Obesity from Mother to Child

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We are pleased to offer you our innovative design, which results from a creative and effective cooperation with *Karger Publishers, Switzerland*.

Prof. Ferdinand Haschke, MD, PhD
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The global epidemic of obesity has generated a lot of interest in the mechanisms resulting in the epidemic and its effects on population health. Towards this end, the effect of maternal nutrition (over- and undernutrition) on fetal and neonatal outcomes is being studied. It is understood that the maternal nutritional status is not simply a question of supply but also the effects of confounding factors such as infections and workload. Maternal nutrition before, during and after birth can all influence the long-term outcomes of the infant.

In this supplement issue of *Annals of Nutrition and Metabolism*, Dr. Yajnik elegantly examines the transmission of obesity-adiposity and noncommunicable diseases from the mother to the baby. He reminds us that ‘we sometimes forget that an individual is born at conception and not at delivery’. Citing multiple studies including his own, he describes the mechanisms of methylation of DNA, chemical modifications of histones and transcription and miRNAs that interfere with the translation of mRNA. Dr. Yajnik discusses a modifiable component of the intergenerational transmission of health and disease traits and suggests that the model of ‘primordial’ prevention offers an opportunity to reduce the burden of noncommunicable diseases, obesity, diabetes and so on by influencing the lifestyle of young women in the preconceptional and periconceptional window.

In their paper entitled ‘Fast growth of infants of overweight mothers: can it be slowed down?’, Haschke et al. then analyze observational studies demonstrating that breastfed infants are protected from obesity in later life. They further describe the nutritional content of human breast milk and its changes with lactation using data from recent studies attempted to answer two questions: first, is there an effect of maternal obesity/overweight on infant growth? And second, does feeding a formula with a lower-than-traditional content of protein decrease the growth trajectory of the infant and, therefore, confer a benefit? From the pooled data, the authors found that infants born to overweight/obese mothers demonstrate accelerated growth during the first year of life even when they were breastfed. Further, they argue that reducing the protein content of follow-on formulas should be a goal for the formula industry by demonstrating that infants fed formulas with a lower protein content (closer to that of breast milk) while supporting adequate growth can ‘slow down fast growth of infants of overweight/obese mothers’. This decrease in growth acceleration may reduce the risk for future obesity.

Dr. Vickers from New Zealand addresses the issue of developmental programming and transgenerational transmission of obesity. Arguing that little attention has been paid to the role of developmental plasticity and alterations in phenotypic outcomes resulting from environmental perturbations in the early-life period, he elegantly describes current evidence suggesting that developmental programming is a transgenerational phenomenon. Data
from both human and animal studies as well as the mechanisms involved are presented. Transgenerational epigenetic transmission of traits allows future generations to be maximally competitive in their environment; however, evidence suggests that environmental exposures such as malnutrition in early life result in maladaptive parental responses that can be passed on to the offspring. Dr. Vickers concludes that ‘understanding the mechanisms of transgenerational inheritance is essential for the development of future intervention strategies to modulate not only that of the immediate adult phenotype but also that of the offspring, grandoffspring and beyond’.

Lastly, Michael G. Ross and Mina Desai continue on the theme with a review entitled ‘Developmental programming of appetite/satiety’ by using data from studies of maternal/fetal under- and overnutrition demonstrating the increased risk for obesity caused by hyperphagia. Further, infants small for gestational age or those born to obese mothers who consume a high-fat diet are also at an increased risk for adult obesity. The authors provide epidemiological evidence for perinatal appetite programming and discuss appetite regulation and mechanisms. Through complex interplay of multiple factors, they conclude that ‘the maternal environment can program fetal/newborn appetite/satiety pathways resulting in newborn and adult hyperphagia’.

In summary, this issue of Annals of Nutrition and Metabolism brings to light new information of transgenerational programming, mechanisms altering appetite and the epigenome as well as the effect of overnutrition in the offspring through postnatal feeding and offers a possible prevention strategy aimed at the future generation of mothers.

Jatinder Bhatia
We sometimes forget that an individual is born at conception and not at delivery.

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Transmission of Obesity-Adiposity and Related Disorders from the Mother to the Baby
by Chittaranjan S. Yajnik

Key insights
The most widely investigated mechanisms of the fetal programming of obesity-adiposity and non-communicable diseases (NCDs) are maternal-fetal nutrition and metabolism. The maternal nutritional status, deranged metabolism, infections, maternal stress and environmental factors have a major influence on fetal growth and programming. In this respect, the periconceptional, intrauterine and postnatal periods are the most influential in programming the health status of the following generation.

Current knowledge
The regulation of gene expression and function depends not only on the inherited DNA sequence, but also on the differential regulation of genes by environmental factors at different times in the life course of an individual. The main mechanisms involved in this process are DNA methylation, which has a ‘silencing’ effect on gene transcription, chemical modifications of histones, which alter chromatin structure and transcription, and miRNAs, which interfere with mRNA translation. The risk of obesity-adiposity-related NCDs is profoundly influenced by intrauterine and postnatal environmental modifications of the epigenome.

Practical implications
The identification of these regulatory mechanisms reveals modifiable components in the intergenerational transmission of health and disease. This offers potential means for curtailing the epidemic of NCDs worldwide. Both undernutrition and overnutrition contribute to NCDs, and in developing countries that are undergoing rapid socioeconomic and nutritional transition, the two cycles may operate sequentially within one lifetime, producing even more detrimental effects. Given the difficulty in implementing long-term lifestyle modifications in adults, an attractive alternative is to influence the lifestyle of young girls and pregnant women in the relatively short window of the periconceptional and gestational periods.

Recommended reading
Transmission of Obesity-Adiposity and Related Disorders from the Mother to the Baby

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Key Messages

- A conventional approach to non-communicable disease prevention targets unhealthy adult lifestyle.
- Epigenetic fetal programming is a novel idea.
- Human and animal studies support an ‘intrauterine programming’ of obesity, diabetes and other non-communicable diseases.
- Maternal nutritional imbalance, a deranged metabolism, stress and other factors contribute to fetal programming.
- Improving the health of young mothers will contribute to a ‘primordial’ prevention of non-communicable diseases in future generations.

Key Words
Obesity · Adiposity · Diabetes · Fetal programming · Intergenerational transmission · Primordial prevention · Mother and baby

Abstract

The conventional aetiological model of obesity and diabetes proposes a genetic predisposition and a precipitation by an unhealthy adult lifestyle. This hypothesis was challenged by David Barker who proposed that the intrauterine environment influences the risk of non-communicable diseases (NCDs). The original idea was based on fetal undernutrition because lower birth weight was associated with a higher risk of diabetes and heart disease. However, soon it was clear that the association was U shaped, and that the increased risk in large babies was driven by maternal obesity and diabetes. A number of human and animal studies have refined our ideas of ‘fetal programming’, which is now thought to be related to acquired chemical changes in DNA (methylation), histones (acetylation and other) and the role of non-coding miRNAs. Maternal nutritional disturbances are the major programming stimulus, in addition to a deranged metabolism, infections, maternal stress, extreme atmospheric temperature, etc. The first demonstration of a link between fetal ‘starvation’ and future ill-health was in the Dutch Hunger Winter studies. In the prospective Pune Maternal Nutrition Study, we found that small and thin Indian babies were more adipose compared to larger English babies, and their higher risk of future diabetes was reflected in higher insulin and leptin and lower adiponectin concentrations in the cord blood. This phenotype was partly related to a deranged 1-carbon metabolism due to an imbalance in vitamin B12 (low) and folate (high) nutrition, which was also related to insulin resistance in the offspring. Maternal obesity and diabetes have made an increasing contribution to childhood obesity and diabetes at a young age. This was prominently shown in Pima Indians but is now obvious in all other populations. The best window of opportunity to prevent fetal programming of NCDs is in the periconceptional period. This is
Transmission of Obesity-Adiposity and Related Disorders from the Mother to the Baby

There is a rapidly growing epidemic of obesity in the world, both in developed and in developing countries [1]. Obesity is a risk factor for a large number of health problems including diabetes, hypertension and cancer, collectively called non-communicable diseases (NCDs). The treatment of obesity is ineffective and not without side effects. Unless a preventive strategy is quickly outlined, obesity and the NCD epidemic will have substantial adverse effects on the development of the world population.

Recent ideas have focused on the ‘first 1,000 days’ of life as the most important window in the programming of health and disease.

Since the description of the human genome, a number of genetic associations have been described for obesity and related disorders. This generated a lot of hype and hope that this will help us deal with the problem. The findings are, however, unlikely to contribute to the prevention of the epidemic. The search for modifiable risk factors has therefore expanded, and a number of risk factors (diet, inactivity, stress) are already described. Many clinical trials have shown a beneficial effect of correcting some of these risk factors, but this effect is usually small and short lived. A recent discovery that offers hope for preventing the burgeoning epidemic of obesity and NCDs is the recognition of the importance of ‘intrauterine programming’ [2]. This thought started with the demonstration that those exposed to the Dutch Hunger Winter in utero had an altered risk of obesity as adults [3]. This was followed by the ‘fuel-mediated teratogenesis’ idea in diabetic pregnancies [4] and was finally established with the ‘thrifty phenotype’ hypothesis from Southampton (UK) [5, 6]. Recent ideas have focused on the ‘first 1,000 days’ of life as the most important window in the programming of health and disease and offer hope that an intervention in early life could help prevent these disorders. We will discuss the scientific basis, observational and interventional evidence as well as future directions in this new paradigm called ‘developmental origins of health and disease’ (DOHaD) in relation to obesity and related NCDs. This article is not proposed to be a comprehensive review but an attempt to discuss some basic concepts.

Background

We sometimes forget that an individual is born at conception and not at delivery. The genome of the conceptus contains all the information for the development into a new individual. The information flows from the DNA to the RNA to the protein. The factors governing this flow contribute to the development and the differentiation of cells, tissues, organs and systems which contribute to the phenotype of the individual. Waddington [7] described all these processes which link the genotype to the phenotype at birth as ‘epigenetic’. Modern epigenetics investigates the molecular basis of the regulation of the flow of information, involving gene expression and function. This does not depend on the sequence of bases in the DNA molecule which is inherited from the parents, but on the differential regulation of genes by environmental factors at different times in the life course of an individual. Currently, three mechanisms are believed to be involved in this process: (1) methylation of DNA which prevents transcription, i.e. ‘silences’ the gene; (2) chemical modifications of histones (acetylation, methylation, etc.) which alter the chromatin structure and transcription, and (3) miRNAs which interfere with the translation of the mRNA. The DOHaD theory (fig. 1) proposes that the risk of obesity-adiposity-related NCDs is substantially influenced by intrauterine and postnatal environmental modifications of the epigenome. Occurring during crucial periods of the development, there is a permanent change in the structure and function of the organism (phenotype), which is called programming.

‘Early life programming’ offers hope that investment in early life could help prevent non-communicable disorders.

Periconceptional, intrauterine and postnatal periods are the most influential in programming. The periconceptional period encompasses the natural ‘wiping out’ of the inherited epigenome and the ‘reestablishment’ of a new one [8]. This period also covers vital processes such as...
as gametogenesis, fertilisation, implantation, morphogenesis, embryogenesis, organogenesis and placentation, all of which have a profound influence on the growth, development, differentiation and phenotype of an individual [9] (fig. 2). If the environment is not ideal (for example, maternal nutritional imbalance, altered metabolism, infections, exposure to environmental pollutants, etc.), this might lead to undesirable programming and induce the risk for later disease.

Barker’s [2, 10] ideas about intrauterine programming and DOHaD were based on retrospective cohorts in the UK, Finland and other countries. The most frequently available measure of intrauterine nutrition and growth was birth weight with very direct measurements of maternal nutrition. These studies showed that low birth weight was a predictor of future diabetes, hypertension, coronary heart disease and other NCDs [10]. The majority of these are associated with obesity (excess weight for a given height). It is to be appreciated that the BMI, the most frequently measured index of obesity, is not a measure of body fat but only a surrogate. A higher percent of body fat is more appropriately called ‘adiposity’ and necessitates direct measurements of body fat (skinfolds, bioimpedance, isotopic dilution, DXA, CT scan, MRI, etc.). It is important to distinguish between these measurements because the interpretation could be misleading, especially when comparing two different populations [11].
**Maternal Nutrition**

The most investigated causes of fetal programming of obesity-adiposity and NCDs are maternal-fetal nutrition and metabolism. Intriguingly, both undernutrition and overnutrition may be responsible.

The first indication that intrauterine undernutrition may be linked to future obesity and related disorders came from the follow-up of individuals who were in utero at the time of the Dutch Hunger Winter, when the Dutch population (including pregnant women) had to survive on a few hundred calories a day for many months. When maternal undernutrition occurred in the first and second trimesters of intrauterine life, the offspring were more likely to be obese as young adults, whereas those exposed in the third trimester were less likely to be obese [3]. It was postulated that in the first two trimesters, undernutrition affected the development of hypothalamic appetite centres, leading to overeating and obesity in postnatal life, while the reduced risk of obesity observed with third-trimester undernutrition was related to a reduction in the number of fat cells which develop during this period. A number of Dutch Winter Hunger studies have linked in utero undernutrition to a variety of outcomes such as diabetes, coronary heart disease, schizophrenia, etc. [12, 13].

The fetal undernutrition theory gained prominence with the publication of the ‘thrifty phenotype’ hypothesis of Hales and Barker [5]. They observed that low birth weight was a risk factor for diabetes, independent of the adult BMI and family history, and proposed that ‘diabetes was a result of the fetus having to be thrifty during its intrauterine life and in infancy’. This caused a considerable upset in the field because it challenged the dogma that diabetes resulted from genetic predisposition and an unhealthy adult lifestyle. Even though fetal nutrition depends on a complex supply line [14], maternal nutrition is the ultimate source of fetal nutrition. Therefore, improvement in the health and nutrition of women in their reproductive age might provide an intergenerational solution to the rapidly escalating epidemic of obesity and diabetes.

The original studies of the thrifty phenotype were all conducted in European populations. Barker [personal communication] predicted that the link between fetal undernutrition and diabetes may be even more important in a country like India, where there is a history of multigenerational undernutrition. It is well recognised that macrosomic infants of diabetic mothers are at a high risk of obesity and diabetes. The low birth weight story was a revelation. However, diabetes was rapidly increasing in India even in the absence of the conventional risk factor of obesity, and ‘malnutrition-related diabetes’ was reported from different parts of India [17, 18]. In collaboration with Prof. Barker and Caroline Fall, we set up a study in 4-year-old children born in our hospital (Pune Children’s Study) [19]. The average birth weight was 2.8 kg. Lower birth weight in these children was associated with higher glucose and insulin concentrations after glucose load, suggesting insulin resistance. When we followed up these children at 8 years of age, we were able to confirm the original observation and additionally found that the children who were born small but grown big at 8 years had the highest levels of adiposity, central obesity, insulin resistance and other cardiovascular risk factors [20]. This was the first demonstration of DOHaD in a developing population which suggested that a mismatch between intrauterine and postnatal experiences was conducive to an increased risk of NCDs. We therefore set up the Pune Maternal Nutrition Study (PMNS) to investigate the influence of maternal nutrition on fetal growth and the future risk of diabetes and other NCDs in 6 villages near Pune. This study has provided some notable findings.

The newborn Indian babies were small and thin (average birth weight = 2.7 kg, ponderal index = 2.4). However, a comparison of detailed anthropometric measurements of newborns in Pune and Southampton (UK) revealed an interesting story. The Indian babies were, on average, 800 g lighter than the English babies but had almost similar subscapular skinfold measurements, suggesting that the Indian babies had a low lean mass but a relatively high fat mass, what we called the ‘thin-fat’ Indian baby (fig. 4) [21]. This observation was similar to the findings in adult Indians and Europeans [11] and demonstrated that the adipose Indian body composition, which predisposes to a higher risk of diabetes and related disorders, may be linked to future obesity and related disorders.
disorders, originates in intrauterine life, and that it is not a result of an unhealthy adult lifestyle. The use of MRI revealed that the Indian babies have a very small abdominal circumference (a marker of intrauterine growth restriction) but higher abdominal fat in both the subcutaneous and intra-abdominal compartments compared to the English babies [23]. Thus, visceral adiposity, a major risk factor for diabetes, is present in Indians from their intrauterine life, suggesting that the interventions to reduce the risk of diabetes should start intergenerationally. It has been suggested that this characteristic body composition of Indians may be due to genetic factors. This might be true to a certain extent, but as yet we have found only small differences in the genetic predisposition to diabetes and obesity-adiposity between Indians and Europeans. The major implication of our finding is that the nutrition of the fetus may be a modifiable risk factor to reduce the risk of NCDs in Indians.

The PMNS analysed the association between maternal nutrition and fetal growth and body composition. The mothers investigated lived in rural areas, were predominantly vegetarians and consumed on average 1,800 calories and 45 g protein per day, which is much below the recommended intakes. There were only a few associations between maternal macronutrient intake and neonatal size. On the other hand, the intake of micronutrient-rich foods (green leafy vegetables, milk and fruits) was strongly related to birth size (fig. 5) [24].

In this predominantly vegetarian rural population, folic acid deficiency was rare, but two thirds of the mothers were vitamin B12 deficient. Circulating folate, vitamin C, vitamin D and iron were associated with fetal growth. Maternal circulating homocysteine concentration (a marker of 1-carbon metabolism) predicted fetal growth restriction [25]. Further, at 6 years of age, maternal folate status in pregnancy was a predictor of the child's adiposity and insulin resistance [26]. The most insulin-resistant children were born to mothers who had low vitamin B12 but high folate levels, suggesting that a balance between these two vitamins is essential. The importance of folate in fetal growth has been well demonstrated in non-vegetarian European populations, but the role of vitamin B12 is less clear. Studies in Bangalore [27], Nepal [28] and Mysore [29] have supported different aspects of our findings in Pune. Folate as well as vitamins B12, B6 and B2 regulate maternal 1-carbon metabolism (usually assessed by circulating homocysteine concentration), which influ-

![Fig. 3. The association between birth weight and risk of diabetes is U shaped, though in individual studies an inverse or direct association may be seen due to preponderance of maternal under- or overnutrition and diabetes [15, 16].](image1)

![Fig. 4. The concept of a thin-fat Indian baby: despite their smallness in all the anthropometric measurements, Indian babies have a relatively higher fat mass in comparison to English babies [21]. Modified from [22].](image2)
ences cellular growth by helping synthesis of nucleic acids and providing the essential amino acid methionine. In addition, methylation of DNA is one of the major mechanisms of epigenetic regulation, thus influencing the genetic regulation of growth and differentiation. The role of 1-carbon metabolism and these vitamins in fetal growth and programming has been recently reviewed [30–32].

**Maternal Metabolism**

In addition to nutrition, maternal metabolism exerts a major influence on fetal growth and programming. Substantial evidence has now accumulated that maternal diabetes and obesity (causing fetal overnutrition) influence future obesity and diabetes in the child. The common perception is that this mechanism must be genetic, but a series of studies have shown a prominent role for non-genetic mechanisms.

The central observation in this field was made by Pedersen [33], who suggested that an excess transfer of glucose and other fuels to the baby in a diabetic pregnancy stimulates fetal islets and causes hyperinsulinaemia. Insulin is a major growth hormone in utero and promotes the growth of insulin-sensitive tissues and organs, leading to fetal adiposity and macrosomia at birth. Freinkel [4] expanded this idea to suggest that the increased risk of postnatal obesity and diabetes was a ‘fuel-mediated teratogenesis’ by comparing these common long-term outcomes with rare disfiguring birth defects (for example, neural tube defects). He proposed that this was mediated by a ‘stable change in gene expression’, which we now call epigenetic programming, and not on the inherited genetic trait (fig. 6).

The Pima Indian studies [34–37] critically investigated the genetic-versus-intrauterine environmental aetiology of obesity and diabetes in the offspring of diabetic mothers. The Pima study has an unparalleled design where every member of the community more than 5 years of age, including all pregnant women, if not already diabetic, was serially investigated with an oral glucose tolerance test every 2 years. This allowed a more confident classification of the in utero exposure of the fetus to maternal diabetes.
The first investigation showed that the children exposed to maternal diabetes in pregnancy were more likely to be obese (and glucose intolerant) compared to children born to prediabetic and non-diabetic mothers [35]. This suggested that the intrauterine diabetic environment could be more important than the genetic factors in the aetiology of obesity and diabetes in the children of diabetic mothers. Interestingly, the risk was independent of macrosomia at birth and manifest as early as 5 years of age. To investigate the possibility that the mothers who were diagnosed as having diabetes at a younger age will transmit a stronger genetic predisposition, a subsequent study [36] investigated the risk of obesity and diabetes in the siblings (expected to inherit similar amounts of diabetes and obesity genes) who were discordant both for the exposure to intrauterine hyperglycaemia (born before or after the development of maternal diabetes) and outcome (obese or non-obese, normal glucose tolerant or hyperglycaemic). This study showed that the risk of obesity and diabetes was higher in the siblings who were born after the diagnosis of maternal diabetes.

In all these studies, there was no corresponding association with paternal obesity and diabetes, which supports a stronger role for the intrauterine diabetic environment compared to genetic predisposition in the aetiology of obesity and diabetes in the children of diabetic mothers. It is estimated that up to 70% of diabetes in Pima Indian children is due to maternal diabetes [37]. It is of note that most of the diabetic Pima Indian women had pregestational rather than gestational diabetes, and, therefore, the children were exposed to an abnormal metabolic mi-

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*Fig. 6.* ‘Fuel-mediated teratogenesis’ refers to an excess transfer of fuels (glucose, amino acids and fatty acids) across the placenta to the developing fetus which causes ‘stable change in gene expression’ affecting not only perinatal but also long-term outcomes in the offspring (obesity and diabetes) [4, 31]. IGT = Impaired glucose tolerance; DM = diabetes mellitus. Modified from [4].

*Fig. 7.* Intrauterine programming of obesity by maternal hyperglycaemia in Pima Indians. Children born to diabetic mothers had a much higher prevalence of obesity compared to children born to prediabetic and non-diabetic mothers [35]. Reproduced with permission from [34].
lieu periconceptionally rather than only late in the pregnancy. In this context, it may also be important to note that the subsequent risk of obesity and diabetes was independent of birth size which is largely ‘fueled’ in the last trimester. The follow-up of children born to mothers with gestational diabetes in the Parthenon study in Mysore, India, has shown an increased risk of obesity and prediabetes below 10 years of age especially in girls [38]. A Danish study which followed children of type 1 and type 2 diabetic mothers as well as children born to mothers with gestational diabetes showed that all these children had a higher risk of diabetes in later life [39]. Many of the diabetic mothers in the Chicago Diabetes Pregnancy studies were also pregestationally diabetic [40].

Other Intrauterine Exposures
In addition to maternal nutrition and metabolism, a number of other factors have been implicated in the regulation of fetal growth and for programming of NCDs in the offspring. These include maternal smoking, exposure to extreme (high) atmospheric temperature, pollutants and toxins (especially the 'endocrine disruptors'). Another major programming exposure may be maternal stress. All these findings suggest that even subtle alterations in the maternal intrauterine environment can have profound effects on the development of the fetal systems and that these effects last for the rest of the child’s life.

The importance of the time of exposure during pregnancy (window) can be easily understood by the sequence of fetal development and has been described for the periconceptional period earlier in the article.

Synthesis
It is now well established that, in addition to the conventional genetic inheritance (the so-called fixed inheritance), there are additional ‘malleable’ epigenetic influ-
ences (the so-called soft inheritance) which shape the future of the growing fetus. These mechanisms translate the environmental messages to the structure and the function of the developing fetus and leave a permanent mark. They are described under the general term of ‘epigenetic’ and represent gene-environment interactions. These new exciting findings describe a modifiable component of the intergenerational transmission of health and disease traits and offer hope of curtailing the rapidly expanding epidemic of NCDs across the world populations. Specific evidence is available for susceptibility to obesity-adiposity, diabetes, cardiovascular disease, neuropsychiatric disorders and cancers. The life-course evolution of these disorders includes a series of changes in the epigenome which start from the periconceptional period and continue to occur in the later life, producing an evolving phenotype which finally ends in a clinically recognisable disorder (fig. 8). Both undernutrition and overnutrition contribute to these processes. In the developing countries undergoing rapid socioeconomic and nutritional transition, the two cycles could operate one after the other in one life time, producing an even more detrimental effect on the phenotype (dual teratogenesis) (fig. 9). This may contribute to the high prevalence of these conditions. Preventive measures will depend on identifying specific exposures, demonstrating an effect of acting on them. Given the difficulties in identifying specific exposures among the multitude as well as the long latency between exposure and disease manifestation, this is not an easy task. Despite these limitations, substantial progress has been made in the last 25 years in this field both in animal and human models. The science of DOHaD is now well established, and preventive trials are in progress. Considering the difficulty of implementing long-term lifestyle modifications in adults to control the risk of NCDs, the model of primordial prevention by influencing the lifestyle of young girls and pregnant women in the relatively short period of periconceptional and gestational windows offers a more attractive alternative. The success of such interventions will be an appropriate tribute to the brilliant research of pioneers in this field over the last many decades.

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Infants born to overweight/obese mothers show accelerated growth during the first year of life even when breastfed, and fast growth is known to be an antecedent of later obesity.

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Fast Growth of Infants of Overweight Mothers: Can It Be Slowed Down?
by Ferdinand Haschke et al.

Key insights
Infants born to overweight or obese mothers show accelerated growth during the first year of life even when breastfed. The effect of maternal obesity is most marked during the first 6 months after birth, coinciding with the time when breast milk provides the majority of nutrients. This suggests that the effect of maternal obesity is mediated through the composition or amount of breast milk. Traditional formulas for infants older than 3 months of age contain unnecessarily high amounts of protein. Formulas with a low protein content closer to that of breast milk may slow down the growth of infants of overweight or obese mothers, reducing the risk of future obesity.

Current knowledge
Breastfeeding may exert its protective effect against later obesity by slowing growth and diminishing adiposity during infancy. Since growth during infancy has a potentially large impact on long-term health, it is important to understand the factors that influence growth in infancy. This article pools the data from 3 recent studies in order to determine whether there is an effect of maternal overweight/obesity on infant growth, and whether a low formula protein content affects infant growth.

Practical implications
Infants born to and breastfed by overweight or obese mothers were larger than the benchmark WHO standards. Weight gain of breastfed infants was significantly higher if the mothers were overweight or obese. In formula-fed infants, the protein concentration of infant formulas had a significant influence on weight gain in the first year of life. Infants fed low-protein formulas still showed lower weight gain than infants fed high protein formula. Reducing the protein content in formulas for infants beyond 3–4 months of age should be a goal of the industry.

Recommended reading
Fast Growth of Infants of Overweight Mothers: Can It Be Slowed Down?

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Introduction

Obesity is a growing health problem worldwide, and there is great interest in preventive measures. As obesity may have its beginning in fetal life or in infancy, early-life events are receiving increasing attention. Rapid growth in infancy is associated with later overweight and obesity [1–8]. Many observational studies have shown that breastfed infants are protected from obesity later in life [7, 9–13]. It is a well-established fact that breastfed infants grow slower than formula-fed infants [14–17], and breastfed infants have been claimed to be leaner than formula-fed infants [18], although body composition studies do not seem to confirm this finding [19]. It is therefore widely thought that breastfeeding exerts its protective effect against later obesity by slowing growth and diminishing adiposity during infancy [20]. The strong belief that breastfeeding is conducive to improved health later in life led the WHO to establishing its new growth standards based on data from infants breastfed for most of the first year of their life [21]. Avoidance of excessive growth during infancy is therefore believed to be desirable and conducive to good health later in life.

The protein needs of the normal infant may be estimated by the factorial method [22]. They may also be derived from the protein intake of the breastfed infant on the assumption that breastfeeding meets the protein re-
quirements of the infant [22, 23]. Estimates by the two approaches agree closely, supporting the notion that breastfeeding meets the protein needs of the infant. The protein content of breast milk decreases from a high of 2.09 g/100 kcal in the first month after birth to about 1.28 g/100 kcal by 3–4 months and to 1.24 g/100 kcal by 9–12 months [22]. Feedings such as formulas would be expected to meet the protein needs of infants if the quality of the protein were similar to that of breast milk and its concentration slightly higher.

Figure 1 illustrates the decreasing protein concentration of breast milk and contrasts it with the fixed protein concentration of formulas. As indicated, the protein concentration of formulas needs to be somewhat higher than that of breast milk commensurate with a possible lower quality of formula protein. Figure 1 also shows the range of protein content in infant formulas currently (as of 2014) commercially available worldwide. The important difference between breast milk and formulas is that the formula protein concentration is fixed and does not decrease as the infant’s needs decrease.

Figure 1. Protein concentration in breast milk [22, 23] and in infant and follow-up formulas. The arrow indicates the big gap from 3 months onwards.

Until about 15 years ago, formulas for infants aged from 4 to 12 (36) months (follow-up formulas [24]) ranged in protein content from 2.5 to 4.6 g/100 kcal. The effect of such high protein feedings on weight and adiposity of infants was assessed in a prospective randomized trial conducted in the context of the European Obesity Project [25]. Feeding formulas with a high protein content (4.60 g/100 kcal) led to increased weight with increased adiposity. The protein content of the high-protein formula used in that trial [25] was representative of the traditional high protein content of formulas for older infants (follow-on formulas). Further, epidemiologic evidence links high protein intakes in infancy to obesity in childhood [26–29].

Since growth during infancy has a potentially large impact on long-term health benefits, it is important to understand the factors that influence growth in infancy. We had the opportunity to use pooled data from 3 recently completed studies [30–32] to try to answer the following questions: (1) Is there an effect of maternal overweight/obesity on infant growth? (2) Does a low formula protein content affect (slow down) infant growth?

The important difference between breast milk and formulas is that the formula protein concentration is fixed and does not decrease as the infant’s needs decrease.

Methods
In this analysis, we used individual data from 3 recent randomized controlled trials where breastfed reference groups were also included. One study each was conducted in France, Chile, and the USA [30–32]. The 3 studies that provided data for the present pooled analysis of weight gain in breastfed infants involved a total of 225 normal infants who were followed from birth to 12 months. Two studies in formula-fed infants (n = 366; Chile and USA) compared weight gain between 3 and 12 months when different amounts of protein were provided with the study formulas. The pre-pregnancy BMI of the mothers was categorized as normal (<25), overweight (25–30), or obese (>30). In the study from Chile, all mothers had a pre-pregnancy BMI ≥25, whereas the study in France involved mothers who had a pre-pregnancy BMI <25. The study conducted in the USA involved unselected mothers. Enrollment criteria and study procedures are described elsewhere [31, 32]. Infants were enrolled at birth, and in the Chilean and US studies formula-fed infants were randomized at 3 months.

Maternal Overweight – Breastfed Infants
To examine whether maternal pre-pregnancy BMI has an effect on infant weight gain from 0 to 6 and from 0 to 12 months, we examined the relationship between maternal BMI and infant weight gain (g/day) by spline interpolation (ITT population). As a second
Can Fast Growth of Infants of Overweight Mothers Be Slowed Down?

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step, we employed a regression analysis with factor selection by employing Akaike’s Information Criteria (AIC). The dependent variable was weight gain between 0 and 12 months. Independent variables considered in the model were birth weight, maternal BMI >25, and gender.

Protein Intake – Formula-Fed Infants

Data of infants who were enrolled at birth in Chile and the USA were considered (ITT population). Infants whose mothers chose to discontinue breastfeeding before 3 months of age were randomized at 3 months. In Chile, infants were randomized to formulas providing either 1.65 or 2.7 g protein/100 kcal (n = 86/86), and in the USA (n = 32) to formulas with 1.61 or 2.15 g protein/100 kcal (n = 97/97). Study formulas were fed exclusively from 3 to 6 months and continued with complementary foods from 6 to 12 months. The low-protein formulas in the 2 studies were whey-based and had amino acid profiles similar to those of breast milk [31]. The formulas with a higher protein concentration were also whey-based and corresponded to international recommendations [24]. In both studies, caloric intakes with formulas were similar in the groups receiving the low- and high-protein formulas [31, 32]. Growth data were analyzed by a regression analysis, in which weight gain between 3 and 6 and between 3 and 12 months were the dependent variables. Independent variables were birth weight, maternal BMI, gender, formula protein content, and the infants’ weight gain between birth and 3 months.

Results

Maternal Overweight/Obesity and Growth of Breastfed Infants

In the study from Chile, there were 76 breastfed infants, of whom 89% were exclusively breastfed at 4 months and 66% at 6 months. Their weight for age z-scores at birth and at 6 and 9 months of age were +0.55, +0.89, and +1.0, respectively. This shows that infants born to and breastfed by overweight/obese mothers are larger than stated in the WHO standards [21, 33]. In the larger cohort of breastfed infants (n = 225), weight gain in infants of mothers with a BMI between 18 and 25 was below (0–6 months) or similar (0–12 months) to the WHO standards [34] as shown in figure 2a and b. Weight gain increased significantly as maternal BMI increased beyond 25. The mean weight gain between 0 and 6 months was greater by almost 5 g/day if the mother’s BMI was 30 compared to 25, and the weight gain between 0 and 12 months was greater by 3 g/day. The fact that the effect of maternal obesity was most marked for the 0- to 6-month interval, a time when breast milk provides the majority of nutrients, is consistent with the notion that the effect of ma-

Fig. 2. Weight gain of breastfed infants between 0 and 6 months (a) and between 0 and 12 months (b) in relation to maternal BMI. Data are from 3 studies (ITT population). Spline curves and 95% CI indicate significant relationships (0–6 months, p < 0.001; 0–12 months, p < 0.001). The horizontal lines indicate the WHO standards (50th percentiles).
ternal obesity is mediated through the composition or amount of breast milk. As the infant becomes older, other foods increasingly contribute nutrients and the influence of maternal milk becomes diluted.

In the regression model (table 1), boys gained weight more rapidly than girls. A higher maternal BMI resulted in a significantly higher weight gain. If the maternal BMI was >25, weight gain between birth and 12 months was 346 g higher than if the maternal BMI was <25. Birth weight and interactions between independent variables were not significant.

### Table 1. Factors which have an influence on weight gain (g/day) of breastfed infants between 0 and 12 months

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Difference</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>1.85</td>
<td>1.12–2.60</td>
<td>0.000001</td>
</tr>
<tr>
<td>Maternal BMI</td>
<td>0.19</td>
<td>0.01–0.28</td>
<td>0.00006</td>
</tr>
<tr>
<td>Male gender</td>
<td>1.80</td>
<td>1.14–2.64</td>
<td>0.000001</td>
</tr>
<tr>
<td>Maternal BMI &gt;25</td>
<td>1.04</td>
<td>0.29–1.77</td>
<td>0.000001</td>
</tr>
</tbody>
</table>

1 Continuous variable.

### Table 2. Factors which have an influence on weight gain (g/day) of formula-fed infants during the periods 3–6 and 3–12 months

<table>
<thead>
<tr>
<th>Weight gain 3–6 months</th>
<th>Difference</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain 0–3 months</td>
<td>0.18</td>
<td>0.09 to 0.26</td>
<td>0.00005</td>
</tr>
<tr>
<td>Formula protein (g/100 kcal)</td>
<td>1.22</td>
<td>0.00 to 2.44</td>
<td>0.05</td>
</tr>
<tr>
<td>Maternal BMI &gt;25</td>
<td>0.09</td>
<td>−0.02 to 0.19</td>
<td>0.09</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Weight gain 3–12 months</th>
<th>Difference</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain 0–3 months</td>
<td>0.05</td>
<td>0.00 to 0.11</td>
<td>0.05</td>
</tr>
<tr>
<td>Formula protein (g/100 kcal)</td>
<td>1.04</td>
<td>0.26 to 1.82</td>
<td>0.009</td>
</tr>
<tr>
<td>Maternal BMI &gt;25</td>
<td>0.03</td>
<td>−0.03 to 0.10</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Factors with a significant influence on weight gain of formula-fed infants are listed in table 2. In the regression model, the protein concentration of infant formulas had a significant influence on weight gain between 3 and 6 and between 3 and 12 months. Formulas with protein concentrations between 1.61 and 2.7 g/100 kcal were included in this analysis. An increase in the protein content of formulas of 1 g/100 kcal caused an increase in weight gain of 287 g between 3 and 12 months. Weight gain from birth to 3 months was the only other independent variable that exerted a significant effect on weight gain between 3 and 12 months. There was a trend of maternal BMI leading to higher weight gain, but it did not reach statistical significance. The interaction of maternal BMI and formula protein did not influence weight gain. It is of interest that among formula-fed infants the influence of gender was small and not significant. Weight gain between 3 and 12 months in the subgroup of formula-fed infants whose mothers had a BMI >25 was significantly influenced by the formula protein content (+1.0 g/day; 95% CI 0.25–1.87; p = 0.02) but not by maternal BMI (+0.09 g/day; 95% CI −0.01 to 0.18; p = 0.07). The influence of the formula protein content between 3 and 12 months was therefore sim-

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Fig. 3. Weight gain (g/day) between 0 and 12 months of infants who were fed low-protein formulas between 3 and 12 months in relation to maternal BMI. Only 25% of the mothers in the 2 studies had a BMI <25. The spline curve shows no significant relationship (p < 0.09). The horizontal line indicates the WHO standard (50th percentile).
ilar in the overall infant cohort and the subcohort of infants whose mothers had a BMI >25.

**Discussion**

The reasons why infants of overweight mothers gain more weight than infants of leaner mothers are still not clear. Plagemann and Harder [35] reviewed the literature in 2005 and concluded that clarification is needed whether breastfeeding might even have negative consequences for the risk of overweight and diabetogenic disturbances in the offspring when the mother suffers from a metabolic disorder. One possibility is intrauterine metabolic programming [36], because newborns of mothers with a BMI >25 already have birth weights that are 0.6 z-scores higher than the WHO standards [33]. A recent meta-analysis [37] has indicated that pre-pregnancy overweight/obesity increases the risk of the newborn infant being large for gestational age (OR 1.53; 95% CI 1.44–1.63, and OR 2.08; 95% CI 1.95–2.23), having a high birth weight (OR 1.53; 95% CI 1.44–1.63, and OR 2.00; 95% CI 1.84–2.18), and suffering from macrosomia (OR 1.67; 95% CI 1.42–1.97, and OR 3.23; 95% CI 2.39–4.37). In addition, the risk of subsequent offspring overweight/obesity during childhood (OR 1.95; 95% CI 1.77–2.13, and OR 3.06; 95% CI 2.68–3.49) is higher. Differences in the fatty acid composition [38, 39], protein and energy content [40], and the microbiome [41] of breast milk of normal- and overweight mothers have been found. Fields and Demerath [42] showed that the concentrations of insulin, leptin, and other components were high in breast milk of obese mothers and correlated with the growth of their infants. It is therefore possible that, in addition to intrauterine and postnatal metabolic programming [36], nutritional factors contribute to the higher weight gain of breastfed infants if the mother is overweight or obese.

Our data show that infants fed low-protein formulas still grow somewhat faster than breastfed infants of mothers with a BMI between 18 and 25 (exemplified by the WHO standards [34]). This is consistent with the protein intake being above requirement. Contrary to breastfed infants, in infants fed low-protein formulas there was no noticeable influence of maternal overweight on the growth of the infants. Perhaps this was so because the growth-slowing effect occurred mostly in the fastest growing infants, thus obliterating the effect of high maternal BMI. However, this finding is also consistent with the notion that the fast growth of infants who are breastfed by overweight mothers is mediated through the composition or quantity of the breast milk of these mothers. Whatever the mechanism may be, the fact that growth was slowed by low-protein formulas makes these formulas an important tool in the efforts to slow fast growth of infants.

The high protein content of follow-on formulas is contributing to the very high protein intakes that have been documented in several localities during the latter part of infancy [9, 26, 42, 44]. High protein intakes that greatly exceed the required intakes have been linked by epidemiologic evidence to obesity in infancy and childhood [26–28] and have been shown in prospective studies to cause fast growth and increased adiposity [10, 25]. Recently, metabolic profiling of stool and urine samples has indicated specific metabolic signatures that were associated with increasing protein content in formulas [45]. Data from the 2 clinical trials analyzed in the present report provide further evidence that high protein intakes from formulas might be harmful.

Reducing the protein content in formulas fed after 3–4 months should be a goal of the industry. In the meantime, formulas which have regulatory approval still have substantially higher protein content than breast milk after 3 months.

**Conclusion**

Infants born to overweight/obese mothers show accelerated growth during the first year of life even when breastfed, and fast growth is known to be an antecedent of later obesity.

**Disclosure Statement**

F. Haschke is a board member of the Nestlé Nutrition Institute and receives consulting fees. E.E. Ziegler declares no conflict of interest. D. Grathwohl is an employee of the Nestlé R&D (Nestec Ltd.) organization.
References

The transgenerational epigenetic transmission of traits allows future generations to be maximally competitive in their environment. Under this assumption, adaptive gene programs acquired during the parental life span persist in the subsequent generation.

Key insights
The effects of developmental programming may be transmitted not only from parent to offspring, but to generations beyond. Data from human and animal studies indicate that metabolic disorders and adverse environmental influences may be perpetuated to the F2 and F3 generations via both the maternal and paternal lineages.

Current knowledge
Environmental factors can modulate the activity of the genome through several mechanisms. The most widely studied mechanism is epigenetics, with the classic example being the influence of the maternal phenotype and intrauterine environment on the regulation of fetal energy metabolism via altered DNA methylation of specific genes. However, environmental influences during key periods in development can also alter the germline of the fetus, thereby affecting the F2 offspring and beyond.

Practical implications
Exposure to environmental stressors such as poor early-life nutrition can result in maladaptive epigenetic traits that are passed on to the offspring. Over several generations, these have the potential to manifest as a population-wide phenotype. The rapid perpetuation of conditions such as obesity is especially relevant to populations undergoing the transition between traditional and Western lifestyles. There is an urgent need to fine the mechanisms that underpin the transmission of developmental programming in order to modulate the phenotype of future generations.

Recommended reading
Developmental Programming and Transgenerational Transmission of Obesity

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Key Words
Developmental programming · Transgenerational transmission · Obesity · Epigenetic inheritance · Germline · Methylation · Maternal nutrition

Abstract
The global obesity pandemic is often causally linked to marked changes in diet and lifestyle, namely marked increases in dietary intakes of high-energy diets and concomitant reductions in physical activity levels. However, far less attention has been paid to the role of developmental plasticity and alterations in phenotypic outcomes resulting from environmental perturbations during the early-life period. Human and animal studies have highlighted the link between alterations in the early-life environment and increased susceptibility to obesity and related metabolic disorders in later life. In particular, altered maternal nutrition, including both undernutrition and maternal obesity, has been shown to lead to transgenerational transmission of metabolic disorders to the F3 generation through both the paternal and maternal lineage following a range of altered maternal (F0) environments.

Key Messages
• Experimental and human evidence to date suggests that developmental programming is a transgenerational phenomenon. A number of potential mechanisms underlie the transmission of metabolic traits, including epigenetic effects via the germline, a suboptimal reproductive tract environment or altered maternal adaptations to pregnancy.
• Evidence from human cohorts is limited, although paternal line transmission of ill-health has been reported through to the F2 generation; data from animal studies describe transgenerational transmission of metabolic disorders to the F3 generation through both the paternal and maternal lineage following a range of altered maternal (F0) environments.
• Many studies reported to date are up to the F2 generation, whereas true transgenerational transmission is the F3 generation and beyond where there is no exposure to the initial environmental challenge; however, data in the F3 generation are limited and often variable depending on the model used.
The mechanisms responsible for these transgenerational effects remain poorly understood; evidence to date suggests a number of potential mechanisms underpinning the transgenerational transmission of the developmentally programmed phenotype through both the maternal and paternal lineage. Transgenerational phenotype transmission is often seen as a form of epigenetic inheritance with evidence showing both germline and somatic inheritance of epigenetic modifications leading to phenotype changes across generations. However, there is also evidence for non-genomic components as well as an interaction between the developing fetus with the in utero environment in the perpetuation of programmed phenotypes. A better understanding of how developmental programming effects are transmitted is essential for the implementation of initiatives aimed at curbing the current obesity crisis.

Background

The developmental programming hypothesis suggests that the early-life environment influences offspring characteristics in later life, including susceptibility to the development of obesity and related metabolic disorders. Growing evidence now indicates that the effects of developmental programming may also be transmitted to future generations in the absence of further environmental stressors. The developmental programming hypothesis has opened up a new research paradigm for understanding chronic disease risk that moved beyond the simplistic explanations based on genetic and lifestyle influences. A more integrated approach has developed examining the interaction between genetic inheritance and lifestyle factors (including diet) but also incorporating the role of developmental plasticity – the ability of changes in gene function to generate a range of phenotypic outcomes based on environmental exposures [1].

The transmission of developmental programming effects is often viewed as a form of epigenetic inheritance, either via the maternal or the paternal line. Evidence exists for both germline and somatic inheritance of epigenetic modifications which may be responsible for phenotypic changes in further generations [2]. It must be noted, however, that the definition of ‘transgenerational’ is not straightforward in the setting of developmental programming [2, 3]. As per figure 1, when a perturbation is applied to the mother (F0, e.g. altered maternal diet), it directly effects the developing fetus in utero (F1) and also the germ cells that will form the F2 generation. Thus, strictly speaking, only later generations (F3 and beyond) can truly be deemed ‘transgenerational’ and not a consequence of the initial early-life exposure [2, 4]. However, except for a few rodent studies (with varying and, in some cases, conflicting data), very limited data are available on the F3 generation and beyond.

The initial epidemiological studies by Barker [5] and other researchers linked fetal growth restriction to later disease, implying that fetal nutritional deprivation may be a strong programming stimulus. This led to a range of experimental animal models that primarily utilised maternal dietary manipulations to induce fetal growth restriction, e.g. maternal calorie, protein or macronutrient deficiency during critical periods of development, to examine transgenerational phenotype transmission. However, in many societies, maternal and postnatal nutrition are now either sufficient or excessive. As a result, excessive weight gain and/or maternal obesity are the more common nutritional problems complicating pregnancy in developed countries. Thus, in view of the rising prevalence of obesity in pregnancy and its association with gestational diabetes, there is now also an increasing interest in the detrimental influence of maternal obesity and excess maternal nutrition on the risk of disease in the F1 generation and beyond. Of note, both ends of the maternal nutrition spectrum can elicit similar phenotypic outcomes in the offspring with both maternal undernutrition and maternal obesity leading to increased adiposity and related metabolic disorders in the offspring; nevertheless, whether the mechanisms are similar remains poorly defined.

Human Populations

A systematic search of the literature for both human population and animal studies was recently undertaken by Aiken and Ozanne [2]. Data to date are primarily derived from rodent models due to the short timeframe required to generate offspring. Human evidence is under-
standably limited due to long generation times for prospective studies and the quality of data records for retrospective studies. Two well-cited human population studies are those of the Dutch Famine cohort [6–8] and the Avon Longitudinal Study of Parents and Children (ALSPAC) and Overkalix cohorts, where both cohorts have been linked to transmission of ill-health into the F2 generation [9].

The study by Painter et al. [6] on the Dutch Famine cohort did not find transgenerational effects of prenatal exposure to famine on birth weight nor on cardiovascular and metabolic disease rates. However, F1 famine exposure in utero was associated with increased F2 neonatal adiposity and poor health in later life. Offspring of prenatally undernourished fathers, but not mothers, were heavier and more obese than offspring of fathers and mothers who had not been undernourished prenatally [7]. In a recent study on this cohort, no evidence of transgenerational effects of grandmaternal undernutrition during gestation on the health of the F2 offspring were reported, but it was suggested that the observed increases in adiposity in the F2 offspring of prenatally undernourished fathers may lead to increased chronic disease rates in the future [7]. Of note, reports from the Dutch Famine cohort have been varied. One study observed that F1 offspring were born smaller and that these effects persisted to the F2 generation [8]; however, a subsequent report by the same authors failed to reproduce some of the initial findings [10].

In the ALSPAC/Overkalix cohorts from 1890, 1905 and 1920, male-line transgenerational responses were reported; the paternal grandfather’s food supply was only linked to the mortality risk ratio of grandsons, while the paternal grandmother’s food supply was only associated with the granddaughters’ mortality risk ratio. These transgenerational effects were observed with exposure during the slow-growth period (both grandparents) or fetal/infant life (grandmothers) but not during either grandparent’s puberty [9]. Although these studies suggest transgenerational effects induced by environmental factors, molecular evidence to date does not support a direct transfer of epigenetic information via the gametes, and it is possible that transgenerational effects of this type could be explained by societal factors [11].

**Animal Models**

Evidence for the transgenerational programming of adverse metabolic outcomes has been shown in a number of experimental paradigms, primarily in the rodent. These include challenges such as nutrient restriction or overfeeding during pregnancy and lactation, restriction of uterine blood flow, intrauterine exposure to high levels of glucocorticoids and experimental gestational diabetes. Although a number of primary research papers have used small animal models of developmental programming to examine at least through to the F2 offspring, only a few have examined obesity as an endpoint, with many focusing on insulin sensitivity and glucose tolerance, cardiovascular outcomes or changes in DNA methylation [2].

While a large number of animal studies have shown the effects of undernutrition during fetal/perinatal development on glucose metabolism in exposed animals (F1) in adulthood [12], several studies have reported that glucose metabolism is also altered in the offspring (F2) of F1 females undernourished in utero, even when the F1 females have been well-nourished after weaning [13, 14]. Maternal protein restriction in the rat adversely affects the glucose metabolism of male and female F2 offspring in a gender- and developmental time window-specific manner (i.e. gestation and/or lactation) [15]. The offspring and grandoffspring of female rats fed low-protein diets during pregnancy and lactation, but fed nutritionally adequate diets thereafter, exhibit altered insulin sensitivity in adulthood [16]. However, Benyshek et al. [17] showed that maternal energy restriction did not consistently program reduced insulin sensitivity in offspring over three consecutive generations. The reasons for this remain unclear, although it is possible that the transgenerational transmission of de-
velopmentally programmed insulin resistance is determined in part by the relative insulin sensitivity of the mother during pregnancy/lactation.

Furthermore, it has been reported that the glucose metabolism of the grandoffspring (F3) of female rats malnourished during development is also adversely affected, but these effects are diminished as compared to those observed for the F2 generation [17]. These data may suggest a normalization in the F3 generation when the maternal diets of F2 dams and post-weaning diets of F3 animals were adequate, and may provide further evidence of an eventual intergenerational ‘resolution’ of the altered glucose-insulin metabolism [17–19]. Whether such an intergenerational normalization can be accelerated by manipulating the diet of insulin-resistant F2 dams remains to be seen. A meta-analysis examining transgenerational effects of maternal caloric restriction on appetite revealed a weak and statistically non-significant overall effect on offspring’s appetite [20]. However, it also showed that a lower protein content of restricted diets was associated with higher food intake in female offspring. Importantly, these data suggest that a main source of variation among studies arises from whether, and how, food intake was adjusted for body mass.

Maternal undernutrition during pregnancy in the mouse programs reduced birth weight, impaired glucose tolerance and obesity in both F1 and F2 offspring. The sex-specific transmission of these phenotypes suggests complex mechanisms including alterations in the maternal metabolic environment (transmaternal inheritance of obesity), gene expression mediated by developmental and epigenetic pathways (transpaternal inheritance of low birth weight), or both (impaired glucose tolerance) [21]. Using a model of intrauterine hyperglycaemia, Ding et al. [22] reported transgenerational glucose intolerance with Igf2/H19 epigenetic alterations in mouse islets. In this model, a high risk of impaired glucose tolerance appeared as early as 3 weeks in F2 offspring and progressed through both parental lineages, particularly the paternal line. In a model of placental insufficiency, fetal exposure to maternal hypertension and hypoleptinaemia was associated with altered leptin and growth patterns in mature female offspring in the F1 generation but was not perpetuated to the F2 generation [23]. First-generation female diabetic offspring of F0 rats treated with streptozotocin during pregnancy had F2 offspring with altered glucose and carbohydrate metabolisms. These studies suggest that the mechanisms involved in developmental programming are likely epigenetic rather than due to DNA sequence mutations [24].

Other studies have reported a transgenerational passage of effects resulting from treatment of pregnant rats with dexamethasone (DEX) by either the maternal or paternal lineage. Male offspring of female rats that had been prenatally exposed to DEX, but not manipulated in their own pregnancy, had reduced birth weight, glucose intolerance and elevated hepatic PEPCK activity. Similar transgenerational programming was observed in off-
spring of male rats prenatally exposed to DEX mated with control females. However, these effects were resolved in the F3 generation [18]. Experimental models in the guinea pig (maternal DEX and low-protein diet) have shown DNA methylation changes, altered hypothalamic-pituitary axis function and cardiovascular impairment as well as delayed neurodevelopment in the F2 offspring [25, 26]. Maternal diets deficient in micronutrients have also been shown to result in F2 phenotypes including vitamin D and zinc deficiencies [27–29].

As reported in the rodent undernutrition models, the work by Pentinat et al. [30] also suggests a partial resolution of the phenotype in subsequent generations in models of early-life overnutrition. A model of neonatal overfeeding (ON) in the F0 generation was used to examine the development of the metabolic syndrome in male offspring (ON-F1) and grandoffspring (ON-F2) of ON-F0 male mice, which were not overfed during lactation. ON-F1 mice developed fed and fasting hyperinsulinaemia, hypertriglyceridaemia, insulin resistance and glucose intolerance, but not obesity, by the age of 4 months. In contrast, ON-F2 male mice showed a more moderate phenotype and only developed fasting hyperglycaemia and glucose intolerance at 4 months of age [30].

The impact of paternal obesity in transgenerational inheritance has also been reported in recent work by Fullston et al. [31]. Paternal obesity was shown to initiate metabolic disturbances in two generations of mice albeit with incomplete penetrance to the F2 generation. Diet-induced paternal obesity modulated the sperm microRNA content and germ methylation status, which are potential signals that program offspring health and initiate the transmission of obesity to future generations. Dunn and Bale [32] have previously reported that maternal high-fat diet exposure in mice results in an increase in body size and reduced insulin sensitivity that persisted to the F2 generation via both maternal and paternal lineages. However, as described above, as the first generation’s primordial germ cells may be affected by gestational exposure, analysis of phenotype transmission into the F3 generation is necessary to determine whether stable epigenetic programming has occurred. Further work looking at the F3 generation in this model revealed that only females displayed an increased body size at F3, and this effect was only passed on via the paternal lineage. The finding of a paternally transmitted phenotype to F3 female offspring further supports a stable germline-based transgenerational mode of inheritance, thus suggesting that imprinted genes may be involved in such epigenetic programming [33].

In addition to studies in the rat and mouse, studies in large animal models have also reported transgenerational programming effects including those in the sheep, pig and primate [34–36]. In the sheep, maternal DEX administration to F0 mothers abolished the neonatal leptin peak in female offspring possibly by inhibition resulting from elevated cortisol levels in the DEX F2 offspring. The DEX F2 offspring displayed hyperphagia, increased weight gain and adiposity during an ad libitum feeding challenge concomitant with a decreased insulin responsiveness following a glucose tolerance test [36]. Using methylation micronutrient-supplemented diets in the pig, gene expression, DNA methylation and carcass composition differences were observed between F2 offspring of supplemented and non-supplemented groups, with the F2 control offspring tending towards increased adiposity compared to the F2 offspring of supplemented pigs. These effects were transmitted through the male lineage [34]. In the primate, also utilising a maternal DEX model, F2 and F3 offspring presented higher cholesterol levels, with significantly more low-density lipoprotein cholesterol, although body weights were not affected [35].

**The mechanisms underlying transgenerational effects in the setting of developmental programming remain poorly understood.**

**Mechanisms**

The mechanisms underlying transgenerational effects in the setting of developmental programming remain poorly understood. Human studies of transgenerational programming are observational in nature and therefore have limited value in determining the mechanisms underlying these phenomena [37]. Data derived from animal models suggest a number of potential mechanisms which may underpin the transgenerational transmission of the programmed phenotype, including persistence of the abnormal environment across generations, programmed effects on maternal physiology and the transmission of epigenetic information through the germline (fig. 2) [2, 19, 38].

Although evidence to date indicates that multiple mechanisms are in play in the interaction between nutritional imbalance and the transgenerational transmission of obesity and related metabolic phenotypes, it is the role
of epigenetics that has gained increasing momentum in recent years [21]. Environmentally induced transgenerational epigenetic inheritance of adult-onset disease involves a variety of phenotypic changes, suggesting a general alteration in genome activity. Combined observations demonstrate that all tissues derived from the epigenetically altered germline develop transgenerational transcriptomes unique to the tissue, but common epigenetic control regions in the genome may coordinate the regulation of these tissue-specific transcriptomes [39]. Data from Burdge et al. [40] suggest that the regulation of energy metabolism during pregnancy and lactation within a generation is influenced by the maternal phenotype in the preceding generation and the environment during the current pregnancy. These transgenerational effects on phenotype are associated with altered DNA methylation of specific genes in a manner consistent with a de novo induction of epigenetic marks in each generation.

In environmentally mediated inheritance, it is suggested that the epigenetic marks in the parents modify their behaviour in a way that causes the same epigenetic marks in their offspring (e.g. altered hypothalamic DNA methylation), and that these behavioural changes recreate the epigenetic marks de novo at each subsequent generation [38]. An example of this are alterations in maternal care where there is evidence for the behavioural transmission of postpartum behaviour from mothers to female offspring [41, 42]. The mechanisms underpinning this transmission have been explored in rats and implicate oestrogen-oxytocin interactions and the differential methylation of hypothalamic oestrogen receptors [41]. However, a recent study has also shown that programmed obesity in offspring of obese mothers is independent of the level of maternal care, although only first-generation effects were investigated [43].

In germline epigenetic inheritance, environmental influences during the period of developmental plasticity leads to an epigenetic change within the first-generation offspring’s germline that is then transmitted to F2 offspring and beyond [38]. In this setting, a number of environmental factors (e.g. toxicants) have now been shown to promote the transgenerational epigenetic inheritance of disease and phenotypic variation [44]. A well-cited example of this relates to exposures to endocrine disruptors such as bisphenol A. Perinatal exposure of male rats to bisphenol A leads to perturbations in the expression profile of testicular steroid receptor co-regulators through to the F3 generation [45]. Of note, maternal behaviour may also be affected by bisphenol A exposure [46], thus potentially leading to behaviour-mediated effects on future generations [41]. Ancestral exposure to the insecticide dichlorodiphenyltrichloroethane in the rat has been reported to promote transgenerational epigenetic inheritance of obesity through to the F3 generation where over 50% of males and females developed obesity [47]. The transgenerational transmission of disease was through both the female and male germlines, and the F3 generation displayed sperm epimutations and differential DNA methylation regions. Further, perinatal exposure of 4-nonylphenol, at environmentally relevant doses, can lead to obesity in both male and female F1 offspring. This effect progresses to the F2 offspring through the maternal line [48].

Mutations in folate metabolism can cause epigenetic instability and transgenerational effects on development. Embryo transfer experiments have revealed that methionine synthase reductase deficiency (necessary for the utilisation of methyl groups from the folate cycle) in mice leads to two distinct, separable phenotypes: adverse effects on their wild-type daughters’ uterine environment, leading to growth defects in wild-type grandprogeny, and the appearance of congenital malformations independent of maternal environment that persist for five generations, likely through transgenerational epigenetic inheritance [49]. The data on maternal folate status are less clear, with some evidence that maternal folate supplementation can lead to insulin resistance and transgenerational transmission of respiratory disorders in offspring [50, 51].

In addition to epigenetic effects, the contribution of the uterine tract environment or maternal adaptations to pregnancy may be critical to programming inheritance via the maternal line. Suboptimal nutrition in utero causes DNA damage and accelerated aging of the female reproductive tract [52]. Aiken and Ozanne [2] suggested that developmental programming effects could be propagated through the maternal line de novo in generations beyond F2 as a consequence of development in a suboptimal intrauterine tract and not necessarily through directly transmitted epigenetic mechanisms. Further, as the effects of age exacerbate the programmed metabolic phenotype, advancing maternal age may increase the likelihood of developmental programming effects being transmitted to future generations.

Suboptimal nutrition in utero causes DNA damage and accelerated aging of the female reproductive tract.
The de novo regeneration of the programming phenotype via the maternal line has been referred to as the ‘vicious’ cycle of developmental programming, e.g. obesity begets obesity (fig. 3). It is well established from epidemiologic and experimental studies that offspring of obese mothers are at increased risk for obesity and metabolic disorders in later life [53, 54]. Thus, increasing rates of maternal obesity translate to birth of offspring that are themselves predisposed towards obesity in their reproductive years, hence perpetuating the obesity cycle. Models in mice with a genetic tendency for obesity show that the effects of maternal obesity accumulate over successive generations and shift the population distribution towards an increased adult body weight. These data suggest that epigenetic mechanisms are involved in this process, and it has been shown that methyl donor supplementation, i.e. to induce DNA hypermethylation during development, can potentially prevent this transgenerational amplification of obesity [55].

**Summary**

The experimental and human evidence to date suggests that developmental programming should be regarded as a transgenerational phenomenon. The transgenerational epigenetic transmission of traits allows future generations to be maximally competitive in their environment [33]. Under this assumption, adaptive gene programs acquired during the parental life span persist in the subsequent generation, enabling future generations to better exist in a potentially adverse environment. However, evidence suggests that environmental exposures such as poor early-life nutrition result in maladaptive parental responses that can be passed to offspring. These epigenetic traits have the potential to result in a population-wide manifestation of a phenotype over several generations – such transmission can exacerbate the rapid onset of phenotypes such as obesity currently observed in human populations [33].

To date, very few studies have examined the transgenerational transmission of obesity in the context of developmental programming, with most focusing on glucose tolerance, cardiovascular outcomes or methylation status. Further, few studies have examined a phenotype in the F3 generation, argued to be a true marker of transgenerational inheritance. For example, confirmation of a germline-based mechanism requires both the analysis of the F3 generation to rule out any direct effects of programming via maternal diet and the transmission through the paternal lineage to avoid the confounding contributions of maternal factors such as altered in utero environment or behaviour [33]. Those F3 data that have been reported have found varying and, in some cases, conflicting outcomes. These outcomes are confounded by the range of models and interventions used (e.g. low-protein high-energy diet, zinc deprivation) and the application of interventions in the F1 and subsequent generations [2].

Many rodent models have investigated the ‘parent of origin’ question, i.e. are the F2 effects produced via the maternal line, the paternal line, or both. This is an important component given the known sex differences programmed in the F1 generation in many developmental programming models [56]. Human epidemiological evidence and rodent studies suggest that transgenerational effects can be passed down the paternal line [18, 22, 33]. These effects are proposed to act via germline epigenetic modification despite evidence of extensive demethylation during germ cell formation and zygotic development. A maternal high-fat diet has been shown to program a true germline-based transgenerational phenotype in the male gametes [33]. Studies in F1 sperm have shown a role for altered IGF2 and H19 expression in the transmission of a phenotype to the F2 offspring [22]. However, not all studies reporting a paternal line transmission have reported epigenetic alterations in the F1 sperm [57]. Work by Rafford et al. [58] failed to find any evidence that the epigenetic reprogramming of imprinting control regions in the germline was susceptible to nutritional restriction, thus
implying that mechanisms other than direct germline transmission are responsible.

Transgenerational programming via the maternal line is more complex to define as there are a number of possible interacting mechanisms by which the mother can exert programming effects on the subsequent generation [2]. These include the role of the intrauterine environment, effect of age of pregnancy, somatic epigenetics and ooplasmic (mitochondrial) programming.

Although a number of studies have now reported transmission through to the F2 lineage, transmission up to the F3 or subsequent generation is less clear, with some studies reporting a resolution of the phenotype by the F3 generation. In the meta-analysis by Aiken and Ozanne [2], of 9 studies carried through to F3, 5 failed to show any effect. Defining the mechanisms underpinning the transmission of developmental programming is an area urgently requiring further research and is particularly relevant to populations in transition between traditional and Western lifestyles. The fact that some traits appear to be resolved where others persist suggests that divergent mechanisms of transmission are involved and that those metabolic traits that do persist are capable of being transmitted via the male germline [33]. However, human evidence remains largely unsubstantiated with the strongest argument for transgenerational epigenetic inheritance in humans being data derived from the rodent [11]. Understanding the mechanisms of transgenerational inheritance is essential for the development of future intervention strategies to modulate not only that of the immediate adult phenotype but also that of the offspring, grandoffspring and beyond.

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Appetite and satiety demonstrate a remarkable heterogeneity among humans, with a spectrum contributing to both anorexia and hyperphagia.

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Developmental Programming of Appetite/Satiety
by Michael G. Ross and Mina Desai

Key insights
Obesity in childhood and in later life may be the result of developmentally programmed hyperphagia. Recent data strongly suggest that maternal and/or fetal under- or overnutrition predisposes the offspring to become hyperphagic. Infants born small for gestational age and those born to obese mothers are at risk.

Current knowledge
Appetite is regulated by a complex circuit of hypothalamic nuclei involved in the generation of appetite versus satiety signals. The arcuate nucleus (ARC) is the predominant appetite regulatory site in the brain and receives signals from different areas of the brain and other tissues. The ARC contains at least two populations of neurons with opposing actions on food intake. Specific alterations in the fetal metabolic/energy environment can alter the equilibrium between orexigenic and anorexigenic neuronal systems, thus affecting appetite and satiety.

Practical implications
Imbalances in maternal nutrition (under- or overnutrition, low-protein or high-fat diets) result in a cascade of genetic, biochemical and cellular effects that can ultimately bias the appetite regulatory networks of the offspring towards hyperphagia. Specific epigenomic studies are necessary in order to pinpoint the genomic sites that act as targets for early nutritional effects.

Maternal nutrition affects appetite in the offspring. Perturbations in maternal nutrition can alter nutrient sensors, neuroendocrine levels and signaling, neurogenesis and neuropeptide levels. These pathways interact and ultimately influence appetite.

A greater understanding of the development of appetite regulatory pathways opens the door for novel interventions for the prevention and treatment of obesity.

Recommended reading
Developmental Programming of Appetite/Satiety

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**Key Messages**
- The fetal and newborn nutritional environment may alter the development of appetite and satiety pathways.
- Childhood and adult obesity may be a consequence of developmentally programmed hyperphagia.
- Both maternal undernutrition and maternal obesity increase the risk of offspring hyperphagia and obesity.

**Key Words**
Hyperphagia · Obesity · Fetal origins

**Abstract**
Obesity is often attributed to a Western lifestyle, a high-fat diet and decreased activity. While these factors certainly contribute to adult obesity, compelling data from our laboratory and others indicate that this explanation is oversimplified. Recent studies strongly argue that maternal/fetal under- or overnutrition predisposes the offspring to become hyperphagic and increases the risk of later obesity. Both infants small for gestational age (SGA) or infants born to obese mothers who consume a high-fat diet are at a markedly increased risk of adult obesity. Specific alterations in the fetal metabolic/energy environment directly influence the development of appetite regulatory pathways. Specifically, SGA infants demonstrate (1) impaired satiety and anorexigenic cell signaling, (2) enhanced cellular orexigenic responses, (3) programmed dysfunction of neuroprogenitor cell proliferation/differentiation, and (4) increased expression of appetite (NPY) versus satiety (POMC) neurons. In both hypothalamic tissue and ex vivo culture, SGA newborns exhibit increased levels of the nutrient sensor SIRT1, signifying reduced energy, whereas maternal high-fat-exposed newborns exhibit reduced levels of pAMPK, signifying energy excess. Via downstream regulation of bHLH neuroproliferation (Hes1) and neurodifferentiation factors (Mash1, Ngn3), neurogenesis is biased toward orexigenic and away from anorexigenic neurons, resulting in excess appetite, reduced satiety and development of obesity. Despite the developmental programming of appetite neurogenesis, the potential for neuronal remodeling raises the opportunity for novel interventions.

**Introduction**
Currently, 66% of adults in the United States are overweight and more than one third are obese (BMI >30), representing a modern-day health crisis. Globally, it is observed in both developed and developing countries and may be a reflection of societal, economic and cultural problems. Obesity and its related diseases are the leading causes of death in Western society, with associated risks of hypertension, cardiovascular disease, stroke and diabetes. As childhood obesity is a major risk factor for adult obe-
sity [1], the increasing incidence of childhood obesity would predict a continued adult obesity epidemic. The manifestation of obesity is impacted by numerous factors including culture, genetic predisposition, economic factors and perinatal environment. The rapid increase in the prevalence of obesity has been partly attributed to environmental factors, with increasing availability of highly palatable foods and 'modern' lifestyles involving less physical work creating a more obesigenic environment. However, as obesity results from an imbalance in energy intake versus expenditure, individuals with dysfunctional appetite regulation (i.e., overeating) are at high risk of developing obesity.

**The manifestation of obesity is impacted by numerous factors including culture, genetic predisposition, economic factors and perinatal environment.**

Appetite regulation develops perinatally, and hence a suboptimal environment during critical periods of development programs appetite and satiety mechanisms, thereby altering infant, childhood and adult ingestive behavior. Numerous epidemiological and animal studies have demonstrated that in utero perturbations of the nutritional, hormonal and/or metabolic environment as well as exposure to environmental toxins (e.g., endocrine disrupters) may alter the development of the appetite regulatory system, resulting in an increased risk of adult obesity.

**Epidemiological Evidence for Perinatal Appetite Programming**

Epidemiological studies have conclusively demonstrated associations between the early nutritional environment, patterns of postnatal growth and adult metabolic syndrome. Paradoxically, low-birth-weight infants are at increased risk of adult obesity, in part a result of programmed hyperphagia and adipogenesis. The Dutch famine in 1944/45 provided an opportunity to determine the effects of perinatal malnutrition on babies that would later be exposed to caloric surfeit. Offspring of mothers exposed to the famine during the first two trimesters of pregnancy had a higher incidence of obesity than the general population [2].

Studies indicate that 25–63% of adult diabetes, hypertension and coronary heart disease can be attributed to the effects of low birth weight with accelerated newborn-to-adolescent weight gain [3]. Recent interest has focused on the generational effect of maternal obesity and the effect of maternal overnutrition on fetal programming. Thus, there is a U-shaped curve for the relationship between birth weight and adult metabolic disease such that those born small or large for age show an increased risk of obesity later in life [4]. Together, these processes have contributed to the population shift toward obesity. Briefly, normal-weight mothers most commonly gave birth to normal-weight infants who develop into normal-weight adults (fig. 1). The increased incidence of prematurity and intrauterine growth restriction during the last half century, combined with improved neonatal survival, overfeeding, formula feeding and exposure to a Western diet, has resulted in an increase in offspring programmed for adult hyperphagia and obesity. This process has, to a significant degree, accounted for the ongoing population shift toward an obese phenotype, with these second-generation (obese) mothers at risk of giving birth to macromomic newborns, who themselves are at risk of adult obesity.

Recent studies have indicated that children who show a rapid weight gain may be at a higher risk of adult diseases regardless of their birth weight [5]. Formula feeding appears to predispose offspring to obesity [6] as several meta-analyses show a greater propensity to adulthood obesity amongst infants who were formula-fed versus breastfed [7]. Whether this increased weight gain is a result of formula composition or is secondary to the process of bottle- versus breastfeeding itself is controversial. The risk of disease in adulthood is already higher in individuals born thin, but this effect is compounded if they experience a rapid weight gain and early adiposity rebound in childhood [8]. Therefore, catch-up growth in the first few weeks of postnatal life appears to be particularly disadvantageous in terms of the propensity to develop hyperphagia and obesity in adulthood. In contrast to the Dutch famine, offspring of mothers exposed to the longer (28-month) Leningrad famine did not exhibit adult obesity, most likely because offspring were exposed to famine throughout pregnancy and early childhood, preventing the rapid catch-up growth [9].

**Animal Models of Perinatally Programmed Hyperphagia**

Manipulation of maternal and/or newborn nutrition has been done in various animal models, though the neuroendocrine regulators of appetite have been best
characterized in rodents. Notably, perinatal protein restriction, undernutrition and overnutrition program offspring appetite and adiposity. Both maternal undernutrition and protein restriction during rat pregnancy result in small for gestational age (SGA) newborns. When nursed normally, SGA newborns exhibit a rapid catch-up growth with hyperphagia and adult obesity [10]. Further, SGA offspring are more susceptible to stress-induced binge eating [11] and, when weaned to a postnatal high-fat diet, show exacerbated hyperphagia and adiposity [12]. Maternal obesity resulting from a high-fat diet [13] and neonatal overnutrition (induced by nursing a smaller litter) also leads to rapid postnatal growth followed by hyperphagia and adult obesity [14]. Despite the diversity of these perinatal nutritional disturbances, the similar outcome of adult obesity suggests that the common underlying mechanism(s) may be programmed hyperphagia.

**Appetite Regulation**

_Hypothalamic Sites of Appetite Regulation_

Appetite is regulated by a complex circuit of hypothalamic nuclei involved in synthesis of appetite/satiety signals, action areas where messengers act and regulatory sites. The predominant appetite regulatory site, the arcuate nucleus (ARC), receives input from peripheral (brain, pancreas and adipocytes) and central sources [15]. The ARC contains at least two populations of neurons with opposing actions on food intake: medial ARC orexigenic [NPY (neuropeptide Y) and AgRP (agouti-related protein)] and lateral ARC anorexigenic neurons [POMC (proopiomelanocortin) and CART (cocaine- and amphetamine-regulated transcript)]. Many of the ARC NPY/AgRP and POMC/CART neurons project to downstream neurons in the periventricular nucleus (PVN).

_Hypothalamic Neuropeptides (NPY and POMC)_

POMC neurons mediate anorexigenic responses by the release of alpha-melanocyte-stimulating hormone (α-MSH) which binds to PVN neurons expressing melanocortin-3 and -4 receptors (MC3/MC4-Rs). The orexigenic property of AgRP results from its competition with α-MSH at MC3/MC4-Rs. Among the five subtypes of NPY receptors, NPY-1R, which is expressed on PVN neurons, is primarily responsible for NPY-induced increases in food intake. In addition to the PVN, the ARC interacts with additional hypothalamic nuclei including the ventromedial nucleus, the lateral hypothalamic, the dorsomedial nucleus and brainstem sites (e.g., locus coeruleus, nucleus of the solitary tract) [16, 17].

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Fig. 1. Population shift toward obesity due to programming. IUGR = Intrauterine growth restriction.
Peripheral Anorexigenic Hormones (Leptin and Insulin)

In addition to neural inputs, ARC neurons respond to blood-born signals (e.g., leptin, insulin and ghrelin). Leptin, which is secreted primarily by fat, is transported across the blood-brain barrier into the cerebrospinal fluid via a saturable transporter system in the choroid plexus, mediated by a short form of the leptin receptor (ObRa) [18]. Insulin, which is secreted by the pancreas, gains access to the hypothalamus by means of a saturable receptor-mediated process and diffusion from the median eminence [19]. Both are anorexigenic factors that stimulate the POMC/CART neurons and inhibit the NPY/AgRP neurons. Accordingly, ARC neurons exhibit high leptin and insulin receptor expression. Genetic mutations in the obese (ob) gene, which codes for leptin, or the diabetes (db) gene, which codes for the leptin receptor, lead to hyperphagia and obesity. Central insulin deficiency is a cause of hyperphagia. The reduction of food intake caused by central insulin is blocked by a POMC antagonist.

ARC Neurogenesis

ARC development is a relatively late process in rodents, rhesus monkeys and humans, with maturation not achieved until later stages of postnatal development [20]. Key hypothalamic nuclei only begin to be populated during fetal life, with continued neural development during the neonatal period [21, 22]. POMC becomes detectable in rat hypothalamic neurons from gestational day E12.5, and NPY neurons first appear in the ARC at E14.5 [23], though POMC and NPY neurons do not acquire their terminal peptidergic phenotype until the postnatal period. Coinciding with ARC neuronal maturation, ARC projections in rodents are formed beginning in the second week of postnatal life [24].

The ARC neurons arise from neurogenic regions within the hypothalamus during fetal/neonatal life. During development, neuroprogenitor cells (NPCs) undergo extensive proliferation (two daughter NPCs), self-renewal (one NPC and one differentiated cell) and ultimate terminal division into cells destined for neuron, astrocyte or oligodendrocyte fate [25], which migrate and populate the hypothalamic nuclei. NPC differentiation to neurons, and ultimately to appetite or satiety neurons, is regulated by a complex spatial/temporal interplay of pathways, including cell communication factors (Notch/Hes1), energy/nutrient sensors (SIRT1, AMPK) and a series of neuroregulatory basic helix-loop-helix (bHLH) transcription factors.

Fig. 2. NPCs. a NPCs can self-renew or differentiate to neuronal or glial cells. b Hes1 promotes NPC proliferation and inhibits Mash1 and NPC differentiation. c Reduced Hes1 suppresses NPC proliferation and alleviates Mash1 inhibition, allowing NPC differentiation.

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factors, such as Mash1 and Neurogenin-3 (Ngn3) [26], among others.

**Neuronal Differentiation to POMC or NPY**

Once NPCs differentiate to neurons, those cells destined for the ARC further differentiate to express orexigenic (NPY, AgRP) or anorexigenic (POMC, CART) peptides. The bHLH factor Mash1 is required for the normal development of POMC neurons [27]. Downstream from Mash1, Ngn3 also promotes the development of POMC neurons, while inhibiting NPY expression (fig. 2). Accordingly, Mash1(−/−) mice demonstrate ARC hypoplasia with minimal expression of POMC neurons, Mash1(+/-) mice actually overexpress NPY neurons and Ngn3(−/−) mice express markedly reduced POMC but increased NPY ARC neurons [28]. Thus, perturbations in Mash1 and Ngn3 expression may markedly alter the development of appetite regulatory neurons [28]. Hes1, an upstream regulator which promotes NPC proliferation while inhibiting differentiation, also acts as a transcriptional regulator (together with corepressors) of both Mash1 and Ngn3.

**Neuronal Growth Factors (Insulin and Leptin)**

Although insulin and leptin regulate adult ingestive behavior, these factors have important roles in appetite neurogenesis. Exogenous insulin promotes cell growth and serves as a trophic factor in fetal neuron cell culture [29]. In rat fetal brain cell culture, axonal growth is stimulated in insulin medium. Leptin also has been recognized as a major neurotrophic factor during the development of the rodent brain, partly because of the initial observation that animals that are either leptin-deficient or -insensitive have decreased brain size and development [30].

**Neurogenesis and Energy Sensors (AMPK and SIRT1)**

Emerging evidence indicates that energy metabolism during development is a critical regulator of NPC proliferation/differentiation [31], providing insight into a putative mechanism for nutrient programming of offspring hyperphagia. Two key proteins which serve as central energy/nutrient sensors are AMP-activated protein kinase (AMPK) and SIRT1, an NAD+-dependent histone deacetylase, both of which have the potential to influence ARC neuronal differentiation and expression of POMC and NPY neurons.

AMPK is a heterotrimer complex which is activated by phosphorylation (pAMPK) in response to reduced energy levels. In adult life, hypothalamic AMPK plays a key role in the control of whole-body energy homeostasis [32], as pAMPK increases the expression of NPY and reduces the expression of POMC. During development, AMPK levels modulate neurogenesis, as the AMPK activator AICAR inhibits NPC proliferation, which is rescued, in part, by AMPK inhibition (compound C). Conversely, a complete absence of AMPK (AMPKβ1−/− mice) reduces NPC proliferation.

Sirtuins are NAD+-dependent (class III) histone deacetylases which express greater activity under low-energy (high NAD+) conditions. Among the mammalian sirtuins, only SIRT1 is directly implicated in NPC biology. SIRT1 is highly expressed in metabolically active sites (e.g., in the ARC) and orchestrates key metabolic responses to nutrient alterations [33]. During development, SIRT1 and Hes1 form a complex that binds to and deacetylates histones at the Mash1 promoter [34], decreasing Mash1 expression. Similarly, SIRT1 and Hes1 may inhibit Ngn3 expression, and SIRT1 may inhibit Hes1. Consistent with this mechanism, NPCs prematurely differentiate [35] in Hes1-deficient brains, reducing the NPC pool.

**Mechanisms of Perinatal Appetite Programming**

Mechanisms of perinatally programmed appetite resulting from altered maternal nutrition (undernutrition, low-protein diet, overnutrition, high-fat diet) may involve multiple interacting factors/pathways that influence offspring hyperphagia. These include altered hypothalamic ARC neuropeptides, neuroendocrine signaling, neurogenesis, neurotrophic factors, energy sensors and/or epigenetics as discussed below (fig. 3).

**Hypothalamic Appetite-Regulating Network**

Altered maternal nutrition biases the offspring regulatory network toward hyperphagia by increasing the production of and the sensitivity to the appetite peptide NPY and/or decreasing those of the satiety peptide POMC.
POMC. Specific studies on rats demonstrate increased levels of hypothalamic NPY mRNA in fetuses [36] and adult offspring as well as in other regions (PVN and lateral hypothalamic area) [37]. Other studies demonstrate decreased mRNA of ARC POMC [38]. In addition, exposure to gestational and lactational maternal diabetes also increases offspring NPY and AgRP as well as decreases POMC and α-MSH [39]. Overall, in response to maternal malnutrition, offspring exhibit an increased ratio of appetite/satiety gene expression. Nonetheless, there are subtle differences dependent upon maternal and post-weaning diets. For example, maternal obesity combined with a post-weaning high-fat diet increases ARC NPY signaling (PVN NPY1R), reduces POMC expression [40] and decreases sensitivity to leptin [41], whereas offspring born to dams fed a high-carbohydrate diet demonstrate increased NPY release in the PVN [42]. In maternally undernourished offspring, brain slice electrophysiology techniques demonstrate enhancement of NPY inhibition of hypothalamic ARC anorexigenic neurons [43].

**ARC Neuronal Development**

Newborns born SGA due to maternal undernutrition have NPCs that exhibit impaired migration in vivo as well as reduced proliferation and neuronal differentiation in vitro [44, 45]. Consistent with this finding, 1-day-old SGA newborns express decreased Hes1 and Ngn3 both in the hypothalamic tissue and NPCs cultures. Importantly, even in culture media outside the fetal environment, NPCs of SGA offspring are programmed to preferentially differentiate to appetite (NPY/AgRP) as compared to satiety neurons.

**Hypothalamic Nutrient Sensors**

Consistent with their energy-deprived status, SGA newborns express increased hypothalamic and NPC SIRT1 expression which persists to adulthood. Notably, SIRT1 silencing via transfection with SIRT1-specific siRNA promotes NPC proliferation with increased Hes1 protein expression in control NPCs, suggesting a SIRT1 regulation of Hes1-mediated NPC proliferation.

Similar to SIRT1 changes, SGA adults allowed ad libitum access to food exhibit upregulated ARC AMPK activity with reduced Akt activity [46]. As these changes correspond to fasting conditions, SGA offspring have an enhanced appetite drive that contributes to hyperphagia and obesity.

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**Fig. 3. Perinatally programmed appetite.** Perturbations in maternal nutrition (undernutrition, low-protein diet, overnutrition, high-fat diet) can alter nutrient sensors, neuroendocrine levels and signaling, neurogenesis and/or neuropeptide levels. These pathways can interact and ultimately impact appetite.

**Leptin and Insulin Neuroendocrinology**

Both high-fat feeding during gestation and lactation as well as a maternal food restriction-induced SGA condition result in offspring with impaired leptin sensitivity [41]. In growth-restricted fetuses and SGA newborns, plasma leptin and insulin levels are decreased [10, 47]. Moreover, SGA newborns show dysregulated hypothalamic leptin and reduced insulin signaling. When nursed normally, obese SGA adults show increased plasma leptin and insulin levels [10]. An examination of the anorexic effects of sibutramine revealed a significantly lower reduction in body weight in SGA offspring. Nonetheless, SGA offspring were leptin- and insulin-resistant as evidenced by a lack of response to peripheral leptin and oral glucose treatment [48]. Maternal high-fat diets during gestation cause offspring to have an impaired hypophagic response to leptin and insulin as adults [49]. Additionally, adult rats that are obese due to neonatal overfeeding demonstrate a reduced ARC neuronal response to leptin and insulin [50, 51]. Interestingly, rats that have a genetic predisposition to develop diet-induced obesity also have a preexisting reduction in central insulin sensitivity, and high-fat diets further reduce the sensitivity to insulin [52].

Treatment of *ob/ob* leptin-deficient mice with leptin does not restore ARC projections to the PVN unless it occurs during postnatal days 4–12 [53]. Recent evidence also indicates that, in offspring of rat dams 70% nutrient-deprived throughout gestation, leptin supplementation from postnatal day 3 to postnatal day 13 prevents programming of high susceptibility to diet-induced obesity.
[54]. However, in control pups, leptin given between postnatal days 1 and 10 causes adult hyperleptinemia, leptin resistance, increased food intake and excess body weight [55]. Furthermore, studies have demonstrated that maternal food restriction during both pregnancy and lactation, which results in further newborn growth restriction, prevents offspring hyperphagia and obesity [10]. Thus, neonatal leptin excess can induce obesity, while fetal/newborn leptin deficiency can prevent obesity. Therefore, further research is needed in this area to determine just how leptin levels during the early postnatal period affect long-term appetite regulation.

Neurotrophic Effects of Leptin and Insulin

Studies have shown that both leptin and insulin induce NPC proliferation and differentiation. Insulin potentiates greater proliferation and astrocyte lineage than leptin. In contrast, leptin promotes neuronal lineage. NPCs of SGA newborns have reduced responses to leptin and insulin [45]. There is also evidence that leptin specifically promotes the development of ARC neuronal projections, consistent with a role in brain development. ob/ob mice have been shown to have significantly higher levels of oligodendrocyte precursor cells than wild-type mice [56], confirming the role of leptin as a regulator of neural progenitor fate. Gestational diabetes that interferes with the ability of insulin to act as a neurotrophic factor causes offspring to have impaired neuronal development specifically in the hypothalamic nuclei responsible for the regulation of appetite. These animals exhibit decreased neuronal cytoplasm in the ARC, the ventromedial nucleus and the parvo cellular division of the PVN, as well as an increase in the glia-to-neuron ratio in the periventricular region of the hypothalamus [57].

Epigenetics

The demographic shift of populations toward a more obese phenotype in a relatively short period of one or two generations argues against a major genetic contribution and instead in favor of environmental or epigenetic mechanisms underlying this phenomenon. The demographic shift of populations toward a more obese phenotype in a relatively short period of one or two generations argues against a major genetic contribution and instead in favor of environmental or epigenetic mechanisms underlying this phenomenon. Genome-wide epigenetic modification occupies a critical role in regulating gene expression at critical stages of embryonic development. Epigenetics more specifically refers to an environmental factor that leads to the chemical modification of DNA or chromatin such that it is fixed in the ‘on’ or ‘off’ position. Major mechanisms associated with epigenetic changes are post-translational modifications (acetylation, methylation or phosphorylation) of core histones and DNA methylation. For example, methylation of CpG-rich clusters (called CpG islands) – which often span the promoter regions of genes – is associated with transcriptional repression, whereas hypomethylation of CpG islands is associated with transcriptional activity. Epigenetic modifications such as these are hypothesized to be the primary basis for the programming phenomenon, and the structural and regulatory effects on many organ systems are likely to be secondary to these environmentally induced changes in gene expression.

Data are now emerging to suggest that alterations in DNA methylation and other epigenetic processes are central to developmental programming in multiple physiological systems. Maternal food restriction that causes offspring to have hyperphagia that is exacerbated by exposure to a high-fat diet also causes increased promoter methylation of energy balance-related receptors, namely the glucocorticoid receptor and the peroxisome proliferator-activated receptor in the liver [12, 58]. In addition, enhanced maternal care early in the postnatal period decreases CpG island methylation of the glucocorticoid receptor, increasing its expression in the brain [59]. It is possible that perinatal environmental perturbations could cause similar DNA modifications in the promoters of genes related specifically to appetite in hypothalamic neurons, although this has not been studied to date. However, one study has investigated a potential epigenetic mechanism for alterations in leptin sensitivity in a cell culture model. Hypermethylation of CpG islands of the functional SOCS3 promoter correlates with transcriptional silencing, and this leads to the constitutive activation of the JAK/STAT pathway in cell culture [60]. Should the perinatal environment also influence the SOCS3 methylation status, then this could be a mechanism for programmed obesity. Only by applying such epigenomic approaches will we eventually be able to determine, in animal models, the specificity and breadth of genomic sites that act as targets for early nutritional effects.
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**Conclusion**

Although food intake is a critical survival function, appetite and satiety demonstrate a remarkable heterogeneity among humans, with a spectrum contributing to both anorexia and hyperphagia. Whereas obesity was often viewed as dietary indiscretion combined with reduced energy expenditure, it is now recognized that enhanced appetite may predispose to obesity, and obesity itself may impair satiety. The maternal and thus fetal nutritional environment may respond to altered energy/nutrient levels during critical embryological and developmental periods so as to alter neurogenesis. Through the interplay of extracellular signaling factors, intracellular transcription responses and nutrient-induced epigenetic alterations, the maternal environment can program fetal/newborn appetite/satiety pathways resulting in newborn and adult hyperphagia. An understanding of the timing, mechanism and potential plasticity of appetite pathway development offers the opportunity for novel interventions for the prevention or treatment of obesity.

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**References**


