British Journal of Nutrition (2022), 128, 802–827 doi:10.1017/S0007114521003834

Rodent models of metabolic disorders: considerations for use in studies of neonatal programming

Kasimu Ghandi Ibrahim[1,2]*, Dawoud Usman[1,2], Muhammad Bashir Bello[2,3], Ibrahim Malami[2,4], Bilyaminu Abubakar[2,5], Murtala Bello Abubakar[1,2] and Mustapha Umar Imam[2,6]

[1]*Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Usmanu Danfodiyo University, P.M.B. 2254, Sokoto, Nigeria*
[2]*Centre for Advanced Medical Research and Training, Usmanu Danfodiyo University, P.M.B. 2346 Sokoto, Nigeria*
[3]*Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, P.M.B. 2346 Sokoto, Nigeria*
[4]*Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, P.M.B 2346 Sokoto, Nigeria*
[5]*Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, P.M.B 2346 Sokoto, Nigeria*
[6]*Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Usmanu Danfodiyo University, P.M.B. 2254 Sokoto, Nigeria*

(Submitted 20 March 2021 – Final revision received 18 August 2021 – Accepted 15 September 2021 – First published online 23 September 2021)

Abstract

Epidemiologically, metabolic disorders have garnered much attention, perhaps due to the predominance of obesity. The early postnatal life represents a critical period for programming multifactorial metabolic disorders of adult life. Though altricial rodents are prime subjects for investigating neonatal programming, there is still no sufficiently generalised literature on their usage and methodology. This review focuses on establishing five approach-based models of neonatal rodents adopted for studying metabolic phenotypes. Here, some modelled interventions that currently exist to avoid or prevent metabolic disorders are also highlighted. We also bring forth recommendations, guidelines and considerations to aid research on neonatal programming. It is hoped that this provides a background to researchers focused on the aetiology, mechanisms, prevention and treatment of metabolic disorders.

Key words: **Altricial: Rodents: Animal models: Metabolic disorders: Neonatal programming: Suckling**

Obesity and non-communicable diseases like type 2 diabetes mellitus constitute global health concerns due to their high morbidity and mortality[1]. These and other metabolic diseases arise due to one or more abnormalities in the components of cellular metabolism. Studies have implicated not only genetic variations like polymorphisms and mutations of certain genes but also prenatal and postnatal environmental factors that influence the expression of the genes responsible for the metabolism of carbohydrates and lipids[2].

The term 'developmental programming' describes adaptive phenotypic changes that occur in later life following environmental exposure to certain factors, termed 'stressors' or 'cues', at critical periods of early life. The intrauterine and neonatal life represent critical and sensitive periods of programming for cellular metabolism. In some cases, the subsequent reprogramming of neonatal life outweighs the programmed developments of intrauterine life[3,4]. Despite the need for more scientific investigations on developmental programming of neonatal life, several factors, such as ethical considerations, genetic heterogeneity, differential disease outcomes, intensive care treatment and methodological constraints, limit direct investigations on the human neonate. Consequently, studies have predominantly adopted the rodent model to study neonatal exposure and adult health[5]. These animal models are useful for the study of malleable environmental factors such as diets, acting predominantly through epigenetic routes[6].

A number of reviews identify the general use of adult animal models programmed for specific pathologies such as metabolic

Abbreviations: PND, postnatal day; RMS, rodent milk substitutes.

* **Corresponding author:** Kasimu Ghandi Ibrahim, email ghandi.kasimu@udusok.edu.ng

syndrome[7,8], diabetes and obesity[2,9]. Some others focus on the use of single faceted nutritional models to neonatal programming[5,10–12]. However, the predominant use of rodents (rats and mice) for neonatal programming lacks full evaluation. Also, emphasis has been raised for the documentation of newer models and clarification of the considerations and caveats for their selection[2,8,9]. Herein, we employed a pioneer attempt to consolidate the altricial models of neonatal programming and document their suitability in the prevention and treatment of metabolic disorders. Furthermore, we present some guidelines and confounding points for model selection and animal experimentation.

Neonatal programming

Previously, the 'Foetal Origins of Adult Disease' hypothesis was used to define the relationship between gestational exposure to stressors and the emergence of adult metabolic disorders[13–15]. This phenomenon was termed 'fetal programming' due to its recognition of the fetal life as a period of plasticity. Subsequently, it was established that exposure to stressors in the postnatal (suckling) period also programme the outcome of adult health[16]. This recognition of 'neonatal programming' led to a shift in the hypothesis to 'the Developmental Origins of Adult Health and Disease'. The overall phenomenon thus became 'Developmental programming'[17,18].

While the stressors that trigger developmental programming could be either exogenous, endogenous or both[19], they are not always detrimental, and the outcomes of developmental programming could be inherited intergenerationally or transgenerationally[20]. For instance, Benyshek *et al.*[21] demonstrated transgenerationally altered glucose metabolism of F3 descendants of rats (F1) undernourished during lactation. Furthermore, exposure to stressors could either be gestational, triggering fetal programming, or may occur in the early postnatal period, causing alterations in adult metabolism due to neonatal programming[22].

Neonatal programming occurs during the suckling period. This period for humans, if allowed, can naturally extend from the neonatal phase of development (birth to 1 month) into infancy (1 month to 2 years)[23]. Comparatively, the suckling period for laboratory altricial rodents (rats and mice) extends to 3 weeks (day 21) after birth and weaning is marked by separation of pups from the dams. However, this may also extend naturally past the 21 d period. The suckling period for rodents comprises of a neonatal phase (birth to 1 week) and an infancy phase (week 1 end to week 3 end) [24,25].

Mechanistic bases for the metabolic programming in early life

With respect to Developmental Origins of Adult Health and Disease, metabolic alterations rely on the mechanistic 'retention' of the impact made by an exposure at early life. So far, such mechanisms are accounted for by some morphological memories and epigenetics[26]. As outlined later on, morphological changes (particularly of the gut) are peculiar to the suckling period. These include tissue remodelling, altered cell numbers, cell distribution or cell selection which may alter in response to nutrition and environment. Accumulating evidence also shows that epigenetics is a primary mechanism by which developmental programming influences and prepares an organism for adult life[27]. This includes changes in the DNA methylome, histone modifications and regulatory noncoding RNA, all affecting gene expression. Notably, the DNA methylome is particularly re-established in early life. During fertilisation and implantation, huge portions of the mammalian DNA methylation are lost, but later regained during the developmental periods (intra-uterine life, suckling period and puberty)[26]. This re-methylation can be modified by nutrition or environment, and a number of reports have demonstrated the role of DNA methylome to neonatal programming. For instance, Plagemann *et al.*[28] illustrated that over-nourished rat pups possessed a dose-dependently increased methylation of their hypothalamic insulin receptor promoter sites, predisposing them to diabesity in later life. We believe, at least anecdotally, that the nutritional influence on epigenetics is partly a function of the one-carbon cycle, which generates the methyl groups needed for DNA methylation. This is because nutrients, such as vitamin B_{12}, folic acid, choline and betaine present in milk, are relevant for the maintenance of this cycle[29]. Further investigations are required to fully understand these mechanisms and possibly uncover new ones.

Epigenetic changes alongside morphological memories (such as cellular distribution/tissue remodelling) may influence metabolic activity, the development of hormonal systems and trigger programming to a degree dependent on timing, type, magnitude and duration of stressor exposure, from conception to the first 1000 d of human life[18,26]. This period of life stretches from the beginning of gestation to the end of the early postnatal life. Notably, prematurity presents a classic case of exposure to adversity during these 1000 d, both gestational and postnatal[19].

Neonatal programming usually primes the subject to cope with future adversity such as a scarcity of nutrients. This 'predictive adaptive response' is an anticipatory process involving developmental adjustments after early-life exposure to stressors[30]. It occurs to match and adapt an organism's phenotype against later life exposure[31]. However, stress results when the predictive adaptive response is mismatched with the future condition resulting in cardiovascular and metabolic disorders[27]. In line with this concept of mismatch, studies relating the neonatal environment to developmental conditions reveal that offspring suckled by diabetic, obese or malnourished mothers are predisposed to metabolic disorders in later life[32–36]. Even so, the collective mechanisms by which such responses develop are yet to be fully elucidated. Notwithstanding, this critical neonatal period is a potent window for developing some targeted metabolic studies and possible preventive measures[37].

Altricial rodents

The nidicolous or altricial rat and mouse are subsets of the largest mammalian order, Rodentia, whose blind pups display absolute dependency on the dams, including a need for stimulation to micturate and defecate[38,39]. For reasons we shall discuss, these altricial mammals present a stream of species with conditions

that translate to the human state and may prove useful in investigating and understanding the fundamental mechanisms involved in neonatal programming. Research with these animal models targets some environmental and/or even genetic events that may shape the metabolic development of a suckling pup, at a period of developmental plasticity. Furthermore, beside the traditional approaches to disease treatment using these animal models[40,41], some novel attempts at disease prevention have been established. For instance, neonatal intake of *Hibiscus Sabdariffa* was shown to protect against fructose-induced dyslipidaemia[42], while neonatal intake of oleanolic acid was reported to repress the development of fructose-induced non-alcoholic fatty liver disease in adult rats[43].

Why rats and mice represent good animal models for investigating the neonatal development of metabolic disorders?

Altricial rodents are the most widely used laboratory animals due to their relatively short life span, little space for maintenance, fecundity, short gestation period and well-defined genetic background, completely sequenced genome of mice (2002: Mouse Genome Sequencing Consortium) and rats (2004: Rat Genome Consortium) [38,44,45]. Models of these rodents are useful in evaluating maternal interventions that exert influence on the metabolic development of the neonate. Their known genomic status provides foundation for genetically modified forms that can be purposely bred to investigate specific disorders[46].

During the neonatal period, the maternal milk, via nutrients and bioactive factors, has the major influence on pup development. Though the pups are blind at birth, they are attracted to the mother's nipples by certain odoriferous cues that coat them[47]. Milk-laden factors, antigens, immunoglobulins, bacteria and macromolecules are absorbed across a highly permeable enteric epithelium. For rats and mice, this permeability to macromolecules completely halts after weaning, a process called 'gut closure' which limits the absorption some dietary inclusions[48,49]. Like humans, suckling rodents have a hardwired age-related increase in receptor number, sensitivity and signal transduction than weaned rodents. This comes with associated consequences. For instance, the increased receptors and sensitivity for enterotoxins of *Escherichia coli* and cholera in the suckling period increase neonatal susceptibility to diarrhoea in man and rat[50–53]. A similar increase with development is noted for certain transporters such as sodium-glucose transporter 1, apical sodium-dependent bile acid transporter, water and Na transport channels in neonatal man, rats and mice[49]. Also, a precociously increased expression of other transporters like GLuT-2 and GLuT-5 can occur through transcriptomic influence in response to early dietary exposure such as a high fructose intake[49]. This has been adopted for the neonatal induction and accelerated development of adult metabolic disorders in these rodents[54,55].

Furthermore, altricial rodents are characterised by low forms of developmental maturity surrounding aspects of gastrointestinal nutrition and evacuation, thermoregulation, locomotion with most ontogeny finishing after birth. The periodical similarity of rodent-human ontogeny of several organs or systems makes suckling rodents suitable targets for neonatal research directed at specific metabolic conditions. Underneath this descriptive similarity of the suckling period of rodents to humans are certain changes that mirror the different phases of human development. For instance, some underdevelopments of the brain and gut show similarity to human premature babies and third trimester of human gestation[5]. The enteric maturation occurring within the suckling period of altricial rodents relates to *in utero* intestinal development for humans. The time lapse is perhaps due to the shorter gestational period for rodents as compared with the longer gestational periods in man[49]. More so, the developmental increase in disaccharidases (sucrase, isomaltase) for humans occurs from the 12th week of gestation while that for rats and mice is increased at weaning[56–58].

Like humans, suckling rodents express critical development in white adipose tissue[59]; immune system and adaptation[11]; establishment of neural networks (hypothalamus) and astrocyte development[60] and permanent metabolic programming of appetite and growth dynamics. All these become useful targets and biomarkers for neonatal programming which are leveraged upon by researchers to test hypotheses and bridge scientific gaps. Specifically, these similarities found in suckling altricial rodents have created opportunities to assess food composition/quality and natural products (for prophylaxis)[61,62]; understand gut microbes and mucosal homoeostasis[63]; conduct immune programming (immuno-nutrition) and genetic studies (advantaged in toe clipping)[11,64] among others.

Despite some marked differences from humans and a major setback in repeated blood sampling due to low blood volume, altricial rodents retain benefits to their undersize and underdevelopment. Rodent pups bear underdeveloped guts and brains that mature during lactation, before weaning and prove useful in the shared similarity with that of a premature human infant[5]. Though adult rodents can be surgically manipulated, the diminutive pup size, particularly of mice, presents a key obstacle for studies beginning at postnatal day 1. Regardless of this challenge, scientists the world over have developed and are still brainstorming upon newer techniques that rightly fit for neonatal programming. This has come with some potent breakthroughs[36,65–71].

Notably, no animal species possess complete similarities to human, along enzymatic form and activity. However, some enzyme isoforms acting upon specific metabolites show similarity in humans and rodents. For instance, the human cytochrome P450, subfamily 1A enzyme (CYP1A), has shown similarity with mice, while human cytochrome P450, subfamily 3A enzyme (CYP3A) exists similarly in both mice and male rats[72]. On the other hand, certain specie-specific differences also exist with some other enzyme isoforms, their organ specificity, expression and/or catalytic action. Marcella *et al.*[72] gave a detailed comparison of enzymes critical to oxidative metabolism in mice, rats and humans. This knowledge is essential for extrapolation of data to humans particularly in studies for preclinical drug development, metabolism and/or drug interactions. Additionally, altricial rodents share similarities with humans in many genes and biochemical pathways[5]. Consequently, experimental methods into the metabolic programming of neonatal rodents are useful in establishing developmental mechanisms and assessing natural products that potentially prevent deficits of early life from triggering the metabolic adversities of later life.

British Journal of Nutrition

Models of altricial rodents for neonatal programming

Animal models are useful representations of humans that allow for extensive hypotheses testing and interventions. The animal models for neonatal programming study direct or indirect postnatal exposure to stressors in the suckling stage of life. These animal models have specific focus on either postnatal exposure and neonatal programming or on the combinational effects of a longitudinal exposure from the pre-conceptional or *in utero* period to the postnatal period[4,55]. In this review, we categorised the models into five, based on the approaches used to trigger neonatal programming (see Tables 1–5 for summary of some articles). These models include altered litter size models, models of altered lactational conditions of the dams, models of altered condition of the pups (diet, photic experience, smoking and maternal separation), foster models and genetic models. These models alongside control groups are useful, singly or in conjunction, to study the development of metabolic phenotype. Using these models, structural changes in the brain, GIT, skeletal muscle, liver and plasma, alongside epigenetic events have been identified in the development of metabolic disruptions[60,63,73–76].

Altered litter size model

The suckling period is a time of intense dependency of the offspring on the mother for caregiving. As this secondary mother–offspring interaction occurs at a periodical hallmark of development for vital organs, studying variations programmable at different stages in this period is commonplace. Also, modelling in this form is a potent tool for comparing and deepening knowledge about rodents and humans. The maternal, paternal, littermate, environmental and predatory events are associations with varying contributions to neonatal programming[77]. Studying one factor effectively involves altering the variable while normalising the others.

For long, nursing of fewer human offspring is believed to facilitate weight gain and predispose to other disorders of adult life[78]. Consequently, altering the litter has been used singly or in combination with other models[79], to study neonatal programming. Following its pioneer usage by Widdowson and McCance[78], modelling alterations in litter size of maternally reared pups (littermate) are currently employed in investigations of under- (large litter) and over-nutrition (small litter) on the programming of new born animals (see Table 1). Techniques such as this use carefully planned mating strategies. For instance, in the pioneer study, female rats were placed in groups of six across five cages and one male was introduced per cage. This ensured that at least two litters were born on same day. Once pregnant, the rats were moved to separate cages where they delivered. Same-day-old pups were immediately mixed and redistributed into small and large litters for maternal rearing. It should be noted that while some studies count the day of birth as postnatal day (PND) 1[80], others mark the day of parturition as PND 0[81]. Though the use of PND 0 predominates (see Table 1), whatever protocol is adopted should be indicated.

The mothers' milk contains essential micronutrients and components which are substrates for metabolism and basis for development. The female rats and mice have six and five pairs of mammary glands, respectively[82]. Adjustments in the litter size affect milk availability. A reduction in litter size below normal creates an increased availability of milk for the pups[83]. Such offspring subjected to increased feeding by the 'litter size effect' have been reported to come up with weight gain and developmental changes through adult life. Small litter offspring of both rats and mice commonly develop overweight as the resulting phenotype and as such are used for investigating obesity[40,84]. Notably, suckling generates a directly proportional surge in oxytocin, and this endogenous oxytocin increases milk supply via the milk let-down reflex[85]. Given the maximum nipple occupation for mice and rats are five and six, respectively, only small litters below these numbers are useful for initiating litter size effects. Also, caution must be exercised in the use of exogenous oxytocin to stimulate milk release as this affects the study of litter size. Aside obesity, small litters have also been used in the study of Ca metabolism, the homoeostatic endocannabinoid system[79,86].

In contrast to the small litter, increased litter size results in an initial rise in milk volume until a limit is reached (approximately fourteen pups), beyond which milk deprivation occurs. Large litter models of numbers beyond this limit may be used to investigate growth retardation and telomere shortening[87]. The length of chromosomal telomeres is significant indicators of early life exposure to multiple forms of stress, and they have been reported to shorten in disease states associated with stress exposure such as obesity, diabetes mellitus, CVD and more[88]. Furthermore, large litters have also been used in the study of obesity and insulin resistance[40,73]. Large litters are associated with lesser nursing time and selective killing of runts between postnatal days 3 and 7[89,90]. Ranges for small, normal and large litter studies have been described[91] (see Fig. 1). With large litter sizing, groups with death count of two or more pups are excluded to avoid auxological development, which may present similar outcomes as small litters[79]. In addition, the type of diet, the class and animal species are equally important in investigating for emergence of metabolic disorders resulting from feeding[92].

Overall, the litter size correlates with variable diets in little or excess. A small litter may initiate overnutrition while a large litter may initiate undernutrition. Consequently, investigations that adopt other neonatal models for programming take measures to avoid the 'litter size effect' by adjusting/controlling litter size to an average number (commonly 8–12) per dam, a term known as 'culling' or 'to standardise'. This helps ensure that any observed phenotypic change is due to the experimental variable and not masked by the litter size. Additionally, culling has been adopted in balancing and avoiding biased sexual ratios[85,93]. However, variability in culling protocols may result in variability in results and as such care must be taken when choosing a protocol. The optimum number of rodents to cull ranges from 6 to 10 pups[85,94]; usually on the first[81], second[87], third[40], fourth, fifth or sixth PND[85].

Model of altered lactational condition of the dam

Most investigations in the lactational period of the nursing dam involve nutritional perturbations. Additional studies examine some other perturbations like maternal disorders, surgical

Table 1. Summary of some publications using the altered litter size model for neonatal programming

Model class	Rodent stock/ strain	Neonatal trigger	Metabolic disorder/related condition of concern	Methods	Sample used	Target biomarkers	Findings	Comment/considerations	Ref.
Altered litter size	CB6F1 hybrid mice (C57BL/ 6 + Balb/c)	Altered population density and milk availability	Endocannabinoid (eCB) system, active metabolising tissues (fat and liver)	Sizing and shuffling the litter to three groups; 3, 6 or 10 per dam on PND 1. Redistributing at PND 21 (4F/cage, 1M/cage). Assessed at PND 50–100	Tissues (liver, visceral fat, hypothalamus)	Body weight, length, surface area, BMI, Gene analyses	3 per dam group showed increase of certain eCB, less eCB receptor transcripts and less sociability compared with 10 per dam group. Differ also in length, weight, BMI, fat mass, hepatic IGF-1 expression and eCB enzyme expression	Excluding group with ≥ 2 deceased pups. 12/12 h light/dark. Temp; 22°C	Schreiner *et al.*[79] (2017)
	Sprague-Dawley rats	Excess milk availability	Insulin resistance and muscle epigenetics	At PND 3; CL (12 F/dam) *v.* SL (3 F/dam). Assessed on PND 21	Blood, tissue (skeletal muscle (SK), liver), body weight, food intake	Glucose, insulin, leptin, DNA, muscle mRNA, protein, methylated CpG sites	Over-nourished pups developed obesity, hyperphagia, increased insulin, glucose and leptin levels. In SK muscle, IRS1 and GLUT 4 genes had increased CpG methylation at promoter sites and decreased mRNA levels. SK muscle IRS1 was low and with serine phosphorylation. SK muscle epigenetics could trigger insulin resistance	Temp 23°C 12 h light Culled to 12/ dam; PND 1. Only females	Liu *et al.*[73] (2013)
	Sprague-Dawley rats	Preweaning overfeeding	Obesity	At PND 3–21; normal litter-NL (12 M/dam) *v.* small litter-SL (3 M / dam). Postweaning; mild CR (pair-feeding – 14 %) *v.* moderate CR (24 %). Assessed on PND 140	Blood, tissue (hypothalamus), weight, food intake	Insulin, leptin, RNA, DNA-CpG methylation	Moderate CR triggers sustainably reversed weight, insulin and leptin to normal for SL rats. Also, reverses effects on the hypothalamic appetite regulation (reduced hyperphagia) which limits weight gain	Temp; 23°C 12 h light culled to 12/ dam; PND 1. Only males	Liu *et al.*[40] (2013)

CL, control litter; CR, energetic restriction; GLUT, glucose transporter; ipGTT, intraperitoneal glucose tolerance test; ipITT, intraperitoneal insulin tolerance test; IRS, insulin receptor substrate; NL, normal litter; PND, postnatal day; SL, small litter.

Table 2. Summary of some publications using the model of altered lactational condition of the dam for neonatal programming

Model class	Rodent stock/strain	Neonatal trigger	Metabolic disorder/related condition of concern	Methods	Sample used	Target biomarkers	Findings	Comment/considerations	Ref.
Altered lactational condition of the dam	Wistar rats	Milk of malnourished dam	Glucose-insulin homoeostasis	4 % low protein (LP) diet; PND 1–14 and 20·5 % normal protein (NP) diet; PND 15–21. Terminal assessment after weaning	Milk, weight blood, vagus nerve, tissue (pancreatic islets, visceral adipose)	Glucose, ipGTT, insulin, ipITT, vagal depolarisation, corticosterone, leptin, weight, M3 Ach receptor function	LP pup had low vagal tone, glucose, insulin and leptin. Normal corticosterone. Increased milk leptin of LP dams. Less responsive M3AChR, pancreatic insulin inadequacy and glucose intolerance	Culled to 6/dam; PND 0. Preference for male. Euthanised at end of light cycle after fasting overnight. Temp 22 ± 2°C, humidity 55 ± 5 %. 12 h light	Mathias *et al.*[95] (2020)
	Wistar rats	Milk of high-fat fed dam	Visceral adiposity and epigenetics	High fat (60 % kcal fat) *v.* control diet (10 % kcal fat). Assessed at PND 12(peak lactation). Weaned to control diet. HFD or C from 3–6 month Assessed after 3rd and 6th month	Milk, blood and tissue (WAT)	Glucose (oGTT), glucose, TG, insulin, leptin, adiponectin, milk FA, epididymal and inguinal adipocyte cell number, mRNA, DNA chromatin CpG methylation – SCD1	Altered milk FA content. Reduced methylation and increased stearoyl-CoA desaturase-1 (SCD1) expression. Increase WAT cellularity and mass	Culling to 8 (4/4) preference for male Temp & humidity-controlled environment. 12 h light	Butruille *et al.*[59] (2019)
	Wistar rats	Milk of dam fed low protein diet	Obesity and insulin resistance	Dam: normal protein-NP (20·5 %) *v.* low protein. diet-LP (4 %) from 0 to 14th PND. PND 21–60; standard diet. Offspring. 60–90 PND: normal fat (4·5 %) *v.* HFD diet (35 %). Assessed at PND 90	Weight, length, food intake, blood, tissue (pancreas), vagus	Lee index, ivGTT, ipITT, insulin, glucose, leptin, TAG, total cholesterol, HDL-cholesterol, vagal activity	LP/HFD offspring had not as much increased weight, fat leptin, altered glucose homoeostasis and insulin levels compared with the NP/HFD group. Protein restriction counters obesogenic outcomes	Temp; 23 ± 2°C); 1 week adaptation; cull 8/dam (day 0); preferentially male 12 light	Martins *et al.*[147] (2018)
	Primiparous Sprague–Dawley rats	Milk of DHA diet fed dams	Inflammation and immune maturation	Dams (2 d pre-parturition) control (0 % DHA of FA) *v.* DHA (0·9 % DHA of FA). Female pups (week 3–6); control *v.* DHA diet. Assessed after week 3 & 6	Tissue (spleen, intestine), milk	Immune cells (count, phenotype), intestinal length, cytokine, FA's (milk & spleen)	DHA-supplemented diet increases the ALA and DHA of milk. It triggers improved anti-inflammatory response and favours immune maturation, without significant effect on growth	Controlled temp, humidity 12 h light culled to 10/dam used female rats, however, recommended male study	Richard *et al.*[99] (2016)
	Wistar rats	Milk of low protein fed dams	Obesity and insulin resistance	Nursing dams; control (15 % protein) *v.* treatment groups (2 % arginine). PND 21–6 week; normal diet (15 % protein) at week 6; normal diet (12·5 % fat) *v.* HFD (35 % fat)	Blood, tissue (liver muscle, fat), milk	Weight, oGTT, glucose, insulin, CT scan, TG, aa and hormones (milk and dams' plasma), food intake	Offspring of arginine fed dams become prone to obesity and insulin resistance. Milk supplied less threonine and glycine	Culled to 8/dam; PND 4. Preference for male	Otani *et al.*[98] (2016)
	Sprague–Dawley rats	Milk of HFD fed dams	Cerebrovascular stroke	Dams (10 d preG + G + L); normal (5·3 % fat) or HFD (25·7 % fat). Offspring from PND 21–120; control *v.* HFD diet forming four groups	Tissue (artery, brains), blood	Weight, food intake, cerebral blood flow, stroke volume, behaviour, glucose, insulin, corticosteroid, mRNA	HFD/C group had increased chance of ischaemic injury, increased BDNF, glucocorticoid conc. and receptor expression. Adrenalectomy reverses effects on stroke	12 h light Temp; 22 ± 2°C culled to 8/dam; PND 1. Preference for male	Lin *et al.*[81] (2016)

Table 2. (*Continued*)

Model class	Rodent stock/strain	Neonatal trigger	Metabolic disorder/related condition of concern	Methods	Sample used	Target biomarkers	Findings	Comment/considerations	Ref.
	C57BL/6J mice	Milk of undernourished dams	Reproduction (brain and gonadal function)	F1 from birth – PND21: control (C: F0 dam 22 % fat) *v.* lactational undernutrition (LUN: F0 dam fed 50 % food ate by C the previous day). Normal diet (11 % fat) post-weaning. F2 has four groups; CC, CL, LC, LL (Mating C *v.* LUN at week 8). No lactational restriction. Birth to PND21: all control diet (22 % fat). Postweaning normal diet (11 % fat)	Tissues (brain), body NMRI	Body weight, adiposity, composition, puberty markers (VO, VE, BPS), RNA	Postnatal malnutrition triggers delayed puberty, alter gene expression and body composition, disturbed repro. of F1 (& F2 – depend on parental history and sex)	Culled to 8/dam included both sex	Kaczmarek *et al.*[101] (2016)
	C57BL/6 J mouse	Milk of undernourished dams	Hormonal repro metabolism	F0; nursing dams with undernutrition F1; lactating pup (weaned and grown on normal diet to 8 week) – no alteration F2: lactating pup (no alteration), weaned	Brain, body weight and composition	Hypothalamus (mRNA – RT-qPCR, microarray), adiposity, puberty	Undernutrition affects development of hypothalamus in F1 (altered gene expression, body composition and delayed puberty) depending on sex and parental malnutrition	Sexual dimorphism	Kaczmarek *et al.*[101] (2016)
	Swiss mice	Milk of HFD dam	Insulin signalling	Dams (3 weeks preG + G + L); St. chow (C) *v.* HFD (45 %). Pup groups: C/C, H/H. weaned at 18. SC from 18–28 or 42. Then HFD or not till PND 82	Food intake, tissue (liver, muscle, fat brain), blood	GTT, ITT, PTT, glycogen, TNF-alpha, leptin, proteins, RNA	HFD offspring gained weight, were hyperphagic with impaired insulin signalling at central and peripheral sites.	12 h light Culled to 8/dam Weaned on PND18 Preference for male Temp: 22 ± 1°C	Fante *et al.*[32] (2016)
	Wistar rats	Milk of malnourished dam	Glucose metabolism	4 % low protein (LP) diet from PND 1–14, *v.* 23 % normal protein (NP) diet from PND 15–21. normal diet for 60 d; assessed after PND 81	Blood, vagus nerve and sympathetic branch of sup. cerv. ganglai, tissue (pancreatic islets, adrenal medulla, adipose)	Glucose, ivGTT, insulin, ipITT, vagal depolarisation, weight (lee index), chow consumption, receptor function (M and α_2), AChesterase activity, catecholamine secretion	LP adult pup had low vagal tone, glucose, insulin and leptin	Litter size effect (6/dam); preferential to male pups; temp. (22 ± 2 °C) and light (12 h	de Oliveira *et al.*[148] (2011)
	C57BL/6 mice	Milk of high-fat fed dam	Non-alcoholic fatty liver disease	Dams: standard chow *v.* HFD (G, L, GL). Offspring from PND 21–3rd month; HFD	Weight, food intake, blood, tissue (liver, fat)	oGTT, insulin, microscopy, liver enzymes, TNF-α, proteins, liver stereology	Increased fatty liver content and expression of sterol regulatory binding protein-1c due to maternal HFD	12 h light culled to 6/dam. PND 0	Gregorio *et al.*[94] (2010)

ACh, acetylCholine; ALA, α-linolenic acid; BDNF, brain-derived neurotrophic factor; BPS, balano preputial separation; C/C, control-control diet; C, control; C57BL/6, C fifty-seven black six mice; F0/1/2, filial generation 0/1/2; FA, fatty acid; G, gestation period; GL, gestation and lactation period; H/H, HFD to HFD dam; HFD, high-fat diet; ipITT, intraperitoneal insulin tolerance test; ivGTT, intra-venous glucose tolerance test; L, lactation period; LUN, lactation undernutrition condition; M, muscarinic; NMRI, nuclear magnetic resonance imaging; o/ip-GTT, oral/intraperitoneal-GTT; PND, postnatal day; PTT, pyruvate tolerance test; SCD1, stearoyl-CoA desaturase-1; VE, age of vaginal oestrous; VO, age of vaginal opening; WAT, white adipose tissue.

Table 3. Summary of some publications using the model of altered lactational condition of the pup for neonatal programming

Model class	Rodent stock/ strain	Neonatal trigger	Metabolic disorder/ related condition of concern	Methods	Sample used	Target biomarkers	Findings	Comment/con-siderations	Ref.
Altered lacta-tional condi-tion of the pup	Sprague-Dawl-ey rats	Fructose and ursolic acid (UA)	NAFLD	Pups from PND6–20; fructose (50 %) and/or ursolic acid (0·5 %). PND70; water or fruc-tose (20 %)	Weight, food & fluid intake, fat,	ALT, ALP, ALB, NAFLD activity score	UA abrogates fructose induced fat accumulation in the liver	Double hit. Used both male and female	Mukonowenzou *et al.*[149] (2020)
	NMRI mice	Resveratol (RSV) and nicotinamide riboside (NR)	Adipogenesis	Pups from PND 2–20; 10–15 µl water (vehicle) *v.* RSV *v.* NR. Standard chow; PND 21–75. NFD (10 %); PND 75–90. HFD (45 %); PND 90–164	iWAT, tissue	RNA, DNA, methylation	RSV & NR supplementation supports white adipocyte beiging via epigenetic change	Only males Culled to 12/ l Euthanized while being fed; within 2 h of light cycle	Serrano *et al.*[75] (2020)
	Sprague Dawl-ey rats	Oleanolic acid (OA)	Oxidative stress and metabolic syndrome	PND 6–14; vehicle *v.* oleanolic acid *v.* High fructose (V, OA, HFrc, OAHF)	Blood, tissue (muscle), weight	Visceral & bone morphome-try, cholesterol, glucose, phosphate, Ca^{2+}, renal, lipid, hepatic and protein profiles, enzyme marker. Antioxidants, lipid peroxi-dation and nitrite assays	Early postnatal OA protects against oxidative damage associated with MetS or precocious entero-ontol-ogy	Limit to PND 14 when eyes open. Oral gavage temperature 25 ± 2°C 12 h Light	Nyakudya *et al.*[104] (2019)
	Sprague Dawl-ey rats	Oligosaccharide	GIT homeostasis and feeding	PND 5–14/15; FOS, GOS/In mix, αGOS or a mix of the monomers resent in the OS solu-tions. Assessed at PND14/15 and PND124/126	Blood, tissue (ileum, colon), feeding pat-tern & pref-erence	GLP-1, PYY, D&L-lactates, immunochemistry, RNA, DNA	Oligosaccharides (OS) affect intestinal, endo-crine features but have no influence on satiety peptides or food intake	12 h light, Temp; 22 ± 2°C culled to 8/ dam	Le Dréan *et al.*[62] (2019)
	Sprague Dawl-ey Rat pups	Fructose and curcumin	Glucose & fructose metabolism. Fructose induced bone diseases	P1; oral administration of curcumin singly and combined with Fructose from PND 6–21. P2; PND 21–63 Fructose- second hit	Blood, femur, tibia	Plasma osteocalcin, radiog-raphy (bone density) and morphometry (length, mass & seedor index)	Curcumin triggers significant decrease in plasma osteocalcin (in both male and female)	Used orogas-tric tube for gavage. Avoided the litter-size effect temp: 26 ± 2°C, 12 h light	Ibrahim *et al.*[54] (2019)
	Wistar rats	Retinyle ester (RE) or β-carotene (BC)	Adipogenesis	Pups from PND 1–20; control *v.* vitamin A (as RE) *v.* vitamin A (as BC)	Tissue	RNA, DNA, CpG methyla-tion	Vitamin A supplement as retinyle ester (RE) or β-carotene (BC) differen-tially impacts epigenetic methylation in WAT	Temp: 22°C, 12 h light. Culled to 10/ dam at PND1. Pipette gav-age	Arreguín *et al.*[106] (2018)
	Wistar rats	Milk substitution	Obesity	Pups; From PND 14: (rat milk, RM) *v.* (cow's milk, CM) *v.* (cow's milk + casein protein,	Blood, milk, tissues, weight	Fat and lean mass, protein, adipocyte morphometry, hormones, lipid profile, glucose, insulin, RNA	CM males had low fat mass while CM females had high fat and hyperphagia. CM-H males had high corticosterone and low	Temp: 25 ± 1° C, 12 light. Culled to 6/ dam at PND	Rodrigues *et al.*[80] (2018)

Table 3. (*Continued*)

Model class	Rodent stock/ strain	Neonatal trigger	Metabolic disorder/ related condition of concern	Methods	Sample used	Target biomarkers	Findings	Comment/con-siderations	Ref.
				CM-H). Assessed on PND 21, 180			protein mass while CM-H females had high fat, hyperphagia, and low insulin	0. 1:1 sex ratio	
	Sprague Dawl-ey rats	Oleanolic acid and high fruc-tose	Non-alcoholic fatty liver disease	Pups: PND 7–13: DW *v.* OA *v.* HFD *v.* OAHF. P2: PND56–112: stan-dard *v.* HFD. Assessed on PND 112	Blood, tissue (liver)	Lipid profile, micrographs, histology	Neonatally administered oleanolic acid protects against hepatic lipid accu-mulation caused by high fructose diet	Temp: 25 ± 2° C, 12 h light. Avoided dam-effect bias	Nyakudya *et al.*[43] (2018)
	Sprague-Dawl-ey rats	Oligosaccharide (FOS, GOS/ lcF mix, AOS) and amoxicil-lin	GI microbiota	Pups from PND 5–14: FOS *v.* GOS/lcF mix (9/1) *v.* AOS, *v.* Controls (+/–) *v.* Amx or a mix of monomers. Weaned to St. chow. Assessed on PND 131	Caecal con-tent	Bacteria, DNA	All treatment particularly amoxicillin altered gut microbiota. All effects fade at adulthood except for that induced by GOS/ lcF mix	Culled to 8/ dam (M/F-6:2)	Morel *et al.*[128] (2015)
	C57BL/ 6J mice	Photic experi-ence; light & darkness	Long-term neuro-chemical and structural altera-tions of SCN and circadian system	PND0–21; L/L, L/D, D/D (using white fluores-cent source) then LD; PND21–50	Brain	Astrocyte by observing the glial fibrillary acidic pro-tein (GFAP) in SCN	Pup exposure to light has long-term effects on the astrocytic population of the SCN	Post-gravid considera-tion Other conditions; ambient temp, water and food	Canal *et al.*[60] (2009)
	Sprague Dawl-ey rats	High carbohy-drate (HC) milk formula	Metabolic profile (insulin, leptin homeostasis, obesity, hypo-thalamic cir-cuitry) and inheritance	F1: PND4–24 (3 weeks), AR (56 % HC) *v.* MR (natural milk, 8 % carbohydrate). F2: mating F1 females + naïve male (PND 80).	Intake, weight, blood, brain	Insulin, leptin, interleukin (IL) −6 & −12, monocyte chemoattractant protein (MCP)-1, macrophage migration inhibitory factor (MIF)-α, and vascular endothelial growth factor (VEGF), lipid peroxides, neuropeptide Y (NPY), agouti-related polypeptide (AgRP), galanin (GAL), and insulin receptor (IR)-β	HC milk formula triggers immediate postnatal lep-tin resistance and hyper-phagia in F1. Also predisposes to later life insulin resistance, oxida-tive damage and elevated proinflammatory signa-tures in F1. F2 showed increased susceptibility to obesity and metabolic deficits.	Temp: 25 ± 2° C, 12 h light. Female pref-erence for the transmis-sion of HC phenotype. Culled to 11 pups/dam.	Srinivasan *et al.*[120] (2008)
	ICR mice	*n*-3 essential fatty acid restriction *v.* supplementa-tion (DHA)	Fatty acid homoeostasis in the cardio-vascular, neural and other organ systems	PND2–15: AR (*n*-3 fatty acid adequate (3·1 % + 1 % DHA) *v.* deficient (0·06 %)) *v.* MR (3·1 %).	Brain, liver, heart, plasma and retina	Lipid profile, phospholipid molecules, fatty acid	*n*-3 fatty acid restriction low-ers adult tissue levels of DHA. Adequacy reveals phosphatidylethanola-mine, phosphatidylcholine and the novel cardiolipin as major repositories for DHA.	Temp: 23 ± 1° C, 12 h light. First applica-tion of AR to mouse pup nutrition.	Hussein *et al.*[117] (2009)
	Sprague Dawl-ey rats	High carbohy-drate (HC) milk formula	Hyperinsulinaemia and pancreatic ontogeny	PND4–12; AR (56 % carbohydrate) *v.* MR (natural milk; 8 % carbohydrate) *v.* AR	Islets	Preproinsulin, insulin, upstream stimulatory fac-tor-1 (USF-1), phosphati-dylinositol 3-kinase (PI3-	HC elevates expression of preproinsulin, insulin, PDX-1, its stimulatory fac-tor and downstream	Culled 12/dam. Short term program-ming using	Srinivasan *et al.*[150] (2001)

Table 3. (*Continued*)

Model class	Rodent stock/ strain	Neonatal trigger	Metabolic disorder/ related condition of concern	Methods	Sample used	Target biomarkers	Findings	Comment/considerations	Ref.
				(high fat milk formula; similar to rat milk with 8 % carbohydrate). Endpoint; PND12		k), stress activated protein kinase-2 (SAPK-2), pancreatic duodenal homeobox factor-1 (PDX-1) (mRNA and protein)	kinases. This adaptation was independent of hyperglycemia.	the pup-in a cup model. Carbohydrate derived from polycose	
	Sprague Dawley rats	High carbohydrate (HC) milk formula	Hyperinsulinemia and long-term pancreatic function	PND4–24; AR (56 % carbohydrate) *v.* MR (natural milk; 8 % carbohydrate) *v.* AR (high fat milk formula; similar to rat milk with 8 % carbohydrate). Endpoint; PND100	Blood, pancreas	Plasma (Insulin, GLP-1, Glucose, TAG, FFA), Islets (preproinsulin, upstream stimulating factor-1 (USF-1), glucose transporter-2 (GLUT-2), phosphatidylinositol 3-kinase (PI 3-kinase), and stress-activated protein kinase-2 (SAPK-2)	HC triggers an adapted elevation of insulin secretion in adulthood.	Culled 12/dam. Long term programming using the pup-in a cup model.	Aalinkeel *et al.*[39] (2001)
	Sprague-Dawley rats	High carbohydrate (HC) milk formula	Long term pancreatic islet ontogeny	PND4–18; AR (56 % carbohydrate) *v.* MR (natural milk; 8 % carbohydrate) *v.* AR (high fat milk formula; similar to rat milk with 8 % carbohydrate). Endpoints; PND6,12,14,18 and 24	Pancreas, blood	Insulin, IGF-I, and IGF-II.	HC trigger decrease in islets size and α cells. While islets were apoptotic, ductal epithelia had elevated proliferation. HC also warrants decreased IGF-II expression. Elevated insulin levels at PND24	Both male and female pup in a cup model.	Petrik *et al.*[119] (2001)

Abbreviations: ALB = albumin; ALP = alkaline phosphatase; ALT = alanine aminotransferase; Amx = amoxicillin; AOS = pectin-derived acidic oligosaccharides; AR = artificial rearing; D/D = dark/dark; DHA = docosahexanoic acid; DW = Distilled water; F0/1/2 = filial generation 0/1/2; FOS = fructo-oligosaccharides; GLP-1 = glucagon like peptide; GLP-1 = glucagon like peptide; GOS/In = beta-galacto-oligosaccharides/inulin mix; GOS/lcF = galacto-oligosaccharides/long-chain fructan mix; HFD = high fat diet; HFrc = high fructose; IGF = insulin growth factor; iWAT = inguinal white adipose tissue; L/D = light/dark; L/L = light/light; MR = maternal rearing; NAFLD = non-alcoholic fatty liver disease; NFD = normal fat diet; NR = nicotinamide riboside; OA = oleanolic acid; OA = oleanolic acid; OAHF = oleanolic acid and high fructose; PND = postnatal day; PYY = peptide YY (tyrosine tyrosine); RSV = resveratrol; SCN = suprachiasmatic nucleus; V = vehicle; αGOS = alpha-galacto-oligosaccharides.

Table 4. Summary of some publications using cross-foster or genetic models for neonatal programming

Model class	Rodent stock/ strain	Neonatal trigger	Metabolic disorder/ related condition of concern	Methods	Sample used	Target biomarkers	Findings	Comment/ considerations	Ref.
Cross-foster models	Wistar rats	Milk content and yield of mono-sodium L-glutamate (MSG) dam	Obesity	F0 mated. F1: MSG (4 mg/g) to female's *v.* control (saline) during first 5 PND for obesity. For F2 mate 70D old F1 with lean rat. F2 male: cross fostered (four groups pup/dam; C/C, C/MSG, MSG/C, MSG/MSG). Standard diet from PND21–120	Tissues (fat, hypothalamus), weight, food intake, milk, plasma	ivGTT, glucose, lipid profile, protein, leptin, insulin, protein expression	Cross mitigates obesity and normalised food intake, leptin signalling, lipid profiles and insulinaemia but not glucose homoeostasis or insulin secretion	Cull to 8/dam at PND1 12 h light Temp: 21 ± 2°C	Miranda *et al.*[35] (2016)
Genetic models	Goto-Zakizaki rats	Milk of GK rat + adversity of cross	T2D	Crossbreeding Wistar (W) and with GK	Plasma, pancreas (beta-cell)	Glucose tolerance (ivGTT) insulin, beta-cell mass, body weight	W/GK pup had defective pancreatic beta-cell function even after lactation by W dam, suggesting a paternal genetic influence	Culled to 8–10/dam at PND1. Preference for male pups	Calderari *et al.*[14] (2006)
	C57/BL6 × DBA mouse (transgenic mouse dams)	DHA-rich milk of transgenic dams	FA metabolism	PND 1–21 suckling	Toe, milk, plasma, brain	DNA, FA – DHA, total lipids	Pups suckling under DHA-rich milk transgenic Dams have increased brain DHA, which promotes development of neural structures	Toe clipping	Kao *et al.*[34] (2006)

FA, fatty acid; ivGTT, intra-venous GTT; PND, postnatal day; T2D, type II diabetes.

Table 5. Summary of some publications using combined models for neonatal programming

Model classes	Rodent stock/ strain	Neonatal trigger	Metabolic disorder/related condition of concern	Methods	Sample used	Target biomarkers	Findings	Comment/considerations	Ref.
Altered litter size, altered lactational condition of the dam, altered lactational condition of the pup and cross-foster	Wistar kyoto (WKY) rats	Uteroplacental insufficiency. Milk changes	Ca homoeostasis and cardiometabolism	Normal or altered (restricted) milk dams with unadjusted (7–15) or reduced litter size (5–6) pups	Blood, whole body, plasma, stomach content, milk, weight, tissue (mammary, bone)	Tissue RNA, protein, lactose, Ca, PTHrP, corticosterone, Na, K, milk FA's, BP, iaGTT, bone morphometry, density and content, glucose	Reduced litter trigger altered Ca homoeostasis, elevated BP, increased w3 and reduced w6-fatty acids	12 h light; temp 19–22°C; culling to SL and standard litter (unaltered surrogate); maternal separation	Briffa *et al.*[86] (2019)
Altered litter size and altered lactational condition of the dam	Wistar rats	Milk content and availability from activity-modulated dams	Obesity	Dam: G + L; exercised (E) *v.* sedentary (S). Pup: PND 3; small (3/dam) *v.* normal litter (9) making a total four groups	Body weight, milk, tissue (nerve, fat)	Lipid profile, ivGTT, insulin, nerve activity, weight	SL of E mothers had low fat, improved glucose tolerance, insulin sensitivity, insulinaemia and glycaemia. Exercise protects against metabolic dysfunctions	Preference for male pups	Ribeiro *et al.*[84] (2017)
	Wistar rats	Milk of low protein fed dam + litter size	Telomere growth and ageing	Dam; gestation (G) + lactation (L) period; 3 groups; control protein (20 % for G & L + NL), recuperated (8 % for G, 20 % for L + SL) and low protein (8 % for G & L + LL) diet groups. Weaned into control diet	–	Telomere DNA, length and degradation	Growth retardation of postnatal life is proportional to telomere and life span lengthening. While protein restriction of fetal life (growth retardation) followed by normal protein (catch-up growth) during neonatal life is proportional to their shortening	Temp 22°C; light 12 h; culling to 8, 4 and 14 for three groups, respectively (PND2)	Jennings *et al.*[87] (1999)
Altered lactational condition of the dam and cross-foster	Wistar rat pups	Adversity of cross fostering (healthy to obese dams)	Adiposity and insulin resistance	PND4–21; cafeteria diet-induced maternal obesity, cross-fostering	Tissue (liver, gastrocnemius, perirenal and gonadal adipocytes) plasma, peritoneum	Glucose clearance, adipose tissue mass and mRNA expression. Fasting glucose and insulin, glucose tolerance test (ipGTT)	Hyperglycaemia, insulin resistance, increased adiposity, etc., i.e. maternal obesity during lactation programmed offspring adiposity and insulin resistance	Light *v.* dark cycle (12/12). Showed some elements of sexual dimorphism	George *et al.*[3] (2019)
	Sprague Dawley rats	Milk of protein restricted dams and cross	Plasma metabolomics and liver lipidomics	Dam (G + L period): control prot. (20 %) *v.* low protein (8 %). Crossed at birth (4 groups). Weaning – 10th week; standard diet (17 % protein, 43 % CHO, 41 % fat). C/R starts from PND16	Blood (plasma), tissue (liver)	mRNA, FA's, ketone bodies, carnitine and acylcarnitine, metabolic and lipidomic markers of phenotype	C/R pups had increased FA β-oxidation and BCAA (branched -chain amino acid) while R/R & R/C pups had impaired β-oxidation	Culling 8 male/ dam at PND0. Preference for male. Sacrificing at same period	Martin Agnoux *et al.*[74] (2018)
	Albino Wistar rat	Milk of dams fed high-fat/high-	Adiposity	Control or CAF dam with control or CAF litter.			CAF litter developed increased body fat	12 h light; room temp. 25°C;	

Table 5. (*Continued*)

Model classes	Rodent stock/ strain	Neonatal trigger	Metabolic disorder/related condition of concern	Methods	Sample used	Target biomarkers	Findings	Comment/considerations	Ref.
		sugar cafeteria diet		House same sex 3–4 after weaning. Assessed at 3rd & 6th week	Weight, blood, tissue (adipose)	ipGTT, glucose, FA, insulin, leptin, mRNA	(neutralises at 6th week), PPAR-alpha, adiponectin and leptin expression at weaning (independent of birth parent). Lower SREBP-1C expression in CAF litter at weaning (M) or 6th week (F)	acclimatisation period (1 week); culled to 8/dam (4/4)	Vithayathil *et al.*[4] (2018)
	Albino Wistar rats	Milk of high fructose and 10 % sucrose fed dams and cross	Obesity and T2DM	Dams (4 weeks PreG + G + L); control diet *v.* 10 % sucrose or HFCS-55 diet. Weaned to control diet. Assessed at week 3 and 12	Blood, tissues	IpGTT, glucose, TAG, total C, HDL-cholesterol, FFA, insulin, leptin, liver lipids, weight, fat stores	Pronounced increase in FA's for S/S and C/H groups. S/C and H/C groups had persistently high FA's at 12th week.	12 light Temp; 22° C 1 week acclimatisation Culled to 8/dam (4/4, PND 0)	Toop *et al.*[55] (2017)
	ICR mice	Milk of high fat fed dam + cross	Milk transcriptome	Dams (4 weeks PreG + G + L); HFD (60 % fat, 7 % sucrose) *v.* control (10 %F, 7 %S). Cross-fostered to give 4 groups (CC, CH, HC, HH). Assessed at PND 12	Milk, blood.	Weight, length, BMI, milk and serum (protein, lactose, fat, mi- & mRNA analyses)	High lactose & fat in HFD dams. mRNA and miRNA increased expression in milk of HFD dams. Increased weight in CH and HH compared to HC and CC groups	Cull to 10/dam (PND 2) 12 h light	Chen *et al.*[151] (2017)
Altered lactational condition of the dam and altered lactational condition of the pup	Wistar rats	Cigarette smoking	Obesity	P1; PND 3–21 (smoking machine) P2; assessed every 4 d PND 180	Tissue (liver) adipocytes, serum	Imaging, TAG, cholesterol, hormones (25(OH) D), protein analysis, mRNA expression	Increased abdominal fat, HPA axis dysfunction (not GC changes), less severe in females, lipogenesis and vitamin D more affected in males	Sexual dimorphism Temp; 23–24°C 12 h light (artificial) 1 week acclimatisation culled to 6(3/3)	Novaes Soares *et al.*[124] (2018)
	Albino Wistar rats	Milk of junk food (JF) fed dams and JF diet for pups	Opioid signalling system	Dams (2 weeks preG + G + L); control (C) *v.* junk food (JF). Post-weaning pup diet same as dam until PND 28	Tissue (brain, liver, fat), blood, morphometrics	DNA, histology, total body fat mass, imaging, MuR expression	Female offspring of JF group had reduced mu-opioid receptor (MuR) expression and ligand binding. No effect on male offspring	1 week acclimatisation Temp; 25°C 12 h light culled to 10/dam sex ratio 1:1 assessed at same phase of day (morning)	Gugusheff *et al.*[107] (2016)
	Wistar rats	Maternal separation + milk of HFD fed dams	Metabolic stress	Dams (G + L): standard diet – SD (12 %) or HFD (40 %). F = Vegetable oil. Pups; control *v.* MatSep groups. MS pup = PND 2–14 (180 min/d). C pup = undisturbed with dam.	Blood, plasma, tissue (brain)	Genes, mRNA, behavioural tests, hormones (leptin, insulin, etc.)	HFD prevented MatSep endophenotypes of adulthood, reduced anxiety and improved maternal care in stressed dams. MS-triggered changes in genes of	12 h light (artificial) Temp; 22° C (controlled) culled to 8–10 (PND 0) equal litter males and females during	Rincel *et al.*[102] (2016)

Table 5. (*Continued*)

Model classes	Rodent stock/ strain	Neonatal trigger	Metabolic disorder/related condition of concern	Methods	Sample used	Target biomarkers	Findings	Comment/considerations	Ref.
				Assessed PND 11 to 8th month			PFC which are attenuated by HFD	Sep. pups at temp; 28 ± 2°C	
	Wistar rats	Milk of diet restricted dam and supplementation	adipogenesis	Dam (G: 1–12): standard chow *v.* energetic restriction (20 %). PND 1: CR *v.* CR-leptin (murine leptin). Assessed on PND 24	Blood, Tissue	RNA, glucose, insulin, T3, protein expression, adipose morphometry and micrography	Leptin reverses the malprogrammed events of WAT	Temp; 22°C, 12 h light 1:1 male: female	Konieczna *et al.*[152] (2015)
Altered lactational condition of the pup and cross-foster	BALB/c mice	WPH in artificial milk formula	Development of infant immunity	PND2–14. AR pups: crossed to ovariectomized dam with *v.* without 5 % WPH supplement.MR: natural dam	Weight, milk, spleen, thymus	Body weight gain rate, feed efficiency and protein efficiency, thymocyte number and viability	WPH counters AR dependent weight loss, improves feed and protein efficiency and positively elevates some immune signatures. VRTPEVDDE represents the most immunomodulatory peptide of WPH.	Intermittent hand feeding (every 3 h) for AR pups. Temp: 23 ± 3°C, 12 h light.	Takeda *et al.*[114] (2020)
Altered lactational condition of the pup and genetic models	C57BL6	PPARα ligand (Wy)	Epigenetics and Obesity	G (14–18) + L (PND 2–16); Wy postweaning (week 4–14); HFD (60 %). PPARα-KO mice (as WT control). FGF21-deficient (KO) mice (G (14–18) + L (PND 2–16)) with HFD 10 weeks (4–14 weeks)	Milk, tissue (liver, adipose), blood	Milk (lipid) DNA, CpG methylation, RNA, GTT, histology (slides)	Ligand-induced PPARα activation triggers demethylation of Fgf21, thus facilitating its expression that persists and may affect obesity of later life	Culled to 5–6/ dam at PND 0	Yuan *et al.*[76] (2018)

AR, artificially reared; BP, blood pressure; C/H, control-HFCS diet; C/R, control dam-low protein dam; CH, control to HFD dam; CHO, carbohydrate; CR, energetic restriction; FGF21, fibroblast growth factor-21 gene; G, gestation period; GC, glucocorticoid; H/C, HFCS-control diet; HC, HFD to control dam; HFCS, high fructose corn syrup; HFD, high-fat diet; HH, HFD to HFD dam; HPA, hypothalamo–pituitary–adrenal axis; ia/ip/oGTT, intra-arterial/intraperitoneal/oral-GTT; L, lactation period; LL, large litter; MatSep, maternal separation; MR, maternally reared; NL, normal litter; P1, phase one; P2, phase two; PFC, pre-frontal cortex; PND, postnatal day; PPAR, peroxisome proliferator activator receptor; PPARα-KO, peroxisome proliferator activator receptor alfa – knock out; PTHrP, parathyroid hormone-related protein; R/C , low protein dam – control dam; R, protein-restricted dam; S/C, sucrose-control diet; S/S, sucrose–sucrose diet; SL, small litter; SL, small litter; SREBP-1c, sterol regulatory element binding protein-1c; T3, triiodothyronine; WAT , white adipose tissue; WPH, whey protein hydrolysate; WT, wild type.

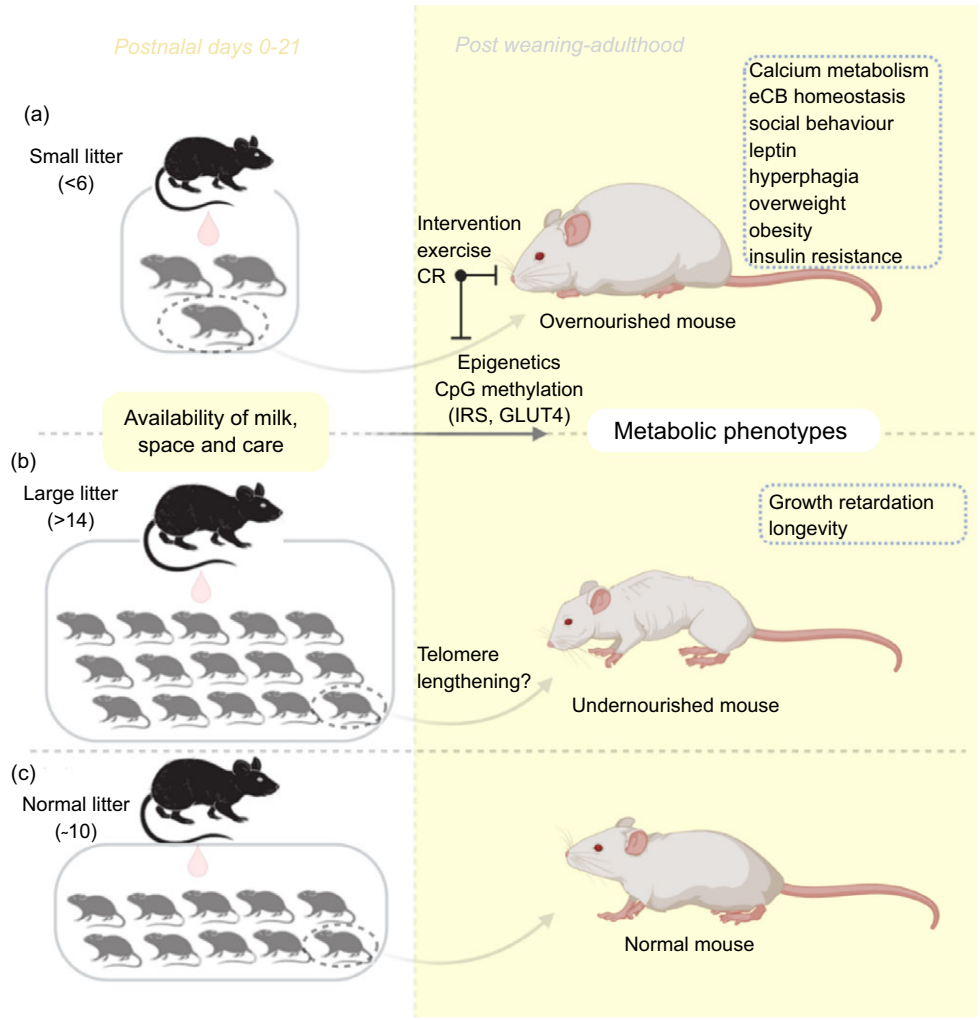

Fig. 1. An illustration of the litter size model useful for metabolic programming at early postnatal life. Adjustments in litter size (a) small or (b) large; determine milk availability and trigger the development of metabolic phenotypes with age. Using this model, several behaviours and phenotypes have been studied (trace boxes). CR, energetic restriction; eCB, endocannabinoid; GLUT4, glucose transporter 4; IRS, insulin receptor substrate.

alterations and even exercise on neonatal development[84,86,95]. Pups suckled by diabetic and obese mothers are also common (see Table 2). Using this approach, the nursing mother is influenced, and the response is sought in the milk fed offspring. For instance, the maternal postnatal undernutrition during suckling has been studied and is implicated in the programming of metabolic diseases via changes in milk composition[36,96]. Also, nutritional protocols such as the maternal low-protein diets have been developed[97] (see Fig. 2). These exposure of dams to several cues during lactation represents the commonest of models for usage in neonatal programming and like other models can be adopted singly or in combination[98]. With this model, it is common to quantitatively and qualitatively assess milk production. Consequently, several milk measurement strategies have been developed and adopted to determine the immediate effects of maternal disruptions to milk yield and content in mice and rats[36,66,67,70,71]. Some investigators also adopt milk collection from the pup stomach[99]. In addition, variabilities in milk volume have been documented, with decreased amounts being collected from inbred and primiparous compared with outbred and multiparous dams[67]. Consequently, we recommend that comparative group studies should use dams of same stock/strain and parity. Furthermore, aside the weight-suckle-weight method of quantifying milk output, a water turnover method has been established[100].

Models of altered lactational condition of the pup

Modifications in pup diet and other direct neonatal experiences can programme the development of adult metabolic phenotypes. Consequently, investigators commonly adopt the model of altered lactational condition of the pup to test for metabolic phenotypes[84,98,101,102] and examine prophylactic agents which may protect from adversities of later life[42,61,75,103,104]. Here we highlight models that employ diet, maternal separation, photic experience or cigarette smoking in the investigation of neonatal programming (see Table 3).

British Journal of Nutrition

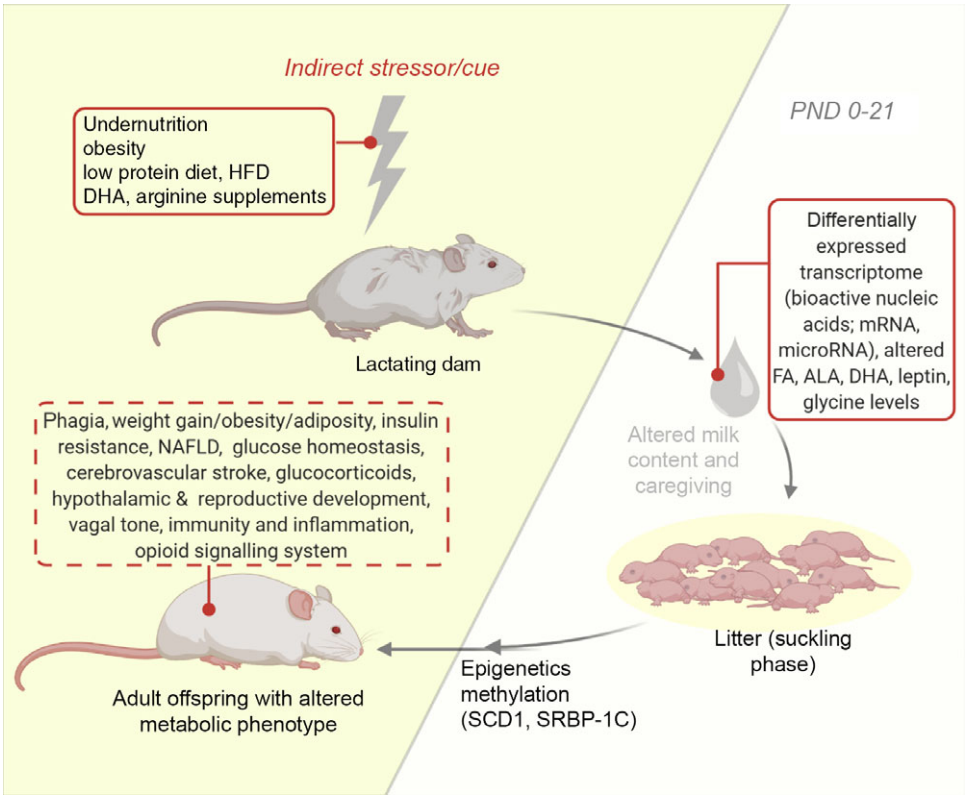

Fig. 2. An illustration of the model of altered lactational condition of the dam. Development of the offspring metabolic health following lactation is influenced by maternal exposure and health during lactation via notable changes in milk content (untraced boxes). Using this model, several behaviours and metabolic phenotypes have been studied (traced box). ALA, α-linolenic acid; DHA, docosahexaenoic acid; FA, fatty acid; HFD, high-fat diet; NAFLD, non-alcoholic fatty liver disease; PND, postnatal day; SCD1, stearoyl-CoA desaturase-1; SREBP-1C, sterol regulatory element-binding protein-1c.

Dietary inclusions

Altered diet models provide means for assessing the outcomes of several administrations or intakes – nutrients, toxins, food composition, energy – on premature gut development and metabolism. Though the mouse and rat widely differ from humans in bioavailability of nutrients, the differences on how they metabolise, utilise and dispose of nutrients are well documented and suited for research[105]. Consequently, the neonatal mice and rats have been adopted for varying dietary protocols. Additionally, due in part to their lack of emesis and increased intestinal permeability of pups as compared with adults[49], we are able to examine the influence of several dietary inclusions on neonatal development and adult metabolic health[5].

Dietary inclusion as a model of altered lactational condition of the pup may involve either maternally or artificially reared pups. The dietary inclusions may be supplementations to maternal suckling or synthesised substitutes for artificial rearing. Unlike the altered litter size models, a precise control of neonatal nutrition in terms of composition or amount can be achieved by artificial rearing. Also, aside gavage techniques, commonly adopted for supplementing littermate (maternally reared, MR) pups, intragastric feeding tubes are employed when administering rodent milk substitutes (RMS) to artificially reared pups. Feeding procedures include the 'pup in a cup' technique as established by Hall[68] for artificially reared pups and a hand-feeding technique first described by Hoshiba[69] for MR pups. Using comparable RMS formula to natural milk composition, artificially reared pups have been proven to exhibit non-distinct growth and development from MR pups[65,68]. Some investigators of neonatal programming limit dietary exposure to the PND 14 based on the fact that pups gain sight and begin to forage/nibble upon ambient substances aside the dams' milk, i.e. pelleting[62,102,104]. However, others extend the period of exposure to PND 21 (weaning) as all the pups are still exposed to the same confounding variables[54,75,80,106]. Some others even extend further (PND 28) [107].

Rodent milk substitute. Unlike maternal rearing, the artificial rearing of pups requires alternatives to the natural milk. The common alternative regimes use synthetic modifications that are comparable with the natural rodent milk composition and are termed RMS. RMS are chemically derived from diary or non-diary formula under aseptic conditions for the artificial rearing of rat[108–110] and mice pups[111]. So far, RMS include several diet models: high-carbohydrate diet model, low-protein diet[97] and high-fat diary diet model[92]. Some of these diets, like the high-fat diary diet, have proven useful in investigating ameliorants of metabolic phenotypes[75], as well as protective strategies[102]. Notably, modifications to enrich or deplete specific composition(s) may be made to a RMS in order to study specific interventions[112].

British Journal of Nutrition

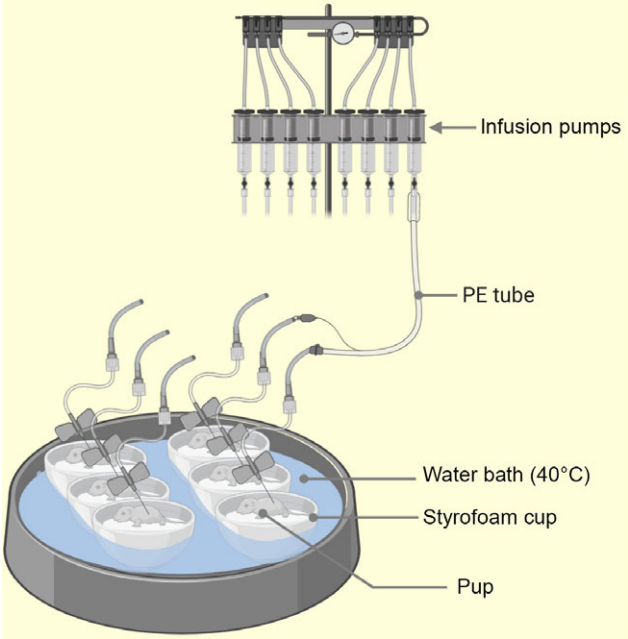

Fig. 3. Illustration of the pup-in-a-cup model used for breeding artificially reared (AR) pups. The pups in warm moist incubators (floating Styrofoam cups insulate the pups from direct contact with heat source, i.e. water bath) water at 40˚C from 2 to 13 postnatal day (PND) to keep pup axillary temp at 32–36˚C. The intragastric cannula (PE leads) emerge from the lids of the cups (not shown) and pass across to syringe mounted infusion pumps[68]. PE, polyethylene.

The pup in a cup; a technique for artificial rearing. The rat 'pup' in a 'cup' technique developed in 1975 by Hall[68] involves the transfer of few day old rat pups into several styrofoam cups floating on a thermo-regulated water bath[5]. A miniaturised intragastric feeding cannula and an incubator housing arrangement are the two major components of this technique[68] (see Fig. 3). The introduction of intragastric feeding cannula (a polyethylene tubing) for mouse by Beierle *et al.*[65] opened doors to the permissive usage of nutritional manipulations with a mouse pup in a cup. Thus, pup in a cup method is currently useful for both rats and mice and has proven helpful with rearing transgenic rodents[5]. The insertion of intragastric cannulas employ a surgical technique for mice[65] and nonsurgical technique for rats[68]. A useful comparison of these techniques is found in the paper by Patel *et al.*[110]. However, such neonatal handling methods reportedly produce reproductive deficits[83]. Further descriptions of artificial rearing and the pup in a cup, and their usefulness to nutritional research, has also been provided by Patel *et al.*[110] and Patel and Hiremagalur[113]. Using artificial rearing, several research works have examined the immediate metabolic outcomes of neonatal nutrition in mice – body weight, immunomodulation[114], – and rats – hyperinsulinaemia and glucose metabolism[115,116]. Also, certain long-term metabolic consequences of specific neonatal nutrition have been established for mice – cardiovascular[117] – and rats – hyperinsulinaemia and adult-onset obesity[39,118], glucose and insulin homoeostasis[119] and leptin homoeostasis[120].

Hand-feeding technique for maternal rearing. Rodents are omnivores and several commercial diets are available for their intake[121]. Oral gavage represents the classical involuntary and most common way of administering liquid compounds to conscious pups. Of the different types of gavage needles (curved or straight, flexible or rigid), we recommend the use of the curved flexible kind due to lower risks of oesophageal damage and since they cannot be chewed by the pups (see Fig. 4). Also in use is a hand-feeding technique first described by Hoshiba[69]. This uses a surrogate nipple for artificially reared mouse pups, which despite tediousness and time devotion, enables post-gravid study of pups and prevents physical injuries. Several nipple sizes have been described for mice and rats[69]. With this method, mouse pups receive calibrated amounts of nutrients with relatively reduced chances of physical trauma as inherent in the native gavage method of larger rats[122].

Maternal separation

Some studies have adopted maternal separation (MatSep) as a model for simulating neglect and predisposing to metabolic disorders[102]. The suckling pups are typically separated from the dam for 2–8 h daily across several days[83]. A similar procedure to MatSep called 'neonatal handling' involves lesser timed separations of pup or litter from the dam and has been described elsewhere[123]. Furthermore, with MatSep, the outcomes appear to be more prominent with inbred rat strains than outbred stocks. Lastly, when conducting MatSep, it is important to consider the age at initiation, short (<24 h) or long term.

Other models of altered pup condition; photic experience and smoking

Though uncommon, pup exposure to light has been investigated and shown to account for phenotypic changes associated with circadian rhythm such as related to astrocytes[60]. The effect of cigarette smoking has also been studied on pup development[124].

Cross-foster models

Cross-fostering involves the transfer of nursing pups from control dams to either metabolically compromised, stressor exposed or even healthy dams during the period of lactation. This is illustrated in Fig. 5. It is useful in the nutritional, hormonal or behavioural investigation of metabolic phenotypes[125]. This model is most advantageous in delineating the outcomes of neonatal/lactational programming from gestational programming[35].

Though sometimes useful as an adversity in itself[125], cross-fostering of offspring from metabolically compromised dams (e.g. obese dams) to normal dams has reportedly mitigated the adverse effects of programming[35,126]. Conversely, cross-fostering of pups from a healthy to a malprogrammed dam (such as protein restricted, diabetic dams) triggers metabolic disruptions of adult life[74,127]. When cross fostering, investigators avoid biased maternal care that may result from co-housing of biological and fostered pups. This is by ensuring none of the new born animals remains together with the biological dam[79]. Furthermore, the systematic cross-fostering of pups involves the distribution of pups across dams of the same status and has been adopted in culling pups[128]. Following cross-fostering,

British Journal of Nutrition

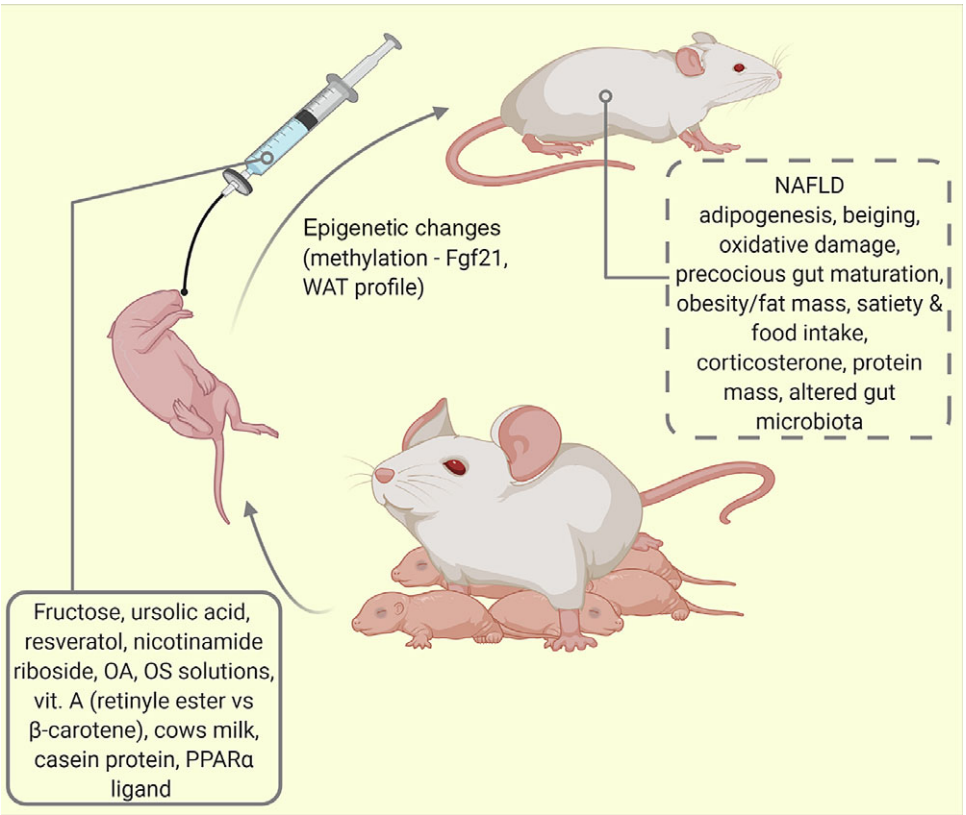

Fig. 4. Oral supplementation/intermittent feeding of maternally reared (MR) pups. Using this model of altered lactational condition of the pup, several behaviours and phenotypes have been studied (traced box). Fgf21 = fibroblast growth factor-21; NAFLD, non-alcoholic fatty liver disease; OA, oleanolic acid; OS, oligosaccharide; PPAR-α, peroxisome proliferator activated protein -alpha; WAT, white adipose tissue.

the pup/dam cages are sometimes maintained for an extended period (e.g. PND 35) to account for natural weaning that may be longer than standard weaning at PND 21[126]. Additionally, this model has also been adopted alongside other models such as the genetic model[41], models of altered pup condition – MatSep[129] (see Table 4).

Genetic models

More so in mice than rats, rodents are resourceful models in the study of the genetic aetiology of some complex human diseases[38]. Using potent advances in reproductive and genetic technology, researchers have been able to understand and dissect the molecular basis behind certain metabolic conditions[130]. Genetically modified rodents could be modelled for diseases (resistant or predisposed to the condition) or for identifying/validating new drugs. They include inbred and hybrid (F1 and F2) strains, most of which are products of mutations, transgenic modifications (gain) or gene inactivation (loss of function)[130,131]. These models are powerful tools that give room to tease apart the influence of metabolic primers or other selected variables on neonatal development (see Table 4).

Genetic models may serve several roles in neonatal programming. First, as a means of examining the effects of wet nursing in cross-fostering paradigms. Modified rodents are introduced as nursing dams to suckle pups cross-fostered from a control lineage. As like other models, Table 5 shows the combinational utility of this model alongside others. Using such genetic models in combination with cross-fostering, Reifsnyder and co-workers have been able to demonstrate that the maternal environmental influence is due to early obesity-inducing factors present in the milk of obese dams[132]. In this scenario, the rodents may be engineered to possess a known metabolic disorder. Such studies are usually aimed at elucidating the independent influences the malprogrammed dam has on the suckling pup. In another usage, the offspring are genetically predisposed to or even exempted from a disorder[76], while testing for the therapeutic impacts that neonatal interventions have on the inheritance and/or development of the adult phenotype, that is, they serve as control subjects in neonatal programming[76].

Following the development of the first knockout mouse in 1987[133], and the first knockout rat in 2009[134], genetic models are gradually permeating investigations in neonatal programming. Interestingly, genetically engineered mice have found use in the development of milk substitutes[34] and aid the study of genes in lactational programming[135]. An example of genetically engineered mice used for neonatal metabolic programming include C57B/6J, New Zealand obese mouse pups (a genetic animal model for obesity and type 2 diabetes)[132]. Examples of genetically modified rats used for neonatal metabolic programming include ZDF (Zucker rat), GK (Goto-Zakiki rat) – for type 2 diabetes mellitus and Otsuka Long

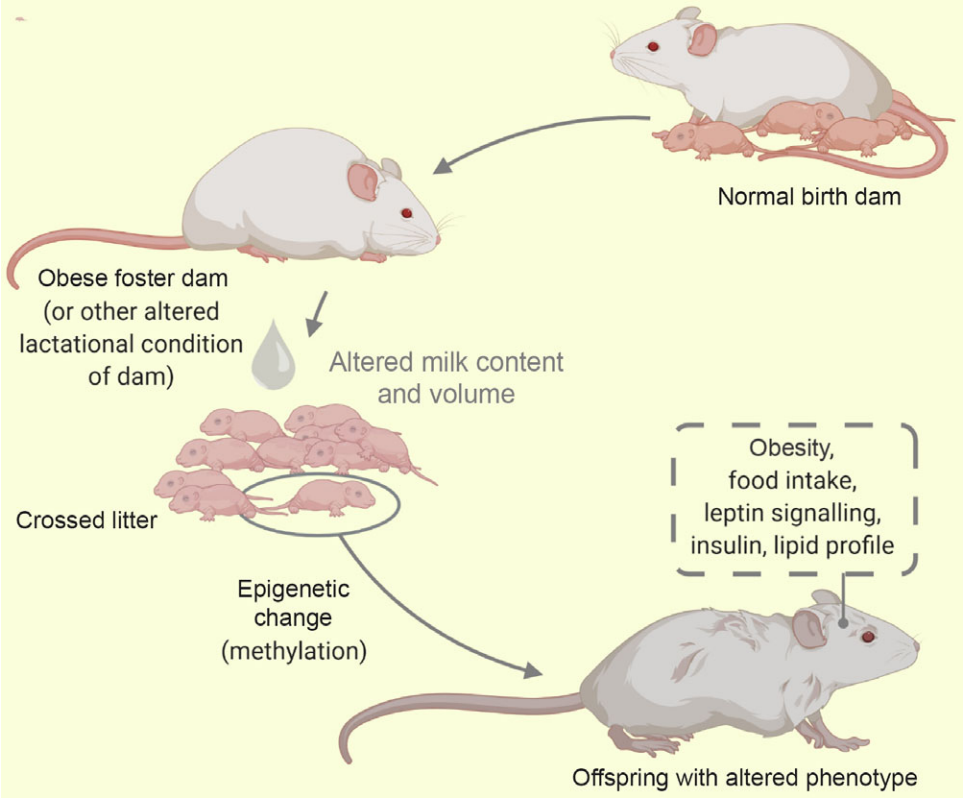

Fig. 5. A cross-foster of litter from a healthy dam to an obese dam. Using the cross-foster model some behaviour and phenotypes have been studied (traced box). Alterations in milk composition influences the offspring metabolic phenotype via epigenetics.

Evans Tokushima Fatty (OLETF) rats (diabetes, obesity and CCK_1 receptor)[41].

Considerations when selecting altricial rodents for neonatal programming

Despite the extensive degree of investigations that can be conducted using pups even of inbred status, there remains some potential to derail an experiment, creating bias that hinder the initial focus of experimentation. These biases could arise from methodology chosen and are prevented when pertinent caveats and certain factors such as history, physiology, identification, sample collection, sexual dimorphism and breeding are taken into account. When selecting suitable models that suit research objectives, these principal considerations must be made.

Historical profile

Thorough knowledge of the rodent history is crucial for research. Perhaps, a vital criterion for the examination of an accurate pup phenotype is to obtain a healthy parent. It is important to consider the genetic background of the dam and fetal environment of the pup. Through the right enquiry, the source of the animal, age, stock or strain and breeding profile (previous environment) could be ascertained[136]. Furthermore, it is important to enquire about housing conditions and feeding/drinking paradigms (diet type – for open-formula diet, diet source and water source/supply method) of laboratory rodents to avoid interference with research methods particularly with neophobic strains. An indication of rodent history and origin is primary to the replicability of a research. In addition, during procurement, we recommend that the rodent undergoes relative physical examination for diseases (general and zoonotic).

Rodent physiology

The laboratory mouse and rat may sometimes exhibit coprophagy and cannibalism[136], which affects the outcome of research. These we believe arise when dams are stressed. Consequently, care and close monitoring must be adopted to reduce stress. Noteworthy, rodent's eyes are exophthalmic and bear a periorbital harderian gland for the secretion of porphyrin, predominantly when stressed[38]. This secretion gives tears a reddish tinge which could be mistaken for bleeding. Though rodents lack sweat glands and emetic centre, they possess scent glands[38], which hypothetically could detect foreign cues on pups post-handling and may trigger cannibalism via an unknown mechanism. Furthermore, Table 6 highlights some variable physiologic parameters of rats and mice. These may show deviations based on rodent source, stock or strain.

Sexing of the pups

Though other methods exist, sexing of the pups is commonly done by comparing the anogenital distance of male and female littermates, as shown in Fig. 6. This distance is longer in males[137]. Also, pre-weaned male pups have undescended

Table 6. Some physiological parameters to be noted for neonatal programming[11,38,153]

Feature	Rat	Mouse
Length of gestation (days)	21–23	19–21
Lactation/suckling period (days)	0–21	0–21
Natural litter size	8–14	4–12
Life expectancy (years)	2·5–3	1–3
Birth weight (g)	5–6	1–1·5
Weaning weight male/female (g)	55–90/45–80	18–25/16–25
Pelleting (solid diet beginning) (days)	12	10
Puberty male/female (weeks)	6/6–8	4–6/5
Eyes open (days)	10–13	12–14

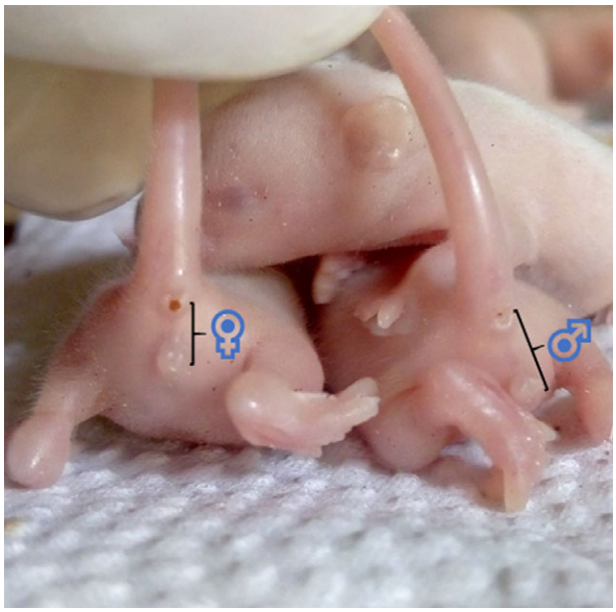

Fig. 6. Showing the anogenital distance of 7 d old Swiss mice (littermates). ♀, anogenital distance of a female pup ≈ 2·5 cm. ♂, anogenital distance of a male pup ≈ 4 cm.

testes, while the females have rudimentary mammae (appearing at PND 8–9) and an occluded vaginal opening[82,138].

Sexual differences (sexual dimorphism)

Several studies of metabolic programming have demonstrated that same stimulus elicits variable long-term effects based on the sex of the rodent offspring[3,80,101,124]. The molecular mechanisms behind this dimorphism are not well understood. However, this unavoidable sexual differences appear long before embryo implantation, gonadal development, sex hormone differentiation or even programming[19]. This could be a result of unidentical sex chromosomes (i.e. Y and X chromosomal genes), which give rise to dissimilar transcripts whose expression can also alter the transcription of autosomal genes (in a process of genetic imprinting: in which only a paternal or maternal copy of an allele is exclusively expressed), thus amplifying the sexual difference[139]. Possibly, the early differential expression of proteins influences molecular pathways (glucose and protein metabolism) and impacts on epigenetic mechanisms (especially DNA methylation). The result is a sex-based difference with susceptibility to environmental cues leading to distinct outcomes in adult health independent of neonatal programming. Consequently, a number of outcomes have been attributed to sexual dimorphism. For instance, though runts are rare, male pups are slightly heavier than female littermates and maternal behaviour has been shown to also vary with the sex of the pup. Due to such dimorphisms, several studies show sex preference for male than female pups[73,75,95,140]. However, sexual bias should be used only with genuine justifications, as new discoveries could be masked by sex.

In altered diet models of low protein, researchers tend to use male pups due to sexual differences in insulin levels and glucose tolerance that have been observed in earlier studies[95]. Others prefer male rodents due to susceptibility to changes in adiposity and body weight in the context of metabolic programming[59]. Conversely, some articles specific for either male or female sex fail to report appropriate justification for usage[32]. This raises serious concerns, as no satisfactory background is laid. Consequently, single sex research should carry appropriate justification for usage.

Handling of the pups

When investigators handle pups, the goal is to accomplish the ideal task with the least amount of restraint to avoid agitation, reduce stress and avoid experimental variations by relaxed handling. It is also important that appropriate personal protective equipment's are used to minimise exposure to hazardous agents and allergens. Unlike adult rodents, the neonates are rather fragile and a major concern when handling is the contact which may incite cannibalism by the dam[123]. Also, males and females are housed separately after weaning to avoid aggression and dominance[101].

Identification of the pups

Besides the use of cage and nontoxic tail colour tags for identifying pup groups[43,104], researchers also adopt toe clipping and tattooing. Toe clipping represents the removal of digits for identification and genotyping[34]. It is useful for identifying genetically modified mice at the suckling stage. Though, perceived to be painful for routine usage, toe clipping has proven to be a fast, safe and easy method. Along established protocols, short- or long-term effects of toe clipping are shown to be completely avoidable[64]. Manual and micro-tattoo systems create tattoo markers on the tail, toes and footpads of pups to aid identification. Electronic microchip devices are also available[121]. Pup identification is important for determining the origins of unexpected breeding errors.

Sample collection

Several methods of neonatal euthanasia have been described[141–143]. Acceptable techniques include the injection of chemical anaesthetic, cervical dislocation and decapitation[144]. Euthanasia is done at the same period of the day to avoid the circadian influence on metabolic outcomes[74]. Some articles fail to clearly highlight the samples obtained after

euthanasia[87,106], which is detrimental to research reporting. Furthermore, the collection of specimens such as blood, milk (from pup stomach), is unavoidable parts of most developmental research. Consequently, given that neonatal rats and mice are perceptive to pain, suitable procedures that initiate the least possible harm are encouraged.

Blood represents a vast repertoire of developmental parameters and is a window to the physiological status of the neonate. Considerations regarding the method of blood collection include anaesthesia to be used and impact of the assessment to be conducted[121]. Given the difficulty associated with serial blood sample collection, particularly of mice, decapitation is most commonly adopted[74,75,98]. However, cardiac puncture has been specially adopted for collecting blood samples from neonatal rats by Grazer[145]. Notably, though neonates metabolise drugs slowly, they display relative resistance to hypoxia[144]. Thus, for anaesthesia, age represents another consideration because neonatal rodents show relatively greater resistance to CO_2 compared with adults. Such resistance causes delayed time of death (sometimes 50 min at PND0 for mice), which gradually decreases with increasing age; 3 min decrease per day between PND 0–10 for rats[142,143]. Consequently, moderate considerations must be made when euthanising the suckling mice or rats with CO_2. In addition, to confirm death following anaesthesia, secondary techniques such as bilateral pneumothorax and immersion in liquid nitrogen (for hairless neonates) may be used[144]. The weight and genetic background (stocks and strains) of neonatal rodents are also important considerations when euthanising[142–144].

Breeding

To facilitate timed and synchronised births, prime considerations are made about the reproduction of pups from parental rodents. Knowledge of how to identify and control rodent mating, gestation and parturition is priceless in this pursuit. For the selection of a mating regime, it is advisable to consider three conditions of the dams: the Whitten effect (synchronised oestrus when a new male is added), the Bruce effect (delayed embryo implantation when a new male is added) and the Lee-Boot effect (absent oestrus when females are caged together). All three conditions are more pronounced with mice, while the Whitten effect is completely lacking in rats[137]. In addition, due to their prolific nature, these rodents exhibit fertile postpartum oestrus and special assistance is needed to curb overpopulation. Notably, normal gestation that lasts about 21 days is extended by suckling. Also, under the right conditions, a pair of housed mice can generate a progeny of approximately 50,000 members in 1 year[136].

Conclusions

An appropriate understanding of the differences that lie in model usage and protocols is gainfully essential. The five models recognised in this review are useful to varying degrees for the investigation of neonatal programming and several variabilities accompanying their usage have been identified. They may be used singly or in combination, and the metabolic outcomes from one model may vary from that of another. For instance, neonatal programming for adult-onset obesity had been shown to be reversible, by Liu *et al.*[40] and not reversible by energy restriction by Srinivasan *et al.*[146] Explanation for these opposing findings may lie in the models adopted as well as different levels of caloric restriction employed in these two studies. While Liu *et al.*[40] adopted the 'altered litter size model', Srinivasan *et al.*[146] adopted the 'model of altered pup diet', both arriving at scientifically acceptable results. That the animal models differ but prime for similar metabolic outcomes such as obesity, advocates for improved mechanistic evaluation to elucidate outcome differences. Also, choosing the right model for research should be accompanied with relevant considerations which when clearly stated aid the reproducibility of an investigation and allow for the interpretation of comparative reports and experimental variability. Furthermore, it is evident that the rat and mouse are excellent models for investigations focused at understanding the metabolic consequences of genetics, epigenetics, nutrient availability, natural lactation and/or substituted suckling. Thus, for example, given that prematurity is associated with neonatal programming[19], studies using these models could help in developing optimal nutritional strategy for preterm mammals. Overall, these models are useful for studying the pathogenesis of metabolic disorders and developing strategies for their prevention and treatment.

Statements of Significance

According to DOHaD (developmental origins of adult health and disease), the neonatal period is a critically responsive time to cues that may trigger adaptation of the metabolic phenotype.Rodent-based investigations remain the predominant means of studying the results of this 'neonatal programming' on metabolic health.Here we establish, for the first time, five approach-based models of rodents useful to neonatal programming.Novel considerations to guide the design and conduct of newer experiments are also highlighted.This will provide background and guide to biomedical and nutritional researchers focused on the aetiology, mechanisms, prevention and treatment of metabolic disorders.

Acknowledgements

We appreciate the intellectual support and resource provided by staff and postgraduate members of the Centre for Advanced Medical Research and Training (CAMRET), Usmanu Danfodiyo University, Sokoto, Nigeria. D. U. acknowledges the postgraduate scholarship awarded to him (CAMRET/2019/MSc/SCH003) by CAMRET.

No specific funding was received to support this work.

K. G. I. conceptualised the review. D. U.: composed the outline, drafted the manuscript and drew the figures. K. G. I., M. B. B., I. M., B. A., M. B. A. and M. U. I.: edited drafts and contributed to the organisation of the manuscript. K. G. I., M. B. A. and M. U. I.: validated the review and revised the manuscript. The manuscript was written through contributions of all authors. All authors have read and approved the final manuscript. Figures 1–5 were created using Biorender.

The authors have no relationship with industry and no potential financial conflicts of interest relevant to the submitted manuscript.

References

1. Saklayen M (2018) The global epidemic of the metabolic syndrome. *Curr Hypertens Rep* **20**, 1–8.
2. Hernández-Granados MJ, Ramírez-Emiliano J & Franco-Robles E (2018) Rodent models of obesity and diabetes. In *Experimental Animal Models of Human Diseases – An Effective Therapeutic Strategy*, pp. 105–121 [B Ibeh, editor]. London: IntechOpen. www.doi.org/10.5772/intechopen.74595
3. George G, Draycott SAV, Muir R, *et al.* (2019) Exposure to maternal obesity during suckling outweighs in utero exposure in programming for post-weaning adiposity and insulin resistance in rats. *Sci Rep* **9**, 10134.
4. Vithayathil MA, Gugusheff JR, Ong ZY, *et al.* (2018) Exposure to maternal cafeteria diets during the suckling period has greater effects on fat deposition and Sterol Regulatory Element Binding Protein-1c (SREBP-1c) gene expression in rodent offspring compared to exposure before birth. *Nutr Metab* **15**, 17.
5. Puimana P & Stolla B (2008) Animal models to study neonatal nutrition in humans. *Curr Opin Clin Nutr Metab Care* **11**, 601–606.
6. Cheng Z, Zheng L & Almeida FA (2018) Epigenetic reprogramming in metabolic disorders: nutritional factors and beyond. *J Nutr Biochem* **54**, 1–10.
7. Panchal SK & Brown L (2011) Rodent models for metabolic syndrome research. *J Biomed Biotechnol* 1–14. https://doi.org/10.1155/2011/351982
8. Wong SK, Chin K, Suhaimi FH, *et al.* (2016) Animal models of metabolic syndrome: a review. *Nutr Metab* **13**, 1–12.
9. Alquier T & Poitout V (2018) Considerations and guidelines for mouse metabolic phenotyping in diabetes research. *Diabetologia* **61**, 526–538.
10. Flamm EG (2013) Neonatal animal testing paradigms and their suitability for testing infant formula. *Toxicol Mech Meth* **23**, 57–67.
11. Pérez-Cano FJ, Franch À, Castellote C, *et al.* (2012) The suckling rat as a model for immunonutrition studies in early life. *Clin Dev Immunol* **2012**, 537310.
12. McClain RM, Keller D, Casciano D, *et al.* (2001) Neonatal mouse model: review of methods and results. *Toxicol Pathol* **29**, 128–137.
13. Barker DJ & Osmond C (1986) Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet* **1**, 1077–1081.
14. Calderari S, Gangnerau M, Meile M, *et al.* (2006) Is defective pancreatic beta-cell mass environmentally programmed in Goto-Kakizaki rat model of type 2 diabetes? Insights from crossbreeding studies during suckling period. *Pancreas* **33**, 412–417.
15. Hales CN & Barker DJ (2001) The thrifty phenotype hypothesis. *Br Med Bull* **60**, 5–20.
16. Koletzko B (2005) Developmental origins of adult disease: barker's or Dorner's hypothesis? *Am J Hum Biol* **17**, 381–382.
17. Armitage JA, Taylor PD & Poston L (2005) Experimental models of developmental programming: consequences of exposure to an energy rich diet during development. *J Physiol* **565**, 3–8.
18. Mandy M & Nyirenda M (2018) Developmental origins of health and disease: the relevance to developing nations. *Int Health* **10**, 66–70.
19. Reichetzeder C, Dwi Putra SE, Li J, *et al.* (2016) Developmental origins of disease – crisis precipitates change. *Cell Physiol Biochem* **39**, 919–938.
20. Xu Y, Wang W, Chen M, *et al.* (2019) Developmental programming of obesity by maternal exposure to concentrated ambient PM2.5 is maternally transmitted into the third generation in a mouse model. *Part Fibre Toxicol* **16**, 27.
21. Benyshek DC, Johnston CS & Martin JF (2006) Glucose metabolism is altered in the adequately – nourished grand – offspring (F3 generation) of rats malnourished during gestation and perinatal life. *Diabetologia* **49**, 1117–1119.
22. Aiken CE, Tarry-Adkins JL & Ozanne SE (2016) Transgenerational effects of maternal diet on metabolic and reproductive ageing. *Mamm Genome* **27**, 430–439.
23. Kramer MS & Kakuma R (2012) Optimal duration of exclusive breastfeeding. *Cochrane Database Syst Rev* **2012**, Cd003517.
24. Barrow PC, Barbellion S & Stadler J (2011) Preclinical evaluation of juvenile toxicity. *Methods Mol Biol* **691**, 17–35.
25. Picut C, Dixon D, Simons M, *et al.* (2014) Postnatal ovary development in the rat. *Toxicol Pathol* **43**, 343–353.
26. Fall CHD & Kumaran K (2019) Metabolic programming in early life in humans. *Philos Trans R Soc Lond B Biol Sci* **374**, 20180123.
27. Goyal D, Limesand SW & Goyal R (2019) Epigenetic responses and the developmental origins of health and disease. *J Endocrinol* **242**, T105–T119.
28. Plagemann A, Roepke K, Harder T, *et al.* (2010) Epigenetic malprogramming of the insulin receptor promoter due to developmental overfeeding. *J Pe&x0301;rinat Med* **38**, 393–400.
29. Lillycrop KA & Burdge GC (2012) Epigenetic mechanisms linking early nutrition to long term health. *Best Pract Res Clin Endocrinol Metab* **26**, 667–676.
30. Wells JC (2007) Flaws in the theory of predictive adaptive responses. *Trend Endocrinol Metab* **18**, 331–337.
31. Bateson P, Gluckman P & Hanson M (2014) The biology of developmental plasticity and the predictive adaptive response hypothesis. *J Physiol* **592**, 2357–2368.
32. Fante TD, Simino LA, Reginato A, *et al.* (2016) Diet-induced maternal obesity alters insulin signalling in male mice offspring rechallenged with a high-fat diet in adulthood. *PLoS One* **11**, e0160184.
33. Gomes RM, Bueno FG, Schamber CR, *et al.* (2018) Maternal diet-induced obesity during suckling period programs offspring obese phenotype and hypothalamic leptin/insulin resistance. *J Nutr Biochem* **61**, 24–32.
34. Kao BT, DePeters EJ & Van Eenennaam AL (2006) Mice raised on milk transgenically enriched with *n*-3 pufa have increased brain docosahexaenoic acid. *Lipids* **41**, 543–549.
35. Miranda RA, da Silva Franco CC, de Oliveira JC, *et al.* (2016) Cross-fostering reduces obesity induced by early exposure to monosodium glutamate in male rats. *Endocrine* **55**, 101–112.
36. Wattez JS, Delmont A, Bouvet M, *et al.* (2015) Maternal perinatal undernutrition modifies lactose and serotranferrin in milk: relevance to the programming of metabolic diseases? *Am J Physiol Endocrinol Metab* **308**, E393–401.
37. Lewis AJ, Galbally M, Gannon T, *et al.* (2014) Early life programming as a target for prevention of child and adolescent mental disorders. *BMC Med* **12**, 33.
38. Otto GM, Franklin CL & Clifford CB (2015) Biology and diseases of rats. In *Laboratory Animal Medicine*, pp. 151–207 [J Fox, L Anderson, G Otto, *et al.* editors]. Amsterdam,

Netherlands: Elsevier Inc. https://doi.org/10.1016/B978-0-12-409527-4.00004-3
39. Aalinkeel R, Srinivasan M, Song F, *et al.* (2001) Programming into adulthood of islet adaptations induced by early nutritional intervention in the rat. *Am J Physiol Endocrinol Metab* **281**, E640–E648.
40. Liu HW, Srinivasan M, Mahmood S, *et al.* (2013) Adult-onset obesity induced by early life overnutrition could be reversed by moderate caloric restriction. *Am J Physiol Endocrinol Metab* **305**, E785–E794.
41. Schroeder M, Shbiro L, Moran TH, *et al.* (2010) Maternal environmental contribution to adult sensitivity and resistance to obesity in Long Evans rats. *PLoS One* **5**, e13825.
42. Ibrahim KG, Chivandi E, Mojiminiyi FBO, *et al.* (2017) The response of male and female rats to a high-fructose diet during adolescence following early administration of Hibiscus sabdariffa aqueous calyx extracts. *J Dev Orig Health Dis* **8**, 628–637.
43. Nyakudya TT, Mukwevho E, Nkomozepi P, *et al.* (2018) Neonatal intake of oleanolic acid attenuates the subsequent development of high fructose diet-induced non-alcoholic fatty liver disease in rats. *J Dev Orig Health Dis* **9**, 500–510.
44. Gibbs RA, Weinstock GM, Metzker ML, *et al.* (2004) Genome sequence of the Brown Norway rat yields insights into mammalian evolution. *Nature* **428**, 493–521.
45. Waterston RH, Lindblad-Toh K, Birney E, *et al.* (2002) Initial sequencing and comparative analysis of the mouse genome. *Nature* **420**, 520–562.
46. Vandamme TF (2014) Use of rodents as models of human diseases. *J Pharm Bioallied Sci* **6**, 2–9.
47. Aïn S, Belin L, Schaal B, *et al.* (2013) How does a newly born mouse get to the nipple? Odor substrates eliciting first nipple grasping and sucking responses. *Dev Psychobiol* **55**, 888–901.
48. Teichberg S, Wapnir RA, Moyse J, *et al.* (1992) Development of the neonatal rat small intestinal barrier to nonspecific macromolecular absorption. II. Role of dietary corticosterone. *Pediatr Res* **32**, 50–57.
49. Xu H, Collins J & Ghishan F (2012) Molecular physiology of gastrointestinal function during development. *Physiol Gastrointestinal Tract* **1**, 415–449.
50. Cohen MB, Guarino A, Shukla R, *et al.* (1988) Age-related differences in receptors for Escherichia coli heat-stable enterotoxin in the small and large intestine of children. *Gastroenterology* **94**, 367–373.
51. Cohen MB, Moyer MS, Luttrell M, *et al.* (1986) The immature rat small intestine exhibits an increased sensitivity and response to Escherichia coli heat-stable enterotoxin. *Pediatr Res* **20**, 555–560.
52. Seo JK, Chu SH & Walker WA (1989) Development of intestinal host defense: an increased sensitivity in the adenylate cyclase response to cholera toxin in suckling rats. *Pediatr Res* **25**, 225–227.
53. Swenson ES, Mann EA, Jump ML, *et al.* (1996) The guanylin/STa receptor is expressed in crypts and apical epithelium throughout the mouse intestine. *Biochem Biophys Res Commun* **225**, 1009–1014.
54. Ibrahim KG, Wright HL, Chivandi E, *et al.* (2019) The potential developmental programming effect of oral curcumin on the bone health and plasma total osteocalcin of male and female rats fed a high-fructose diet during suckling and post weaning. *Gen Physiol Biophys* **38**, 435–444.
55. Toop CR, Muhlhausler BS, O'Dea K, *et al.* (2017) Impact of perinatal exposure to sucrose or high fructose corn syrup (HFCS-55) on adiposity and hepatic lipid composition in rat offspring. *J Physiol* **595**, 4379–4398.
56. Antonowicz I & Lebenthal E (1977) Developmental pattern of small intestinal enterokinase and disaccharidase activities in the human fetus. *Gastroenterology* **72**, 1299–1303.
57. Boudreau F, Rings EH, van Wering HM, *et al.* (2002) Hepatocyte nuclear factor-1 α, GATA-4, and caudal related homeodomain protein Cdx2 interact functionally to modulate intestinal gene transcription. Implication for the developmental regulation of the sucrase-isomaltase gene. *J Biol Chem* **277**, 31909–31917.
58. Ménard D & Pothier P (1987) Differential distribution of digestive enzymes in isolated epithelial cells from developing human fetal small intestine and colon. *J Pediatr Gastroenterol Nutr* **6**, 509–516.
59. Butruille L, Marousez L, Pourpe C, *et al.* (2019) Maternal high-fat diet during suckling programs visceral adiposity and epigenetic regulation of adipose tissue stearoyl-CoA desaturase-1 in offspring. *Int J Obes* **43**, 2381–2393.
60. Canal MM, Mohammed NM & Rodriguez JJ (2009) Early programming of astrocyte organization in the mouse suprachiasmatic nuclei by light. *Chronobiol Int* **26**, 1545–1558.
61. Gumede NM, Lembede BW, Nkomozepi P, *et al.* (2020) β-Sitosterol mitigates the development of high-fructose diet-induced nonalcoholic fatty liver disease in growing male Sprague-Dawley rats. *Can J Physiol Pharmacol* **98**, 44–50.
62. Le Dréan G, Pocheron AL, Billard H, *et al.* (2019) Neonatal consumption of oligosaccharides greatly increases l-cell density without significant consequence for adult eating behavior. *Nutrients* **11**, 1967.
63. Steegenga WT, Mischke M, Lute C, *et al.* (2017) Maternal exposure to a Western-style diet causes differences in intestinal microbiota composition and gene expression of suckling mouse pups. *Mol Nutr Food Res* **61**, 1600141.
64. Paluch L-R, Lieggi CC, Dumont M, *et al.* (2014) Developmental and behavioral effects of toe clipping on neonatal and preweanling mice with and without vapocoolant anesthesia. *J Am Assoc Lab Anim Sci* **53**, 132–140.
65. Beierle EA, Chen MK, Hartwich JE, *et al.* (2004) Artificial rearing of mouse pups: development of a mouse pup in a cup model. *Pediatr Res* **56**, 250–255.
66. DePeters E & Hovey R (2009) Methods for collecting milk from mice. *J Mammary Gland Biol Neoplasia* **14**, 397–400.
67. Gómez-Gallego C & Ilo T, Jaakkola UM, *et al.* (2014) A method to collect high volumes of milk from mice (mus musculus). *Anales Veterinaria Murcia* **29**, 55–61.
68. Hall WJ (1975) Weaning and growth of artificially reared rats. *Science* **190**, 133–1315.
69. Hoshiba J (2004) Method for hand-feeding mouse pups with nursing bottles. *Contemp Topics* **43**, 50–53.
70. Paul HA, Hallam MC & Reimer RA (2015) Milk collection in the rat using capillary tubes and estimation of milk fat content by creamatocrit. *J Visualized Exp: JoVE* **106**, e53476.
71. Willingham K, McNulty E, Anderson K, *et al.* (2014) Milk collection methods for mice and Reeves' muntjac deer. *J Visualized Exp: JoVE* **89**, 51007.
72. Marcella M, Groothuis GMM & Kanter R (2007) Species differences between mouse, rat, dog, monkey and human CYPmediated drug metabolism, inhibition and induction. *Expert Opin Drug Metab Toxicol* **2**, 875–893.
73. Liu HW, Mahmood S, Srinivasan M, *et al.* (2013) Developmental programming in skeletal muscle in response to overnourishment in the immediate postnatal life in rats. *J Nutr Biochem* **24**, 1859–1869.
74. Martin Agnoux A, El Ghaziri A, Moyon T, *et al.* (2018) Maternal protein restriction during lactation induces early and lasting plasma metabolomic and hepatic lipidomic signatures of the

offspring in a rodent programming model. *J Nutr Biochem* **55**, 124–141.
75. Serrano A, Asnani-Kishnani M, Couturier C, *et al.* (2020) DNA methylation changes are associated with the programming of white adipose tissue browning features by resveratrol and nicotinamide riboside neonatal supplementations in mice. *Nutrients* **12**, 461.
76. Yuan X, Tsujimoto K, Hashimoto K, *et al.* (2018) Epigenetic modulation of Fgf21 in the perinatal mouse liver ameliorates diet-induced obesity in adulthood. *Nat Commun* **9**, 636.
77. Lucion AB & Bortolini MC (2014) Mother–pup interactions: rodents and humans. *Front Endocrinol* **5**, 17.
78. Widdowson EM & McCance RA (1960) Some effects of accelerating growth. I. General somatic development. *Proc Royal Soc London Series B Biol Sci* **152**, 188–206.
79. Schreiner F, Ackermann M, Michalik M, *et al.* (2017) Developmental programming of somatic growth, behavior and endocannabinoid metabolism by variation of early postnatal nutrition in a cross-fostering mouse model. *PLoS One* **12**, e0182754.
80. Rodrigues VST, Moura EG, Bernardino DN, *et al.* (2018) Supplementation of suckling rats with cow's milk induces hyperphagia and higher visceral adiposity in females at adulthood, but not in males. *J Nutr Biochem* **55**, 89–103.
81. Lin C, Shao B, Zhou Y, *et al.* (2016) Maternal high-fat diet influences stroke outcome in adult rat offspring. *J Mol Endocrinol* **56**, 101–112.
82. Brower M (2018) Overview of the reproduction of laboratory mice and rats. In *Encyclopedia of Reproduction*, **6**, 711–721 [MK Skinner, editor]. Oxford: Academic Press. https://doi.org/10.1016/B978-0-12-809633-8.20619-5
83. Boersma GJ, Bale TL, Casanello P, *et al.* (2014) Long-term impact of early life events on physiology and behaviour. *J Neuroendocrinol* **26**, 587–602.
84. Ribeiro TA, Tófolo LP, Martins IP, *et al.* (2017) Maternal low intensity physical exercise prevents obesity in offspring rats exposed to early overnutrition. *Sci Rep* **7**, 7634.
85. Agnish ND & Keller KA (1997) The rationale for culling of rodent litters. *Fundam Appl Toxicol* **38**, 2–6.
86. Briffa JF, O'Dowd R, Romano T, *et al.* (2019) Reducing pup litter size alters early postnatal calcium homeostasis and programs adverse adult cardiovascular and bone health in male rats. *Nutrients* **11**, 118.
87. Jennings BJ, Ozanne SE, Dorling MW, *et al.* (1999) Early growth determines longevity in male rats and may be related to telomere shortening in the kidney. *FEBS Lett* **448**, 4–8.
88. Ridout KK, Ridout SJ, Goonan K, *et al.* (2017) Telomeres and early life stress. In *Stress: Neuroendocrinology and Neurobiology*, pp. 185–193 [G Fink, editor]. San Diego: Academic Press.
89. Gandelman R & Simon NG (1978) Spontaneous pup-killing by mice in response to large litters. *Dev Psychobiol* **11**, 235–241.
90. Grota LJ & Ader R (1969) Continuous recording of maternal behaviour in Rattus Norvegicus. *Animal Behav* **17**, 722–729.
91. Patel MS & Srinivasan M (2011) Metabolic programming in the immediate postnatal life. *Ann Nutr Metab* **58**, 18–28.
92. Ehrampoush E, Homayounfar R, Davoodi SH, *et al.* (2016) Ability of dairy fat in inducing metabolic syndrome in rats. *Springer Plus* **5**, 1–8.
93. Suvorov A & Vandenberg LN (2016) To cull or not to cull? Considerations for studies of endocrine-disrupting chemicals. *Endocrinology* **157**, 2586–2594.
94. Gregorio BM, Souza-Mello V, Carvalho JJ, *et al.* (2010) Maternal high-fat intake predisposes nonalcoholic fatty liver disease in C57BL/6 offspring. *Am J Obstet Gynecol* **203**, 495–498.
95. Mathias PCF, Miranda GDS, Barella LF, *et al.* (2020) Cholinergic-pathway-weakness-associated pancreatic islet dysfunction: a low-protein-diet imprint effect on weaned rat offspring. *J Dev Orig Helath and Dis* **11**, 1–8. https://www.doi.org/10.1017/S2040174420000215
96. Martin Agnoux A, Antignac JP, Boquien CY, *et al.* (2015) Perinatal protein restriction affects milk free amino acid and fatty acid profile in lactating rats: potential role on pup growth and metabolic status. *J Nutr Biochem* **26**, 784–795.
97. Ma D, Ozanne SE & Guest PC (2017) A protocol for producing the maternal low-protein rat model: a tool for preclinical proteomic studies. *Adv Exp Med Biol* **974**, 251–255.
98. Otani L, Mori T, Koyama A, *et al.* (2016) Supplemental arginine above the requirement during suckling causes obesity and insulin resistance in rats. *Nutr Res* **36**, 575–585.
99. Richard C, Lewis ED, Goruk S, *et al.* (2016) The content of docosahexaenoic acid in the suckling and the weaning diet beneficially modulates the ability of immune cells to response to stimuli. *J Nutr Biochem* **35**, 22–29.
100. Sevrin T, Alexandre-Gouabau MC, Darmaun D, *et al.* (2017) Use of water turnover method to measure mother's milk flow in a rat model: application to dams receiving a low protein diet during gestation and lactation. *PLoS One* **12**, e0180550.
101. Kaczmarek MM, Mendoza T & Kozak LP (2016) Lactation undernutrition leads to multigenerational molecular programming of hypothalamic gene networks controlling reproduction. *BMC Genom* **17**, 333.
102. Rincel M, Lépinay AL, Delage P, *et al.* (2016) Maternal high-fat diet prevents developmental programming by early-life stress. *Transl Psychiatr* **6**, e966.
103. Muhammad N, Lembede BW & Erlwanger KH (2021) Zingerone administered neonatally prevents the subsequent development of high dietary fructose-induced fatty liver in sprague dawley rats. *J Med Food* **24**, 944–952.
104. Nyakudya TT, Isaiah S, Ayeleso A, *et al.* (2019) Short-term neonatal oral administration of oleanolic acid protects against fructose-induced oxidative stress in the skeletal muscles of suckling rats. *Molecules* **24**, 661.
105. Neal-Kluever A, Fisher J, Grylack L, *et al.* (2019) Physiology of the neonatal gastrointestinal system relevant to the disposition of orally administered medications. *Drug Metab Dispos* **47**, 296–313.
106. Arreguín A, Ribot J, Mušinović H, *et al.* (2018) Dietary vitamin A impacts DNA methylation patterns of adipogenesis-related genes in suckling rats. *Arch Biochem Biophys* **650**, 75–84.
107. Gugusheff JR, Bae SE, Rao A, *et al.* (2016) Sex and age-dependent effects of a maternal junk food diet on the mu-opioid receptor in rat offspring. *Behav Brain Res* **301**, 124–131.
108. Kanno T, Koyanagi N, Katoku Y, *et al.* (1997) Simplified preparation of a refined milk formula comparable to rat's milk: influence of the formula on development of the gut and brain in artificially reared rat pups. *J Pediatr Gastroenterol Nutr* **24**, 242–252.
109. Auestad N, Korsak RA, Bergstrom JD, *et al.* (1989) Milk-substitutes comparable to rat's milk; their preparation, composition and impact on development and metabolism in the artificially reared rat. *Br J Nutr* **61**, 495–518.

110. Patel MS, Vadlamudi S & Johanning GL (1994) Artificial rearing of rat pups: implications for nutrition research. *Annu Rev Nutr* **14**, 21–40.
111. Yajima M, Kanno T & Yajima T (2006) A chemically derived milk substitute that is compatible with mouse milk for artificial rearing of mouse pups. *Exp Anim* **55**, 391–397.
112. Motoki T, Naomoto Y, Hoshiba J, *et al.* (2011) Glutamine depletion induces murine neonatal melena with increased apoptosis of the intestinal epithelium. *World J Gastroenterol* **17**, 717–726.
113. Patel MS & Hiremagalur BK (1992) Artificial-rearing technique: its usefulness in nutrition research. *J Nutr* **122**, 412–419.
114. Takeda S, Harauma A, Okamoto M, *et al.* (2020) Effects of whey protein hydrolysate on growth promotion and immunomodulation in mouse pups in artificial rearing system. *Anim Sci J = Nihon Chikusan Gakkaibo* **91**, e13395.
115. Haney PM, Estrin CR, Caliendo A, *et al.* (1986) Precocious induction of hepatic glucokinase and malic enzyme in artificially reared rat pups fed a high-carbohydrate diet. *Arch Biochem Biophys* **244**, 787–794.
116. Aalinkeel R, Srinivasan M, Kalhan SC, *et al.* (1999) A dietary intervention (high carbohydrate) during the neonatal period causes islet dysfunction in rats. *Am J Physiol* **277**, E1061–E1069.
117. Hussein N, Fedorova I, Moriguchi T, *et al.* (2009) Artificial rearing of infant mice leads to *n*-3 fatty acid deficiency in cardiac, neural and peripheral tissues. *Lipids* **44**, 685–702.
118. Vadlamudi S, Hiremagalur BK, Tao L, *et al.* (1993) Long-term effects on pancreatic function of feeding a HC formula to rats during the preweaning period. *Am J Physiol* **265**, E565–E571.
119. Petrik J, Srinivasan M, Aalinkeel R, *et al.* (2001) A long-term high-carbohydrate diet causes an altered ontogeny of pancreatic islets of Langerhans in the neonatal rat. *Pediatr Res* **49**, 84–92.
120. Srinivasan M, Dodds C, Ghanim H, *et al.* (2008) Maternal obesity and fetal programming: effects of a high-carbohydrate nutritional modification in the immediate postnatal life of female rats. *Am J Physiol Endocrinol Metab* **295**, E895–E903.
121. Duke Boynton F, Dunbar M & Koewler N (2020) *General Experimental Techniques*. London: Academic Press. https://doi.org/10.1016/B978-0-12-814338-4.00019-2
122. Halpern MD, Khailova L, Molla-Hosseini D, *et al.* (2008) Decreased development of necrotizing enterocolitis in IL-18-deficient mice. *Am J Physiol-Gastrointest Liver Physiol* **294**, G20–G26.
123. Raineki C, Lucion AB & Weinberg J (2014) Neonatal handling: an overview of the positive and negative effects. *Dev psychobiology* **56**, 1613–1625.
124. Novaes Soares P, Silva Tavares Rodrigues V, Cherem Peixoto T, *et al.* (2018) Cigarette smoke during breastfeeding in rats changes glucocorticoid and vitamin d status in obese adult offspring. *Int J Mol Sci* **19**, 3084.
125. Matthews PA, Samuelsson AM, Seed P, *et al.* (2011) Fostering in mice induces cardiovascular and metabolic dysfunction in adulthood. *J Physiol* **589**, 3969–3981.
126. Romano T, Wark JD, Owens JA, *et al.* (2009) Prenatal growth restriction and postnatal growth restriction followed by accelerated growth independently program reduced bone growth and strength. *Bone* **45**, 132–141.
127. Fahrenkrog S, Harder T, Stolaczyk E, *et al.* (2004) Cross-fostering to diabetic rat dams affects early development of mediobasal hypothalamic nuclei regulating food intake, body weight, and metabolism. *J Nutr* **134**, 648–654.
128. Morel FB, Oozeer R, Piloquet H, *et al.* (2015) Preweaning modulation of intestinal microbiota by oligosaccharides or amoxicillin can contribute to programming of adult microbiota in rats. *Nutrition* **31**, 515–522.
129. Huot RL, Gonzalez ME, Ladd CO, *et al.* (2004) Foster litters prevent hypothalamic-pituitary-adrenal axis sensitization mediated by neonatal maternal separation. *Psychoneuroendocrinology* **29**, 279–289.
130. Agca Y (2020) Assisted reproductive technologies and genetic modifications in rats. In *The Laboratory Rat*, pp. 181–213 [MA Suckow, FC Hankenson, RP Wilson, *et al.*, editors]. London: Academic Press. https://doi.org/10.1016/B978-0-12-814338-4.00007-6
131. Hedrich HJ (2006) Taxonomy and stocks and strains. In *The Laboratory Rat*, pp. 71–92 [MA Suckow, editor]. Amsterdam, Netherlands: Elsevier Inc. https://doi.org/10.1016/B978-012074903-4/50006-6
132. Reifsnyder PC, Churchill G & Leiter EH (2000) Maternal environment and genotype interact to establish diabesity in mice. *Genome Res* **10**, 1568–1578.
133. Mansour S, Thomas K & Capecchi M (1988) Disruption of the proto-oncogene int-2 in mouse embryo-derived stem cells: a general strategy for targeting mutations to non-selectable genes. *Nature* **336**, 348–352.
134. Geurts AM, Cost GJ, Freyvert Y, *et al.* (2009) Knockout rats via embryo microinjection of zinc-finger nucleases. *Science* **325**, 433–433.
135. Cowley M, Garfield AS, Madon-Simon M, *et al.* (2014) Developmental programming mediated by complementary roles of imprinted Grb10 in mother and pup. *PLoS Biol* **12**, e1001799.
136. Daviau J (1999) Clinical evaluation of rodents. *Vet Clinics North Am* **2**, 429–445.
137. Lohmiller JJ, Swing SP & Hanson MM (2020) Chapter 6 – reproduction and breeding. In *The Laboratory Rat*, pp. 157–179 [MA Suckow, FC Hankenson, RP Wilson, editors]. London: Academic Press. https://doi.org/10.1016/B978-0-12-814338-4.00006-4
138. Vidal JD (2017) The impact of age on the female reproductive system. *Toxicol Pathol* **45**, 206–215.
139. Bermejo-Alvarez P, Rizos D, Lonergan P, *et al.* (2011) Transcriptional sexual dimorphism during preimplantation embryo development and its consequences for developmental competence and adult health and disease. *Reproduction* **141**, 563–570.
140. Richard C, Lewis ED, Goruk S, *et al.* (2016) A dietary supply of docosahexaenoic acid early in life is essential for immune development and the establishment of oral tolerance in female rat offspring. *J Nutr* **146**, 2398–2406.
141. Danneman PJ & Mandrell TD (1997) Evaluation of five agents/methods for anesthesia of neonatal rats. *Lab Anim Sci* **47**, 386–395.
142. Pritchett K, Corrow D, Stockwell J, *et al.* (2005) Euthanasia of neonatal mice with carbon dioxide. *Comp Med* **55**, 275–281.
143. Pritchett-Corning KR (2009) Euthanasia of neonatal rats with carbon dioxide. *J Am Assoc for Lab Animal Sci* **48**, 23–27.
144. Valentim AM, Guedes SR, Pereira AM, *et al.* (2016) Euthanasia using gaseous agents in laboratory rodents. *Lab Anim* **50**, 241–253.
145. Grazer FM (1958) Technic for intravascular injection and bleeding of newborn rats and mice. *Proc Soc Exp Biol Med* **99**, 407–409.
146. Srinivasan M, Mahmood S & Patel MS (2013) Metabolic programming effects initiated in the suckling period predisposing for adult-onset obesity cannot be reversed by calorie restriction. *Am J Physiol Endocrinol Metab* **304**, E486–494.

147. Martins IP, de Oliveira JC, Pavanello A, *et al.* (2018) Protein-restriction diet during the suckling phase programs rat metabolism against obesity and insulin resistance exacerbation induced by a high-fat diet in adulthood. *J Nutr Biochem* **57**, 153–161.
148. de Oliveira JC, Scomparin DX, Andreazzi AE, *et al.* (2011) Metabolic imprinting by maternal protein malnourishment impairs vagal activity in adult rats. *J Neuroendocrinol* **23**, 148–157.
149. Mukonowenzou NC, Dangarembizi R, Chivandi E, *et al.* (2020) Administration of ursolic acid to new-born pups prevents dietary fructose-induced non-alcoholic fatty liver disease in Sprague Dawley rats. *J Dev Orig Health Dis* **12**, 1–12. https://www.doi.org/10.1017/S2040174420000124
150. Srinivasan M, Song F, Aalinkeel R, *et al.* (2001) Molecular adaptations in islets from neonatal rats reared artificially on a high carbohydrate milk formula. *J Nutr Biochem* **12**, 575–584.
151. Chen Y, Wang J, Yang S, *et al.* (2017) Effect of high-fat diet on secreted milk transcriptome in midlactation mice. *Physiol Genomics* **49**, 747–762.
152. Konieczna J, Palou M, Sánchez J, *et al.* (2015) Leptin intake in suckling rats restores altered T3 levels and markers of adipose tissue sympathetic drive and function caused by gestational calorie restriction. *Int J Obes* **39**, 959–966.
153. Whary M, Baumgarth N, Fox J, *et al.* (2015) Biology and diseases of mice. In *Laboratory Animal Medicine*, pp. 43–149 [J Fox, L Anderson, G Otto, *et al.*, editors]. Amsterdam, Netherlands: Elsevier Inc. https://doi.org/10.1016/B978-0-12-409527-4.00003-1