Chapter 9

Perinatal and Postnatal Determinants of Brain Development: Recent Studies and Methodological Advances

Sarah J. Spencer and Trisha A. Jenkins

Abstract

Perinatal diet is an important factor in programming brain development and susceptibility to obesity. There are currently several elegant and simple prenatal and postnatal animal models in use to mimic the effects of early life overfeeding and to study its impact on brain and metabolic development. In this chapter we will discuss the background to some of these models, with a specific focus on manipulating rodent litter sizes to alter the early life nutritional environment.

Key words Diet, Fostering, Hypothalamic–pituitary–adrenal axis, Lipopolysaccharide, Litter size, Obesity, Perinatal programming, Suckling

1 Introduction

1.1 The Importance of Perinatal Diet in Programming Obesity, Metabolic Dysfunction, and Appetite in the Offspring

Obesity has become epidemic in our society and, as such, obesity in parents at conception and throughout pregnancy has become very common. Around 60 % of women of childbearing age are classified as overweight or obese in the US and Australia [1, 2]. In addition to the effects of diet and obesity at conception and during pregnancy, a baby may also have to contend with an inappropriate diet in the days, weeks, and months following birth. Diet during these vulnerable early programming stages of life can have significant influences on feeding, satiety, and metabolic circuitry in the brain, but also on brain development in general, including on cognitive function [3, 4].

The first dietary influence to potentially affect baby's brain development is an indirect one; the effect of paternal diet on the sperm. There is currently limited evidence for the extent of this effect on brain development, but we do know paternal obesity in humans influences sperm concentration and motility and can damage sperm DNA [5]. When other factors are controlled for, paternal obesity at conception leads to increased adiposity in daughters (but not sons) when they reach adolescence [6]. In rat models, paternal

David W. Walker (ed.), *Prenatal and Postnatal Determinants of Development*, Neuromethods, vol. 109,
DOI 10.1007/978-1-4939-3014-2_9,

obesity is linked to pancreatic beta-cell dysfunction and a predisposition to diabetes, with, again, these effects being manifest in female but not male offspring [7].

Perhaps more intuitively obvious is the maternal effect on the offspring. Maternal metabolic state during pregnancy can have pronounced effects on offspring brain development. Overweight or obese mothers are significantly more likely to have or develop type 2 or gestational diabetes and these conditions predispose the baby to abnormal pancreatic development, insulin resistance, diabetes, and obesity [8–11]. Even in the absence of diabetes, a "junk food" diet, one high in fat, sugar, and salt, during pregnancy can also influence a baby's brain development [12–14]. For instance, a maternal diet high in fat is linked with changes to central reward processing, altering the way the rewarding aspects of food are perceived throughout life, leading to a preference for fatty, sugary foods [3]. Cognitive function in general can be altered in the offspring of obese or high-fatdiet-fed mothers, with these children having poorer psychomotor and cognitive development scores than children from lean mothers [4]. These dietary influences from both the father and mother can occur in the absence of or in addition to any specific genetic effects. Thus, experimental animal models, where the genetic variable is removed, consistently reveal an important early life dietary influence on brain development.

Postnatally, a baby's vulnerability to dietary influences continues. Obese and overweight babies and children are significantly more likely to grow to be obese and overweight adults [15, 16]. There are a variety of factors that contribute to this. Potentially, the most important dietary contribution to a baby's brain development is the speed with which it gains weight after birth [17–19]. Particularly in, but not restricted to, small for gestational age babies, intensive feeding immediately postnatally is important in ensuring appropriate brain and lung development, but can also lead to increased risk of obesity and its associated central dysfunction [17–19]. Stettler and colleagues have found for every 100 g a baby gains in weight in the week after birth, its chances of developing obesity as an adult are increased by 28 % [20]. Factors influencing weight gain include the baby's general health as well as the composition of its diet. Maternal diet can influence the composition and amount of breast milk available. For instance, there are indications that a maternal diet high in conjugated linoleic acid isomers (found in organic meats and dairy) may reduce adiposity and the likelihood of obesity in the baby. At least, conjugated linoleic acid isomers in diet can reduce fat accrual in adults [21, 22] and can also be passed on to the baby through the breast milk [23]. Maternal breast milk omega-3 levels are even significantly correlated with better performance in mathematics tests in the offspring [24]. Similarly, baby formula content may also be important in programming a baby's development throughout life.

High-protein, but not low-protein formulas are associated with obesity long-term [25], and formulas fortified with long-chain polyunsaturated fatty acids are associated with faster processing speeds in cognitive tests later in life than standard formulas [26]. Feeding frequency and the timing of introduction of solid food may also influence a baby's brain development and its propensity to develop obesity [27].

While there are indications that factors such as breast versus formula-feeding and timing of solid food introduction in humans are very important in a baby's development, research in this area is clouded by the huge number of additional variables inherent in any human study of this kind. Socioeconomic and other environmental factors, parental age, definitions of exclusive breast-feeding, breast versus bottle versus different types of formula-feeding, and social stigma encouraging reporting errors all make it difficult to draw solid conclusions from the data [28]. Animal models are therefore essential for us to delineate the effects of early life overfeeding as well as the mechanisms for these changes.

2 Models of Perinatal Overfeeding

Several animal models of perinatal overfeeding are in routine use to study the programming influence of diet on brain and metabolic development [29–33]. These include:

1. Paternal high fat diet prior to and/or at conception.
2. Maternal high fat diet prior to and/or at conception and/or during pregnancy.
3. In utero growth restriction.
4. Maternal high fat/high protein diet during lactation.
5. "Pup-in-a-cup" artificial rearing.
6. Sucking pups in small litters.
7. Combinations of any of the above.

2.1 Equating Developmental Ages and Stages

It is difficult and controversial to accurately equate developmental stages between species. However, higher order mammals do undergo significant development of brain pathways contributing to feeding regulation, satiety, and metabolism in the third trimester of gestation. The rodent reaches a similar stage of development of these pathways in the first and second week of life. For instance, the projections from the arcuate nucleus to the paraventricular nucleus of the hypothalamus (PVN) are essential for body weight regulation and these are functional in primates and other higher order mammals but immature in the rodent at birth [34]. It is thus

generally considered that late gestation and the early postnatal period in the rodent are roughly equivalent to the third trimester of pregnancy in the human, particularly with regard to metabolic systems. For this reason, we will concentrate on postnatal rodent models of early life dietary intervention in this chapter. In particular, we will discuss the impact of suckling rodent pups in small litters to induce neonatal overfeeding.

2.2 Postnatal Models of Overfeeding; Manipulations in Maternal and Pup Diet

Alteration of the maternal diet is a well-recognized technique of changing the nutritional content of milk for lactating rodents. Nutrients can be directly administered to the mother or added to the maternal diet during pregnancy and/or pre-weaning [35]. A well-established model of postnatal overfeeding involves feeding the mother a high fat diet. This causes excessive weight gain in the pup, which is, considering variability due to diet composition and strain, generally maintained until adulthood [36].

Within the literature many diets have been investigated, ranging from chow altered to contain increased amounts of fats and/or carbohydrates to the high-fatcafeteria-style diet diet [37]. Each has their own advantages: the chow diet can be made to contain varying concentrations and types of fats and sugars so consumption of different components is easily calculated; while the cafeteria-style diet, which is the feeding of "human" food, such as pies and cakes, to rodents, is seen to be much more palatable and perhaps more similar to the human condition, but absolute amounts of ingestion of the various elements are sometimes difficult to assess [38].

The effect of maternal obesity on the offspring has been investigated from gestation through to lactation. Offspring from obese mothers gain more weight and exhibit increased adiposity, glucose intolerance, and increases in blood pressure and other metabolic markers, than those from lean dams [39, 40]. Central disturbances in the development of hypothalamic feeding circuits are also apparent [41]. Moreover, this excess weight gain and metabolic disturbance is carried through to adulthood, and is independent of post-weaning diet. Meanwhile, pups born to lean mothers then nursed by obese dams exhibit increases in weight and plasma triglyceride levels compared to pups born and nursed by lean mothers [42]. This example demonstrates the complicated nature of maternal dietary influences, that is maternal obesity at both pregnancy and the time of nursing have influences on programming obesity and metabolic dysfunction in the offspring.

The rodent pup-in-a-cup artificial rearing model allows the researcher to have complete control of dietary content and quantity in the immediate postnatal period. This neonatal overfeeding procedure allows the lipid, protein, and carbohydrate composition of milk to be manipulated by artificially rearing pups in foam cups in a temperature-controlled bath and feeding via tubes implanted directly into the gut [43, 44]. Studies have been performed from

neonatal day one, though results should be considered in the context of the underlying influence of loss of normal maternal and sibling interactions during pre-weaning, absence of ano-genital licking to stimulate digestion, and some suggestion of reduced brain growth [45].

3 Postnatal Models of Overfeeding; Litter Size Manipulation to Induce Overfeeding

An alternative well-accepted rodent model to mimic human overfeeding during the perinatal period is to manipulate the litter size in which the pups are suckled. Pups are suckled in either small litters, where they have greater access to their mother's milk, or in standardized control litters. This type of overfeeding during the early postnatal period leads to increased weight gain and body fat in early life that persists throughout the juvenile period and into adulthood [46–48].

3.1 Materials and Methods

Suckling rat (or mouse) pups in small litters is an extremely simple and effective method of inducing changes in neonatal diet. It will be discussed here for Wistar rats but can be adapted for use in mice and other animals. It requires:

1. At least three time-mated pregnant dams (scheduled to give birth on the same day).
2. Spare cages labeled with the dam's identification code.
3. Scales for weighing.

(a) Observe all dams periodically on the date of birth and commence litter size manipulation 2–3 h after birthing is complete. This timing avoids additional pups accidentally being added to the litter after manipulation and limits stress placed on the dam during birth.

(b) Gently remove all pups from their nests and place them in whole-litter groups in labeled clean cages. Ensure pups remain together in a bunch so they retain as much heat as possible. At this point it is useful to track numbers of pups born and numbers stillborn.

(c) Randomly pick one male and one female from litters other than the natural litter of the foster-dam until the desired litter size is reached. We use small litters of 4 and control litters of 12 [46–49]. We also use this model to induce neonatal underfeeding by creating large litters of 20 pups [49–51].

(d) Weigh the pups in a group and return them to the nest, taking care to disturb the nest site as little as possible. Excess pups should be euthanized in a different room.

(e) Leave the dams and pups undisturbed for as long as possible before cleaning cages or otherwise disturbing them. Cage cleaning, feeding, and all other procedures should be standardized between cages. Pups are generally weaned into same-sex littermate pairs at postnatal day (P)21.

While these litter manipulations are designed to manipulate the amount of food the pup has access to, they are well within the normal physiological range. Wistar rats give birth to an average of 12–15 pups but are known to regularly give birth and raise as many as 18 or as few as 2.

3.2 Troubleshooting

Pup temperature during manipulation: As young neonates, pups behaviorally thermoregulate in the nest, circulating closer to the dam and the centre of the nest to stay warm [52]. Prolonged periods away from the dam lead to a significant drop in body temperature that should be avoided as it potentially contributes to pup rejection by the foster-dam [53]. Keep the pups together as a birth-litter during manipulation and conduct the procedure as quickly as possible to avoid excessive cooling. We typically manipulate only three to six litters at one time to reduce the time the pups are away from the dam to approximately 5 min. If excessive cooling is anticipated and unavoidable, an incubator or heating lamp can be used. Heating blankets are not recommended due to the possibility the pups may overheat.

Pup attrition: Unanticipated pup death occasionally occurs, even in untouched litters, and this can be due to a variety of factors. Occasionally pups are born with congenital abnormalities that mean they are either not viable for long after birth or are less competitive for food and maternal attention. When selecting pups for reallocation, take care to avoid those that are weaker, smaller, or paler in color than their siblings to minimize the chances of selecting an individual with an existing abnormality.

Severe stress can, in some cases, induce the dams to kill and cannibalize healthy pups [54, 55]. One should take extreme care when removing pups from the nest and introducing the new pups to minimize contact with the dam and to avoid disturbing her nest-site. Cages should be left unchanged for as long as feasible after birth/litter manipulation. This will depend upon animal facility policy, cage size, cage ventilation, etc., but we have found careful cage changes 3 days after birth do not adversely affect the dams or their litters.

Fostering issues: Fostering eliminates pregnancy-related variables from the model. Wistar rats are very good foster parents [53, 56]

and we have seen no cases of refusal to care for a new litter. We have also found no differences in crude measurements, including weight and fat pads, in litters that were not fostered but were culled to small size ([46], unpublished). There is some suggestion foster mothers may give more attention to their own pups in a mixed litter or otherwise treat foster-pups differently [57]. For this reason, we ensure no dam receives any of her naturally born pups.

Greater difficulties with fostering may present with other rat strains and with mice. One can minimize rejection by taking great care to not stress the dam. It has also been suggested that rubbing bedding material onto the experimenter's gloves and onto the new pups to disguise foreign smells assists with acceptance [53].

3.3 Comments and Considerations on the Model

Gender balance: The size and gender composition of the litters will depend upon the experimental protocol, but for a standard model to induce overfeeding during the neonatal period we ensure a 50-50 balance of males and females within a litter. There is some evidence dams offer differences in attention to males and females [58, 59] and, although it might be desirable to generate all-male litters when all-male studies are being designed for, we chose to eliminate this variable as it is not possible to do this for small and control litters equally.

Litter representation for statistical analysis: Typically when a whole-litter manipulation is conducted, that litter is then regarded as an "*n*" of one for the purposes of group composition and statistical analysis [60]. In this regard, one also needs to consider the ethically appropriate use of animals in research and how to avoid maximizing information obtained from each animal. We can also consider all our pups are fostered and are therefore not from the same pregnancy. For our experiments we typically take one to two males and one to two females from each litter for allocation to each experimental group, thereby controlling for mothering effects but maximizing appropriate animal use.

Maternal attention and other non-nutritive elements to the model: This model is certainly effective at increasing the food available to the pups suckled in the small litters. Previous studies have shown rats suckled in small litters receive more milk and milk that is higher in fat than those from control litters, despite the dam reducing her milk production [29]. We should note, however, that this model has elements independent of food intake that could also contribute to weight gain and brain development.

Rats raised in small litters are, for instance, given more opportunities for interaction with their dam [29]. Maternal attention is an important component of an animal's development that can permanently influence brain function long-term. For instance, seminal studies by Meaney and colleagues have shown that pups that receive more licking, grooming, and intensive (arched back)

nursing during their suckling period have hyperactive hypothalamic–pituitary–adrenal (HPA) axes [61–64]. These effects of maternal care are likely to be due, at least in part, to the tactile stimulation inducing thyroid hormone and serotonin responses that stimulate nerve growth factor inducible factor A expression, which increases histone acetylation of the glucocorticoid receptor [65]. Increased maternal attention therefore leads (in otherwise untreated pups) to comparatively enhanced glucocorticoid receptor transcription, more efficient glucocorticoid negative feedback onto the HPA axis, and attenuated HPA axis responses to stress [65, 66]. Although there is no question pups raised in small litters receive more maternal attention, it is clear that this is not sufficient to override the nutritive and other effects of the model with respect to its effects on HPA axis function. Thus, neonatally overfed rats have exacerbated, not attenuated, HPA axis responses to stress and immune challenge [46, 47, 67]. How the overfeeding is able to override the long-term effects of maternal care in this way is not known.

Other factors that should be considered with the model are the potential for differences in body temperature regulation in a small litter with respect to a control one. In addition, neonatally overfed pups will receive a diet that is higher in several nutritional elements, not just fat. For instance, they will also receive proportionally more leptin, which can act as a trophic factor in the brain in early life [68–71].

3.4 Typical/ Anticipated Results

Neonatal overfeeding from being suckled in a small litter leads to accelerated growth and weight gain that persists into early adulthood. Thus rats from small litters weigh significantly more as early as P7 and maintain this elevated weight into adulthood of 12 [46–49]. Those from large litters have the opposite phenotype [49–51] (Fig. 1).

Animal models of early life overfeeding have shown us postnatal diet can be extremely important in programming brain development. For instance, rats raised in small litters, where they have greater access to their dam's milk than those in control litters do, have accelerated maturation of their HPA axes. In this case, the excess milk, fat, and other nutrients leads to an adult-like profile of adrenocorticotropic hormone and corticosterone in small litter rats, as well as increases in PVN glucocorticoid receptor mRNA [67]. They also respond with a greater hormonal and central response to stress than controls do [47, 49]. It is likely that this change in HPA axis maturation contributes long-term to the way the animal responds to stress and immune challenge. Thus, neonatally overfed adult female rats have exacerbated neuronal activation in the PVN, the apex of the HPA axis, in response to psychological stress compared with control rats [46]. Neonatally overfed male

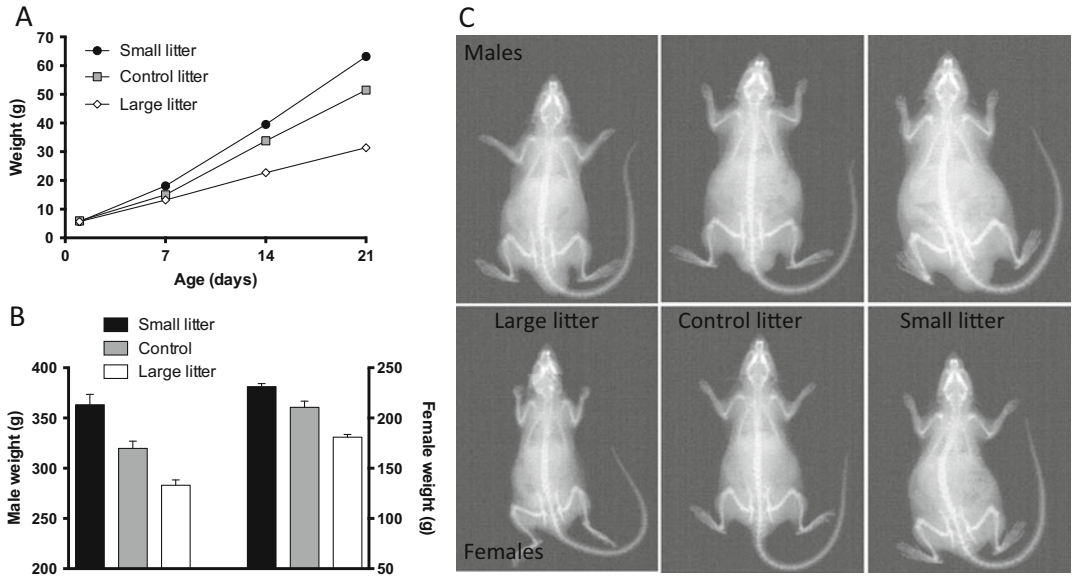

Fig. 1 Representative data showing how litter size affects long-term body weight. (**a**) Pre-weaning weights. (**b**) Adult weights. (**c**) Adult dual-energyX-ray absorptiometry scans showing fat mass. Similar data have been published previously [46–51]

and female adult rats have a similar exacerbated PVN response to an immune challenge with the bacterial endotoxin lipopolysaccharide (LPS) [47]. This latter is likely to be a reflection of slower glucocorticoid negative feedback, culminating in less suppression of the HPA axis response to the immune challenge and also less, or slower glucocorticoid-mediated suppression of nuclear factor κB-dependent cytokine transcription. In short, these changes mean neonatally overfed animals have dysregulated central and peripheral responses to stress and immune challenge. Again, neonatally underfed (large litter) rats have the opposite responses [50, 51].

Neonatal overfeeding in animal models may also contribute to aberrant development of brain pathways regulating feeding, satiety, and metabolism. In the rat and mouse, connectivity between various parts of the hypothalamus occurs at critical stages of development [68–71]. The growth of these connections is stimulated by a surge in circulating leptin. In early life, leptin is obtained principally from the diet. Excessive leptin, as occurs with neonatal overfeeding by raising rats in small litters [48], can potentially signal this hypothalamic connectivity to start developing too soon, or to overdevelop. In either case, the result is a potential impairment in satiety signaling.

This potential for central changes in satiety signaling in neonatally overfed animals is reflected in changes in feeding, and metabolism in these rats. Thus, many groups have seen neonatal overfeeding leads to hyperphagia [72–75]. We have also seen changes in

metabolism [48], and it has been suggested alterations in brown adipose tissue thermogenesis contribute to the phenotype [76].

4 Conclusion

Obesity has become a significant problem in the developing world, and the impact of early life diet on how our children develop is an important factor to explore. There are now several simple and replicable rodent models available that allow us to investigate this question. Litter size manipulation is one of these. This relatively non-invasive technique to alter early life nutrition, as outlined here, allows us to interrogate the neurological changes that occur with early life diet and, ultimately, test strategies to ameliorate the effects of an overweight/obese phenotype initiated in early life.

Acknowledgements

This work was supported by a Discovery Project Grant from the Australian Research Council (ARC) to SJS (DP130100508). SJS is an ARC Future Fellow (FT110100084) and an RMIT University VC Senior Research Fellow.

References

1. Flegal KM, Carroll MD, Ogden CL, Johnson CL (2002) Prevalence and trends in obesity among US adults, 1999-2000. JAMA 288 (14):1723–1727
2. Colagiuri S, Lee CM, Colagiuri R, Magliano D, Shaw JE, Zimmet PZ et al (2010) The cost of overweight and obesity in Australia. Med J Aust 192(5):260–264
3. Ong ZY, Muhlhausler BS (2011) Maternal "junk-food" feeding of rat dams alters food choices and development of the mesolimbic reward pathway in the offspring. FASEB J 25 (7):2167–2179
4. Casas M, Chatzi L, Carsin AE, Amiano P, Guxens M, Kogevinas M et al (2013) Maternal pre-pregnancy overweight and obesity, and child neuropsychological development: two Southern European birth cohort studies. Int J Epidemiol 42(2):506–517
5. Kasturi SS, Tannir J, Brannigan RE (2008) The metabolic syndrome and male infertility. J Androl 29(3):251–259
6. Figueroa-Colon R, Arani RB, Goran MI, Weinsier RL (2000) Paternal body fat is a longitudinal predictor of changes in body fat in premenarcheal girls. Am J Clin Nutr 71 (3):829–834
7. Ng SF, Lin RC, Laybutt DR, Barres R, Owens JA, Morris MJ (2010) Chronic high-fat diet in fathers programs beta-cell dysfunction in female rat offspring. Nature 467 (7318):963–966
8. Aerts L, Holemans K, Van Assche FA (1990) Maternal diabetes during pregnancy: consequences for the offspring. Diabetes Metab Rev 6(3):147–167
9. Silverman BL, Metzger BE, Cho NH, Loeb CA (1995) Impaired glucose tolerance in adolescent offspring of diabetic mothers. Relationship to fetal hyperinsulinism. Diabetes Care 18 (5):611–617
10. Plagemann A, Harder T, Kohlhoff R, Rohde W, Dorner G (1997) Glucose tolerance and insulin secretion in children of mothers with pregestational IDDM or gestational diabetes. Diabetologia 40(9):1094–1100
11. Weiss PA, Scholz HS, Haas J, Tamussino KF, Seissler J, Borkenstein MH (2000) Long-term-follow-up of infants of mothers with type 1 diabetes: evidence for hereditary and nonhereditary transmission of diabetes and precursors. Diabetes Care 23(7):905–911
12. Albuquerque KT, Sardinha FL, Telles MM, Watanabe RL, Nascimento CM, Tavares do

Carmo MG et al (2006) Intake of trans fatty acid-rich hydrogenated fat during pregnancy and lactation inhibits the hypophagic effect of central insulin in the adult offspring. Nutrition 22(7–8):820–829

13. Srinivasan M, Katewa SD, Palaniyappan A, Pandya JD, Patel MS (2006) Maternal high-fat diet consumption results in fetal malprogramming predisposing to the onset of metabolic syndrome-like phenotype in adulthood. Am J Physiol Endocrinol Metab 291(4): E792–E799
14. Ashino NG, Saito KN, Souza FD, Nakutz FS, Roman EA, Velloso LA et al (2012) Maternal high-fat feeding through pregnancy and lactation predisposes mouse offspring to molecular insulin resistance and fatty liver. J Nutr Biochem 23(4):341–348
15. Whitaker RC, Wright JA, Pepe MS, Seidel KD, Dietz WH (1997) Predicting obesity in young adulthood from childhood and parental obesity. N Engl J Med 337(13):869–873
16. Biro FM, Wien M (2010) Childhood obesity and adult morbidities. Am J Clin Nutr 91 (5):1499S–1505S
17. Lucas A (1998) Programming by early nutrition: an experimental approach. J Nutr 128 (Suppl 2):406S
18. Gluckman PD, Hanson MA (2004) Living with the past: evolution, development, and patterns of disease. Science 305 (5691):1733–1736
19. Singhal A, Kennedy K, Lanigan J, Fewtrell M, Cole TJ, Stephenson T et al (2010) Nutrition in infancy and long-term risk of obesity: evidence from 2 randomized controlled trials. Am J Clin Nutr 92(5):1133–1144
20. Stettler N, Stallings VA, Troxel AB, Zhao J, Schinnar R, Nelson SE et al (2005) Weight gain in the first week of life and overweight in adulthood: a cohort study of European American subjects fed infant formula. Circulation 111(15):1897–1903
21. Park Y, Albright KJ, Storkson JM, Liu W, Pariza MW (2007) Conjugated linoleic acid (CLA) prevents body fat accumulation and weight gain in an animal model. J Food Sci 72 (8):S612–S617
22. Racine NM, Watras AC, Carrel AL, Allen DB, McVean JJ, Clark RR et al (2010) Effect of conjugated linoleic acid on body fat accretion in overweight or obese children. Am J Clin Nutr 91(5):1157–1164
23. Rist L, Mueller A, Barthel C, Snijders B, Jansen M, Simoes-Wust AP et al (2007) Influence of organic diet on the amount of conjugated linoleic acids in breast milk of lactating women in the Netherlands. Br J Nutr 97 (4):735–743
24. Lassek WD, Gaulin SJ (2013) Maternal milk DHA content predicts cognitive performance in a sample of 28 nations. Matern Child Nutr
25. Koletzko B, von Kries R, Monasterolo RC, Subias JE, Scaglioni S, Giovannini M et al (2009) Infant feeding and later obesity risk. Adv Exp Med Biol 646:15–29
26. Willatts P, Forsyth S, Agostoni C, Casaer P, Riva E, Boehm G (2013) Effects of long-chain PUFA supplementation in infant formula on cognitive function in later childhood. Am J Clin Nutr 98(2):536S–542S
27. Seach KA, Dharmage SC, Lowe AJ, Dixon JB (2010) Delayed introduction of solid feeding reduces child overweight and obesity at 10 years. Int J Obes 34(10):1475–1479
28. Durmus B, van Rossem L, Duijts L, Arends LR, Raat H, Moll HA et al (2011) Breast-feeding and growth in children until the age of 3 years: the Generation R Study. Br J Nutr 105 (11):1704–1711
29. Fiorotto ML, Burrin DG, Perez M, Reeds PJ (1991) Intake and use of milk nutrients by rat pups suckled in small, medium, or large litters. Am J Physiol 260(6 Pt 2):R1104–R1113
30. West JR (1993) Use of pup in a cup model to study brain development. J Nutr 123(Suppl 2):382–385
31. Vuguin PM (2007) Animal models for small for gestational age and fetal programming of adult disease. Horm Res 68(3):113–123
32. Lukaszewski MA, Eberle D, Vieau D, Breton C (2013) Nutritional manipulations in the perinatal period program adipose tissue in offspring. Am J Physiol Endocrinol Metab 305 (10):E1195–E1207
33. Williams L, Seki Y, Vuguin PM, Charron MJ (2014) Animal models of in utero exposure to a high fat diet: a review. Biochim Biophys Acta 1842(3):507–519
34. Bouret SG, Simerly RB (2007) Development of leptin-sensitive circuits. J Neuroendocrinol 19(8):575–582
35. Perez-Cano FJ, Franch A, Castellote C, Castell M (2012) The suckling rat as a model for immunonutrition studies in early life. Clin Dev Immunol 2012:537310
36. Plagemann A, Heidrich I, Gotz F, Rohde W, Dorner G (1992) Obesity and enhanced diabetes and cardiovascular risk in adult rats due to early postnatal overfeeding. Exp Clin Endocrinol 99(3):154–158

37. Panchal SK, Brown L (2011) Rodent models for metabolic syndrome research. J Biomed Biotechnol 2011:351982
38. Speakman J, Hambly C, Mitchell S, Krol E (2008) The contribution of animal models to the study of obesity. Lab Anim 42(4):413–432
39. Buckley AJ, Keseru B, Briody J, Thompson M, Ozanne SE, Thompson CH (2005) Altered body composition and metabolism in the male offspring of high fat-fed rats. Metabolism 54 (4):500–507
40. Samuelsson AM, Matthews PA, Argenton M, Christie MR, McConnell JM, Jansen EH et al (2008) Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension, and insulin resistance: a novel murine model of developmental programming. Hypertension 51(2):383–392
41. Chen H, Simar D, Morris MJ (2009) Hypothalamic neuroendocrine circuitry is programmed by maternal obesity: interaction with postnatal nutritional environment. PLoS One 4(7):e6259
42. Desai M, Jellyman JK, Han G, Beall M, Lane RH, Ross MG (2014) Rat maternal obesity and high fat diet program offspring metabolic syndrome. Am J Obstet Gynecol 211(3):237. e1–237.e13
43. Hall WG (1975) Weaning and growth of artificially reared rats. Science 190 (4221):1313–1315
44. Beierle EA, Chen MK, Hartwich JE, Iyengar M, Dai W, Li N et al (2004) Artificial rearing of mouse pups: development of a mouse pup in a cup model. Pediatr Res 56(2):250–255
45. Diaz J, Moore E, Petracca F, Schacher J, Stamper C (1982) Artificial rearing of rat pups with a protein-enriched formula. J Nutr 112(5):841–847
46. Spencer SJ, Tilbrook A (2009) Neonatal overfeeding alters adult anxiety and stress responsiveness. Psychoneuroendocrinology 34 (8):1133–1143
47. Clarke MA, Stefanidis A, Spencer SJ (2012) Postnatal overfeeding leads to obesity and exacerbated febrile responses to lipopolysaccharide throughout life. J Neuroendocrinol 24 (3):511–524
48. Stefanidis A, Spencer SJ (2012) Effects of neonatal overfeeding on juvenile and adult feeding and energy expenditure in the rat. PLoS One 7 (12), e52130
49. Smith JT, Spencer SJ (2012) Preweaning over- and underfeeding alters onset of puberty in the rat without affecting kisspeptin. Biol Reprod 86(5):145, 141–148
50. Bulfin LJ, Clarke MA, Buller KM, Spencer SJ (2011) Anxiety and hypothalamic-pituitary-adrenal axis responses to psychological stress are attenuated in male rats made lean by large litter rearing. Psychoneuroendocrinology 36 (7):1080–1091
51. Clarke M, Cai G, Saleh S, Buller KM, Spencer SJ (2013) Being suckled in a large litter mitigates the effects of early-life stress on hypothalamic-pituitary-adrenal axis function in the male rat. J Neuroendocrinol 25(9):792–802
52. Farrell WJ, Alberts JR (2007) Rat behavioral thermoregulation integrates with nonshivering thermogenesis during postnatal development. Behav Neurosci 121(6):1333–1341
53. Suckow MA, Weisbroth SH, Franklin CL (2005) The laboratory rat. Academic, New York, NY
54. Lane-Petter W (1968) Cannibalism in rats and mice. Proc R Soc Med 61(12):1295–1296
55. DeSantis DT, Schmaltz LW (1984) The mother-litter relationship in developmental rat studies: cannibalism vs caring. Dev Psychobiol 17(3):255–262
56. http://www.arc.wa.gov.au
57. Cierpial MA, Murphy CA, McCarty R (1990) Maternal behavior of spontaneously hypertensive and Wistar-Kyoto normotensive rats: effects of reciprocal cross-fostering of litters. Behav Neural Biol 54(1):90–96
58. Sharpe RM, Morris A, Wyatt AC (1973) The effect of the sex of litter-mates on the subsequent behaviour and breeding performance of cross-fostered rats. Lab Anim 7 (1):51–59
59. Moore CL, Morelli GA (1979) Mother rats interact differently with male and female offspring. J Comp Physiol Psychol 93 (4):677–684
60. Lazic SE (2010) The problem of pseudoreplication in neuroscientific studies: is it affecting your analysis? BMC Neurosci 11:5
61. Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A et al (1997) Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science 277(5332):1659–1662
62. Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky PM, Meaney MJ (1998) Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. Proc Natl Acad Sci U S A 95(9):5335–5340
63. Francis DD, Meaney MJ (1999) Maternal care and the development of stress responses. Curr Opin Neurobiol 9(1):128–134
64. Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR et al (2004) Epigenetic programming by maternal behavior. Nat Neurosci 7(8):847–854

65. Hellstrom IC, Dhir SK, Diorio JC, Meaney MJ (2012) Maternal licking regulates hippocampal glucocorticoid receptor transcription through a thyroid hormone-serotonin-NGFI-A signalling cascade. Philos Trans R Soc Lond B Biol Sci 367(1601):2495–2510
66. Champagne F, Meaney MJ (2001) Like mother, like daughter: evidence for non-genomic transmission of parental behavior and stress responsivity. Prog Brain Res 133:287–302
67. Boullu-Ciocca S, Dutour A, Guillaume V, Achard V, Oliver C, Grino M (2005) Postnatal diet-induced obesity in rats upregulates systemic and adipose tissue glucocorticoid metabolism during development and in adulthood: its relationship with the metabolic syndrome. Diabetes 54(1):197–203
68. Schmidt I, Fritz A, Scholch C, Schneider D, Simon E, Plagemann A (2001) The effect of leptin treatment on the development of obesity in overfed suckling Wistar rats. Int J Obes Relat Metab Disord 25(8):1168–1174
69. Bouret SG, Draper SJ, Simerly RB (2004) Formation of projection pathways from the arcuate nucleus of the hypothalamus to hypothalamic regions implicated in the neural control of feeding behavior in mice. J Neurosci 24 (11):2797–2805
70. Bouret SG, Draper SJ, Simerly RB (2004) Trophic action of leptin on hypothalamic neurons that regulate feeding. Science 304 (5667):108–110
71. Bouret SG, Simerly RB (2006) Developmental programming of hypothalamic feeding circuits. Clin Genet 70(4):295–301
72. Oscai LB, McGarr JA (1978) Evidence that the amount of food consumed in early life fixes appetite in the rat. Am J Physiol 235(3): R141–R144
73. Lopez M, Tovar S, Vazquez MJ, Nogueiras R, Seoane LM, Garcia M et al (2007) Perinatal overfeeding in rats♀ results in increased levels of plasma leptin but unchanged cerebrospinal leptin in adulthood. Int J Obes 31(2):371–377
74. Rodrigues AL, De Souza EP, Da Silva SV, Rodrigues DS, Nascimento AB, Barja-Fidalgo C et al (2007) Low expression of insulin signaling molecules impairs glucose uptake in adipocytes after early overnutrition. J Endocrinol 195(3):485–494
75. Rodrigues AL, de Moura EG, Passos MC, Dutra SC, Lisboa PC (2009) Postnatal early overnutrition changes the leptin signalling pathway in the hypothalamic-pituitary-thyroid axis of young and adult rats. J Physiol 587(Pt 11):2647–2661
76. Xiao XQ, Williams SM, Grayson BE, Glavas MM, Cowley MA, Smith MS et al (2007) Excess weight gain during the early postnatal period is associated with permanent reprogramming of brown adipose tissue adaptive thermogenesis. Endocrinology 148 (9):4150–4159