs weighing 4.5–5.9 kg were placed on TPN and found to have varying quantities of green mucous-like sludge in the gallbladder (9), similar to the sludge reported to be found in humans on TPN. Bohles et al. (10) placed infant piglets on enteral formula, carnitine free-TPN or TPN supplemented with 1.5 mg/kg/day of L-carnitine. The TPN piglets had low taurine concentrations in liver and brain that were normal in the carnitine-supplemented TPN. In another set of similarly treated infant piglets, Bohles et al. (11) demonstrated decreased respiratory quotient and improved nitrogen retention when TPN was supplemented with carnitine. Our laboratory (12) has shown that the pattern of accretion of carnitine in piglet tissues during gestation is similar to the pattern observed in the human fetus, suggesting that the piglet also would be a good model to study carnitine metabolism during the preterm period.

Neonatal and infant piglets who have received varying amounts of colostrum have been studied in several laboratories, including that of Shulman (13). Cohen et al. (9, 14) placed 53 piglets with a mean weight of 1.8 kg and a mean age of 3.1 days on TPN for periods ranging from 6 to 31 days, yielding a total of 867 TPN piglet days. Clinical complications were similar to those observed in human neonates on TPN,

including 12 piglets with fatty changes in liver, 6 with cholestasis, 10 with lipid in alveolar macrophages and 2 with necrotizing enterocolitis. More recently, Cohen et al. (15) showed that in healthy neonatal piglets with histologically normal lungs, there were no differences in fatty deposition in alveolar macrophages among piglets enterally fed, TPN piglets receiving physiological doses of fat emulsion and TPN piglets receiving pharmacological doses of fat emulsion.

Germ-free colostrum-deprived piglets have been supported by TPN. Mehrazar and Kim (16) delivered miniature piglets 3–5 days preterm, deprived them of colostrum and maintained them on TPN in a germ-free environment for 21 days. Piglets do not receive immunoglobins across the placenta and are dependent on colostrum for all their passive immunity. The piglets were kept colostrum free and in a germ-free environment because the investigators wanted them to be "immunologically virgin" for use in studying the development of the immune system.

Piglet neonatal intensive care unit

Because human neonates in a Neonatal Intensive Care Unit are exposed to a broad spectrum of infectious agents and frequently become septic, our laboratory has developed a colostrum-deprived neonatal piglet model that can be nutritionally supported by TPN without placing them in a germ-free environment. The piglets are delivered at the desired gestational age and admitted to the Piglet Neonatal Intensive Care Unit where they receive the same standard of care as given to human neonates in a Neonatal Intensive Care Unit, including continuous nursing care. On the day of delivery, a catheter is surgically placed in the external jugular and advanced to the aorta for TPN administration, a second in the carotid for arterial blood sampling, a third in the stomach for quantitative administration or collection of fluid and a fourth in the bladder for continuous collection of urine. Catheters exit at the site of the incision and are encased in sterile stretch cloth jackets rather than tunneling under the skin. The catheter used for administration of parenteral nutrition is connected to a swivel, and piglets are permitted free movement. Piglets are housed in individual incubators under thermoneutral and high humidity conditions. TPN is administered as a single mixture of amino acids, dextrose, fat, vitamins and minerals. The TPN is advanced to full strength using a procedure similar to that used for human neonates, with the exception that strength is advanced at 12-hour intervals rather than the 24-hour intervals often used for neonates. Piglets are provided with toys for play and with a pacifier to encourage normal rooting and sucking activity. Although the colostrum-deprived piglets have compromised immunity due to reduced circulating immunoglobins (as do preterm human neonates), they thrive when given the same level of care as provided human neonates. Piglets at earlier gestational ages re-

quire the same respiratory support as provided in Neonatal Intensive Care Units. The labor intensity of maintaining colostrum-deprived preterm piglets in a Piglet Neonatal Intensive Care Unit is similar to that of maintaining human neonates in a Neonatal Intensive Care Unit. The time invested results in an animal model that meets the criteria listed in Table 1.

Potential methods for extrapolating data obtained from the piglet

The major problem with the colostrum-deprived piglet model is simply that a piglet is not a human neonate. Data from any animal model require certain extrapolation before that data can be applied to the human. Because there are different levels of protein, carbohydrate and fat in sow milk and human milk, one should not expect the piglet and the neonate to have identical requirements. **Table 2** lists the parameters that need to be elucidated for each nutrient. If the nutrient of interest is termed "x," we need to determine the concentration of nutrient x in human milk. We also need to determine the concentration of nutrient x in enteral formula and in parenteral formula that will support normal growth and development in the human neonate at different stages of gestational maturity and with different pathophysiologies. At the present time, we have data values for many nutrients in human milk and in enteral formulas that support normal growth and development in term neonates. The nutrient values for enteral formulas designed for preterm neonates need to be refined. The question of

TABLE 2

Nutrient parameters to be elucidated

HMx	Concentration of nutrient x in human milk
HFx	Concentration of nutrient x in formula designed for feeding fullterm neonates
HTPNx	Concentration of nutrient x in parenteral nutrition solutions designed for preterm neonates that supports growth and development
HTPNx/HFx	Ratio of concentration of nutrient x needed in parenteral formula designed for preterm neonates to concentration of nutrient x needed for enteral formula for fullterm neonates
SMx	Concentration of nutrient x in sow milk
SFx	Concentration of nutrient x in formula designed for feeding fullterm piglets that supports normal growth and development
STPNx	Concentration of nutrient x in parenteral formulation designed for preterm piglets that supports normal growth and development in the preterm piglet
STPNx/SFx	Ratio of concentration of nutrient x needed in parenteral formulation designed for preterm piglets to concentration of nutrient x needed for enteral formula for fullterm piglets

interest is: What concentration of nutrient x should be included in parenteral nutrition solutions designed for preterm neonates? We need to determine the unknown parameter HTPNx (Table 2).

The invasive techniques needed to address these areas can be performed directly in the neonatal piglet. We can experimentally determine all three parameters for the piglet and thus solve the equation for the ratio (STPNx)/(SFx) defined in Table 2. The absolute values obtained for the piglet parameters listed in Table 2 would not be expected to be the same values needed by the human neonate. However, the effect of different levels of gestational maturity and superimposed pathophysiologies on the nutrient requirements should be similar in the neonatal piglet and in the neonatal human. Many investigators have cautioned that care is needed when data obtained from animal models are applied to humans. "The results from animal studies should usually be interpreted relatively rather than being used as absolutes, and the utmost care in drawing conclusions must always be exercised" (3).

CONCLUSION

The colostrum-deprived piglet is the first practical model to allow the use of invasive techniques to evaluate parenteral nutrition formulas designed to feed the preterm neonate.

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