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Delayed growth, motor function and learning in preterm pigs during early postnatal life

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Andersen AD, Sangild PT, Munch SL, van der Beek EM, Renes IB, van Ginneken C, Greisen GO, Thymann T. Delayed growth, motor function and learning in preterm pigs during early postnatal life. *Am J Physiol Regul Integr Comp Physiol* 310: R481–R492, 2016. First published January 13, 2016; doi:10.1152/ajpregu.00349.2015.—Preterm birth interrupts normal fetal growth with consequences for postnatal growth and organ development. In preterm infants, many physiological deficits adapt and disappear with advancing postnatal age, but some may persist into childhood. We hypothesized that preterm birth would induce impaired organ growth and function during the first postnatal week in pigs, while motor abilities and behavioral characteristics would show more persistent developmental delay. Cesarean-delivered preterm ($n = 112$, 90% gestation) or term ($n = 56$, 100% gestation) piglets were reared under identical conditions and euthanized for blood and organ collection on *postnatal days* 0, 5, or 26. Body weight gain remained lower in preterm vs. term pigs up to *day* 26 (25.5 ± 1.5 vs. 31.0 ± 0.5 g·kg⁻¹·day⁻¹, $P < 0.01$) when relative weights were higher for brain and kidneys and reduced for liver and spleen. Neonatal preterm pigs had reduced values for blood pH, PO₂, glucose, lactate, hematocrit, and cortisol, but at *day* 26, most values were normalized, although plasma serotonin and IGF 1 levels remained reduced. Preterm pigs showed delayed neonatal arousal and impaired physical activity, coordination, exploration, and learning, relative to term pigs (all $P < 0.05$). Supplementation of parenteral nutrition during the first 5 days with an enteral milk diet did not affect later outcomes. In preterm pigs, many physiological characteristics of immaturity disappeared by 4 wk, while some neurodevelopmental deficits remained. The preterm pig is a relevant animal model to study early dietary and pharmacological interventions that support postnatal maturation and neurodevelopment in preterm infants.

preterm birth; behavior; neurodevelopment

PRETERM BIRTH (<37 WK GESTATION) occurs for ~10% of all live births, and the number is stable or even slightly increasing (23). Especially when born late preterm and following survival in the immediate postnatal period, most preterm infants appear to grow and develop without major deficits (18). Nevertheless, preterm birth interrupts the normal maturational trajectories of organs, like lungs, gut, and brain, potentially leading to increased risks of both short- and long-term complications. Even infants born late in gestation (i.e., 32–36 wk) show higher incidences of complications, such as hypoglycemia, respiratory distress, anemia, and temperature instability (33). Gastrointestinal disorders, such as necrotizing enterocolitis (27), infections

and sepsis, retinopathy of prematurity, and periventricular leucomalacia are also common in preterm infants (44). Technological and clinical advances have increased survival of the most immature neonates (i.e., extremely preterm infants, 24–28 wk gestation), but medium- and long-term deficits after preterm birth are frequently observed (9, 44).

In clinical neonatology, a key aim is for the preterm infant to achieve a satisfactory functional development within the first weeks and months after birth, including neurodevelopment (14). The risk of adverse neurodevelopmental outcomes is inversely related to gestational age (3). Impaired neurodevelopment is associated with increased neonatal morbidity and mortality (32), and even relatively late preterm infants (i.e., > 32 wk gestation) may show psychomotor delay and behavioral, attention, and learning deficits (28, 36, 41, 52). In extremely preterm infants, the neurodevelopmental impairment involves multiple neurological domains (42) and predicts lasting handicaps (10). Epidemiological studies indicate that such developmental deficits, including cerebral cortical dysmaturational, are inversely related to postnatal growth rates (2, 15, 50). Postnatal growth deficits are common among preterm infants (7), and at term-corrected age, preterm infants may show increased body fat proportion, relative to term infants, indicating nutritional and metabolic imbalances (29). These effects may relate to low levels of IGF-1 following preterm birth (24, 25, 53) that have also been associated with a smaller brain volume at term-corrected age (25) and a higher risk of suboptimal neurodevelopment at 2 yr old (26). Until now, a clinically relevant animal model of preterm birth that allows detailed study of organ development and physiology beyond the immediate postnatal period has not been available.

The newborn preterm pig born at 90% gestation has recently been used extensively as a model in neonatal gastroenterology and nutrition (45), and the preterm pig may reflect the deficits in neonatal metabolism and gut function common for moderately preterm infants (45, 46). On the other hand, neurodevelopment may be relatively mature at birth, even in preterm pigs, due to their precocial development. Nevertheless, the pig brain shares many similarities with the human brain in its gross anatomy (34), and the relatively large size at birth allows investigations using similar clinical tools in pigs and infants (8). Importantly, the perinatal brain growth velocity peak is similar (13), suggesting that the brain may be vulnerable to preterm birth also in pigs. Brain function and cognition have been studied in normal-term piglets (12, 17), and in growth-restricted piglets (22, 43), but no studies have compared preterm and term pigs to help describe the postnatal development of brain structure and function, and to investigate whether

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neurodevelopment is persistently delayed following preterm birth.

In pigs, a reduction in gestation length by just 10% results in severe signs of prematurity because many organs, including the lungs, gut, and liver develop rapidly in the last 2–3 wk before normal birth. This likely also affects neurodevelopmental outcomes. Increased mortality and aberrant behavior were recently associated with low levels of serotonin at birth in pigs (11). Serotonin, synthesized from tryptophan mainly in the gut, is an important neurotransmitter that affects the developing brain (4, 39) but also functions outside the central nervous system (CNS), e.g., nutrient metabolism and gut function (16, 51).

We hypothesized that preterm birth would impair the clinical and physiological characteristics in pigs, especially during the first week when the most acute postnatal adaptations occur. Secondly, we hypothesized that body growth and neurodevelopment would be impaired much longer, beyond term equivalent age, as the combined result of immature organs at birth and the postnatal clinical complications associated with prematurity. To test these hypotheses, we compared preterm and term pigs reared, treated, and nourished identically over the first 4 wk of life following our standard care procedures for preterm pigs. During the first 5 days, the pigs were nourished with or without small amounts of enteral milk to stimulate gut function (48), before all pigs were transitioned to the same enteral milk diet that was fed until *day 26*. Before this age, physiological variables, as well as neuromuscular control, physical activity, coordination, exploratory interest, and learning ability, were investigated. We aimed to understand the physiological responses to preterm birth beyond the first week of life and whether the clinical variables and development of brain-related functions make the preterm pig a suitable model in studies of longer-term dietary or pharmacological interventions.

METHODS

Animal procedures, diet, and housing. One-hundred and sixty-eight pigs (Danish Landrace $\times$ Large White $\times$ Duroc) from eight sows were delivered by cesarean section at 90% gestation (106 days, $n = 112$, preterm) or 100% gestation (118 days, $n = 56$, term), as described in detail (45), where anesthesia and analgesia were induced and maintained with zolazepam and tiletamin (Zoletil 50, Virbac, Kolding, Denmark; xylazine: Narcoxyl 20 mg/ml, MSD Animal Health, Ballerup, Denmark); and ketamine (ketaminol 100 mg/ml, MSD Animal Health) and butorphanol (Torbugesic 10 mg/ml; ScanVet, Fredensborg, Denmark). All pigs were immediately transferred for individual rearing in heated and oxygenated incubators (2 l/min for the first ~ 20 h) to stabilize respiration and body temperature. Within 3 h of delivery, while still anesthetized from the cesarean section, the piglets were fitted with a vascular catheter (infant feeding tube 4F; Portex, Kent, UK) inserted into the transected umbilical artery and an orogastric feeding tube (6F, Portex). Both were secured to the skin with sutures, as described elsewhere together with more details for clinical care (45). The piglets were initially stratified according to birth weight and sex and then randomly allocated within each stratum to receive either total parenteral nutrition (TPN; $n = 65$), or parenteral nutrition combined with bovine colostrum administered as minimal enteral nutrition (MEN; $n = 77$). For the TPN group, parenteral nutrition (modified combination of Kabiven, Vitalipid, Soluvit and Vamin, Fresenius Kabi, Bad Homburg, Germany) (45) was given at $96 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ on *day 1*, gradually increasing to $144 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ on *day 5*. For the MEN group, enteral nutrition with

bovine colostrum (Biofiber Damino, Vejle, Denmark) started at $16 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ on *day 1*, increasing to $64 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ on *day 5*, and this was accompanied by a reduction in parenteral nutrition, such that the two dietary regimens both provided similar fluid volumes and were isoenergetic (increasing from 74 to $110 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ over the first 5 days). These two dietary regimens were chosen because of their relevance for common feeding practices for preterm infants. In this setting, parenteral nutrition was infused continuously (45), and enteral feed was provided every 3 h from *day 1* to *day 8* and from *day 23* to *day 26*, whereas from *day 9* to *day 22*, feeding frequency increased during the daytime to a total of 10 feedings per day. On *day 5*, following the critical neonatal period, a subset of randomly selected piglets from each feeding group was euthanized, and tissues were collected. For the remaining pigs, the parenteral nutrition was discontinued, and all piglets were given increasing amounts of raw bovine milk ($64\text{--}150 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$, providing $37\text{--}70 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) from feeding troughs over the next 4 days, and were subsequently transferred to fortified whole milk powder (WMP; $150\text{--}200 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$, Arla Foods, Viby J, Denmark) until *day 26*. One liter of fortified WMP was prepared by reconstituting into tap water 170 g WMP, 25 g whey protein (DI9224), and 30 g lactose (Variolac), both provided by Arla Foods, which provided piglets with $156\text{--}208 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$. All pigs had ad libitum access to water from *days 5–26*.

After catheterization, iron dextran (1 ml, 200 mg Uniferon, Pharmacosmos, Holbaek, Denmark) was injected subcutaneously into the inguinal region, and passive immunization was provided as three boluses of maternal sow plasma coinfused with the parenteral nutrition at 5 h (4 ml/kg), 13 h (5 ml/kg), and 21 h (7 ml/kg) after the cesarean section. All experimental procedures were approved by the National Ethics Committee on Animal Experimentation (protocol no. 2012-15-2934-00193).

During the first 5 days, the piglets were reared individually in incubators with controlled ventilation and heating. From *days 5–12*, they were transitioned to larger home boxes to allow more free movement. The incubators and boxes were equipped with foam pads and diapers that were frequently changed to maximize comfort and hygiene. From *day 12* to *26*, they were further transitioned to larger home cages equipped with a local heat lamp. Cloths, balls, and dog toys were provided for environmental enrichment. Personnel involved in behavioral assessments handled all piglets in the same manner and did not take part in procedures that might expose the piglets to a degree of stress or fear (e.g., blood sampling).

Growth and clinical recordings. Each pig was weighed daily, and nutritional intake was adjusted accordingly. To prevent diarrhea caused by coccidia, a common porcine parasite, piglets received prophylactic toltrazuril by mouth on *day 5* (20 mg/kg; Baycox, Bayer Animal Health, Leverkusen, Germany), and amoxicillin trihydrate (Paracillin Vet 70%, MSD, Animal Health, Ballerup, Denmark) was administered prophylactically in the feed (20 mg/l) on *days 5–15*, to prevent gut bacterial overgrowth, infection, and sepsis. In individual cases of diarrhea, pigs were supplied with an electrolyte mixture (Revolyt, GK Pharma, Kjøge, Denmark), and severe diarrhea with suspected infectious etiology was treated with gentamicin (5 mg/piglet orally, gentamycin, ScanVet Animal Health, Fredensborg, Denmark) and enrofloxacin (5 mg/kg im; Baytril, Bayer Animal Health) for three consecutive days. Finally, diarrhea and dehydration were treated with loperamid hydrochloride plus simeticon (0.025 mg/kg orally; Imodium Plus, McNeil, Birkerød, Denmark), and zinc oxide (1,000 ppm) and active charcoal (1.5 mg/kg; Norit, Medic Team, Allerød, Denmark) were added to the diet for all pigs on *day 12* and *day 15*, respectively. To alleviate pain for a few preterm pigs that experienced ileus, meloxicam was administered (0.4 mg/kg im; Metacam, Boehringer-Ingelheim, Copenhagen, Denmark) on *day 16* ($n = 1$), *day 23* ($n = 1$), and *day 24* ($n = 1$). Further, meloxicam was administered on *day 15* and *19* due to tail lesions in two piglets. Also, metoclopramide (0.5 mg/kg im; Primperan, Sanofi-Aventis, Hør-

holm, Denmark) was used to support stomach emptying in preterm pigs on *day 22* ($n = 1$) and *day 23* ($n = 2$) in individual cases of suspected ileus.

Each piglet was clinically assessed at 9 AM and 6 PM every day throughout the study period and assigned a clinical score between 1 (best) and 4 (worst). Assessment criteria included respiratory distress, cyanosis, cold extremities, lethargy, reduced activity, diarrhea, abdominal distension, vomiting, and skin changes. A specific hydration score was assessed by grasping a skin fold behind the ear and measuring the delay in seconds (1: no delay, 2: 2–5 s, and 3: ≥ 6 s) before the skin fold would return to its normal position. A fecal score was given every day at 9 AM and 6 PM, according to the following criteria: 0, no stools; 1, meconium or firm feces; 2, pasty feces; 3, droplets of watery feces/diarrhea; 4, moderate diarrhea, and 5, severe diarrhea.

Blood samples were collected on the day of cesarean section and on the day of tissue collection (*day 0*, 5, or 26), and immediately analyzed for pH, PCO_2 , PO_2 , Na^+ , HCO_3^- , K^+ , Ca^{2+} , glucose, lactate, and hematocrit (GEM premier 3000, Instrumentation Laboratory, Bedford, MA). A plasma sample from the same time points was further analyzed for concentration of cortisol (cortisol parameter assay, R&D Systems, Minneapolis, MN), serotonin (total and platelet-poor plasma fraction of 5-HT) RE59121, IBL International, Hamburg, Germany] and total tryptophan (HPLC analysis). Finally, also IGF-1 was analyzed in heparinized plasma by automated assays on an Immulite 2000 (Siemens Healthcare Diagnostics, Ballerup, Denmark).

Body composition. All pigs reared until *day 26* were subjected to whole body dual-energy X-ray absorptiometry (DEXA, Lunar Prodigy scanner, GE Healthcare, Little Chalfont, UK). Prior to the scan, piglets were anesthetized with an intramuscular injection of zoletil mixture (Zoletil 50, 125 mg tiletamine and 125 mg zolazepam; 6.25 ml xylazine, 20 mg/ml; 1.25 ml ketamine, 100 mg/ml; 2.5 ml butorphanol, 10 mg/ml; 1 ml/10 kg body wt). After DEXA scanning in ventral recumbency, each pig was euthanized with an intracardiac injection of pentobarbital sodium and tissues were collected.

Acquisition of basic motor skills after birth and home cage activity. Timing of acquisition of neuromuscular control after birth (eyelid

opening, first stand, and first walk) was closely observed and recorded. Home cage activity was recorded by an infrared video surveillance camera placed over each incubator and connected to an HD recorder with a built-in motion detection software. The digital output for each camera allowed recording of the status of individual piglets as active or resting. With the PIGLWin application (Ellegaard Systems, Faaborg, Denmark), activity counts (number of shifts between being active and resting), and the proportion of active time in 1-h bins, were automatically registered. To avoid having minor activities, such as leg twitches counting as active time, a filter of 10 s was applied post hoc when retrieving the data, as the lower threshold of a positive activity count. The surveillance system was actively recording activity throughout the day and night until *day 12*. Individual cameras were turned off when pigs were handled by caretakers. Home cage activity on the day of the cesarean section was excluded from the analyses, and data are analyzed in 3-h bins.

Balance, coordination, and exploration. For all behavioral assessments, individual piglets were carried by the caretakers from the home pen to an adjacent room arranged with gray curtains covering the walls to avoid unintended shift of focus during any of the behavioral tasks. Two separate platforms were used: 1) an open field arena to elucidate selected behavioral and functional domains (Fig. 1, A–C), and 2) a test cage to evaluate learning abilities (Fig. 1D). All equipment was sprayed with ethanol, and, if necessary, cleaned with water and dried between testing two individual pigs. The open field arena (1.20 $\times$ 1.20 m) had wooden black walls and black rubber flooring and was used to assess coordination and open field behavior (*days 4*, 9, 16, and 23, Fig. 1) and novel object recognition (*days 16* and 24) with simultaneous recording by video cameras mounted from the ceiling (bird's eye view to assess distance traveled and pattern) and from the side of the arena (side view for coordination assessments). Piglets were recorded in the open field arena for 3 min on *days 4*, 9, 16, and 23, except the few preterm pigs ($n = 8$) that were still clinically compromised or incapable of getting on their feet by *day 4*. The duration of the recordings were chosen to minimize stress and fatigue for the piglets. From each open field recording, "balance/coordination," "locomotion," and "exploration" observations were

Fig. 1. Flow diagram of study and functional tests showing the open field arena with zones of interest used for tracking (A), assessment of balance and coordination (B), and novel object exploration (C). The test apparatus for learning assessment show touch panels to the left, and the guillotine door that separates the test area from the start and reward zone to the right (D).

scored. To evaluate development in balance and coordination skills, a scoring scale was developed on the basis of observations by four experimenters after thorough review and analyses of a large number of randomly selected videos from age- and gestational age-matched piglets. This rating scale appropriately covers the piglet repertoire in this experimental setting and describes both the variation and improvements in balance and coordination with advancing age. Score characteristics were 0 (piglet was incapable of lifting the anterior or posterior part of the body from the floor), 1 (piglet was able to lift the anterior or posterior part of the body ≥ 3 s but unable to stand), 2 (piglet was able to stand ≥ 3 s and walked but with poor balance, e.g., sideways or backwards), 3 (piglet was able to stand and walk, was dyscoordinated, but had no difficulty in keeping balance while moving around, except for a few failures to keep balance), 4 (piglet gait was primarily well coordinated, with very few failures in balance), and 5 (piglet displayed a smoothly coordinated and effortless gait, without any failures to keep the balance). Color marker tracking analyses of the open field video recordings was done using a commercially available software (EthoVision XT10, Noldus Information Technology, Wageningen, The Netherlands), providing information on distance traveled (locomotion), movement pattern within the arena (general exploratory behavior), and duration of stays in border and center zones, respectively. In a subset of piglets ($n = 9$ preterm and $n = 10$ term), the video recordings were further used for assessing visual function on *days 4, 9, and 16*. In brief, the number of times a piglet collided with the arena wall, indicating poor visual function, was quantified by an observer. The number of unintended contacts, relative to the distance traveled in the arena during a 2-min period, was used as a surrogate marker of visual function. Unintended contacts with the arena wall that were judged to be due to poor balance were not included.

A novel object recognition (NOR) test was applied on *days 16 and 24* to assess both short-term memory and specific exploratory behavior (21), using the same test area as for the open field evaluations (Fig. 1C). The test consisted of a 3-min sample and test phase, separated by a 4-min intertrial interval (ITI), in which the piglets were returned to their home cages. The test relied on the intrinsic curiosity toward novel objects. To avoid the risk of an a priori object preference that would interfere with the test regime, the objects chosen were based on previous findings in pigs (31). In brief, the piglet was placed in the arena with two similar objects and allowed time to explore these. After the ITI, the pig was reintroduced to the arena in which one of the two objects had been replaced, and the time spent exploring the novel object, relative to the old object, was quantified (1).

Learning ability (cognition). Piglet learning ability was assessed in a behavioral test apparatus. The test cage was $185 \times 75 \times 50$ cm (L $\times$ W $\times$ H) and was constructed from clear acrylic material and consisted of a start box and a test area separated by a manually operated guillotine door (GD; Fig. 1D). Opposite to the start box, two touch panels with visual cues connected to a computer were mounted in the test area, eliciting a click when poked by the piglets. The sessions were video recorded for subsequent analyses.

Prior to any learning assessments, an association between the click and the reward (their usual milk replacer) was established. During this training period of 3 days, some of the ordinary feedings were replaced with training sessions in the home cages. To ensure focus on the learning task in the test cage and to minimize reactions toward a novel environment, piglets were introduced to the test cage prior to the first day of acquisition testing. On test days, piglets were tested ~ 3 h after a meal. The order of testing was randomly generated to even out possible influence of fasting time. A "white noise" was played constantly to minimize influence of background sounds. The training consisted of a 5-min session in the test cage in which the piglet was allowed to explore until initially a spontaneous touch evoked a click, which was rewarded with 3 ml milk (their usual milk). The milk was offered to the piglet in a trough introduced into the start area by the trainer and with the door shut to prevent access to the test area (Fig.

1D). When the pig finished the reward, the guillotine door was reopened, and the pig was allowed to move back into the test area for a new poke on the touch panel. Assessments of learning performance were quantified as the time taken from opening of the guillotine door to the first poke-reward, and subsequently the number of poke-rewards within a 5-min training session. Training started on *day 17* with a final session on *day 25*.

The cage was initially constructed to test piglets in a visual delayed match to sample (VDMS) task, assessing both learning abilities and working memory. Preliminary observations showed, however, that even the acquisition phase (learning to poke the touch panels for a milk reward) appeared very challenging for preterm piglets. Within the first four acquisition days, some term piglets were convincingly poking the touch panels, and for these, the complexity of the task was increased, so they had to visually discriminate between symbols placed on the touch panels to elicit the click. During the following training sessions, these pigs displayed signs of frustration such as biting walls, vocalization, and running back and forth from the touch panels to the start box, indicating perseveration. Therefore, it was decided to use only the first four acquisition days to compare the preterm ($n = 22$) and term ($n = 22$) piglets that were tested. Only pigs that were clinically well and free from diarrhea on the days of planned training were evaluated in the test cage.

Statistics. Group comparisons on *days 0, 5, and 26* that included values for blood chemistry, glucose, cortisol, serotonin, and tryptophan levels, body and organ weights, and data from DEXA scans were analyzed using unpaired *t*-tests or Mann Whitney *U*-tests, as appropriate. Group differences in the proportion of pigs with IGF-1 levels ≥ 25 ng/ml on *days 5 and 26* were assessed by Fischer's exact test. Growth curves were analyzed by two-way repeated-measures ANOVA (time, gestational age) for piglets euthanized on *day 26*, and differences in weight gain over the duration of the study were investigated by ANCOVA, initially including sex, litter, and birth weight in the models, and by unpaired *t*-tests of the relative weight gain per day. Differences in basic motor skill acquisition, time to learn how to drink from a trough, and home cage activity indices were analyzed using unpaired *t*-test or Mann Whitney *U*-test. Balance and coordination scores were analyzed by two-way repeated-measures ANOVA. Data from open field and novel object exploration tests were analyzed using ANOVAs initially with litter and sex included in the models. Sex did not show significant effect in any of the models. Finally, Fisher's exact test was used to assess sex and gestational age differences among responders and nonresponders in the test cage, and performance of responders was analyzed by two-way repeated-measures ANOVA. Increase in performance from acquisition *days 1 to 4* was further assessed using paired *t*-tests. Values in text and figures are presented as means $\pm$ SE, unless otherwise stated, and all statistical analyses were performed using Stata 12.0 (College Station, TX) and GraphPad Prism 5.01 for Windows (GraphPad Software, San Diego CA) with statistical significance at $P < 0.05$. All figures were prepared using GraphPad Prism.

RESULTS

Clinical observations and blood chemistry values. Term piglets were euthanized for tissue collection either at birth ($n = 11$), *day 5* ($n = 22$), or *day 26* ($n = 22$). One term pig died spontaneously on *day 3*. Likewise, preterm pigs were euthanized for tissue collection at birth ($n = 10$), *day 5* ($n = 37$), or *day 26* ($n = 34$). Twenty-nine preterm piglets from these litters were euthanized or died spontaneously within the first few days from causes related to their organ immaturities, and autopsies typically revealed partly unexpanded lungs.

At birth, preterm pigs had fused eyelids and reduced muscle tone and required additional heating pads and insulating cover cloths to avoid hypothermia during the first 12–24 h. In

Table 1. *Clinical chemistry values at days 0, 5, and 26 in preterm and term pigs*

	0 days		5 days		26 days		P ₀	P ₅	P ₂₆
	Preterm	Term	Preterm	Term	Preterm	Term			
<i>n</i>	87	52	19	23	34	22			
pH	7.32 ± 0.01	7.48 ± 0.02	7.39 ± 0.01	7.45 ± 0.01	7.49 ± 0.01	7.48 ± 0.01	<0.001	0.0035	0.66
PCO ₂ , mmHg	66.7 ± 1.7	47.7 ± 1.2	51.5 ± 1.4	43.0 ± 1.6	39.1 ± 1.1	38.3 ± 0.7	<0.001	<0.001	0.61
PO ₂ , mmHg	43.2 ± 3.3	96.3 ± 12.7	45.2 ± 6.2	73.2 ± 6.0	90.2 ± 4.5	104.3 ± 3.8	<0.001	0.0025	0.03
Na ⁺ , mmol/l	142.4 ± 0.8	140.6 ± 0.6	140.6 ± 0.8	133.3 ± 1.0	135.9 ± 0.6	137.5 ± 0.5	0.10	<0.001	0.06
HCO ₃ ⁻ , mmol/l	33.6 ± 0.5	36.0 ± 0.6	31.4 ± 0.6	29.9 ± 0.8	29.9 ± 1.0	28.5 ± 0.4	0.002	0.13	0.28
K ⁺ , mmol/l	3.3 ± 0.08	3.3 ± 0.09	3.7 ± 0.07	3.7 ± 0.09	3.6 ± 0.05	3.6 ± 0.06	0.62	0.52	0.46
Ca ²⁺ , mmol/l	1.2 ± 0.02	1.2 ± 0.03	1.4 ± 0.02	1.4 ± 0.03	1.4 ± 0.02	1.3 ± 0.02	0.24	0.94	0.02
Glucose, mmol/l	1.6 ± 0.1	2.6 ± 0.2	2.1 ± 0.2	3.4 ± 0.3	5.0 ± 0.3	5.4 ± 0.2	<0.001	0.001	0.33
Lactate, mmol/l	2.1 ± 0.2	2.8 ± 0.3	1.2 ± 0.1	1.7 ± 0.2	1.5 ± 0.1	1.5 ± 0.2	0.028	0.031	0.87
Hematocrit, %	32.4 ± 0.6	33.9 ± 0.7	21.3 ± 0.7	23.9 ± 1.2	17.2 ± 0.3	21.5 ± 0.5	0.13	0.07	<0.001
Cortisol, ng/ml	47.9 ± 6.7	59.2 ± 10.4	101.6 ± 9.2	153.7 ± 18.0	13.3 ± 1.7	15.6 ± 4.1	0.38	0.01	0.58
PPP 5-HT, ng/ml	17.1 ± 5.2	5.1 ± 0.5	15.7 ± 2.3	70.1 ± 13.6	13.1 ± 1.6	29.5 ± 5.1	0.045	0.003	0.005
Tryptophan, µg/ml	2.81 ± 0.34	3.45 ± 0.21	5.54 ± 0.33	4.84 ± 0.48	3.51 ± 0.48	3.70 ± 0.40	0.13	0.24	0.76

Values are expressed as means ± SE. *P* values represent comparisons between preterm and term pigs at each age (0, 5, or 26 days). PPP 5-HT, platelet-poor-plasma serotonin. Cortisol values are based on *day 0* (*n* = 10 and *n* = 11), *day 5* (*n* = 37 and *n* = 21), and *day 26* (*n* = 33 and *n* = 22) in preterm and term pigs, respectively.

contrast to term pigs, many preterm piglets required resuscitation, and apneic piglets were resuscitated with a manual ventilation bag (pressure 15–20 cmH₂O) to secure lung expansion. To further enhance respiration, atipamezol (Antisedan, Orion Pharma Animal Health, Copenhagen, Denmark) and doxapram (Dopram, Boehringer Ingelheim, Copenhagen, Denmark) were given to individual pigs on indication. The initial respiratory challenges in 0–5 day-old preterm pigs were further illustrated by reduced blood pH, PO₂ and HCO₃⁻ values, and increased PCO₂ (Table 1), and higher relative lung weights (more lung fluid accumulation, Table 2), relative to term pigs. These respiratory deficits in preterm pigs were no longer present by *day 26*, although PO₂ remained slightly lower than values in term pigs (Table 1). Except for a relative hypernatremia on *day 5* in preterm pigs, blood electrolyte levels showed minimal differences from values in term piglets at all time points (Table 1). Preterm pigs showed significant neonatal hypoglycemia and reduced lactate levels at 0–5 days, but values were normalized by *day 26*. Hematocrit and cortisol values were high in all pigs during the first week after birth, but values were lower for

cortisol during the first 5 days and for hematocrit values at *day 26* in preterm pigs (Table 1). Tryptophan levels were comparable between preterm and term pigs and peaked at *day 5* (Table 1). In contrast, both total and the platelet-poor plasma fraction of serotonin were reduced in preterm vs. term pigs (except at *day 0*). For preterm pigs, serotonin levels did not change over time, whereas in term piglets, levels rose and peaked at *day 5* (Table 1, Fig. 2). From *day 9*, there were signs of diarrhea in both groups. Between *days 9* and *14*, mean fecal scores tended to be lower in preterm pigs (1.48 ± 0.09 vs. 1.88 ± 0.18, *P* = 0.07), but after *day 14*, scores were higher than in term pigs (1.41 ± 0.06 vs. 0.50 ± 0.07, *P* < 0.01). This difference was partly explained by the fact that 59% of observations from term pigs during this period were from pigs without any defecation (score 0) in contrast to 14% for preterm piglets. Fecal scores ≥ 3 were observed for 17% of preterm pigs compared with 5% of term pigs.

Immediate effects of TPN versus minimal enteral nutrition during the first 5 days. Overall, the relative weight gain from birth to 5 days of age was similar (15.0 ± 1.6 vs. 20.0 ± 2.0

Table 2. *Body and organ weights at day 0, 5, and 26 in preterm and term pigs*

	0 day		5 days		26 days		P ₀	P ₅	P ₂₆
	Preterm	Term	Preterm	Term	Preterm	Term			
<i>n</i>	10	11	37	22	34	22			
Body weight,* g	839 ± 27	1375 ± 40	917 ± 32	1548 ± 52	2039 ± 90	3332 ± 148	<0.001	<0.001	<0.001
SI length, cm/kg	294 ± 20	283 ± 17	342 ± 10	256 ± 11	307 ± 10	216 ± 10	0.70	<0.001	<0.001
SI weight, g/kg	17.2 ± 0.4	24.7 ± 0.9	25.5 ± 0.5	29.2 ± 0.9	53.9 ± 1.2	52.4 ± 2.5	<0.001	<0.001	0.55
Liver, g/kg	25.5 ± 1.2	28.5 ± 1.7	25.5 ± 0.8	23.5 ± 0.9	29.8 ± 0.4	31.6 ± 0.7	0.18	0.10	0.03
Lungs, g/kg	27.2 ± 2.3	17.0 ± 1.0	23.7 ± 0.8	16.5 ± 0.8	14.4 ± 0.3	13.8 ± 0.2	<0.001	<0.001	0.16
Spleen, g/kg	1.7 ± 0.1	1.1 ± 0.05	1.8 ± 0.1	1.7 ± 0.1	3.0 ± 0.2	4.1 ± 0.2	<0.001	0.72	<0.001
Kidneys, g/kg	8.7 ± 0.4	7.9 ± 0.4	8.7 ± 0.2	8.3 ± 0.3	7.8 ± 0.2	6.9 ± 0.2	0.15	0.30	0.003
Adrenal gland, g/kg	0.06 ± 0.01	0.12 ± 0.01	0.10 ± 0.01	0.12 ± 0.02	0.09 ± 0.01		<0.001	0.24	
Brain, g/kg	32.6 ± 2.7	26.2 ± 1.7	35.0 ± 2.1	23.8 ± 1.3	19.1 ± 0.9	13.3 ± 0.6	0.05	<0.001	<0.001
BMC, g/kg					15.3 ± 0.6	14.0 ± 0.2			0.15
Fat, g/kg					16.6 ± 2.8	9.3 ± 1.6			0.06
Muscle, g/kg					966 ± 2	964 ± 3			0.62

Values are expressed as means ± SE. SI, small intestine; BMC, bone mineral content. *P* values represent comparisons between preterm and term pigs at each age (0, 5, or 26 days). *Body weight on *day 0* represents data from *n* = 112 preterm and *n* = 56 term pigs, while on *day 5*, data are represented from *n* = 86 preterm and *n* = 44 term pigs, and weights of adrenal glands on *day 0* are based on *n* = 9 and *n* = 11; *day 5*, *n* = 4 and *n* = 8; *day 26*, *n* = 9 for preterm and term pigs, respectively.

Fig. 2. Plasma levels of total 5-HT in preterm (white bars) and term (gray bars) on days 0, 5, and 26. *** $P < 0.01$.

g/kg for MEN and TPN, $P > 0.05$). As expected, feeding MEN increased intestinal weight, relative to TPN (28.7 ± 0.6 vs. 24.5 ± 0.7 g/kg, $P < 0.001$), while relative liver weight was reduced (23.7 ± 0.8 vs. 26.0 ± 0.8 g/kg, $P < 0.05$). The relative weights of the lungs (21.4 ± 1.0 vs. 20.4 ± 1.0 g/kg), spleen (1.8 ± 0.1 vs. 1.7 ± 0.1 g/kg), kidneys (8.4 ± 0.2 vs. 8.8 ± 0.2 g/kg), and brain (31.6 ± 2.2 vs. 29.3 ± 2.0 g/kg) were similar for MEN and TPN pigs, respectively. MEN feedings were further associated with reduced glucose levels (2.3 ± 0.2 vs. 3.5 ± 0.4 mmol/l) and higher hematocrit levels (24 ± 1 vs. $21 \pm 1\%$), relative to TPN (both $P < 0.01$), whereas no other clinical chemistry values listed in Table 1 were different between MEN and TPN. Basic motor skills acquisition, home cage activity, balance, and coordination scores and behaviors recorded in the open field arena were also similar between the groups. Because no other endpoints measured in this study were significantly affected by diet during the first 5 days (TPN or MEN), we condensed the study results presented in tables and figures to include only the pooled comparison between preterm and term piglets.

Body and organ weights. For the preterm piglets, the mean litter size was 22 (range: 21–27) with 53% males and birth weights ranging from 271 to 1,468 g. For term piglets, the mean litter size was 19 (range: 14–23) with 45% males and birth weights ranging from 665 to 1,991 g. Throughout the experiment, body weight was consistently lower in preterm vs. term pigs (Table 2, Fig. 3A) and intrauterine growth restriction (IUGR; <10th percentile of gestational age) was comparable (11%) in both groups. During the first 24 h, preterm pigs lost more weight than term pigs (73.8 ± 2.3 vs. 17.1 ± 3.9 g/kg, $P < 0.01$), and they did not reach a plateau in body weight gain until day 4, which was later than in term pigs. Relative weight gain was significantly lower in preterm pigs during the first 5 days (Fig. 3B), the age at which they were weaned to full enteral nutrition. At this age, preterm pigs showed a delayed ability to drink from a trough (56 ± 9 vs. 13 ± 3 h, $P < 0.001$). To ensure that transition to full enteral nutrition would not result in digestive complications, the amount of milk offered was transiently reduced during days 5–8. Therefore, both groups lost weight (relative to their body weight) immediately after the complete transition to enteral feeding on day 5, but term pigs lost more than preterm pigs ($P < 0.001$). Around day 10, following some days without weight gain due to restricted food intake, growth rate stabilized in both groups, but remained

lower in preterm pigs, resulting in a different total weight gain over the 26 days, even after adjustment for litter and birth weight differences ($1,017 \pm 74$ vs. $1,923 \pm 98$ g, $P < 0.001$). At the time around birth, both preterm and term pigs had low levels of circulating IGF-1, as shown by the values below the detection limit of 25 ng/ml (Fig. 3C). Samples from all 5-day-old preterm pigs ($n = 37$) remained below this limit, while a significant proportion of term pigs (27%) had values above this threshold ($P < 0.01$). This difference between groups persisted until 26 days of age ($P < 0.01$), when 45% of preterm and 95%

Fig. 3. Growth curves from birth to day 25 (A) and daily weight gain relative to body weight from days 1 to 5 and 6 to 25 (B), and plasma IGF-1 levels at birth, day 5, and day 26 (C), in preterm (white circles) and term (gray circles) pigs, respectively. Data are expressed as means $\pm$ SE (A and B) or absolute values (C). C: dotted horizontal line represents the quantitative detection limit of the assay (25 ng/ml), and values below this are plotted for visual appraisal (range 2–24 ng/ml). Group differences in the number of samples above the line are based on Fischer's exact test. *** $P < 0.01$.

of term pigs had detectable levels of IGF-1. There were no effects of sex or diet during the first 5 days (TPN or ENT) on IGF-1 levels.

Relative to their body weight, 0–5-day-old preterm pigs had longer but lighter small intestines, resulting in a reduced weight per length, relative to term pigs. At day 26, this difference had disappeared (Table 2). At birth, absolute brain weight was ~25% higher in term vs. preterm pigs, indicating a significant brain growth during the last 10% of gestation. Relative to body weight, however, preterm brain weight remained higher at all postnatal ages (Table 2). Relative weights of the spleen and liver were reduced at day 26 in preterm pigs, while kidney weights were increased, relative to body weight (Table 2). Also at day 26, relative fat mass tended to be increased in the preterm pigs ($P = 0.06$), while bone mineral density was comparable between the groups.

Acquisition of basic motor skills after birth and home cage activity. Acquisition of basic neuromuscular control was significantly delayed in preterm pigs (Fig. 4). Within the first 24 h, most term pigs had opened their eyes, were able to stand and walk inside their home cage, and all animals had acquired these basic motor functions within the first 5 days. Preterm pigs showed a significant delay, with only few pigs demonstrating any of these skills during the first day, and more than half of the pigs achieved these developmental milestones only gradually over the following days (Fig. 4). Preterm pigs (vs. term pigs) required significantly longer time before eyelids were open (52 ± 4 vs. 16 ± 1 h), until their first stand (38 ± 3 vs. 9 ± 1 h) and until their first walk (49 ± 3 vs. 14 ± 2 h). In accordance with this, preterm pigs displayed 40% less activity in their incubators during the first days after birth ($P < 0.01$, Fig. 5), despite similar number of activity bouts. After day 5, a home cage activity of around 15% was observed in both groups, but preterm pigs displayed significantly more activity bouts than term pigs ($P < 0.01$, Fig. 5), resulting in shorter duration of the activity bouts in preterm pigs.

Balance, coordination, and exploration. On day 4 the balance and coordination scores were lower for preterm pigs ($P < 0.01$, Fig. 6). Distance traveled and general exploratory behavior in the open field recordings were significantly lower (both $P < 0.01$), compared with term pigs (Fig. 7, A and B). On day 9, preterm pigs spent more time in the center of the arena (Fig. 7C), with reduced exploration ($P = 0.09$, Fig. 7B), but the distance traveled was the same between the groups (Fig. 7A). Although coordination in preterm pigs was inferior relative to term pigs at all times tested ($P < 0.01$), their performance in the open field improved over time ($P < 0.01$, Fig. 6). Locomotion and open field exploration were comparable between groups on day 16, but on day 23, preterm pigs had fewer zone transitions ($P < 0.05$), indicating decreased exploratory interest. Supporting this, the time spent exploring the specific objects in the novel object recognition test was similar between groups on day 16, but reduced for preterm pigs on day 24 ($P < 0.01$, Fig. 8). Short-term memory assessments were not different on day 16 or day 24 (data not shown). On the basis of the number of unintended contacts in the open field arena, visual function improved over time in both groups ($P < 0.001$), and it tended to be best in term pigs ($P = 0.06$).

Learning ability. Within the time available for assessing learning performance (4 days for term and 8 days for preterm), only ~50% of the pigs learned to snout poke in the test cage,

Fig. 4. Hours before a given percentage of preterm ($n = 95$, white triangles) and term ($n = 45$, gray triangles) pigs have first been observed to open their eyes (A), stand on their feet (B), or walk (C).

as judged by a criterion of ≥ 3 pokes in two consecutive training sessions. This proportion did not differ between preterm and term pigs or between sexes. Within the first four acquisition days, however, only 10% of all preterm pigs had responded to the training by reaching the learning criterion, whereas this proportion was 50% for term pigs (Fig. 9A). Among the subset of pigs that eventually reached the learning criterion ($n = 12$ for both preterm and term piglets, Fig. 9, B–D), the initial latency before pigs entered the test zone was high (Fig. 9B), but improved in both groups during further testing ($P < 0.05$). Preterm pigs were slow to increase their

Fig. 5. Mean home cage activity percentage (A) and counts (B) during days 2–5 and 7–12, in preterm ($n = 18–39$, white bars) and term ($n = 22–33$, gray bars) pigs. Data are expressed as means $\pm$ SE. *** $P < 0.01$.

performance with a longer latency period to the first poke ($P < 0.01$, Fig. 9C) and reduced number of total pokes per session ($P < 0.01$, Fig. 9D), relative to term pigs. Both groups showed improved learning over time, as demonstrated by paired con-

Fig. 6. Open field assessment of development of balance and coordination scores in preterm ($n = 34$) and term ($n = 22$) pigs (means $\pm$ SE). ***Significant differences between preterm and term pigs ($P < 0.001$) and an effect of time ($P < 0.001$).

trasts of the number of pokes per session for both preterm and term pigs from acquisition days 1–4 ($P < 0.01$). Among the responders, learning was markedly reduced in preterm pigs, and these needed four trials more than term pigs to reach the same level of performance.

DISCUSSION

Our data demonstrate that impaired physical activity, motor control, and learning are evident in the postnatal period of preterm pigs. The differences in physical activity and some aspects of motor control were transient, whereas the differences in balance and coordination and learning lasted longer—at least beyond term-equivalent age and until 26 days. Besides the apparent delay in neurodevelopment and motor control, preterm pigs displayed persistent reductions in body

Fig. 7. Open field assessment scores of locomotion (A, distance traveled within 3-min sessions), different zone transitions (B, general exploratory interest) and stay in center of the arena (C) in preterm (white, $n = 22–29$) and term (gray, $n = 22–31$) pigs (least significant means $\pm$ SE). # $P = 0.09$, * $P < 0.05$, *** $P < 0.01$.

Fig. 8. Time spent exploring specific objects in the sample phase of the novel object recognition test on day 16 and 24 in preterm (white bars) and term (gray bars) pigs (LS means $\pm$ SE). *** $P < 0.01$.

growth, liver and spleen weights, and in blood serotonin, hematocrit, and IGF-1 levels. This is the first report of an animal model that provides important baseline data for preterm birth and its associated postnatal comorbidities beyond the immediate neonatal period. We show that many of the normal neonatal functional impairments following preterm delivery adapt within the first postnatal days, while others remain present for a longer period.

In pigs, preterm delivery at 90% gestation is associated with neonatal respiratory function defects that may reflect the complications in preterm infants at 70–80% gestation (5). However, the gastrointestinal tract appears even more immature than in such preterm infants, as indicated by its high sensitivity to necrotizing enterocolitis, if fed infant formula without supportive antibiotic treatment (46, 47). Blood gas values obtained shortly after birth documented impaired respiratory function in preterm pigs, and such defects are likely to be the main explanation for the high mortality in the days just after preterm birth. The mortality was higher in this study than normally observed for preterm pigs at 90% gestation [$<15\%$, (45)], despite that we used our standard rearing procedures, and we ascribe this to large litter sizes and a relatively high proportion of growth-restricted piglets (5). Our procedures did not include intensive mechanical ventilation, cardiovascular support or detailed adjustments of blood glucose and electrolytes. Consequently, the results reflect the combined influences of the immature organs and the postnatal complications that result from this immaturity.

Both infants and pigs show impaired glucose homeostasis after preterm birth. We speculate that the low lactate levels at 0–5 days in preterm pigs result from low levels of available glucose. The low glucose levels may relate to the low liver glycogen stores and gluconeogenic enzyme levels in preterm pigs as a consequence of low exposure to fetal cortisol before birth (19). The prepartum cortisol surge also stimulates maturation of many other organ systems, e.g., lungs, gut, and kidneys (20, 46). In our study, relative adrenal gland weight and plasma cortisol sharply increased in the days after preterm birth, but cortisol levels remained lower in preterm vs. term pigs on day 5. This may play a role for the diminished physiological capacity of preterm pigs to adapt to postnatal

Fig. 9. Percentage of all pigs tested reaching a learning criterion of ≥ 3 pokes in two consecutive training sessions during the first four acquisition days (A). After a further four training days more, 50% of preterm pigs had reached this criterion, and these are collectively designated “the responders”. Preterm “responders” (white triangles; $n = 12$) had an increased latency to enter through the GD door (B), $P < 0.01$, an increased latency to first poke (C), $P < 0.001$, and fewer total number of poke-rewards/session (D), $P < 0.001$, relative to term “responders” (gray triangles; $n = 12$) (means $\pm$ SE).

life. Corticosteroids can also alter tryptophan metabolism via the kynurenine pathway and, thereby, modulate circulating serotonin levels (40). Despite similar levels of tryptophan, plasma serotonin levels were persistently reduced in preterm pigs, which may be related to a functional immaturity of the gut enterochromaffin cells. This could influence both glucose and lipid metabolism (16, 51) and, thereby, aggravate the dysmetabolic phenotype. In the brain, serotonin serves as an important neurotransmitter affecting many brain functions (39), including brain developmental processes (4) and early neural network connectivity (37). In rodent models, experimental manipulation of serotonin levels during prenatal and postnatal life affects later behavior (30). In utero, these processes are thought partly to be under the influence of serotonin derived from outside the CNS (4). Although speculative, disruption of proper maturational signaling from outside the CNS in sensitive periods of development (e.g., before the closure of the BBB) could potentially be important also in relation to preterm birth.

Growth was markedly reduced in preterm vs. term pigs, and at term, corrected-age (12 days postnatally) body weight in preterm piglets remained lower than in newborn term pigs ($1,174 \pm 48$ vs. $1,375 \pm 40$ g, $P < 0.01$). Brain weight was increased throughout the postnatal period, reflecting some brain conservation during extrauterine growth retardation in preterm pigs. The trend toward increased adiposity in preterm pigs is consistent with observations in preterm infants (35), but in contrast to infants, both preterm and term pigs are born with very limited fat depots. Since all pigs were reared and nourished under identical conditions (e.g., same fluid and nutrient intakes per kg body wt), the results suggest that prematurity at birth may induce metabolic changes that start to become evident already within the first weeks after preterm birth. Such changes likely contribute to the reduced growth rate and may relate to the very low IGF-1 levels in preterm pigs. Using the same assay, the majority of IGF-1 levels were also below detection limit in preterm infants during the first month of life (24). The slightly reduced relative liver weight, and increased kidney weight at 26 days, may relate to this metabolic dysregulation, together with changes in other endocrine control mechanisms, e.g., reduced sensitivity to insulin or IGF-1, as shown for growth-restricted piglets (38).

Following normal birth, newborn pigs depend on relatively advanced neurological functions for their survival, including both mature neuromuscular control to support physical movements, together with complex brain functions, such as the cognitive abilities that facilitate essential social interactions with their mother and siblings. Preterm pigs displayed several impairments and delays: neonatal arousal, eyelid opening, and time to stand and walk in their home cages were significantly delayed, relative to pigs born at term. This may be explained by neurological immaturity but could also partly be related to limited energy stores, hypoglycemia, and decreased cortisol influence in preterm pigs. Further, it cannot be excluded that a diminished liver function reduced the clearance of maternal anesthetics and, thereby, inhibited neonatal arousal. Nevertheless, home cage activity, balance and coordination, and open field activity during the first few weeks were all clearly delayed in preterm vs. term pigs.

Most of the functional assessments indicated delays, rather than deficits. The delays in function were shorter than the

reduction in gestation length (12 days), and demonstrate a developmental plasticity in pigs born preterm. Basic motor function, home cage activity, locomotion, and balance and coordination scores suggested delays of about 2, 6, 5, and 11 days, respectively. In the cognitive domain, the preterm pigs displayed reduced performance in the poke-reward test up to day 25, and required an additional 4 days to reach a performance similar to that of term pigs. This suggests impaired learning abilities at this age. In clinical follow-up of ex-preterm infants, the degree of prematurity is corrected for by the use of "corrected age" when assessing psychomotor development in the first years of life. It is unknown whether the delays that we observed would wane with age, although our pigs did show reduced exploratory interest toward the end of the experiment. This could represent the emergence of a permanent functional deficit.

A strength of this animal model is that it incorporates the physiological responses of preterm birth and allows longer-term rearing and evaluation of clinically relevant interventions. The size of preterm pigs is similar to that of extremely preterm infants and allows the use of tools, equipment, and guidelines common in clinical neonatology, including brain-relevant procedures, such as EEG (49, 54) and MRI (8). The fast growth rate and precocial nature of pigs allow assessment of organ growth, function, and cognition, already within a few weeks after preterm birth. At the same time, some important environmental factors (delivery mode, medication, nutrition and rearing conditions) can be controlled for. Although the 90% gestation preterm pig shows respiratory immaturity similar to that in human infants born at 70–80% gestation, the brain is arguably more comparable to late preterm infants. This reflects a discrepancy in specific organ development between pigs and humans, which should be considered when interpreting the data. In late preterm infants, severe metabolic and respiratory complications and associated brain defects are relatively rare. In our study, we cannot exclude that hypoglycemia, hypoxia, or other neonatal complications affected the neurodevelopment in the preterm pigs. Future model adjustments could include prenatal treatment with corticosteroids and postnatal surfactant and glucose administration to study development in the absence of hypoxia and hypoglycemia.

Perspectives and Significance

We have shown that it is possible to rear 90% gestation preterm piglets well beyond the immediate neonatal period. Our results show that preterm birth induces growth deficits, neurodevelopmental delay, and defects in several organ systems at least until 1 mo of life. The exact time frame and organ specificity of postnatal organ maturation may differ between 90% gestation preterm pigs and very preterm infants, but there may be enough similarities to make it relevant to test interventions to reduce long-term consequences of preterm birth in infants. In perspective, the study period could be extended beyond the first month of life to study more long-term effects of early-life interventions. Although rearing through the first month has a high demand for skilled personnel and an advanced infrastructure, we judge that longer-term studies would be realistic to do with the current experimental paradigm.

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DISCLOSURES

Eline van der Beek and Ingrid B. Renes are employed at Nutricia Research.

AUTHOR CONTRIBUTIONS

Author contributions: A.D.A., P.T.S., G.O.G., and T.T. conception and design of research; A.D.A., S.L.M., and T.T. performed experiments; A.D.A., S.L.M., and C.V.G. analyzed data; A.D.A., P.T.S., S.L.M., E.M.v.d.B., I.B.R., C.V.G., G.O.G., and T.T. interpreted results of experiments; A.D.A. prepared figures; A.D.A., P.T.S., and T.T. drafted manuscript; A.D.A., P.T.S., E.M.v.d.B., I.B.R., C.V.G., G.O.G., and T.T. edited and revised manuscript; A.D.A., P.T.S., S.L.M., E.M.v.d.B., I.B.R., C.V.G., G.O.G., and T.T. approved final version of manuscript.

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