Published in final edited form as:

Physiol Behav. 2010 July 14; 100(5): 560–566. doi:10.1016/j.physbeh.2010.04.008.

Perinatal environment and its influences on metabolic programming of offspring

Kellie L. K. Tamashiro and Timothy H. Moran

Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, 720 Rutland Avenue, Ross 618, Baltimore, MD 21205

Abstract

The intrauterine environment supports the development and health of offspring. Perturbations to this environment can have detrimental effects on the fetus that have persistent pathological consequences through adolescence and adulthood. The developmental origins of health and disease concept, also known as the “Barker Hypothesis”, has been put forth to describe the increased incidence of chronic disease such as cardiovascular disease and diabetes in humans and animals exposed to a less than ideal intrauterine environment. Maternal infection, poor or excess nutrition, and stressful events can negatively influence development of different cell types, tissues and organ systems ultimately predisposing the organism to pathological conditions. Although there are a variety of conditions associated to exposure to altered intrauterine environments, the focus of this review will be on the consequences of stress and high fat diet during the pre- and perinatal periods and associated outcomes related to obesity and other metabolic conditions. We further discuss possible neuroendocrine and epigenetic mechanisms responsible for metabolic programming of offspring.

Keywords

prenatal stress; high fat diet; metabolic programming; obesity; diabetes; epigenetic; development

Introduction

Overweight and obesity are at epidemic levels. The health risks associated with obesity include coronary artery disease, type 2 diabetes, hypertension and cancer [1,2]. One of the major factors that influence the likelihood of an individual becoming obese and developing is childhood obesity, which itself continues to escalate worldwide at an alarming rate [3–7]. While genome-wide association studies have identified several genomic loci that are associated with obesity, the overall effect size remains small and cannot account for the tremendous increase in prevalence of overweight and obesity over the last 3 decades [8,9] suggesting that the environment plays a significant role.

*Corresponding author: Kellie L. K. Tamashiro, Ph.D., Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, 720 Rutland Avenue, Ross 618, Baltimore, MD 21205, Phone: 410-955-2996, Fax: 410-502-3769, ktamashiro@jhmi.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Maternal diet and stress

Epidemiological evidence strongly suggests that the intrauterine and early postnatal environments have a significant long-term influence on body weight and energy homeostasis in offspring. Poor maternal nutrition during the Dutch Hunger Winter during World War II in the 1940's resulted in increased incidence of obesity, cardiovascular disease and diabetes in adult males [10–12]. These studies suggesting that programming of metabolism can occur *in utero* led to the development of the “Thrifty Phenotype Hypothesis” which posits that diabetes and the metabolic syndrome result from the postnatal challenge thrust upon an energy balance regulatory system that has been programmed for a thrifty postnatal lifestyle by *in utero* maternal nutrient restriction [13]. In modern society, fetal under nutrition can occur under conditions of hyperemesis gravidum, high altitude pregnancy, or pregnancy in women with eating disorders. Based upon this, animal models of low birth weight or intrauterine growth restriction (IUGR) have been developed and are becoming well-characterized [14–18]. The current environment is more commonly one of nutritional excess, particularly in developed Western countries including the United States.

In fact, human studies now show that exposure to very high nutrition before birth is also associated with obesity in postnatal life particularly in combination with maternal gestational diabetes [19]. The relationship between birth weight and adult fat mass has developed into a “U” shaped curve such that either being very light or very heavy at birth may predispose offspring to increased fat mass and incidence of metabolic syndrome as children and adults. As a consequence of changing diets around the world, greater numbers of women are consuming a fat-rich diet and an increasing proportion of women are overweight when pregnant. Alterations in maternal diet have lead to a two-fold increase in the incidence of maternal overweight and obesity (to over 45%) over the last 20 years in contrast to a 50% decrease in those mothers that have body mass index in the underweight range (~11%) (Table 16D, 2008 Pregnancy Nutrition Surveillance, Centers for Disease Control and Prevention). Maternal high-fat or cholesterol over-feeding during pregnancy and lactation in rodents produces a phenotype that closely resembles the human metabolic syndrome including dyslipidemia, hyperleptinemia, and increased adiposity [20]. Elevated blood glucose and triglycerides in offspring suggest insulin resistance [21] and elevated blood pressure and impaired vascular function are indicative of cardiovascular dysfunction [22–24].

In addition to consumption of high-calorie, high-fat foods, modern day lifestyle now includes little physical activity and increased stress stemming from work and social environments. A growing body of literature in humans as well as in animals strongly suggests that stress has adverse effects on humans and animals and one well documented consequence is altered energy homeostasis resulting in overweight and obesity [25–27]. Human studies indicate that psychosocial and socioeconomic challenges activate the hypothalamic-pituitary-adrenal (HPA) axis causing hypersecretion of cortisol, and this in turn has been related to the development of hypertension, osteoporosis and depression [28]. Increased cortisol levels have also been associated with obesity-related conditions including excessive visceral fat deposition, insulin resistance, dyslipidemia, and cardiovascular disease [27]. The long-term consequences of prenatal stress events on adult rat behavior and metabolic phenotype has been investigated in rodent models [reviewed in [29–31]]. Prenatal glucocorticoid exposure has been implicated in altered stress responsivity [32], increased anxiety-like behavior [33], reduced neurogenesis [34], and has been linked to adult hypertension, hyperglycemia, and features related to the metabolic syndrome [35,36]. The mechanisms responsible for these occurrences, however, remain to be elucidated.

Animal model

In our laboratory we are using a rat animal model to determine the consequences of prenatal stress against a high fat diet background. Pregnant female Sprague-Dawley (SD) rats are maintained on standard chow (CHOW) or 60% high fat (HF) diet throughout gestation and lactation. In addition, half of each diet group was subjected to a variable stress paradigm (STRESS) to determine the short- and long-term metabolic effects of maternal stress during the last week of gestation on offspring (Table. 1). Details have been described previously [37].

In this model we find that prenatal variable stress is non-habituating and has a significant effect in elevating baseline glucocorticoid levels in dams. HF diet feeding results in greater caloric consumption during the first 2 weeks of gestation and produces greater weight gain in HF fed dams than CHOW fed dams. STRESS in the 3rd week of gestation suppresses maternal weight gain. Thus, although maternal HF feeding accelerates weight gain during the first two weeks of gestation, a combination of lower caloric consumption among the HF dams and less weight gain in STRESS dams results in no difference in body weight among the groups by gestation day 21, immediately prior to parturition. HF diet consumption increased plasma leptin levels, but had no effect on blood glucose or plasma insulin indicating that the dams had not developed a diabetes, a condition that by itself produces offspring with gestational diabetes show increased adiposity, impaired pancreatic function, impaired glucose tolerance, and altered hypothalamic development [38–40].

At birth, both male and female pups from STRESS dams weighed more than those from CON moms irrespective of maternal diet. There were no differences in litter sizes or male to female ratios at birth and thus these factors could not account for the differences in birth weight among the groups. From birth on, both prenatal stress and HF diet conditions had significant effects on the pups' weight gain. Pups from dams that were stressed and/or on HF diet weighed more as early as postnatal (PND) 7 compared to those from dams on CHOW diet and the difference in body weight persisted until weaning on PND 21 (Fig. 1). Using whole body NMR after the animals were sacrificed, we determined that the increase in body weight among STRESS and HF offspring was associated with greater adiposity in both male and female pups from HF dams (Table 2) with no difference in visceral vs. subcutaneous distribution (data not shown). Although the HF-STRESS group is heavier than HF-CON, the amount of body adiposity and lean mass appears to be lower in the HF-STRESS group. This non-significant difference is likely due to the fact that body composition analysis was done post-mortem and we also separated the subcutaneous from the visceral fat depot thus introducing some degree of error.

We challenged male and female offspring to an oral glucose tolerance test to assess their ability to clear glucose. Pups from HF dams cleared glucose more slowly and required a greater amount of insulin compared to CHOW pups suggesting that they may be developing insulin resistance. Together these data suggest that maternal HF diet during gestation and lactation has significant metabolic effects on the offspring by the time they are weaned.

Hormones leptin, insulin and corticosterone are critical in the proper development of neural circuitry during the early postnatal period [41]. We collected blood from pups on PND 1, 7, 14 and 21 and the endocrine profile of male offspring is shown in Fig. 2. On PND 1, male pups from the HF-STRESS group already had significantly elevated plasma leptin levels. From PND 7 on both HF-CON and HF-STRESS groups had elevated plasma leptin compared to those pups from CHOW fed dams. Plasma insulin was not different until PND 21 when pups from HF diet fed dams had significantly higher levels of insulin regardless of whether or not they were subject to prenatal stress. Corticosterone levels were lower in both CHOW- and HF-STRESS groups on PND 1 but did not differ through the rest of the suckling period.

When weaned onto CHOW diet, the *in utero* experience did not affect offspring body weight at 70 days of age. However, when weaned onto HF diet, both male and female pups from dams that were stressed and/or on HF diet gained more body weight and this was attributable to greater adiposity. Males weaned on CHOW diet showed no difference in glucose clearance or insulin secretion in an OGTT. However, males weaned on HF diet had impaired glucose tolerance if their dams were stressed or on HF diet.

We had originally hypothesized that prenatal stress against a background of HF diet feeding would result in an additive or synergistic effect on the metabolic profile of the offspring. However, the data at the time points examined suggest that there is no increased effect of the combination of stress and HF diet. There may have been a ceiling effect of either stress or HF diet or both that masked evidence of additivity or synergism. In particular, the HF diet that we used had a 60% fat content and lowering this to 40% would maintain a fat content that is comparable to a standard Western diet. An alternative possibility is that maternal HF diet would have a “beneficial” effect in attenuating the stress response of the dam and, in turn, lessen its impact on the developing fetus. Dallman and colleagues have demonstrated that high fat diet can attenuate the HPA axis response to stress [42]. We did not note either a detrimental or beneficial effect of HF diet on top of the effects of stress.

Potential mechanisms

Overall, our data suggest that the perinatal environment can alter the susceptibility of offspring to future metabolic challenge, in this case HF diet resulting in obesity. Evidence that genetic or environmental factors disrupt early development and produce increased susceptibility to disease has been reported for Parkinson’s disease [43,44] and schizophrenia [45,46]. The “first hit” disruptions that occur during early development set the stage for long-term vulnerability to a “second hit” that occurs later in life that functions to precipitate a pathological condition. Thus, it appears that prenatal stress or maternal HF diet is not the direct cause of increased body weight or disturbances in energy homeostasis, but instead increases future susceptibility. This concept is reminiscent of Alfred Knudson’s “two-hit” hypothesis initially proposed for the development of cancer where genetic mutation alone does not produce cancer until a second factor, a genetic mutation usually derived from environmental exposures (e.g. UV exposure, smoking, diet, or stress), precipitates the condition. Although genetic predisposition is certainly a factor in development of obesity and other metabolic conditions, it is clear that factors other than genetics may play a significant role in predisposing an individual to development of chronic adult disease. Identification of the pathways and mechanisms that produce long-term vulnerability in response to early environmental disruption will facilitate development of clinical intervention and prevention strategies to reduce the incidence of disease. Using the animal model described above we are now able to extend our studies to determine the potential mechanisms through which prenatal stress or HF diet may program systems that regulate body weight and control food intake.

There is evidence that hormones such as corticosterone, insulin and leptin can cross the placental-fetal barrier to potentially affect fetal development [47–49] and, thus, it is likely that fetuses have hormone levels that mirror those of their dams. The significant changes in endocrine parameters in offspring throughout the early postnatal period reported here represent reasonable candidates for “metabolic programming” that occurs in response to maternal HF diet consumption or prenatal stress [50].

Glucocorticoids

Glucocorticoids are prime candidates for perinatal metabolic programming. Maternal corticosterone levels normally increase during pregnancy. Glucocorticoids are critical during development for maturation of tissues and organs, cellular differentiation and lung maturation

at parturition. However, with the prenatal stress there is a reliable increase in basal corticosterone of stressed dams with no evidence of habituation to chronic variable stress as we have used in our model. Indeed, there are protective mechanisms (maternal and placental corticosteroid binding globulin and placental 11 β -hydroxysteroid dehydrogenase Type-1 and -2) in place that normally prevent transmission of excess glucocorticoids to the fetus. However, studies have shown that under chronic stress conditions (maternal stress or exogenous corticosterone administration), these mechanisms become deficient or fail [51,52]. The net result is that fetal glucocorticoid levels in rodents correlate with maternal glucocorticoid levels suggesting that the fetuses of prenatally stressed dams are also exposed to high glucocorticoid levels [48]. Excess glucocorticoid exposure during gestation can result in long-term effects on expression of glucocorticoid sensitive genes in the central nervous system as well as in the periphery. Prenatal stress or maternal dexamethasone (a synthetic glucocorticoid) administration is associated with exaggerated stress responses in offspring that over time can produce adverse neuropsychiatric, metabolic, and cardiovascular conditions [53,54]. Hyperactivity of the HPA axis is mediated by reduced hippocampal glucocorticoid receptor expression resulting in attenuated negative feedback control of HPA axis activity. In the periphery, liver glucocorticoid receptor expression is increased and may drive increases in expression and activity of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) resulting in insulin resistance and glucose intolerance in adulthood [36,55]. Maternal glucocorticoid administration can also affect kidney development by reducing nephron number, increasing local GR expression and producing hypersensitivity to vasoconstrictors affecting blood pressure regulation in offspring [56].

The role of increased corticosterone in the dam in the effects of prenatal on the offspring has been directly assessed. Maternal adrenalectomy with corticosterone replacement at non-stress levels prevents the HPA axis changes in offspring normally seen following prenatal stress. Furthermore, administration of corticosterone in a pattern to mimic that of prenatal stress recapitulates the phenotype [57]. Finally, cross-fostering studies in which pups from stressed dams were raised by non-stressed dams from birth have demonstrated that hypertension and hyperglycemia resulting from prenatal stress was attributable to glucocorticoid effects on the fetus *in utero*, and not to altered maternal postnatal behavior or physiology [58].

Insulin and leptin

Both leptin and insulin are trophic factors that act during the pre- and postnatal periods and can significantly affect development of neural systems important in the maintenance of energy homeostasis [59]. The development of important aspects of the hypothalamic appetite regulatory system in rodents occurs primarily after birth [59,60]. As an example, NPY is present in the fetal ARC as early as gestational day 14, however NPY/AgRP projections between the ARC and the paraventricular (PVN) hypothalamus do not fully develop until postnatal day 15–16 suggesting that both the prenatal and postnatal environments are critical for normal development [59]. As demonstrated by Simerly *et al.*, the development of these projections is under the control of leptin. In ob/ob mice lacking leptin, these connections do not fully develop and providing exogenous leptin rescues the development of these pathways in neonates, but not adults [61]. The timing of leptin replacement during the early postnatal period is also critical as demonstrated in the intrauterine growth restriction (IUGR) model which in mice and rats produces greater susceptibility to diet-induced obesity in adulthood. IUGR mice show a premature leptin surge when cross-fostered to a non-restricted dam during lactation and mimicking the premature leptin increase in normal control mice recapitulates the low leptin sensitivity and obese phenotype of IUGR mice [62]. Thus, not only is the presence of leptin important, but the timing of the surge in leptin is critical for normal development to progress.

Another obesity model is the diet-induced obese (DIO) rat which Levin and colleagues use to study the development of hypothalamic projections in rodents that are susceptible or resistant to development of the metabolic syndrome when provided with a diet with moderate amounts of fat. In this model, neonatal DIO rats have severely reduced density of axons projecting from the arcuate nucleus to the paraventricular nucleus compared to diet-resistant (DR) offspring [63]. Arcuate nucleus explants of DIO offspring are less responsive to leptin in vitro and have a diminished ability to develop neurite outgrowth compared to DR when stimulated with leptin. Thus the developmental deficits in this case are attributed to low hypothalamic leptin sensitivity that is already manifest during the neonatal period. These data indicate that decreased leptin sensitivity can also result in developmental deficits in the hypothalamus.

Insulin also exerts an important influence on hypothalamic development. Injections into pregnant dams between GD 15 and 20 results in obesity in offspring [39,40]. Streptozotocin-induced diabetes produces hyperinsulinemia in offspring and is associated with alterations in hypothalamic neuropeptide expression and neuronal morphology [64,65]. In addition, postnatal injections of insulin are associated with morphological changes in the ventral medial hypothalamus [66]. Together these data support a role for hyperinsulinism during gestation or the early postnatal period in programming hypothalamic development.

In our paradigm, male pups from HF dams are hyperleptinemic beginning on PND 7 and become hyperinsulinemic and hyperglycemic by PND 21. The effect of hyperleptinemia on brain development throughout the critical early postnatal period has been less well studied. As discussed earlier in this section, hypothalamic development can be disrupted by a lack of leptin, an early increase in leptin, or a decrease in hypothalamic leptin sensitivity. In this paradigm, the latter two possibilities appear to be likely mechanisms to be tested. Taken together, elevations in the levels of leptin, insulin and glucose suggest that metabolic systems such as the neuropeptide Y (NPY) and proopiomelanocortin (POMC) pathways may be altered by maternal overnutrition (HF diet or increased caloric intake) in a manner that also predisposes offspring to early weight gain, increased adiposity and impaired glucose tolerance followed by diet-induced obesity in adulthood. Although the effects of high fat feeding on peripheral organ function have been documented, the role of altered development of neural systems involved in controlling energy balance in the etiology of symptoms consistent with metabolic syndrome has not been studied as thoroughly.

Epigenetic programming

Epigenetic modifications represent a potential way that “metabolic programming” occurs and a mechanism through which prenatal stress and maternal diet could result in long-term changes in metabolism. While epigenetics is known to play a critical role in the etiology of many cancers, only recently has the involvement of epigenetic mechanisms in other pathophysiological conditions, including the metabolic syndrome, been recognized [67–69].

Epigenetics refers to post-translational modifications of DNA that result in differential levels of gene expression without altering the DNA sequence itself. Epigenetic modifications are of three basic types. The first is *DNA methylation*, which is the easiest to study, as it involves a modification of cytosine and can be approached by restriction endonuclease analysis or bisulfite DNA sequencing. The second epigenetic modification is *genomic imprinting*, a form of parental origin-specific gene silencing, or comparative silencing of one parental allele over the other, a phenomenon that appears to affect only a very small fraction of genes. The third type is *chromatin modifications* and these are of many types, but they primarily involve post-translational modifications of histones H3 and H4, including methylation, phosphorylation, and acetylation. While alterations in genomic imprinting and chromatin modifications have been associated with metabolic conditions [70–72], we will limit the discussion here to DNA methylation because it is currently our main epigenetic focus.

Environmental factors during gestation can influence the epigenetic state of the genome and this has been shown convincingly for diet. A clear demonstration of this phenomenon comes from studies of the agouti gene in mice which does not carry a parental-specific imprint. Normally black mice can become yellow and obese when the agouti gene spontaneously has a retroviral element inserted into it. When this element is methylated, however, the mice maintain a normal phenotype; only when it is unmethylated do they manifest the yellow and obese phenotype [73]. Interestingly, when mothers are fed a high methyl diet, leading to increased S-adenosylmethionine, they produce a higher than expected number of normal offspring, suggesting that the high methyl diet leads to increased DNA methylation [74]. In a recent study in heterozygous viable yellow agouti (A^{vy}/a) mice, maternal dietary genistein (major phytoestrogen in soy) supplementation during gestation resulted in A^{vy}/a offspring with coat color that was shifted toward pseudoagouti and that had decreased incidence of adult-onset obesity. This phenotype was significantly associated with greater methylation at six CpG sites in the A^{vy} intracisternal A particle retrotransposon [75]. Further, methylation status of the gene is heritable as demonstrated by its transmission to the next generation [76]. Thus, the agouti locus demonstrates that maternal nutrition during gestation can change epigenetic marks (i.e. DNA methylation) and affect the expression of a gene that does not carry a parental imprint.

The glucocorticoid receptor (GR) is an example of a gene that is epigenetically affected by postnatal environmental influences. Offspring of rat mothers that display high levels of postnatal maternal care (licking and grooming behavior and favorable arched back nursing posture, “high-LGAB”) have increased expression of GR in the hippocampus and attenuated HPA axis responses to acute restraint stress in adulthood compared to offspring of dams that show low levels (“low-LGAB”) of the same behaviors [77]. The differences in GR expression are associated with differential methylation within the exon 1₇ GR promoter. Hypomethylation of specific CG dinucleotides within the exon 1₇ promoter promotes binding of the NGFI-A transcription factor and results in increased gene expression in offspring of high-LGAB dams [77]. Although it was initially thought that epigenetic modifications are laid down very early in development, evidence now suggests that this is not strictly the case and that the adult brain retains some degree of plasticity in adulthood opening the door for possible development of pharmaceuticals targeting epigenetic modifications to treat pathological conditions.

Additional genes involved in energy homeostasis have been found to be regulated by DNA methylation and/or histone modifications. Studies *in vitro* have demonstrated that DNA methylation regulates the expression of leptin [78], SOCS3 [79,80], and glucose transporter (GLUT)-4 [81]. Examples of *in vivo* epigenetic regulation include: leptin [82], peroxisome proliferator-activated receptor (PPAR)- α [83–85], PPAR- γ [86], POMC [87], 11 β -hydroxysteroid dehydrogenase (HSD)-2 [88,89], and corticotrophin releasing hormone [90, 91]. Thus, epigenetic modulation of multiple genes encoding peptides involved in energy balance has been demonstrated. While the specific factors responsible for diet mediated epigenetic changes remain to be identified, it is reasonable to hypothesize that similar alterations may occur in offspring exposed to high fat diet.

The fetal and neonatal programming hypotheses do, of course, come with caveats. Epidemiological studies that prompted proposal of the “Developmental Origins of Health and Disease” theory are correlational and at the moment causality has yet to be established. More importantly, thorough examination of the potential mechanisms for differential outcomes is not feasible in humans. Thus, the value of animal models to study effects of environmental perturbations on metabolic programming is clear. Rigorous testing of hypotheses in these models will enable elucidation of mechanisms that underlie metabolic programming and provide opportunities to better treat metabolic abnormalities or prevent metabolic disease from occurring.

Acknowledgments

We gratefully acknowledge Dr. James I. Koenig (Maryland Psychiatric Research Center, University of Maryland) for thoughtful discussions related to the studies described here. We also thank Chantelle E. Terrillion, Ryan H. Purcell, and Jayson Hyun for their technical assistance with the studies described here. Supported by NIH grants HD055030 and DK077623.

References

1. Flegal KM, et al. Prevalence and trends in obesity among US adults, 1999–2000. *Jama* 2002;288(14):1723–7. [PubMed: 12365955]
2. Procopiou M, Philippe J. The metabolic syndrome and type 2 diabetes: epidemiological figures and country specificities. *Cerebrovasc Dis* 2005;20(Suppl 1):2–8. [PubMed: 16276079]
3. Ogden CL, Carroll MD, Flegal KM. Epidemiologic trends in overweight and obesity. *Endocrinol Metab Clin North Am* 2003;32(4):741–60. vii. [PubMed: 14711060]
4. Swinburn BA. The obesity epidemic in Australia: can public health interventions work? *Asia Pac J Clin Nutr* 2003;12(Suppl):S7. [PubMed: 15023592]
5. Tremblay MS, Katzmarzyk PT, Willms JD. Temporal trends in overweight and obesity in Canada, 1981–1996. *Int J Obes Relat Metab Disord* 2002;26(4):538–43. [PubMed: 12075581]
6. Jebb SA, Rennie KL, Cole TJ. Prevalence of overweight and obesity among young people in Great Britain. *Public Health Nutr* 2004;7(3):461–5. [PubMed: 15153277]
7. Gordon FK, et al. High levels of childhood obesity observed among 3- to 7-year-old New Zealand Pacific children is a public health concern. *J Nutr* 2003;133(11):3456–60. [PubMed: 14608058]
8. Ogden CL, et al. Prevalence and trends in overweight among US children and adolescents, 1999–2000. *Jama* 2002;288(14):1728–32. [PubMed: 12365956]
9. Ogden CL, et al. Prevalence of overweight and obesity in the United States, 1999–2004. *Jama* 2006;295(13):1549–55. [PubMed: 16595758]
10. Painter RC, Roseboom TJ, Bleker OP. Prenatal exposure to the Dutch famine and disease in later life: an overview. *Reprod Toxicol* 2005;20(3):345–52. [PubMed: 15893910]
11. Roseboom TJ, et al. Coronary heart disease after prenatal exposure to the Dutch famine, 1944–45. *Heart* 2000;84(6):595–8. [PubMed: 11083734]
12. Ravelli AC, et al. Obesity at the age of 50 y in men and women exposed to famine prenatally. *Am J Clin Nutr* 1999;70(5):811–6. [PubMed: 10539740]
13. Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia* 1992;35(7):595–601. [PubMed: 1644236]
14. Ross MG, Desai M. Gestational programming: population survival effects of drought and famine during pregnancy. *Am J Physiol Regul Integr Comp Physiol* 2005;288(1):R25–33. [PubMed: 15590994]
15. Ozanne SE, Fernandez-Twinn D, Hales CN. Fetal growth and adult diseases. *Semin Perinatol* 2004;28(1):81–7. [PubMed: 15058905]
16. Desai M, Babu J, Ross MG. Programmed metabolic syndrome: prenatal undernutrition and postweaning overnutrition. *Am J Physiol Regul Integr Comp Physiol* 2007;293(6):R2306–14. [PubMed: 17898113]
17. Desai M, et al. The timing of nutrient restriction during rat pregnancy/lactation alters metabolic syndrome phenotype. *Am J Obstet Gynecol* 2007;196(6):555 e1–7. [PubMed: 17547893]
18. Desai M, et al. Programmed hyperphagia due to reduced anorexigenic mechanisms in intrauterine growth-restricted offspring. *Reprod Sci* 2007;14(4):329–37. [PubMed: 17644805]
19. Boney CM, et al. Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. *Pediatrics* 2005;115(3):e290–6. [PubMed: 15741354]
20. Armitage JA, et al. Developmental programming of the metabolic syndrome by maternal nutritional imbalance: how strong is the evidence from experimental models in mammals? *J Physiol* 2004;561(Pt 2):355–77. [PubMed: 15459241]
21. Guo F, Jen KL. High-fat feeding during pregnancy and lactation affects offspring metabolism in rats. *Physiol Behav* 1995;57(4):681–6. [PubMed: 7777603]

22. Koukkou E, et al. Offspring of normal and diabetic rats fed saturated fat in pregnancy demonstrate vascular dysfunction. *Circulation* 1998;98(25):2899–904. [PubMed: 9860793]
23. Ghosh P, et al. Abnormal aortic fatty acid composition and small artery function in offspring of rats fed a high fat diet in pregnancy. *J Physiol* 2001;533(Pt 3):815–22. [PubMed: 11410637]
24. Khan IY, et al. Gender-linked hypertension in offspring of lard-fed pregnant rats. *Hypertension* 2003;41(1):168–75. [PubMed: 12511548]
25. Jayo JM, et al. Effects of exercise and stress on body fat distribution in male cynomolgus monkeys. *Int J Obes Relat Metab Disord* 1993;17(10):597–604. [PubMed: 8242129]
26. Shively CA, Clarkson TB. Regional obesity and coronary artery atherosclerosis in females: a non-human primate model. *Acta Med Scand Suppl* 1988;723:71–8. [PubMed: 3164976]
27. Bjorntorp P. Do stress reactions cause abdominal obesity and comorbidities? *Obes Rev* 2001;2(2):73–86. [PubMed: 12119665]
28. Chrousos GP, Gold PW. A healthy body in a healthy mind--an vice versa--the damaging power of uncontrollable stress. *J Clin Endocrinol Metab* 1998;83:1842–1845. [PubMed: 9626106]
29. Welberg LA, Seckl JR. Prenatal stress, glucocorticoids and the programming of the brain. *J Neuroendocrinol* 2001;13(2):113–28. [PubMed: 11168837]
30. Weinstock M. The potential influence of maternal stress hormones on development and mental health of the offspring. *Brain Behav Immun* 2005;19(4):296–308. [PubMed: 15944068]
31. Maccari S, et al. Prenatal stress and long-term consequences: implications of glucocorticoid hormones. *Neurosci Biobehav Rev* 2003;27(1–2):119–27. [PubMed: 12732228]
32. Henry C, et al. Prenatal stress increases the hypothalamo-pituitary-adrenal axis response in young and adult rats. *J Neuroendocrinol* 1994;6(3):341–5. [PubMed: 7920600]
33. Vallee M, et al. Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress-induced corticosterone secretion. *J Neurosci* 1997;17(7):2626–36. [PubMed: 9065522]
34. Coe CL, et al. Prenatal stress diminishes neurogenesis in the dentate gyrus of juvenile rhesus monkeys. *Biol Psychiatry* 2003;54(10):1025–34. [PubMed: 14625144]
35. Lindsay RS, et al. Prenatal glucocorticoid exposure leads to offspring hyperglycaemia in the rat: studies with the 11 beta-hydroxysteroid dehydrogenase inhibitor carbenoxolone. *Diabetologia* 1996;39(11):1299–305. [PubMed: 8932995]
36. Nyirenda MJ, et al. Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. *J Clin Invest* 1998;101(10):2174–81. [PubMed: 9593773]
37. Tamashiro KL, et al. Prenatal stress or high-fat diet increases susceptibility to diet-induced obesity in rat offspring. *Diabetes* 2009;58(5):1116–25. [PubMed: 19188431]
38. Fahrenkrog S, et al. Cross-fostering to diabetic rat dams affects early development of mediobasal hypothalamic nuclei regulating food intake, body weight, and metabolism. *J Nutr* 2004;134(3):648–54. [PubMed: 14988462]
39. Jones AP, Olster DH, States B. Maternal insulin manipulations in rats organize body weight and noradrenergic innervation of the hypothalamus in gonadally intact male offspring. *Brain Res Dev Brain Res* 1996;97(1):16–21.
40. Jones AP, et al. Maternal hormonal manipulations in rats cause obesity and increase medial hypothalamic norepinephrine release in male offspring. *Brain Res Dev Brain Res* 1995;88(2):127–31.
41. Bouret SG, Simerly RB. Developmental programming of hypothalamic feeding circuits. *Clin Genet* 2006;70(4):295–301. [PubMed: 16965320]
42. Dallman MF, Pecoraro NC, la Fleur SE. Chronic stress and comfort foods: self-medication and abdominal obesity. *Brain Behav Immun* 2005;19(4):275–80. [PubMed: 15944067]
43. Barlow BK, et al. The gestational environment and Parkinson's disease: evidence for neurodevelopmental origins of a neurodegenerative disorder. *Reprod Toxicol* 2007;23(3):457–70. [PubMed: 17350799]
44. Ling Z, et al. Progressive dopamine neuron loss following supra-nigral lipopolysaccharide (LPS) infusion into rats exposed to LPS prenatally. *Exp Neurol* 2006;199(2):499–512. [PubMed: 16504177]

45. Maynard TM, et al. Neural development, cell-cell signaling, and the “two-hit” hypothesis of schizophrenia. *Schizophr Bull* 2001;27(3):457–76. [PubMed: 11596847]
46. Bayer TA, Falkai P, Maier W. Genetic and non-genetic vulnerability factors in schizophrenia: the basis of the “two hit hypothesis”. *J Psychiatr Res* 1999;33(6):543–8. [PubMed: 10628531]
47. Menon RK, et al. Transplacental passage of insulin in pregnant women with insulin-dependent diabetes mellitus. Its role in fetal macrosomia. *N Engl J Med* 1990;323(5):309–15. [PubMed: 2195347]
48. Takahashi LK, Turner JG, Kalin NH. Prolonged stress-induced elevation in plasma corticosterone during pregnancy in the rat: implications for prenatal stress studies. *Psychoneuroendocrinology* 1998;23(6):571–81. [PubMed: 9802128]
49. Lesage J, et al. Maternal undernutrition during late gestation induces fetal overexposure to glucocorticoids and intrauterine growth retardation, and disturbs the hypothalamo-pituitary adrenal axis in the newborn rat. *Endocrinology* 2001;142(5):1692–702. [PubMed: 11316731]
50. Levin BE. Metabolic imprinting on genetically predisposed neural circuits perpetuates obesity. *Nutrition* 2000;16(10):909–15. [PubMed: 11054596]
51. Gitau R, et al. Fetal exposure to maternal cortisol. *Lancet* 1998;352(9129):707–8. [PubMed: 9728994]
52. Welberg LA, Thiruvikraman KV, Plotsky PM. Chronic maternal stress inhibits the capacity to up-regulate placental 11beta-hydroxysteroid dehydrogenase type 2 activity. *J Endocrinol* 2005;186(3):R7–R12. [PubMed: 16135661]
53. Koenig JJ, et al. Prenatal exposure to a repeated variable stress paradigm elicits behavioral and neuroendocrinological changes in the adult offspring: potential relevance to schizophrenia. *Behav Brain Res* 2005;156(2):251–61. [PubMed: 15582111]
54. Levitt NS, et al. Dexamethasone in the last week of pregnancy attenuates hippocampal glucocorticoid receptor gene expression and elevates blood pressure in the adult offspring in the rat. *Neuroendocrinology* 1996;64(6):412–8. [PubMed: 8990073]
55. Ozanne SE, Hales CN. Early programming of glucose-insulin metabolism. *Trends Endocrinol Metab* 2002;13(9):368–73. [PubMed: 12367817]
56. Bertram CE, Hanson MA. Animal models and programming of the metabolic syndrome. *Br Med Bull* 2001;60:103–21. [PubMed: 11809621]
57. Barbazanges A, et al. Maternal glucocorticoid secretion mediates long-term effects of prenatal stress. *J Neurosci* 1996;16(12):3943–9. [PubMed: 8656288]
58. Nyirenda MJ, Welberg LA, Seckl JR. Programming hyperglycaemia in the rat through prenatal exposure to glucocorticoids-fetal effect or maternal influence? *J Endocrinol* 2001;170(3):653–60. [PubMed: 11524246]
59. Grove KL, et al. Development of metabolic systems. *Physiol Behav* 2005;86(5):646–60. [PubMed: 16289141]
60. Grove KL, Smith MS. Ontogeny of the hypothalamic neuropeptide Y system. *Physiol Behav* 2003;79(1):47–63. [PubMed: 12818709]
61. Bouret SG, Draper SJ, Simerly RB. Trophic action of leptin on hypothalamic neurons that regulate feeding. *Science* 2004;304(5667):108–10. [PubMed: 15064420]
62. Yura S, et al. Role of premature leptin surge in obesity resulting from intrauterine undernutrition. *Cell Metab* 2005;1(6):371–8. [PubMed: 16054086]
63. Bouret SG, et al. Hypothalamic neural projections are permanently disrupted in diet-induced obese rats. *Cell Metab* 2008;7(2):179–85. [PubMed: 18249177]
64. Plagemann A, et al. Malformations of hypothalamic nuclei in hyperinsulinemic offspring of rats with gestational diabetes. *Dev Neurosci* 1999;21(1):58–67. [PubMed: 10077703]
65. Singh BS, Westfall TC, Devaskar SU. Maternal diabetes-induced hyperglycemia and acute intracerebral hyperinsulinism suppress fetal brain neuropeptide Y concentrations. *Endocrinology* 1997;138(3):963–9. [PubMed: 9048596]
66. Plagemann A, et al. Morphological alterations of hypothalamic nuclei due to intrahypothalamic hyperinsulinism in newborn rats. *Int J Dev Neurosci* 1999;17(1):37–44. [PubMed: 10219959]
67. Waterland RA, Jirtle RL. Early nutrition, epigenetic changes at transposons and imprinted genes, and enhanced susceptibility to adult chronic diseases. *Nutrition* 2004;20(1):63–8. [PubMed: 14698016]

68. Gallou-Kabani C, Junien C. Nutritional epigenomics of metabolic syndrome: new perspective against the epidemic. *Diabetes* 2005;54(7):1899–906. [PubMed: 15983188]
69. Feil R. Environmental and nutritional effects on the epigenetic regulation of genes. *Mutat Res* 2006;600(1–2):46–57. [PubMed: 16854438]
70. Takahashi M, Kamei Y, Ezaki O. Mest/Peg1 imprinted gene enlarges adipocytes and is a marker of adipocyte size. *Am J Physiol Endocrinol Metab* 2005;288(1):E117–24. [PubMed: 15353408]
71. Kamei Y, et al. Peg1/Mest in obese adipose tissue is expressed from the paternal allele in an isoform-specific manner. *FEBS Lett* 2007;581(1):91–6. [PubMed: 17182038]
72. Nikonova L, et al. Mesoderm-specific transcript is associated with fat mass expansion in response to a positive energy balance. *Faseb J* 2008;22(11):3925–37. [PubMed: 18644838]
73. Michaud EJ, et al. Differential expression of a new dominant agouti allele (Aiap_y) is correlated with methylation state and is influenced by parental lineage. *Genes Dev* 1994;8(12):1463–72. [PubMed: 7926745]
74. Wolff GL, et al. Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/ a mice. *Faseb J* 1998;12(11):949–57. [PubMed: 9707167]
75. Dolinoy DC, et al. Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. *Environ Health Perspect* 2006;114(4):567–72. [PubMed: 16581547]
76. Morgan HD, et al. Epigenetic inheritance at the agouti locus in the mouse. *Nat Genet* 1999;23(3): 314–8. [PubMed: 10545949]
77. Weaver IC, et al. Epigenetic programming by maternal behavior. *Nat Neurosci* 2004;7(8):847–54. [PubMed: 15220929]
78. Melzner I, et al. Leptin gene expression in human preadipocytes is switched on by maturation-induced demethylation of distinct CpGs in its proximal promoter. *J Biol Chem* 2002;277(47):45420–7. [PubMed: 12213831]
79. Stoger R. Epigenetics and obesity. *Pharmacogenomics* 2008;9(12):1851–60. [PubMed: 19072643]
80. Campion J, Milagro FI, Martinez JA. Individuality and epigenetics in obesity. *Obes Rev* 2009;10(4): 383–92. [PubMed: 19413700]
81. Yokomori N, Tawata M, Onaya T. DNA demethylation during the differentiation of 3T3-L1 cells affects the expression of the mouse GLUT4 gene. *Diabetes* 1999;48(4):685–90. [PubMed: 10102682]
82. Milagro FI, et al. High fat diet-induced obesity modifies the methylation pattern of leptin promoter in rats. *J Physiol Biochem* 2009;65(1):1–9. [PubMed: 19588726]
83. Lillycrop KA, et al. Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. *J Nutr* 2005;135(6): 1382–6. [PubMed: 15930441]
84. Lillycrop KA, et al. Induction of altered epigenetic regulation of the hepatic glucocorticoid receptor in the offspring of rats fed a protein-restricted diet during pregnancy suggests that reduced DNA methyltransferase-1 expression is involved in impaired DNA methylation and changes in histone modifications. *Br J Nutr* 2007;97(6):1064–73. [PubMed: 17433129]
85. Lillycrop KA, et al. Feeding pregnant rats a protein-restricted diet persistently alters the methylation of specific cytosines in the hepatic PPAR alpha promoter of the offspring. *Br J Nutr* 2008;100(2): 278–82. [PubMed: 18186951]
86. Fujiki K, et al. Expression of the peroxisome proliferator activated receptor gamma gene is repressed by DNA methylation in visceral adipose tissue of mouse models of diabetes. *BMC Biol* 2009;7:38. [PubMed: 19589179]
87. Newell-Price J, King P, Clark AJ. The CpG island promoter of the human proopiomelanocortin gene is methylated in nonexpressing normal tissue and tumors and represses expression. *Mol Endocrinol* 2001;15(2):338–48. [PubMed: 11158338]
88. Alikhani-Koopaei R, et al. Epigenetic regulation of 11 beta-hydroxysteroid dehydrogenase type 2 expression. *J Clin Invest* 2004;114(8):1146–57. [PubMed: 15489962]
89. Friso S, et al. Epigenetic control of 11 beta-hydroxysteroid dehydrogenase 2 gene promoter is related to human hypertension. *Atherosclerosis* 2008;199(2):323–7. [PubMed: 18178212]

90. Mueller BR, Bale TL. Sex-specific programming of offspring emotionality after stress early in pregnancy. *J Neurosci* 2008;28(36):9055–65. [PubMed: 18768700]
91. McGill BE, et al. Enhanced anxiety and stress-induced corticosterone release are associated with increased Crh expression in a mouse model of Rett syndrome. *Proc Natl Acad Sci U S A* 2006;103(48):18267–72. [PubMed: 17108082]

Fig. 1.

Body weight of male and female offspring through PND 21. Left: Male offspring in each litter were weighed on postnatal days (PND) 1, 7, 14, and 21. Right: Female offspring in each litter were weighed on PND 1, 7, 14, and 21. CHOW-CON ($n = 11$ litters), CHOW-STRESS ($n = 10$ litters), HF-CON ($n = 11$ litters), HF-STRESS ($n = 10$ litters). * main effect of STRESS, $P < 0.05$ vs. CON; # main effect of DIET, $P < 0.05$ vs. CHOW; ** main effect of DIET and STRESS, $P < 0.05$. Modified from [36].

Fig. 2. Endocrine profile in male offspring PND 1-21. Top: Plasma leptin. Middle: Plasma insulin. Bottom: Plasma corticosterone. CHOW-CON (n = 11), CHOW-STRESS (n = 10), HF-CON (n = 11), HF-STRESS (n = 10). * $P < 0.05$ vs. CHOW-CON, CHOW-STRESS, HF-CON; # $P < 0.05$ vs. CHOW-CON, CHOW-STRESS, ** $P < 0.05$ vs. CHOW-CON. Modified from [36].

Fig. 3.

Body weight on PND 70. Body weight adult male (left) and female (right) offspring. Males weaned on CHOW: CHOW-CON (n = 6), CHOW-STRESS (n = 4), HF-CON (n = 4), HF-STRESS (n = 4); Males weaned on HF: CHOW-CON (n = 4), CHOW-STRESS (n = 4), HF-CON (n = 5), HF-STRESS (n = 4); Females weaned on CHOW: CHOW-CON (n = 5), CHOW-STRESS (n = 4), HF-CON (n = 4), HF-STRESS (n = 4); Females weaned on HF: CHOW-CON (n = 4), CHOW-STRESS (n = 4), HF-CON (n = 5), HF-STRESS (n = 4). * $P < 0.05$ vs. CHOW WEAN; # $P < 0.05$ vs. CHOW-CON. Modified from [36].

Table 1

Schedule of variable stress during gestation.

Gestational Day	TIME OF DAY		
	MORNING	NOON	AFTERNOON
14	Restraint–60 min	Swim - 15 min at room	Restraint–60 min
15	Cold exposure (4 °C)–6 hr		
16	Swim–15 min	Restraint–60 min	Swim–15 min
17		Swim–15 min	Lights on during dark
18	Social stress–group housing–12 hr		
19	Restraint–60 min	Swim–15 min	Restraint–60 min
20	Cold exposure (4 °C)–6 hr		
21	Swim–15 min	Restraint–60 min	Swim–15 min

Table 2

Body composition of male offspring on PND 21.

	CHOW-CON	CHOW-STRESS	HF-CON	HF-STRESS
Adipose (g)	11.5 ± 1.1	12.0 ± 0.8	22.3 ± 1.1 *	20.5 ± 1.1 *
Lean (g)	60.6 ± 5.4	66.2 ± 1.6	64.5 ± 1.6 *	62.3 ± 0.1 *
Water (g)	43.8 ± 3.8	48.0 ± 1.2	46.8 ± 1.2 *	45.1 ± 0.2 *

Data are presented as mean ± s.e.m.

* $P < 0.05$ vs. CHOW.