# Animal models to study neonatal nutrition in humans
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**Purpose of review**
The impact of neonatal nutrition on the health status of the newborn and incidence of disease in later life is a topic of intense interest. Animal models are an invaluable tool to identify mechanisms that mediate the effect of nutrition on neonatal development and metabolic function. This review highlights recently developed animal models that are being used to study neonatal human nutrition.

**Recent findings**
In recent years, mice, rats, and pigs have become the most frequently used animal models to study human neonatal nutrition. Techniques for rearing newborn mice, preterm rats, and preterm pigs have been developed. Neonatal mice have great potential for mechanistic and genomic research in postnatal nutrition and related diseases. The neonatal pig model is valuable to study acute and chronic effects of parenteral and enteral nutrition on whole-body metabolism as well as specific tissues. To date, a wealth of information from studies with neonatal pigs has been applied to humans.

**Summary**
Further development of neonatal animal models related to nutrition is required for the advancement of research in early postnatal nutrition. Improvement of nutritional support during this critical period of development will enhance immediate clinical outcomes and possibly prevent diseases later in life.

**Keywords**
animal, model, neonate, nutrition

## Introduction
Le choix intelligent d'un animal... est souvent la condition essentielle du succès d'une expérience et de la solution d'un problème physiologique très important.

(The success of the enterprise clearly depends upon selecting a suitable animal for the investigation)
(Claude Bernard, 1865)

The study of nutritional influences on neonatal growth and functional development is important because of the critical stage of development and potentially long-lasting impact of intervention. Factors that limit the clinical investigation of human neonates, apart from the obvious ethical constraints, include small sample size, methodological difficulties and genetic heterogeneity, as well as differences in disease and (intensive care) treatment. Therefore, animal models are an invaluable tool to study physiological, metabolic, and cellular events related to early nutrition in both neonatal and later life under highly controlled circumstances. Studies of neonatal animals have led to the improvement of nutritional intervention and advanced care of the human neonate. However, limitation of in-vivo animal experimentation lies in the observation that embryology, physiology, and anatomy in animals do not completely resemble that in humans and that frank disease is not easily reproducible. Hence, the purpose of this review is to describe neonatal animal models suitable for studying early postnatal nutrition and to provide guidance for investigators who are new to this field of research.

## Neonatal animal models
Animal models used for human neonatal nutrition include mice, rats, rabbits, guinea pigs, dogs, pigs, and nonhuman primates. Traditionally, studies in this field have been dominated by rodent species like rat and mouse and to a lesser extent rabbit, guinea pig, pig, and baboon. The latter species, that is, nonhuman primates are a good model to study postnatal nutrition in humans because of their close homology in several organ systems to humans. They have been used to study short-term and long-term effects of prenatal and postnatal

(parenteral) nutrition in term as well as preterm neonates, but expensive housing, lifespan, and ethical considerations limit their use [1–8]. In recent years, techniques to artificially rear rodents and methods to investigate the effects of neonatal pig nutrition in health and disease have evolved. At present, rodents and pigs are widely used to study human neonatal nutrition and therefore will be the focus of this review.

## Neonatal rodent models

In the current state of neonatal intensive care, with increasing numbers of premature babies and their rates of survival, neonatal nutrition has to be tailored to specific newborn populations differentiated by their gestational age and health status. To prevent nutritional deficits in this critical developmental period and because neonatal nutrition may determine health in later life, it is important to have appropriate animal models to study the underlying mechanisms involved. Although rodents differ from humans in many ways including developmental, anatomical, and physiological characteristics, they are highly related in terms of similarity of genes and biochemical pathways. Rodent models have the advantage of a relatively uniform genetic background on which environmental effects during gestation or early postnatal life into adulthood can be studied. Low cost and advanced breeding programs in rodents, and especially mice, allow studies that enable the investigation of

mechanistic pathways in genetically modified animals. The length of gestation for rodents, like rat and mouse, is short (19–22 days) and the pups are born very immature with respect to the stage of gut and brain development. In newborn pups, the gut matures gradually during lactation (day 0–21) and a particularly rapid maturation takes place during the short period of transition from milk to solid food (weaning) [9••] (Fig. 1). This maturation includes anatomical, functional, and immunological aspects of the gut. Considering this developmental pattern, rodents represent animal species that are quite different from human infants whose intestinal tract is more mature at birth [9••] (Fig. 1). Therefore, the immature rodent gut resembles more that of a human infant born prematurely. Furthermore, the rapid development of the intestinal tract in rodents has the advantage that nutritional effects can be studied within a relatively short time.

The goal in the clinical management of premature and low-birth-weight infants is to achieve growth at a rate that approximates intra-uterine growth; however, cumulative deficits in energy and protein intakes are still a concern in many neonatal intensive care settings. Limited early nutrition as a result of delayed adequate nutritional support and the inability to meet the high metabolic demand leads to extra-uterine growth restriction. This may have devastating short-term consequences such as increased susceptibility to infection and lack of organ

**Figure 1 Timing of gut maturation in three different groups of mammalian species**
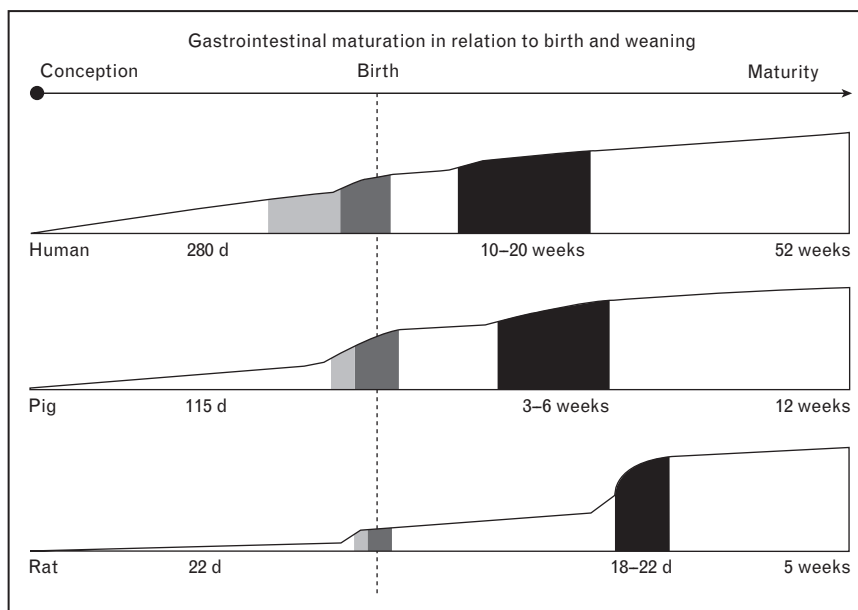


In humans and other primates, gastrointestinal development is slow and maturation starts early (in fetal life). Gut maturation in pigs is intermediate (i.e., maturation is rapid during the period from shortly before birth to shortly after weaning). In most small rodents and carnivorous species, the developmental changes occur relatively quickly and late (postnatally around weaning). Around birth (dark gray areas) and weaning (black areas), maturation is particularly rapid, resulting in a birth and weaning cluster of maturational changes. Birth of viable preterm neonates occurs over a wider range of gestational ages in humans compared with pigs and rats (light gray areas). (▨) Preterm birth of viable newborn. (▨) Birth cluster of gastrointestinal tract maturational changes. (■) Weaning cluster of maturational changes. Reproduced with permission from [9••].

growth, as well as poor neurodevelopmental outcome. Since the seminal studies by Widdowson [10] and McCance [11] on newborn animals, it has become clear that nutrition in early life affects postnatal growth and development. This work applied a straightforward approach to investigate the effects of over nutrition and undernutrition on growth and development by manipulating litter size, and is still used today [12]. However, precise control of volume and nutrient intake can only be obtained by artificial rearing. To date, most studies using artificial rearing to investigate nutrient intake and specific effects on biochemical mechanisms have been performed in rats. One of the most effective approaches is the so-called 'pup-in-a-cup' model, which has been used more extensively in recent nutritional neonatal research. This model, first described by Hall [13], has recently been applied to mouse pups and is a useful adaptation, given the greater availability of transgenic and knockout mice [14••]. Mouse pups in this model are taken from the dam at postnatal day 5 and put individually in Styrofoam cups that float in a temperature-controlled water bath, hence the name 'pup in a cup'. Subsequently, intragastric feeding tubes are placed into the pups that allow for regular infusion of rodent milk substitute (RMS). Recently, a hand-feeding technique using a surrogate nipple for artificial rearing of mouse pups has been developed [15]. Although more time consuming, this technique enables us to study pups shortly after birth and prevents physical injury. Furthermore, hand-feeding permits the use of a nursing box housing multiple pups and mimics natural feeding, which both stimulates physical activity and reduces stress. Newly developed RMS formulae accomplished a comparable weight gain between artificially reared mouse pups and breastfed littermates, although differences in organ weight, precocious gut maturation, and altered immune development have been observed [14••,16–18].

In our opinion, artificial rearing of mouse pups provides a means to study the effects of specific nutrients, food composition, and energy intake on gut development and metabolism within the relatively immature gut. Furthermore, rearing mice from birth onwards offers the opportunity to investigate whether early nutrition can change expression of genes and alter epigenetic markings. So far, only maternal nutrition has shown to induce epigenetic changes in offspring and it is unknown whether early neonatal nutrition can introduce or modify maternal epigenetic alterations [19,20].

## Neonatal piglet model

Compared with rodents, the neonatal pig has more anatomical, physiological, immunological, and metabolic similarities with the human neonate. The piglet has a gestational length of approximately 115 days and, compared with humans, is slightly less mature at birth in several aspects including digestive system and body composition (low fat content). However, during the neonatal period, protein deposition is very rapid, and owing to similarities of postnatal nutrition and intestinal development to humans, the piglet can be viewed as an accelerated model of postnatal growth and development [9••]. As breastfeeding is initiated and maintained, the intestine continues to develop and adapt to enteral food and bacterial colonization. The changes are primarily reflected in rapid intestinal growth and changes in functional parameters like digestive enzyme activity, nutrient absorption, and immune function. Later on, gut development is more gradual than around birth and probably reflects the slow transition from milk-based nutrition toward solid food. The pig is also appropriate for modeling liver function and metabolism, as it has hepatic features similar to that of humans and, unlike the rat, a gallbladder. More importantly, however, owing to its body size, the piglet model allows extensive surgical manipulation, repeated blood sampling, and long-term dietary treatment protocols [21–24].

The piglet model is a long established model used for both enteral and parenteral nutritional studies. We and others have used this model combined with stable isotopic tracer techniques to investigate postnatal intestinal, splanchnic, and whole-body nutrient metabolism [25–28,29••,30]. The indicator amino acid oxidation method (IAAO), initially developed to measure amino acid requirements in growing pigs, has also been applied to enterally and parenterally fed piglets [31••]. Importantly, these studies have provided a wealth of information that formed the conceptual basis for similar studies to be conducted in neonates, children, and adults [29••,31••,32–37] (Table 1). A series of studies in neonatal pigs investigated the mechanisms by which feeding stimulates protein synthesis, a response that decreases with development, particularly in skeletal muscle. These studies led to the development of the hyperinsulinemic–euglycemic–euaminoacidemic clamp technique to examine the role of insulin in the regulation of protein synthesis, independent of changes in circulating amino acids and glucose [38,39]. The piglet has also been

**Table 1 Splanchnic utilization rates of dietary amino acids and glucose as percentage of intake in piglets, human neonates, and human adults**

| Amino acid | Piglets | Neonates | Adults |
|---|---|---|---|
| Leucine | 42 | 42–48 | 21 |
| Phenylalanine | 51 | | 29 |
| Lysine | 43 | 18 | 32 |
| Methionine | 39 | | 33 |
| Threonine | 71 | 70 | |
| Glutamine | | 53 | 64 |
| Glutamate | 92 | 75 | 96 |
| Glucose | 40 | 32 | |

Adapted from [29••,33,36].

proven to be a useful representation of the human neonate when studying lipid nutrition [40,41], including the effect of long-chain n-3 polyunsaturated fatty acids on protein metabolism in the neonate during growth [42•].

In human neonates, total parenteral nutrition (TPN) is a life-saving therapy when enteral nutrition cannot be provided. In addition to the pig, neonatal rabbits [43,44], guinea pigs [45,46], and dogs [47] have been used to study effects of TPN. However, the prominence of the piglet in studies concerning human neonatal nutrition reflects the thought that the pig is most similar to humans compared to other animals and therefore the preferred model. The TPN model has been applied to study specific effects of TPN on intestinal growth, blood flow, digestion, absorptive function, epithelial integrity, and gut barrier function [48–51]. TPN-associated liver injury in piglets resembled that seen in human neonates [52,53•]. These studies suggest that TPN-induced hepatic steatosis is influenced by the source of lipid used [54]. Whether TPN administration during the neonatal period affects health in later life is unknown. Recently, development of insulin resistance and diabetes in adult life has been linked to not only being small for gestational age but also to prematurity alone [55••,56]. The mode of nutrition in this critical window of development may be an underlying factor of this phenomenon. In this respect, TPN in early postnatal life might have a role in metabolic programming. In support of this idea, in our current studies, TPN induced insulin resistance, hepatic steatosis, and greater fat deposition in TPN-fed compared with formula-fed piglets [57].

Most recently, the piglet model has been advanced to the delivery of viable premature piglets that can be studied using enteral and parenteral feeding protocols [58,59]. In comparison to mammals with a long gestation, preterm birth of viable offspring is possible only with a maximum of 10–12 days reduction in pigs (10%) and 1–2 days for rodents (Fig. 1). Considering their overall immaturity at full-term birth, preterm delivery of piglets at 90% of gestation translates into a relatively more premature animal. Preterm piglets have physiological similarities with human preterm infants and, thus, are suitable for studying preterm gut function, effects of parenteral and enteral nutrition, and immunity using novel in-vivo experimental approaches.

### Rodent and piglet models related to necrotizing enterocolitis

Necrotizing enterocolitis (NEC) is the most common gastrointestinal disease in premature infants and is associated with a high rate of morbidity and mortality. The exact cause is unknown, but major risk factors include intestinal immaturity, enteral feeding, and bacterial colonization. Most NEC models are neonatal rat models in which intestinal injury is induced by (over)feeding with formula, induction of pathogenic bacteria or endotoxins (LPS), or exposure to stress via hypoxia and/or hypothermia [60–66]. More recently, these experimental approaches have been extended to mouse models of NEC that have examined the impact of specific gene products in neonatal NEC, namely Toll-like receptor 4 [67,68•]. Asphyxiated rats and piglets have been studied for the role of hemodynamic and vascular changes in the intestine that could lead to NEC [69–71]. However, asphyxia is not believed to be the primary cause of the development of NEC. Studies performed in a new premature piglet NEC model, solely based on prematurity and formula feeding, have shown to mimic pathological changes in the gut, similar to that observed in human infants [72••]. Therefore, this model will enable the use of more invasive experimental approaches to investigate the role of parenteral nutrition, blood flow, and digestive capacity for specific nutrients in the development of NEC.

## Conclusion

In conclusion, neonatal animal models are an effective and valuable tool in understanding the impact of nutrition administered early in life on short-term and long-term functional development and metabolism. Rodents have the advantage of their low cost and provide a mean of using genetically modified animals to study mechanistic pathways. Using (preterm) piglets as a neonatal model favors translation to the human neonate with respect to gut development, nutritional requirements, and neonatal disease. In general, careful choice of an animal model is critical in the design of any study attempting to answer relevant questions.

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