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REVIEW

Epigenomics, gestational programming and risk of metabolic syndrome

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Epigenetic mechanisms are emerging as mediators linking early environmental exposures during pregnancy with programmed changes in gene expression that alter offspring growth and development. There is irrefutable evidence from human and animal studies that nutrient and environmental agent exposures (for example, endocrine disruptors) during pregnancy may affect fetal/newborn development resulting in offspring obesity and obesity-associated metabolic abnormalities (metabolic syndrome). This concept of 'gestational programming' is associated with alterations to the epigenome (nongenomic) rather than changes in the DNA sequence (genomic). Epigenetic alterations induced by suboptimal maternal nutrition/endocrine factors include DNA methylation, histone modifications, chromatin remodeling and/or regulatory feedback by microRNAs, all of which have the ability to modulate gene expression and promote the metabolic syndrome phenotype. Recent studies have shown tissue-specific transcriptome patterns and phenotypes not only in the exposed individual, but also in subsequent progeny. Notably, the transmission of gestational programming effects to subsequent generations occurs in the absence of continued adverse environmental exposures, thus propagating the cycle of obesity and metabolic syndrome. This phenomenon may be attributed to an extrinsic process resulting from the maternal phenotype and the associated nutrient alterations occurring within each pregnancy. In addition, epigenetic inheritance may occur through somatic cells or through the germ line involving both maternal and paternal lineages. Since epigenetic gene modifications may be reversible, understanding how epigenetic mechanisms contribute to transgenerational transmission of obesity and metabolic dysfunction is crucial for the development of novel early detection and prevention strategies for programmed metabolic syndrome. In this review we discuss the evidence in human and animal studies for the role of epigenomic mechanisms in the transgenerational transmission of programmed obesity and metabolic syndrome.

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OBESITY AND METABOLIC SYNDROME EPIDEMIC

Obesity is a public health crisis, contributing to morbidity and mortality throughout the world.[1] Obesity is central to the development of metabolic syndrome, which includes a constellation of metabolic abnormalities comprised of insulin resistance, elevated triglycerides, hypertension and atherosclerosis.[2] Among US adults, 69% are overweight (body mass index (BMI) >25 kg m^{-2}), 35% are obese (BMI $\geqslant 30$ kg m^{-2}) and 24% have at least three features of metabolic syndrome.[3–5] Importantly, 17% of children are obese and thus at increased risk of adult obesity.[6] Among childbearing women, the prevalence of obesity is ~30%. In conjunction with increased maternal obesity, we have witnessed an increase in obesity-associated pregnancy complications and a 25% increase in the incidence of high birth weight babies,[7] which itself represents a risk factor for childhood obesity.[8,9]

The world-wide shift towards an obese phenotype has occurred in a relatively short period of one or two generations (from 15–35.7%).[3,10] This suggests that environmental or epigenetic factors rather than genetic mechanisms play a role in the obesity epidemic. While there is little doubt that Western style, high-fat diets combined with decreased activity levels are strong contributors to the prevalence of obesity, data from our laboratory and others, support the concept that obesity may have its origins *in utero*.[11–15] More importantly, obesity and its associated metabolic abnormalities persist in offspring that are developmentally programmed by suboptimal nutrition *in utero*, despite normal postnatal nutrition, and may be evident in multiple generations. Such transgenerational transmission of obesity may occur as a result of changes to the epigenome. Since epigenetic modifications alter the phenotype rather than the genotype, there is potential for intervention and reversibility. This review focuses on the emerging evidence for the role of epigenetics in the developmental origins of metabolic syndrome and heritable changes in gene expression.

PROGRAMMED OBESITY AND METABOLIC SYNDROME

Epidemiological studies have convincingly demonstrated associations between the early nutritional environment, patterns of postnatal growth and metabolic syndrome in adults.[16,17] The Dutch famine in 1944/45 provided an opportunity to determine the effects of perinatal malnutrition on babies that would later be exposed to a surfeit of calories. Offspring of mothers exposed to the famine during the first two trimesters of pregnancy had lower

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birth weights, though paradoxically a higher incidence of obesity than the general population.[17] In particular, low birth weight infants with rapid catch-up growth in the first several years of life had the highest risk of adult obesity and metabolic syndrome.[18,19] While low birth weight infants develop in a state of relative 'undernutrition', fetal 'overnutrition' also has adverse implications for offspring health. Recent interest has focused on the effect of maternal overnutrition on fetal programming. Specifically, maternal pre-pregnancy obesity and/or increased weight gain during pregnancy are associated with higher birth weight newborns[20,21] and an increased risk of obesity and diabetes in later life.[22] Thus, there is a U-shaped curve for the relationship between birth weight and adult metabolic disease, such that those born small or large have an increased risk of obesity later in life.[23,24] Together, these processes likely contribute, in part, to the population shift toward obesity and to the intergeneration aggregation of obesity (Figure 1).

Animal models of low birth weight, induced using a variety of methods such as maternal nutrient restriction, placental uterine ligation or glucocorticoid exposure, have confirmed an increased risk of offspring adiposity,[25–27] particularly among those who exhibit rapid catch-up growth.[12,28,29] More recent animal models of maternal overnutrition, including maternal obesity and Western, high-fat diets similarly replicate the human experience, in that offspring are predisposed to adult obesity.[13,30,31] These data raise important questions about divergent maternal nutritional exposures that result in differential fetal growth. First, how do both under and overnutrition produce a metabolic syndrome phenotype in the offspring? And second, how does the memory of the short period of insult persist across generations?

EPIGENETIC MECHANISMS OF PROGRAMMED OBESITY AND METABOLIC SYNDROME

During the developmental period, rapidly growing fetuses and neonates are vulnerable to perturbations of the maternal nutritional and non-nutritional milieu, resulting in programmed changes in organ structure, cellular responses and gene expression that impact metabolism and physiology of the offspring. Developmental programming may have immediate effects, for example, impaired organ growth at a critical stage, whereas other programming effects are deferred and altered organ function occurs at a later age. This again raises the question as to how the memory of early events is stored and later expressed, despite continuous cellular replication and replacement. Among the different mechanisms causing obesity, epigenetics has emerged as an important determinant that can influence phenotypic outcomes, even in the absence of genetic or environmental heterogeneity.

Epigenome

Epigenetic phenomena are an essential feature of mammalian development that cause heritable and persistent changes in gene expression without altering the DNA sequence.[32] The three major molecular substrates that are involved in this process are the DNA, proteins that form the core around which the DNA wraps (histones) and a specific form of RNA molecules (noncoding RNA). Epigenetic changes include DNA methylation, chromatin folding and binding, packaging of DNA around nucleosomes and covalent modifications of the histone proteins (Figure 2).[33] The epigenome varies across different cell types and undergoes precise, coordinated changes during a lifetime.[34,35]

Epigenetic regulation of gene transcription occurs, in part, by DNA methylation.[36] DNA methylation is highly dynamic during embryogenesis. Prior to implantation DNA is hypomethylated and following implantation there is a progressive increase in DNA methylation that leads to differentiation and organogenesis.[37,38] During postnatal and adult life, DNA methylation is susceptible to intrinsic and extrinsic factors,[39,40] and with aging there is global loss of DNA methylation.[41] Increased methylation mediated by DNA methyltransferase enzymes (DNMTs) is associated with transcriptional silencing. Abnormal DNA methylation is associated with inappropriate gene silencing, and such changes in epigenetic marks are associated with several human diseases (for example, cancers, neurological disorders and inflammation). As methylation involves the supply and enzymatic transfer of methyl groups, it is

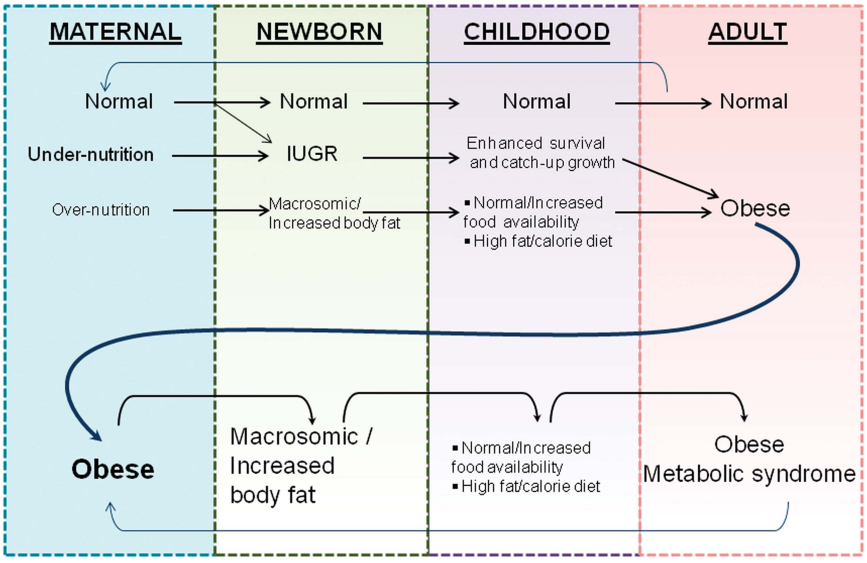

Figure 1. The role of gestational programming in population shifts towards obesity and metabolic syndrome. Normal weight mothers usually give birth to normal-weight infants with normal adiposity. These offspring develop into normal adults with normal body fat content and a normal metabolic profile. The increased incidence of prematurity and intrauterine growth restriction (IUGR) resulting from undernutrition, among other factors, combined with improved neonatal survival, formula feeding and exposure to a Western postnatal diet has resulted in increased offspring obesity and metabolic syndrome. A small portion of obese mothers give birth to newborns with increased body fat, as a result of overnutrition (consumption of a high-fat diet). These processes may contribute to the population shift towards an obese phenotype, with second generation obese women at increased risk for giving birth to newborns with increased body fat content, and who in turn, are at risk of developing obesity and metabolic syndrome.

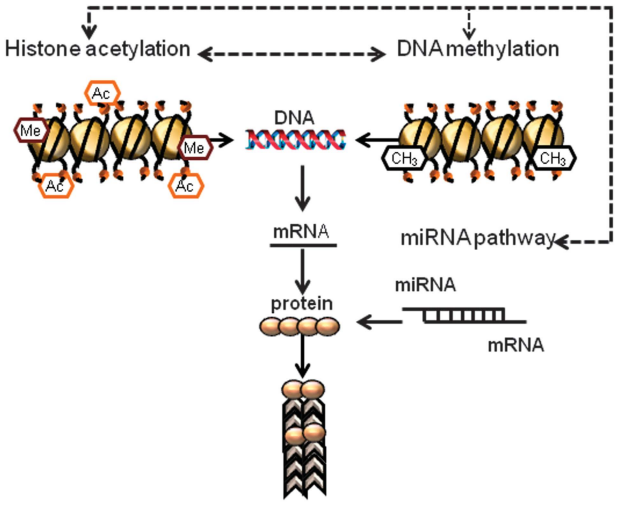

Figure 2. Epigenetic regulation. There are three distinct, interrelated mechanisms of epigenetic regulation: (i) DNA methylation: methyl groups attached to CpG islands regulate gene activity. Methylation renders the DNA inaccessible and thereby suppresses gene expression; (ii) Histone modifications: methylation (Me) or acetylation (Ac) of histones determines the activity of the DNA wrapped around them, and; (iii) microRNA (miRNA): noncoding RNA molecules that affect RNA silencing and post-transcriptional regulation of gene expression.

plausible that *in utero* nutritional (for example, methyl donors), hormonal or other metabolic cues alter the timing and direction of methylation patterns during fetal development.[42,43]

An additional essential mechanism of regulating gene expression and silencing is the packaging of chromatin into open (euchromatic) or closed (heterochromatic) states, respectively. Chromatin consists of DNA packaged around histones. Post-translational modifications of histone tails commonly involve methylation/demethylation and acetylation/deacetylation mediated by histone modifying enzymes. Histone methylation can either repress or activate transcription depending on which lysine is methylated. For example, trimethylation of histone H3 at lysine 4 (H3K4me3) is associated with active gene transcription, whereas dimethylation of histone H3 at lysine 9 (H3K9me2) is associated with transcriptional silencing. Unlike methylation, acetylation potentiates and deacetylation suppresses gene expression.[44,45]

Non-coding RNAs (ncRNAs) also participate in the epigenetic regulation of gene transcription. In general ncRNAs are transcribed from DNA, but are not translated into proteins, and function to regulate gene expression at the transcriptional and post-transcriptional level. Those ncRNAs that are involved in epigenetic regulation comprise the short ncRNAs (<30 nts) and the long ncRNAs (>200 nts). The three major short ncRNAs associated with gene silencing are microRNAs (miRNAs), short inhibitory RNAs and piwi-interacting RNAs.[46,47] Long ncRNAs play a regulatory role during development,[48] exhibit cell type-specific expression[49,50] and are associated with adipogenesis.[51,52] While these noncoding RNAs are usually associated with regulation of gene expression at the translational level, recent work suggests they may be involved in DNA methylation and histone modifications as well, thereby further regulating the transcription of their target genes.[53,54]

It is now recognized that the epigenome is dynamic, changing in response to nutrient availability, physical exercise, weight loss and aging, among other exposures.[55–58] *In utero* nutrition, environmental exposures and other factors (for example, maternal or fetal stress) may permanently alter offspring gene expression via epigenetic mechanisms, and hence alter the structure and function of cells and organs leading to metabolic abnormalities.

Nutrition, the epigenome and obesity

Evidence implicating epigenetic mechanisms as potential mediators of obesity is largely from animal and limited human studies. Notably, nutritional and environmental factors can interact with the genotype by modulating epigenetic markings in the somatic cells and thus impacting the phenotype. This is well-demonstrated in monozygous twins, in which the offspring exhibit divergent DNA methylation and histone acetylation patterns,[57] and in the *agouti* mouse, in which the diet affects methylation status and the animal's coat color.[59,60] To address the phenotypic discordance (that is, anthropomorphic features and susceptibility to disease) in monozygous twins, lymphocyte and muscle biopsy samples were obtained from 3–74-years-old Spanish monozygotic twins (monochorionic and dichorionic). These biopsy samples were analyzed for global and locus-specific DNA methylation and histone acetylation. Older, but not younger, twins showed differences in their epigenetic markings and their gene-expression profile, suggesting an environmental influence on the regulation of gene expression, despite a similar genotype. In agouti mice, the agouti gene is normally methylated and, as a result, the mice are thin and have a brown coat color. However, when the agouti gene is completely unmethylated, the coat color is yellow and the mice are obese and diabetic. Although, the 'fat yellow mice' and 'skinny brown mice' have a similar genotype, the phenotype is different as a result of epigenetic changes. Notably, these methylation patterns can be transmitted across generations.[59,61] More remarkably, 'fat yellow mice' fed a methyl-rich diet during pregnancy produced pups with brown coat without obesity.[62] These results reinforce the interaction between nutrition and the epigenome, and offer opportunities for potential intervention.

While epigenetic modulation of imprinted genes is well-recognized,[63,64] including their impact on body weight (for example, IGF2),[65,66] epigenetic changes also occur in key non-imprinted genes that are involved in energy metabolism. Genes that regulate adipogenesis, glucose homeostasis, inflammation and/or insulin signaling are regulated by epigenetic mechanisms, including genes encoding hormones (for example, leptin), nuclear receptors (adipogenic and lipogenic transcription factors PPARγ and PPARα, respectively) gluconeogenic enzymes (for example, phosphoenolpyruvate carboxykinase (PEPCK)) and transmembrane proteins (for example, uncoupling protein 1).[67–75] Plasma levels of leptin, a satiety hormone produced by adipocytes, can be modulated by diet via epigenetic mechanisms. Consumption of a high-fat diet in rats increased methylation of leptin gene promoter in retroperitoneal adipocytes and this was associated with lower circulating leptin levels, suggesting leptin methylation affects leptin gene expression.[67] Similarly, adipogenesis is driven by adipocyte differentiation and induction of adipogenic transcription factors (PPARγ, C/EBPα) via epigenetic mechanisms. Both histone lysine methylation (H3K4) and acetylation (AcH3) regulate adipocyte differentiation. During terminal differentiation, significant increases in histone trimethylation (H3K4me3) and acetylation (AcH3K9/K14) coincide with upregulation of PPARγ and C/EBPα. Early induction of adipogenic genes as a result of increased H3K4me3 and AcH3K9/K14 contributes to early adipocyte differentiation and obesity.[76–78] Hepatic PEPCK is the rate-limiting enzyme in the metabolic pathway of gluconeogenesis and consumption of a high-calorie diet caused hypomethylation of the PEPCK gene in rats. This was associated with increased PEPCK mRNA levels indicative of increased liver gluconeogenesis.[43] Indeed, these methylation changes were associated with changes in target gene expression and glucose and lipid metabolism.

In humans, global DNA methylation, specific histone methylation (H3K4 and H3K9) and certain miRNAs are positively associated with BMI.[79–81] For example, genome wide methylation analysis showed that human obesity was associated with methylation changes in blood leukocyte DNA.[79] Another study of specific

histone methylation (K4 and K9) in primary human adipocytes from overweight subjects with and without type 2 diabetes revealed 40% lower levels of K4 dimethylation in overweight, non-diabetic individuals. In contrast, trimethylation at K4 was 40% higher in adipocytes from overweight diabetic subjects as compared with normal-weight and overweight non-diabetic subjects. Obese and lean individuals show varying DNA methylation levels in specific genes and cultured preadipocytes and mature adipocytes show differentially expression of miRNAs.[80] Similarly, genome wide miRNA profiling of human subcutaneous adipose tissue biopsies showed differential and dysregulated expression of miRNA in obese versus lean individuals. Moreover, the expression pattern of miRNAs in human adipose tissue was associated with obesity and parameters of glucose metabolism.[81]

Animal studies provide further evidence implicating epigenetic mechanisms in the regulation of adipogenesis and appetite in the development of obesity, though this pertains more specifically to histone modifications and miRNA rather than DNA methylation.[73,82–85] For instance, histone modifications (methylation and acetylation) and miRNA have been linked to the regulation of the principal adipogenic transcription factor (PPAR) and its target genes, resulting in obesity, hyperphagia and hyperlipidemia.[82,86–88] Specifically, maternal obesity/high-fat diet in mice increases the expression of a key transcription factor responsible for adipogenic lineage commitment (Zfp423) during fetal development. Consistent with this, the repressive histone methylation (H3K27me3) was lower in the Zfp423 promoter of fetal tissues from maternal obese mice.[89] Together with increased mRNA expression, alterations in DNA methylation of CpG sites and CGI shores of pro-adipogenic factors (Zfp423 and C/EBP-β) have also been demonstrated in rat offspring (3 week old) from obese pregnancies.[90] In contrast, mice with genetically increased or decreased levels of DNA methyltransferases (DNMT1 and DNMT3b) do not gain weight or have increased adiposity.[91] Although *de novo* DNMT3a was more than doubled in white adipose tissue of obese mice, in that study, a transgenic mouse that had threefold elevated DNMT3a mRNA levels in adipose tissue did not exhibit increased body weight or adiposity.[91] These findings argue against DNA methyltransferase-driven obesity.

Unlike ubiquitous DNA methyltransferases, perhaps it is histone deacetylases that serve specialized functions and have tissue-specific expression that are major contributors. Indeed, specific histone methyltransferases are important for adipogenesis; mice with mutations in the histone H3, lysine 4 methyltransferase MLL3 exhibit reduced adipogenesis and are protected from high-fat diet-induced obesity.[92] In contrast, mice with loss of function of the H3K9-specific demethylase JmjC domain-containing histone demethylase 2A are obese and hyperlipidemic.[82,83] Therefore, there is evidence that mutations in epigenetic-modifiers may contribute to obesity.

Epigenome and programmed obesity

There is also evidence that environmental exposures during early life can induce persistent alterations in the offspring epigenome, which may lead to an increased risk of obesity and metabolic syndrome later in life.

In humans, programmed obesity occurs principally via alterations in DNA methylation, but the involvement of histone modifications and changes in chromatin structure has not yet been demonstrated. Remarkably, exposure to either maternal famine or obesity reduced DNA methylation of the imprinted IGF2 gene in the offspring. In the Dutch Famine (1944–1945) cohort, 60-year-old adults who were prenatally exposed to famine showed hypomethylation of whole blood IGF2 gene, and hypermethylation of two obesity-related non-imprinted genes (TNF, leptin) as compared with their unexposed, same-sex siblings. This association was specific for periconceptional exposure, reinforcing that the early developmental period is crucial for establishing and maintaining epigenetic marks.[93,94] More recently, parental obesity and specifically paternal obesity was associated with IGF2 hypomethylation in umbilical cord blood leukocytes of newborns.[95,96] Specific maternal characteristics, including gestational weight gain and gestational diabetes, have also been associated with signature DNA methylation in cord blood and increased placental leptin gene methylation, respectively.[55,97] Furthermore, maternal carbohydrate intake in early pregnancy was associated with the umbilical cord methylation levels of the nuclear receptor gene, retinoid X receptor-α, which heterodimers with the adipogenic transcription factor PPARγ, as well as with adiposity in later childhood.[98] Human newborn studies are generally limited to determination of umbilical blood leukocytes or placental tissue epigenetic assessments, which may not parallel organ-specific cellular changes.

Animal studies demonstrate that both DNA methylation and histone modification changes are associated with the developmental programming of obesity in rodent models of maternal under or overnutrition (for example, high-fat diet, maternal obesity), and newborn-specific nutritional modifications (for example, litter size manipulation). This has been extensively reviewed recently.[99–103] For example, epigenetic changes have been detected at genes that regulate growth factors,[104] adipogenesis,[67,105] brain appetite and satiety/reward pathways[98,106–109] and glucose homeostasis.[110] Similar to humans, there is some indication that both maternal under and overnutrition may impact similar genes,[90,111] though whether this occurs via similar epigenomic changes remains to be established.

The subtle differences between human and animal findings, including genetically modified mice may partly be attributed to certain factors, such as sampling tissue (umbilical blood versus specific tissue/cells), period and duration of exposure (periconceptual, fetal or neonatal) and in the case of humans, lifestyle. Nonetheless, both human and animal studies provide evidence of programmed metabolic syndrome resulting from early nutritional exposures and suggest that epigenetic modification of non-imprinted genes may be a major contributing factor.

TRANSGENERATIONAL EPIGENETIC INHERITANCE OF OBESITY AND METABOLIC SYNDROME

It is crucial to understand how epigenetic marks become heritable. DNA methylation is highly dynamic during early embryogenesis, but contrary to popular belief, is not completely erased during very early development or gametogenesis.[112–114] As such, some methylated sites survive and are replicated every time a cell divides and the DNA is passed along with associated histones. These markings can then be copied every time the cell divides, influencing gene expression throughout life (Figure 3).[115] Thus, epigenetic modifications provide a mechanism by which early nutritional and environmental exposures affect the offspring phenotype via germline modifications.[116] More importantly, epigenetic changes can be inherited mitotically in somatic cells with long-term effects on gene expression.[117,118] As described by Skinner,[117] an intrinsic transgenerational process requires only one exposure to the environmental factor resulting in germline involvement and, whereas extrinsic transgenerational epigenetic processes involve an epigenetic alteration in a somatic tissue with required (repeat) exposures at each generation. Intergeneration aggregation of obesity (that is, BMI) has been consistently demonstrated with evidence of both familial and geographic clustering. Increased BMI occurs disproportionally among offspring of heavier parents and there is greater familial influence on offspring obesity from mothers than fathers.[119,120] A vicious cycle develops as females born to obese women have an increased risk of obesity[121] and give birth to a subsequent generations with the same risks. In support of this concept, maternal weight loss via

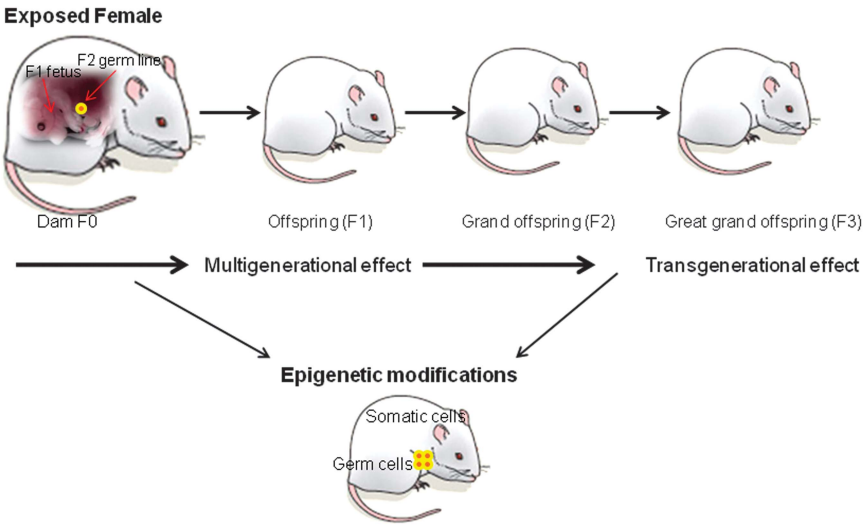

Figure 3. Environmental exposures and transgenerational epigenetic inheritance. Transgenerational inheritance must extend from the F0 to the F3 generation, as the developing fetal germ cells (which will produce the F2 generation) are exposed to the F0 environmental factors. The expression through the F3 generation may occur due to germline transmission.

bariatric surgery reduces obesity in the F1 generation. Children of obese women who underwent bariatric surgery compared with those in children born before maternal surgery, had lower birth weight, reduced prevalence of macrosomia and a threefold lower prevalence of severe obesity at follow-up (2.5–26 years).[122] Epidemiologic studies have largely attributed this phenomenon to an *extrinsic* process resulting from the maternal phenotype and the associated nutrient alterations occurring within each pregnancy, rather than to germline alterations, which persist through generations.

True transgenerational inheritance must extend from the F0 to the F3 generation, as the developing fetal germ cells, which will produce the F2 generation, are exposed to the F0 environment.[123] Therefore, expression through the F3 generation may occur via germline transmission. Notably, nutritional and non-nutritional factors (for example, endocrine disruptors) are independently[59,124,125] and synergistically[126] capable of causing methylation changes in the male germ line. For example, bisphenol A an endocrine disruptor, causes site specific hypomethylation in the agouti mouse in conjunction with the development of offspring obesity,[126] which suggests the potential for transgenerational transmission of obesity. Bisphenol A also impairs male fertility for three generations, inducing transgenerational, tissue-specific changes in the transcriptome,[127,128] suggesting that epigenetic modifications in the germ line may impact secondary epigenetic mechanisms, which regulate somatic cell gene expression.[129] Importantly, maternal nutritional supplements negated the effects of bisphenol A on the offspring phenotype,[126] raising the potential for preventative and therapeutic strategies.

Epigenetic changes in the germ line may affect somatic cell gene expression by changes in inflammatory signaling pathways. Maternal nutrition affects the methylation of offspring genes central to immune responses, adipogenesis and lipogenesis.[8,130] Sub-optimal maternal nutrition also inhibits adiponectin and stimulates the release of inflammatory adipokines, including IL-6 and TNFα from human offspring adipose tissue.[131] Notably, inflammatory mediators can modify DNA methylation, as mastitis silences bovine casein gene expression.[132] Thus, offspring epigenetic modifications induced by suboptimal nutrition may be mediated by inflammation-associated mechanisms. In addition, transcription factors (for example, nuclear receptors), which are susceptible to nutritional modulation, can cause epigenetic changes. Nuclear receptors associate with both positive and negative chromatin modifying complexes to activate or repress gene transcription.[133–135] Similarly, nutritionally mediated changes in hormones (for example, estrogen and androgen) can influence miRNA expression.[136,137] In addition to maternal nutrition, an inappropriate metabolic environment *in utero* may also perpetuate the transgenerational cycle. For example, maternal gestational diabetes and the resultant intrauterine hyperglycemia can transmit the diabetogenic phenotype to the next generation.[138,139] Indeed animal studies in mice have demonstrated that intrauterine hyperglycemia alters imprinted gene expression of the sperm.[140] In summary, evidence supports that adverse nutritional exposure results in F1/F2 obesity and potentially F3 transgenerational obesity.[141]

Transgenerational effects in humans

To date, most of the evidence for transgenerational inheritance pertains to effects on offspring growth, particularly on body weight. However, human and animal studies have shown that increased adiposity can occur despite normal body weight.[13,142] Nonetheless, the human studies described below provide important evidence of transgenerational effects. The earliest study showing transgenerational birth weight effects examined Swedish women and their offspring. Women who were themselves born small for gestational age had an increased risk of giving birth to either intrauterine growth restricted or preterm infants, emphasizing the importance of both maternal factors and the intrauterine milieu in determining the offspring phenotype.[143]

Retrospective cohort studies, the Dutch Hunger Winter Families Study in the western Netherlands, Chinese famine and the Overkalix cohort in Sweden also indicate non-genomic transmission of phenotypic traits and metabolic abnormalities. The Dutch Hunger Winter famine occurred at the end of World War II, from October 1944 to May 1945. The birth cohort that was *in utero* during this time, when nutritional intake plummeted to <500 kcal per day has been extensively studied. The vast majority of studies of developmental programming (F0 to F1) by the Dutch Hunger Winter have demonstrated effects of altered *in utero* and newborn nutrition, with the offspring phenotype often dependent on the developmental stage of exposure. However, reports on transgenerational (F0 to F2, F3) effects are conflicting. An early study reported that offspring born during the famine were smaller than average and that the risk of having smaller babies could last two generations (F1 and F2).[144] Further, the maternal diet during

the F0 pregnancy affected the F2 birth weight, independent of the F1 birth weight.[145,146] However, a subsequent study failed to replicate these findings.[147] Similarly, F1 women who experienced the famine as fetuses had F2 babies with increased neonatal adiposity and poorer adult health,[148] though a more recent study found no evidence of transgenerational effects if the grandmother had been undernourished.[149] Instead, there was evidence of increased adiposity in the offspring of prenatally undernourished fathers.[149] These conflicting results have been attributed to the differing methods of obtaining data. Early studies were based on record retrieval and relied on parents' recall of their offspring's size at birth and later health, whereas in later studies, the offspring were directly contacted to assess their body composition and health.

Relative to the Dutch famine, the Chinese famine was longer (1959–1961), less precisely defined and superimposed on a background of widespread chronic undernutrition. However, studies of the Chinese famine also suggest transgenerational effects of the famine on offspring growth. The offspring of mothers exposed to famine *in utero* and during the first few years gave birth to newborns with larger birth size as compared with those offspring that were not exposed to the famine.[150]

Data from both famines suggest that transgenerational effects of nutrition on offspring phenotype occur via the maternal lineage. However, a historical study of three generations in Overkalix, Sweden revealed that fathers' diets may also play a crucial role. In the early 20th century, this region suffered marked food shortages due to failed harvests, interspersed with periods of great plenty, during which many people would gorge. The fathers who ate a surfeit of food during the pre-adolescence period had sons and grandsons with an increased risk of diabetic mortality. The paternal grandmother's food supply was also linked to the mortality risk ratio of the granddaughters, suggesting sex-specific transmission operating exclusively through the paternal line.[151,152] Recent animal studies further support the finding that maternal high-fat diet-induced obesity extends to the F3 female offspring via the paternal lineage.[153] Although limited, human data suggest that both maternal and paternal nutrition may exert transgenerational effects on the metabolic phenotype of their offspring.

Transgenerational effects in animals

There is evidence of both somatic and germline transmission of metabolic phenotypes via epigenetic alterations. Early rat studies demonstrated that protein restriction for 12 generations has a cumulative effect on fetal growth, causing progressively greater reductions in fetal body and organ weights.[154] As noted above, maternal undernutrition or a high-fat maternal diet predisposes the first-generation (F1) offspring to obesity and metabolic syndrome.[12,13,30,155] Importantly, the F1 generation can transmit similar phenotypes to the second (F2),[141,156–158] and third (F3) generations,[159] despite normal nutrition during pregnancy in the F1 generation. Consumption of a protein-restricted diet during rat pregnancy in the F0 generation resulted in obesity, elevated blood pressure and insulin resistance in the F1 and F2 offspring.[160,161] Notably, transgenerational transmission of the birth weight phenotype occurred via the paternal lineage, transmission of obesity occurred via the maternal lineage and transmission of abnormal glucose homeostasis occurred via both maternal and paternal lineages.[141] The role of epigenetic regulation of gene expression was demonstrated in the male progeny of F1 females that were exposed to the protein-restricted diet. The F2 males showed hypomethylation of the hepatic PPARα promoter to levels comparable to F1 males,[162] suggesting that transmission of a phenotype induced in the F1 generation to the F2 generation preserves levels of DNA methylation of specific genes in somatic cells.[163] A similar effect is seen with *in utero* dexamethasone exposure, where F1 and F2 generation show comparable reduction in birth weight and blood glucose levels.[164] Although these studies emphasize the primary mechanism of transmission between generations via somatic cells, other studies demonstrate transmission through the germ line. Maternal high-fat diet during pregnancy and lactation increased mouse offspring body size in the F1 and F2 generations, with transmission via both the maternal and the paternal lineages. However, subsequent transmission to F3 of the higher body size and weight phenotype was restricted only to females, and was transmitted through the paternal lineage only.[153] Similarly, overfeeding of male mice via culling of litter size, resulted in altered insulin and glucose metabolism of two subsequent generations only in male offspring.[156] These studies highlight the importance of examining both gender-specific programming effects, the expression of which may change with each generation, and gender-specific (maternal versus paternal) lineage effects influencing the phenotype expression. When studying transgenerational models (F0 to F3), the interplay of exposures, lineages and gender may result in a daunting number of subjects. Nevertheless, further mechanistic studies of parental nutrition and transgenerational epigenetic inheritance are required to establish the specificity of germline versus somatic cell transmission of metabolic phenotypes.

Although human and animal studies indicate that transgenerationals effects can be transmitted via both the maternal and the paternal lineages, studies of bariatric surgery in women strongly support the maternal environment as a key factor driving the obesity cycle. Bariatric surgery improves patient metabolic profiles.[165–168] Further, children born after maternal bariatric surgery exhibited lower prevalence of severe obesity, greater insulin sensitivity and improved lipid profile in comparison to siblings born before maternal surgery.[122,169] Several studies have identified changes in genes associated with insulin action after bariatric operations[170,171] and demonstrated that this may be mediated through differential methylation of genes involved in immune and inflammatory pathways.[172,173]

CONCLUSION

Although still preliminary, there is compelling evidence from human and animal studies that altered epigenetic states may contribute, in part, to the obesity epidemic. Nonetheless, many questions remain: (1) is there a causal link between epigenetic modifications and metabolic disease phenotypes?; (2) does analysis of one tissue reflect the epigenetic profile of other tissues?; and (3) are epigenetic changes reversible? Improved understanding of epigenetic pathways, including the potential for reversing epigenetic gene modifications, raises the hope of developing preventive and therapeutic approaches to address transgenerational aggregation of obesity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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