Parenteral Nutrition Compromises Neurodevelopment of Preterm Pigs

Asim F Choudhri, Helen J Sable, Viktor V Chizhikov, Karyl K Buddington, and Randal K Buddington

Abstract

Background: Despite advances in nutritional support and intensive care, preterm infants are at higher risk of compromised neurodevelopment.

Objective: This study evaluated the contribution of total parenteral nutrition (PN) to compromised neurodevelopment after preterm birth.

Methods: Preterm pigs were provided PN or enteral nutrition (EN) for 10 d. Neurodevelopment was assessed by observations of motor activity and evaluation of sensory/motor reflexes, brain weight, MRI, and cerebellar histology.

Results: Despite similar gains in body weight, PN pigs had smaller brains (32 ± 0.4 vs. 35 ± 0.6 g; P = 0.0002) including the cerebellum, as well as reduced motor activity (P = 0.005), which corresponded to underdeveloped myelination (P = 0.004) measured by diffusion tensor imaging. PN resulted in lower serum triglycerides (17 ± 5.9 vs. 27 ± 3.1 mg/dL; P = 0.05), total cholesterol (31 ± 9.6 vs. 85 ± 8.1 mg/dL; P = 0.04), VLDL cholesterol (3.7 ± 1.2 vs. 5.7 ± 0.7 mg/dL; P = 0.04), and HDL cholesterol (16 ± 4.6 vs. 57 ± 7.3 mg/dL; P = 0.03) and nonsignificantly lower LDL cholesterol (10.7 ± 4.4 vs. 22.7 ± 2.9 mg/dL; P = 0.09).

Conclusions: The compromised neurodevelopment caused by total PN is a novel finding, was independent of confounding variables (disease, inconsistent gestational ages, diverse genetics, extrauterine growth retardation, and inconsistent neonatal intensive care unit protocols), and highlights a need to improve current PN solutions. The preterm pig is a translational animal model for improving nutrition support to enhance neurodevelopment of preterm infants requiring PN. J Nutr doi: 10.3945/jn.114.197145.

Introduction

Compromised neurodevelopment among preterm infants is estimated at 40–50%, with the risk inversely related to gestational age at birth (1). Noninvasive imaging technologies have revealed that the brains of preterm infants have delayed development of white and gray matter and altered microstructural development (2, 3). The delays and differences in anatomic development of the preterm brain have been linked to altered cognitive and motor development (4–6). The compromised neurodevelopment of many preterm infants has been attributed to health complications such as necrotizing enterocolitis (NEC) (7, 8), need for prolonged mechanical ventilation (9), and various drugs, including corticosteroids (10). It is possible that nonoptimal nutritional support also contributes to neurodevelopmental deficits observed in preterm infants. However, confounding variables (e.g., in utero events, different protocols for postnatal care, and the health issues mentioned above) have complicated interpretation of the influence of nutrition on neurodevelopment.

The provision of adequate energy, nutrients, and fluid after preterm birth is critical for growth and development and is of paramount importance for neurodevelopment (11). Preterm infants unable to process adequate volumes of enteral inputs require parenteral nutrition (PN). This is particularly true of preterm infants born before 30 wk and weighing <1000 g with immature gastrointestinal tracts who may be reliant on PN for an extended period (12). Even though early advancement to full PN may be safe (13), it is often delayed and the resulting malnutrition and weight loss until full PN is achieved may contribute to poor neurodevelopmental outcomes (14). Although an early transition from PN to enteral nutrition (EN)
improves health outcomes (15), many infants require PN for prolonged periods before they are transitioned to EN.

Despite PN being essential for many preterm infants, there are concerns about adverse impacts. While evaluating the preterm pig as a large animal model of PN-associated liver disease (PNALD; RK Buddington, unpublished data, 2013) we observed differences in brain mass between littermates provided PN and EN. The research presented herein used a multidisciplinary approach to conclusively determine if providing PN compromises brain development. A second objective was to validate the preterm pig as a translational model for exploring the role of nutrition in neurodevelopment after preterm birth.

The preterm pig was selected as the animal model on the basis of recognition of the metabolic, nutritional, and physiologic relevance to human infants (16–18), including neurodevelopment (19), compatibility of preterm pigs with neonatal intensive care unit (NICU) equipment and protocols, availability of timed-pregnancy sows of defined genetic lineage, and large litter sizes that allow comparisons of siblings receiving different nutrition support. Importantly, the use of healthy preterm pigs avoids the confounding variable of disease (e.g., NEC), which requires preterm infants to remain reliant on PN and may influence neurodevelopment independent of nutrition. Our results raise concerns that current PN formulations compromise neurodevelopment. Moreover, the associations between brain growth, myelination, and delayed sensory and motor skills mimic the characteristics of compromised neurodevelopment described for preterm infants and indicate that the pig is a relevant model for studying neurodevelopment after preterm birth and for improving nutritional support.

Methods

Source and care of the preterm pigs. All aspects of the project that involved the care and use of the experimental pigs were approved by the institutional animal care and use committees of participating universities (University of Tennessee Health Sciences Center for cesarean delivery and University of Memphis for care of preterm pigs). Preterm pigs were delivered at 91% term (day 105 of 115 d of gestation) by sterile cesarean delivery (17) from 2 timed-pregnancy sows that were specific-pathogen free, shared a consistent genetic lineage, and had been artificially inseminated by using a consistent source of semen. Because growth responses of pigs may be influenced by birth weight (20), we selected pigs from each litter that were of similar size and included both sexes (n = 8 and 9 for litters 1 and 2). The pigs received 24-h care throughout the entire 10-d period, and observations of general health and motor activity were recorded hourly.

The pigs were transferred after delivery to an advanced-care facility, and within 3 h after delivery each preterm pig had an umbilical artery catheter (UAC) inserted and a feeding tube placed via a small incision made in the cheek. The UAC and feeding tube were secured on the dorsal surface. After placement of the UAC, each pig was provided a single dose of maternal serum (5 mL/kg) prepared from blood collected after the fetal pigs had been removed. The maternal serum provides passive immunity and compensates for the lack of colostrum. Cefazolin (50 mg/kg) was administered via the UAC for the first 2 d after delivery. The pigs were individually housed in incubators maintained at 38–39°C with supplemental oxygen (2–3 L/min) provided for the first 6–8 h after delivery. All of the pigs (n = 17) received PN starting within 3–4 h after delivery at a rate of 8 mL/kg (6 h) by using an all-in-one solution prepared by People’s Custom Pharmacy (Memphis, TN) and based on previous studies (17) (Table 1).

After 24 h of PN, the pigs were weighed and randomly separated into 2 groups. Pigs assigned to the PN groups continued to receive PN at the same rate. The remaining pigs were disconnected from PN and were converted to full EN by using bolus feeding at 24 mL/kg every 3 h for a total of 192 mL/kg (d) and a formula (milk replacer; Animix) formulated to meet the energy and nutrient requirements of newborn pigs (Table 1). On the basis of reported compositions, the Intralipid (Baxter Healthcare, Deerfield, IL) added to the PN solution included linoleic (19:2n−6; 44–62%) and linolenic (18:3n−3; 4–11%) acids, whereas the edible lard used as the lipid source for the EN solution had lower amounts of each (4.95% and 0.45%, respectively). The PN and EN did not provide long-chain omega-3 (n−3) FAs (i.e., DHA and EPA). Body weights of the pigs in both groups were recorded daily, and the volumes of PN and EN were adjusted accordingly to account for growth.

Blood samples were collected on the final day of the study without stopping the PN and without nutrition-depriving the EN pigs. The serum was isolated and submitted for a comprehensive metabolic panel that included lipids. The pigs were then killed (Euthasol; Virbac Animal Health, Ft. Worth, TX; 1 mL/kg; i.v.) for collection of the brain and for observations and measurements of wet weight for other organs. The brain was removed intact immediately after death. After total brain mass was recorded, the mass of the cerebellum was recorded separately. The entire brain was then placed in 10% neutral buffered formalin for analysis of gross and microscopic structure. Because of the fixation procedure, the dry mass and composition of the brain were not determined.

### Table 1 Ingredients and macronutrient composition of the parenteral and enteral solutions

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose (70% solution), g/L</td>
<td>116.3</td>
</tr>
<tr>
<td>Travalos (10%), g/L</td>
<td>60.5</td>
</tr>
<tr>
<td>Intralipid (30%), g/L</td>
<td>31.3</td>
</tr>
<tr>
<td>Electrolytes, mL/L</td>
<td>3.3</td>
</tr>
<tr>
<td>Pediatric Intruvite</td>
<td>4.4</td>
</tr>
<tr>
<td>Pediatric MTE-4</td>
<td>20</td>
</tr>
<tr>
<td>Lactose</td>
<td>323</td>
</tr>
<tr>
<td>Milk protein isolate</td>
<td>376</td>
</tr>
<tr>
<td>Dry fat 7/60</td>
<td>241</td>
</tr>
<tr>
<td>Vitamin and mineral mix</td>
<td>22.6</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>17.4</td>
</tr>
<tr>
<td>LEC/STAR487/MO 8i</td>
<td>20</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>116.3 (22.3)</td>
</tr>
<tr>
<td>Enteral</td>
<td>70 (13.4)</td>
</tr>
<tr>
<td>Protein or amino acids</td>
<td>60.5 (11.6)</td>
</tr>
<tr>
<td>Enteral</td>
<td>83 (15.9)</td>
</tr>
<tr>
<td>Lipids</td>
<td>31.3 (6.0)</td>
</tr>
<tr>
<td>Enteral</td>
<td>47.5 (9.1)</td>
</tr>
</tbody>
</table>

1 Multivitamin source (Baxter Healthcare).
2 Multi-trace element additive (Baxter Healthcare).
3 NZMP total milk protein 1220 (Forterra, New Zealand).
4 Spray-dried fat powder (Animix).
5 Reported composition (%) of the vitamin and mineral mix: anhydrous dicalcium phosphate (85.13); ferrous sulfate 20% (2.596); Bioplex zinc 15% (2.462); Sel-Plex 2000 (Alitech) as a source of selenium (2.278), taurine (1.56), dl-tocopherol acetate 50% (1.225), vitamin C (1.02), zinc sulfate (1.008), carnitine 50% (0.6117), Bioplex, Altitech, Nicholasville, KY) copper 10% (0.5606), bismuth 2.0% (0.4575), retinol acetate (0.2857), nicinamide (0.2222), calcium pantothenate (0.1464), Bioplex manganese 15% (0.1213), menadione 33% (0.0618), riboflavin (0.0611), cholecalciferol (0.0671), manganese sulfate (0.0488), vitamin B-12 1% (0.0260), pyridoxine hydrochloride (0.0295), thiamin monohydrate (0.0188), calcium pantothenate (0.0195), and folic acid (0.0037).
6 An emulsifier consisting of a blend of polyoxyethylene glycol (400 mono and dioleate), lecithin, and mineral oil (Animix).
Activity index and behavioral measures. There was not enough detail about activity level in the records of clinical observations for the first litter to accurately assign an activity score for each testing day. After the potential differences were recognized for the first litter, for the second litter caregivers were instructed to include more detail in their clinical observations. The qualitative remarks about activity and responsiveness by the caregivers were evaluated by a reviewer blind to treatment group (PN: \( n = 5 \); EN: \( n = 4 \)) and assigned a score corresponding to the following rating scale: 0 = lethargic, nonresponsive to touch, or unable to awaken; 1 = difficult to wake up but responsive to touch with some movement; 2 = can be awoken but cannot right itself (i.e., cannot sit up, remains on back or side); 3 = awake and can right itself but cannot stand for 2 s without assistance; 4 = can stand 2 s unassisted but cannot locomote 50 cm without assistance; 5 = can locomote 50 cm unassisted but locomotion is labored; and 6 = unassisted active locomotion >50 cm, very alert (often playing, jumping, tail wagging, and/or vocalizing). The highest score obtained within each 24-h period was the score assigned to each pig for each of the 10 d after delivery.

On the basis of the differences in activity and brain structure from MRI for the PN and EN pigs in the first litter, it was decided to perform quantitative (rather than qualitative) behavioral assessments by using representative pigs from the PN \( (n = 3) \) and EN \( (n = 2) \) groups of the second litter before collection of blood and killing. Because of the time necessary to perform the assessments, it was not possible to evaluate all of the pigs from each group during the period of time allotted for the necropsies. Although the limited sample sizes reduced statistical power and precluded statistical analysis, these assessments were considered important for designing future studies. For the Galant Reflex test, the pig was held on the ventral side with legs dangling. One side of the back was stroked with the end of a wooden stick. Proper reflexive movement was rapid lateral flexion of the trunk toward the stimulated side. Thus, each pig received a rating of 0 = no movement, 1 = some movement of trunk, or 2 = movement of trunk in direction of stimulus. For the Rooting Reflex, the pig’s cheek was stroked below the eye with one finger, starting at the posterior opening of the mouth. A proper response involved the pig turning toward the stimulus and making rooting behaviors with the snout/mouth. Each pig received a rating of 0 = no movement, 1 = incomplete or slow movement to stimulus, or 2 = rapid head turn toward finger, sucking response. To evaluate the Swallowing Reflex, a pipettor was used to slowly dispense 2 mL of a very palatable 0.5-mol/L sucrose solution into the mouth. Each pig received a score of 0 = all fluid lost, 1 = successful attempt to swallow some solution but some also lost, or 2 = all fluid swallowed, none lost. Finally, for the Stepping Reflex, each pig was held up behind the shoulders with rear legs hanging down. The pig was then lowered slowly with the rear legs/feet approaching a table top. If the reflex is present, the pig will make a stepping/walking motion as the rear legs make contact with the table top. Each pig received a score of 0 = no leg movement, 1 = leg became hypotonic as feet contacted table top, or 2 = stepping motion as feet contacted table top.

MRI of the brain. The fixed brains were scanned while in the formalin-filled container by using a 3.0 Tesla MRI scanner (Siemens Verio; Siemens AG) with a 32-channel coil. Scanning parameters included a volumetric T1W sequence with a slice thickness of 0.5 mm, a matrix size of 256 × 256 mm, and a 12.8-cm field of view (FOV; 0.5-mm isotropic voxels). Axial and coronal T2-weighted images were performed with a slice thickness of 0.8 mm (FOV, 12.8 cm; matrix, 256 × 256 mm). Axially acquired diffusion tensor imaging (DTI) was performed with 20 directions of encoding (slice thickness, 1 mm; matrix, 128 × 128 mm; FOV, 12.8 cm). Image sets were assigned code numbers and transferred to a research picture archiving computer system using an open-source image archive software.

Images were evaluated by a neuroradiologist (AFC) blinded to the nutrition status of the specimen. Analysis of myelination was performed in brain locations associated with motor pathways (corticospinal and corticobulbar tracts, including the posterior limb of the internal capsule) because of observations of reduced motor development in PN pigs. For each anatomic location, myelination was assessed on a 5-point Likert scale (0 = unmyelinated, 1 = early myelination, 2 = partial myelination, 3 = mostly myelinated, 4 = near complete/fully myelinated) following published reports (22) and was based on the extent of hypointense signal on T2-weighted imaging. Hypointense T2 signal is associated with more mature myelination, related to decreased water content.

Histology of the cerebellum. Because of the delayed development of motor skills seen in the PN pigs, we performed histologic analysis of the cerebellum, which is a major center of motor coordination and other higher brain functions but can be subject to developmental delays in preterm infants (23). After fixation in the 10% formalin for 6 wk at room temperature, the cerebella were dehydrated in ethanol and embedded in paraffin. Paraffin blocks were sagittally serially sectioned at 7 μm and stained with hematoxylin and eosin. Multiple cerebellar sections from 3 EN and 3 PN pigs were examined by using an Olympus SXZ16 microscope, and images were taken with the use of an Olympus DP72 camera.

Statistical analysis. Differences between the 2 groups for body weight, weights of the brain and other organs, and serum biochemistry values were detected by using t tests. The activity index data gleaned from the medical records were analyzed by using a 2 (treatment group) × 10 (day) mixed ANOVA where treatment group (EN vs. PN) was a between-subjects factor and day was a repeated-measures factor. The extent of myelination between the PN and EN groups detected by DTI was compared by using Wilcoxon’s rank-sum test. For all comparisons, \( P < 0.05 \) was accepted as the critical value for significance.

Results

General observations. All 17 pigs survived the 10-d experimental period, gained weight, and physically appeared normal at necropsy. At the start of nutrition support, body weights were comparable for both groups (PN = 1010 ± 67.0 g, EN = 1080 ± 44.0 g; \( P = 0.17 \)). The immediate provision of PN at 8 mL/kg⋅h prevented a growth decrement [extrarenal growth retardation (EUGR)] during the first 24 h after delivery. PN resulted in greater daily weight gains than did EN for days 2 and 3 (Figure 1). Although growth was similar thereafter, the initially greater growth resulted in the PN pigs gaining 70% of birth weight after 10 d relative to the 55% gained by EN pigs (\( P = 0.09 \)), corresponding to a nearly 100 g greater gain in body weights. Despite this, the PN pigs had smaller brains, whether expressed relative to body weight (Table 2) or for absolute weights of the intact brain (31.6 ± 0.37 vs. 35.3 ± 0.58 g; \( P = 0.0002 \)) and for comparisons of the cerebrum (28.0 ± 0.08 vs. 32.4 ± 0.63 g; \( P = 0.003 \)) and cerebellum (2.91 ± 0.08 vs. 3.47 ± 0.10 g; \( P = 0.01 \)), separately.

Differences were also detected between the PN and EN groups for the weights of other internal organs (Table 2); values are normalized to body weight to account for individual and group differences in final body weights. Pigs receiving PN had larger livers, whereas EN resulted in longer small intestines that were heavier for the proximal, mid, and distal regions. Interestingly, the EN pigs did not have a heavier stomach. The 10 d of nutrition support, body weights were comparable for both groups (PN = 1010 ± 67.0 g, EN = 1080 ± 44.0 g; \( P = 0.17 \)). The immediate provision of PN at 8 mL/kg⋅h prevented a growth decrement [extrarenal growth retardation (EUGR)] during the first 24 h after delivery. PN resulted in greater daily weight gains than did EN for days 2 and 3 (Figure 1). Although growth was similar thereafter, the initially greater growth resulted in the PN pigs gaining 70% of birth weight after 10 d relative to the 55% gained by EN pigs (\( P = 0.09 \)), corresponding to a nearly 100 g greater gain in body weights. Despite this, the PN pigs had smaller brains, whether expressed relative to body weight (Table 2) or for absolute weights of the intact brain (31.6 ± 0.37 vs. 35.3 ± 0.58 g; \( P = 0.0002 \)) and for comparisons of the cerebrum (28.0 ± 0.08 vs. 32.4 ± 0.63 g; \( P = 0.003 \)) and cerebellum (2.91 ± 0.08 vs. 3.47 ± 0.10 g; \( P = 0.01 \)), separately.

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Blood biochemistries. Electrolyte concentrations (sodium, potassium, chloride, phosphorus, calcium) were in the normal range and did not differ between the PN and EN pigs. Total protein concentrations were similarly low in both groups (3.0 ± 0.2 and 2.9 ± 0.2 g/dL for PN and EN, respectively), with both groups having low and comparable values for albumin (1.5 ± 0.14 and 1.4 ± 0.03 g/dL) and globulin (1.5 ± 0.09 and 1.6 ± 0.19 g/dL).
Kidneys, g/kg 9.6
Spleen, g/kg 3.32
Pancreas, g/kg 2.36
Heart, g/kg 10.6
Small intestine, cm/kg
Liver, g/kg 36.7
Cerebellum, g/kg 1.68
Cerebrum, g/kg 17.3

7.3 mg/dL; P

Total brain, g/kg 18.9

EN, enteral nutrition; PN, parenteral nutrition.

*Different from EN at that time, P < 0.05.

FIGURE 1 Cumulative body weight gains of preterm pigs provided PN (n = 9) or formula (EN; n = 8) for 10 d. Values are means ± SEs.

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Bilirubin was low in both groups (0.40 ± 0.25 vs. 0.11 ± 0.04 mg/dL; P = 0.11). Alkaline phosphatase was higher for EN pigs (1110 ± 250 vs. 45 ± 49 IU/L; P = 0.04), whereas the groups did not differ for alanine aminotransferase (10.3 ± 0.3 and 13.7 ± 2.7 IU/L; P = 0.14) and aspartate aminotransferase (12 ± 3.5 vs. 13 ± 2.5 IU/L; P = 0.41). EN pigs had higher creatinine values (0.48 ± 0.03 vs. 0.30 ± 0.02 mg/dL; P = 0.006), whereas PN pigs had higher blood urea nitrogen (23 ± 4.5 vs. 7.3 ± 3.3 mg/dL; P = 0.04). Blood glucose values were in the normal range and were similar for both groups. Serum lipids differed between groups, with PN pigs having lower TGs (17 ± 5.9 vs. 27 ± 3.1 mg/dL; P = 0.05), total cholesterol (31 ± 9.6 vs. 85 ± 8.1 mg/dL; P = 0.04), HDL cholesterol (16 ± 4.6 vs. 57 ± 7.3 mg/dL; P = 0.03), VLDL cholesterol (3.7 ± 1.2 vs. 5.7 ± 0.7 mg/dL; P = 0.04), with slightly lower LDL (10.7 ± 4.4 vs. 22.7 ± 2.9 mg/dL; P = 0.09).

Behavioral measures and activity index. Analyses of the activity index data revealed a significant treatment × day interaction [F(9,63) = 2.102, P = 0.042]. There was also a main effect of nutrition source on activity [F(1,7) = 5.62, P = 0.05].

TABLE 2 Birth and final body weights, relative organ weights, and small intestine length of preterm pigs

<table>
<thead>
<tr>
<th></th>
<th>PN</th>
<th>EN</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight, g</td>
<td>1020 ± 40.0</td>
<td>1080 ± 44.0</td>
<td>0.32</td>
</tr>
<tr>
<td>Final body weight, g</td>
<td>1670 ± 80.0</td>
<td>1540 ± 85.0</td>
<td>0.44</td>
</tr>
<tr>
<td>Total brain, g/kg</td>
<td>18.9 ± 0.83</td>
<td>23.8 ± 1.36</td>
<td>0.01</td>
</tr>
<tr>
<td>Cerebrum, g/kg</td>
<td>17.3 ± 0.77</td>
<td>21.5 ± 0.130</td>
<td>0.008</td>
</tr>
<tr>
<td>Cerebellum, g/kg</td>
<td>1.68 ± 0.10</td>
<td>2.04 ± 0.09</td>
<td>0.10</td>
</tr>
<tr>
<td>Liver, g/kg</td>
<td>36.7 ± 1.6</td>
<td>27.9 ± 2.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Small intestine, cm/kg</td>
<td>131 ± 9.9</td>
<td>169 ± 7.5</td>
<td>0.05</td>
</tr>
<tr>
<td>Length</td>
<td>24.4 ± 1.8</td>
<td>40.5 ± 1.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Weight</td>
<td>10.6 ± 0.6</td>
<td>7.9 ± 0.5</td>
<td>0.05</td>
</tr>
<tr>
<td>Heart, g/kg</td>
<td>16.1 ± 1.0</td>
<td>17.7 ± 0.8</td>
<td>0.24</td>
</tr>
<tr>
<td>Lungs, g/kg</td>
<td>2.36 ± 0.12</td>
<td>3.73 ± 0.05</td>
<td>0.001</td>
</tr>
<tr>
<td>Pancreas, g/kg</td>
<td>3.32 ± 0.30</td>
<td>3.08 ± 0.21</td>
<td>0.002</td>
</tr>
<tr>
<td>Spleen, g/kg</td>
<td>9.6 ± 0.5</td>
<td>7.6 ± 0.2</td>
<td>0.03</td>
</tr>
</tbody>
</table>

1 Values are means ± SEs. Pigs provided PN (n = 9) or formula (EN; n = 8) for 10 d. EN, enteral nutrition; PN, parenteral nutrition.

Advances in nutrition support have improved postnatal survival and growth of preterm infants born at earlier stages of development, and the goals have shifted more to improving long-term health outcomes after preterm birth. Of particular importance is the relation between neurodevelopment and nutrition. For example, essential FAs have been established as being critical for brain development (24) and n–3 FAs are now added to infant formula (25). The differences between the PN and EN pigs highlight how the role of nutrition in neurodevelopment after preterm birth remains an issue of concern and an area in need of improvements.

The differences between PN and EN pigs in brain growth, anatomic maturation, and the functional outcomes existed without confounding variables such as EUGR, disease, inconsistent gestational ages, diverse genetics, and different nutritional support and NICU protocols (e.g., antibiotics, ventilation support). These findings reveal a heretofore unrecognized need for concern about PN. The risks are probably higher for smaller preterm infants who are reliant on PN for longer periods (12).

Growth and health status. Most newborn preterm infants suffer malnutrition and experience weight loss until full PN or EN is achieved (11,26). This period of EUGR is a risk factor for compromised neurodevelopment (27) and is a confounding variable for studies in preterm infants (28). The aggressive introduction of full PN to all of the pigs avoided EUGR and resulted in positive growth during the first 24 h after birth, which is critical for neurodevelopment (29). Therefore, EUGR did not contribute to the compromised neurodevelopment of the PN pigs. It is unknown why the pigs converted to EN realized a transient growth decrement before growth recovered to match the rate achieved with PN. It remains to be determined if infants reliant on PN who grow well also suffer from compromised neurodevelopment.

All of the pigs remained healthy, and at necropsy blood chemistries were within normal ranges, although concentrations of blood urea nitrogen, serum lipids, and glucose were elevated in PN pigs. There was no obvious evidence of sepsis or other
PN-associated complications. Although the livers of the PN pigs were larger, overt PNALD was not present, as we previously observed (RK Buddington, unpublished data, 2012). If disease was a contributing factor, the sudden transition from PN to EN on day 2 would be considered by many to be a risk factor for NEC and other adverse gastrointestinal responses that could compromise neurodevelopment. Yet, neurodevelopment was more advanced for the EN pigs.

Brain weight at different gestational ages is relatively independent of fetal body weight, except for extremely-small-for-gestational age pigs, whereas other organs grow in proportion with body weight (16; RK Buddington, unpublished data, 2012). Similarly, MRI-based volume measurements of normal and intrauterine growth-restricted human fetuses revealed organ weights grow in proportion with body weight, with the exception of the brain (30). Collectively, these findings imply that brain growth is “hard wired” independent of body weight gain. Yet, the PN pigs had smaller brains, a less mature myelination pattern, and neurodevelopmental delays. This indicates that the PN disrupted the normal neurodevelopment process and emphasizes an immediate need to improve PN support regimens used for preterm infants to reduce the incidence and severity of compromised neurodevelopment.

The amounts per kilogram of carbohydrate, protein, and fat provided to the EN pigs were 61%, 137%, and 152% of the amounts received by the PN pigs, respectively. Similarly, commercial formulas for preterm infants provide less carbohydrate (75–80%), more lipid (~180%), and comparable amounts of amino acids than PN. For as yet unknown reasons, PN was adequate for general weight gain but not for neurodevelopment. A limitation of the present study is that it was not determined if the increased growth was related to differences in hydration status or body composition.

The enhanced neurodevelopment of the EN pigs corresponded with higher serum lipids, which can be partly explained by the 1.6-fold higher lipid intake compared with PN [9.1 g/(kg·d) vs. 6.0], but not by a higher intake of essential FAs. The PN provided more linolenic acid and linoleic acid, but neither contributed the long-chain essential PUFAs that are added to infant formulas to enhance neurodevelopment (25). The higher serum lipids and more advanced neurodevelopment of the EN pigs and particularly the >3-fold higher HDL-cholesterol concentrations correspond with the importance of HDL cholesterol for delivery of lipids to the adult brain (31) and during development (32), the positive correlation between HDL concentrations measured in preterm infants and gray matter development (33), and the importance of lipoproteins and associated receptors for neurodevelopment (34). The higher concentrations of lipoproteins measured in the EN pigs may also enhance surfactant synthesis by the immature lungs (35).

Plasma lipoprotein profiles are responsive to the source and amount of lipid, as well as route of delivery (36). Although lipoprotein synthesis and the responses to PN and EN are not well understood for the preterm infant, fetuses develop the capacities to synthesize chylomicrons in anticipation of birth and the need to process milk lipids (37, 38). Hence, the provision of EN may have increased intestinal and hepatic synthesis of lipoproteins and thereby increased delivery of lipids to the developing brain. It remains to be determined if provision of the PN enterally rather than systemically would have elicited improved neurodevelopment.

**Behavioral measures and activity indices.** Abnormal general movements by children who were born very preterm are associated with white matter abnormality (39) and a reduction in the transverse diameter of the cerebellum (6). In addition, the quality of general movements in the months after preterm birth is predictive of later motor deficits (5, 40–43). The reduced activity and responses of the PN pigs were similarly related to differences in cortical volume and myelination, and reduced cerebellar size compared with the EN pigs. Importantly, the differences between PN and EN were dramatic and developed rapidly.

The PN pigs took longer to accomplish major motor milestones (e.g., righting, standing, locomoting), with some never achieving the later milestones by the end of the 10-d study. The reflexes examined in this study (e.g., Galant, rooting, swallowing, stepping) are also important for survival. Many of these reflexes were not yet completely developed in some PN pigs, whereas siblings that received EN had fully developed these reflexes by the end of the study. Because these motor abilities and reflexes are critical for survival, the deficits detected in the PN pigs hint that prolonged reliance on PN could contribute to the classification of a child as “failure to thrive.”
Collectively, this study suggests that prolonged reliance on PN is a risk factor for delayed motor function. Furthermore, the results indicate that nutrition regimens that result in higher body weight gain do not necessarily improve behavioral health outcomes. Maintaining preterm infants exclusively on PN to increase body weight, to avoid risk associated with EN (e.g., NEC), or because of necessity may actually be detrimental to motor and behavioral development.

**MRI of the brain.** The increasing availability and use of advanced imaging technologies have revealed altered global and regional patterns of neurodevelopment among preterm infants (44). The imaging results are consistent with the 40–50% incidence of abnormal neurodevelopment among preterm infants (1, 45, 46) and have been instrumental in early diagnosis of compromised neurodevelopment. The delayed myelination of motor nerve tracts among the PN pigs detected by DTI was associated with compromised motor skills. This is similar to findings for preterm infants (47). However, the delayed motor skills for the pigs were evident within 1–2 d of PN, whereas they are not evident among infants for several months.

The studies that reported abnormalities in preterm infants that are detected by advanced MRI techniques attributed or associated the abnormalities with NEC or other complications, the use of drugs (e.g., antenatal and postnatal steroids, caffeine), or various necessary NICU procedures (mechanical ventilation). However, the infants who develop life-threatening diseases or who require intense NICU care and interventions are the infants most likely to be reliant on PN for extended periods: for example, extremely preterm, extremely low-birth-weight infants who require PN for longer periods of time (12). To our knowledge, the potential of PN as a risk factor for abnormal brain development has not been recognized.

**Impact of PN on other organs.** Although PN is essential for very preterm infants, long-term PN increases the risk of sepsis, PNALD (48), and slower bone growth and mineralization (49) and is associated with imbalances of trace minerals (50). The higher blood urea nitrogen values in the PN pigs, despite lower amino acid loads, suggests that enteral delivery of nutrients alters nutrient metabolism and utilization.

Intestinal growth and maturation of preterm pigs are delayed during PN (present study) and the risk of NEC is increased (17). The larger hearts and kidneys of the PN pigs were unexpected findings. Although renal excretory output differs between preterm infants receiving PN and EN (51), there are no reports of PN increasing kidney growth relative to EN. The larger hearts of the PN pigs is a novel finding and unexplained, and the long-term health consequences are as yet unknown. The larger pancreas of the EN pigs is consistent with the increased demands placed on the exocrine pancreas to produce digestive enzymes, and the larger spleens may be in response to the greater antigenic load in the gastrointestinal tract associated with EN.

**The preterm pig as a translational model.** Fetal research using large animal models has been dominated by baboons and lambs (52). The decreasing availability and use of baboons and other nonhuman primates has created a need for an alternative to lambs. Pigs are recognized as a relevant model for pediatric nutrition and metabolism (17, 53), and they have a pattern of brain development more similar to humans than that of sheep (16). Importantly, preterm pigs are compatible with NICU protocols and nutrition support. The compromised neurodevelopment of the PN pigs reproduces the delayed growth and cognitive skills of 40–50% of preterm infants and was detectable structurally and functionally within 10 d and without the confounding variables that complicate clinical studies, which require several years before definitive behavioral and cognitive assessments can be made (54). The preterm pig model will enable efforts to determine the risks of PN, develop and test improved PN solutions, improve nutrition support after preterm birth, and evaluate potential mechanisms underlying the compromised neurodevelopment during PN. This includes altering the gut microbiome (55) and the developing gut-microbiome-brain axis (56).
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References

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