

Intrauterine Programming of Diabetes and Adiposity

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Abstract The prevalence of diabetes and adiposity has increased at an alarming rate and together they contribute to the rise in morbidity and mortality worldwide. Genetic studies till date have succeeded in explaining only a proportion of heritability, while a major component remains unexplained. Early life determinants of future risk of these diseases are likely contributors to the missing heritability and thus have a significant potential in disease prevention. Epidemiological and animal studies show the importance of intrauterine and early postnatal environment in programming of the fetus to adverse metabolic outcomes and support the notion of Developmental Origins of Health and Disease (DOHaD). Emerging evidence highlights the role of epigenetic mechanisms in mediating effects of environmental exposures, which in certain instances may exhibit intergenerational transmission even in the absence of exposure. In this article, we will discuss the complexity of diabetes and increased adiposity and mechanisms of programming of these adverse metabolic conditions.

Keywords Diabetes · Intermediate traits · Fetal programming · Fuel- and nutrient-mediated teratogenesis · Mendelian randomization · Thrifty epigenotype

Introduction

Diabetes mellitus is the most prevalent metabolic disease characterized by hyperglycemia due to primary defect in insulin secretion and/or function. Type 1 diabetes mellitus (T1D) is characterized by lack of insulin secretion due to autoimmune-mediated destruction of pancreatic beta cells, and type 2 diabetes mellitus (T2D) is featured by marked insulin resistance and relative insulin deficiency. A third and emerging class of diabetes is gestational diabetes (GDM) where hyperglycemia presents during pregnancy and usually resolves postpartum. With close to 387 million people affected globally, diabetes has now reached pandemic proportions. Majority of people affected with diabetes are type 2 diabetics, and this phenomenal increase can be traced to rapid rise in obesity and lifestyle factors like physical inactivity [1, 2]. While T1D has rather clear autoimmune origin, T2D does not have a known single identifiable cause. Multi-factorial etiology of T2D has been substantiated by numerous studies which revealed effects of risk factors like age, sex, ethnicity, physical inactivity, obesity, insulin resistance, diet, smoking, family history, and gestation. Chronic exposure to stress, low socioeconomic status, and psycho-social factors are also established risk factors from recent environment-wide association studies. T2D patients spend a long asymptomatic period (prediabetes) characterized by undetectable hyperglycemia, sufficient to cause pathological changes [3]. Resistance to insulin action in tissues like the liver, skeletal muscle, and adipose tissue leads to other characteristic features such as dyslipidemia, visceral and/or

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abdominal adiposity, obesity, and rise in pro-coagulant and pro-inflammatory factors [4].

Diabetes and Adiposity: “Partners in Crime”

Of the above risk factors, obesity, simply defined as fat deposition, is considered to be a major contributor for the global rise in the number of T2D patients. Conventionally, obesity is defined as per classification metrics based on body mass index (BMI) [5]. However, BMI is currently considered a weak measure and other anthropometric measurements such as increased waist circumference (WC) and waist-to-hip ratio (WHR) are being adopted as more accurate components of obesity related to metabolic abnormalities [6]. On the other hand, “adiposity” is defined as the degree of body fat accumulation and is a condition where increased fat deposition occurs in adipose tissues. Percent body fat, fat free mass, and fat mass are generally used as adiposity measures [7]. While adiposity per se is not a pathological condition but plays critical roles in energy homeostasis, reproduction, and immune function, it is the excess fat mass deposited in specific regions leading to visceral and abdominal adiposity that is associated with insulin resistance [8]. Increased fat mass results in increased plasma free fatty acid (FFA) levels and elevated intracellular fatty acid metabolites such as diacylglycerol, fatty acyl CoA, and ceramides. Over all, these imbalances result in inhibition of glycolysis, impaired insulin signaling, and imbalance between the pro- and anti-inflammatory cytokines leading to insulin resistance [9]. Thus, as opposed to sole focus on increased fat mass, dysfunction of adipose tissue results in various metabolic abnormalities, which together encompass the phenotype of diabetes.

Diabetes: Complexity Compounded

Diabetes is now established as a complex disorder where genetic and/or environmental risk factors interact in causal and reverse-causal manner through various intermediate traits and lead to a phenotype that is highly heterogeneous depending upon the extent of interaction. The intimate association between T2D and obesity led to coinage of the term “diabesity” [10]; however, this close association is not universally true [11]. It provides a glimpse of phenotypic complexity in T2D where not just BMI, but several other intermediate anthropometric and glycemic traits like WHR, WC, fasting plasma glucose (FPG), fasting insulin, 2-h glucose, glycosylated hemoglobin (HbA1c), homeostatic model of assessment for insulin resistance, and homeostatic model of assessment for pancreatic beta cell function act in concert and contribute to the disease progression.

Several lines of evidence support the principle of inherited genetic susceptibility as an important risk factor for T2D. High concordance rate in monozygotic twins suggests a strong

genetic component, and the offspring of a parent with T2D are known to face a 40 % lifetime risk of developing T2D, which increases to 70 % when both parents have T2D [12]. Furthermore, 40 % of first-degree relatives of T2D patients develop diabetes as compared to 6 % in the general population [13]. However, twin studies and family studies are confounded by environmental influences (intrauterine and postnatal). Progress in understanding genetics of T2D started with familial linkage studies and candidate gene analyses, substantial knowledge has been with the advent of genome wide association studies (GWAS) and next generation sequencing approaches like whole genome and exome sequencing [14]. Independent studies and meta-analyses have identified more than 80 loci associated with T2D risk, of which transcription factor 7-like 2 (*TCF7L2*) is the most strongly associated gene to date [15]. Several loci influencing glycemic traits like FPG, fasting insulin, 2-h glucose, and HbA1c levels were identified from studies of the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) of which few also influence risk of T2D [16•]. Among the 53 glycemic traits-associated loci identified from GWAS in Europeans, 33 are T2D-associated and explain ~4.8 % variance in fasting glucose and ~1.2 % in fasting insulin. Studies from the Genetic Investigation of ANthropometric Traits (GIANT) consortium identified common genetic variants at hundreds of genetic loci that modulate obesity-related traits like height, BMI, WC, WHR, and WHR adjusted for BMI [16•]. Of all, association of fat mass and obesity-associated (*FTO*) locus is most strongly associated with BMI and increases T2D risk in European population and is considered as an obesity-susceptibility gene [17].

However, many but not all the variants identified in Europeans have been replicated in non-Europeans and among replicated loci, differences in allelic frequencies and effect sizes have been observed. For example, among Europeans, *FTO* shows strong association with BMI, adiposity, and T2D, but it is strongly associated with T2D independent of BMI and adiposity in Asian and African ancestry populations [18•, 19]. These results highlight similarity in genetic susceptibility as well as draw attention to ethnic differences in genotype-phenotype correlation. In addition, these loci explain only a small proportion (less than 20 %) of overall heritability. The area under “Receiver Operating Characteristic” curve from genetic risk score (GRS) of identified loci ranges from 0.54 to 0.63 predicting small effect, and addition of GRS to risk based on conventional factors adds minimal but statistically significant risk [20]. This could be because of small effect sizes of the genetic loci identified from GWAS where only common genetic variants are analyzed. Adding to it, the non-coding nature of variants has proven to be extremely challenging in establishing causality of the identified loci. Together, these limitations raise questions over the “missing heritability” [21]. Several hypotheses have been put forward to

explain this missing heritability which include overestimates of heritability, unexplored regions of the genome, copy number variants, high-penetrant rare-variants, heritable epigenetic changes, and gene-gene (G-G) and gene-environment (G-E) interactions [22, 23]. Of these, modulation of genetic predisposition by environmental factors has received substantial attention owing to some of the strongest evidences for G-E interactions. For example, people who carry the obesity-susceptibility gene, *FTO*, have a higher risk of obesity, but the risk appears to be reduced in people who are physically active. This serves as a classical example where prevalent risk allele is powerfully influenced by the environment and is regulated by modifications beyond the DNA sequence [24]. These regulations by the environment are mediated by epigenetic mechanisms like DNA methylation, histone modification, and microRNAs. Of all, DNA methylation is considered the most stable and best understood epigenetic mechanism by which environmental factors modify genetic predisposition [25, 26].

Fetal programming: An Alternative Explanation

Epidemiological data over the last two decades makes it obvious that genetic factors alone cannot explain the rapid spread of the “diabesity” epidemic. Hence, the most common perception is that of interplay between genes and environment. Early line of thinking for this perception comes from the hypothesis put forward by James Neel who suggested that “thrifty genotype” involves quick insulin trigger which reduces urinary glucose loss and promotes fat deposition under conditions of better nutrient availability and allows their reutilization under starvation. It presumably evolved due to positive selection pressure in ancestral “feast and famine” environment [27]. However, in the modern environment with adequate availability of nutrients, these “thrifty” genes are disadvantageous as they promote fat deposition causing widespread of obesity and diabetes among individuals carrying the risk genotype. The hypothesis suggests that the metabolic fate of an individual predisposed to diabetes is decided before birth and thus makes an attempt to explain why individuals from some populations are prone to diabetes [28]. However, it is based on disputable assumptions like evolution of humans through multiple famines, presence of circulating insulin antagonists, and ignores more important aspects like physical activity [29, 30]. Though there is reasonable evidence for selection at specific type 2 diabetes-associated loci like *TCF7L2*, peroxisome proliferator activated receptor gamma coactivator 1-alpha (*PPARGC1A*), and neurogenic locus notch homolog protein 2 (*NOTCH2*), a consistent pattern of selection that can provide conclusive confirmation of the “thrifty genotype” hypothesis is lacking [31, 32••].

While the “thrifty genotype” hypothesis was put forward highlighting the role of evolution and natural selection as a

cause for the current prevalence of obesity, the “thrifty phenotype” hypothesis proposed a link between poor early fetal and postnatal growth to an increased cardio-metabolic risk in later adult life as a manifestation of adaptation to nutrient deprivation or environmental stress rather than any genotypic effect. It provides justification to the epidemiological findings that an individual’s metabolic profile is determined not just by their genetic composition, rather by the genetic factors modulated by environmental cues during early periods of life [33]. Epidemiological studies on birth and death records by David Barker and colleagues led to the premise that babies who are born lighter and survived infancy might have an increased risk of developing coronary heart disease later in life. This led to the “Barker hypothesis” or “fetal programming hypothesis” which states that disturbed intrauterine and postnatal growth will have long-term effects on adult health which includes increase in incidence of hypertension, coronary heart disease, impaired glucose tolerance, insulin resistance, and T2D [34]. In support of this hypothesis, several studies have explicitly shown an inverse relationship between infantile birth weight and increase in metabolic and cardiovascular disease risk in later life. Furthermore, these associations are not found to be affected by confounding variables like socio-economic conditions and are replicated among different populations [35–39, 40••].

Barker’s hypothesis stimulated worldwide interest to understand the relationship between low birth weight, cardiovascular disease risk, and development of impaired glucose tolerance under the term fetal origins of adult diseases (FOAD) [41]. This concept soon incorporated diabetes during pregnancy and evolved into a new modified paradigm—the Developmental Origins of Health and Disease (DOHaD) [42]. The DOHaD notion describes how environmental factors during early life, such as at conception, and/or during fetal life, infancy, and early childhood, induce changes in development and result in long-term impact on later health and disease risk [43]. The Dutch “Hunger Winter” is one of the earliest and best examples of DOHaD paradigm where during World War II, scarcity in food supply caused famine in western Netherland’s population during the 1944–1945 winter. The pregnancies during that period resulted in “fetal starvation” which ultimately had long-term ill effect on the health of the newborn [44]. Thus, of all stages, in utero fetal development is a complex process and intrauterine insults or deviation from normal development during critical phases are shown to affect birth weight and lead to permanent changes in certain tissues such as adipocytes, myocytes, pancreatic β -cells, and neurons with long-term consequences such as cardio-metabolic syndrome [45]. This phenomenon is known as “intrauterine or fetal programming” [46] and is exemplified by numerous studies that show a J- or U-shaped relationship between birth weight and disease risk where babies born with both low or high birth weight are at increased disease risk [47]. Many recent studies

favor in utero programming over genetic predisposition as a major driver of chronic metabolic and cardiovascular diseases.

Thus, obesity and T2D which manifest in adult life are related to two seemingly opposing factors encountered in early stages of life: (a) deprivation of micronutrients during critical period of fetal growth due to undernourished mother and (b) excess availability of fuel (glucose or fats) due to diabetic pregnancies and/or postnatal availability of excess nutrients to a low birth weight infant. These factors restrict the developmental plasticity (where a genotype can produce multiple forms and behaviors in response to environmental conditioning) and lead to programming (restriction of plasticity) and have been called “nutrient mediated teratogenesis (NMT)” and “fuel mediated teratogenesis (FMT)” respectively to explain the relationship between birth weight and disease risk.

Nutrient-Mediated Teratogenesis

One of the first evidences for the association of intrauterine undernutrition to future metabolic disorders came from the Dutch “Hunger Winter” study, when the Dutch population including pregnant women had to survive on a few hundred calories a day for many months. Follow-up of individuals who were in utero at the time of the Dutch “Hunger Winter” showed long-lasting effects of maternal undernutrition during different periods of gestation on fetal development and later adult life [44]. Fetal undernutrition during the first and second trimesters of intrauterine life was associated with obesity in adulthood, raised circulating lipids, blood clotting factors, and greater prevalence of coronary heart disease compared to individuals who are exposed to this insult during the third trimester [48–50]. Undernutrition during mid- and late gestation was found to be associated with impaired renal function and impaired glucose metabolism, respectively [51–53]. These observations are well supported by a large body of animal studies where maternal undernutrition, especially of proteins resulted in a “programming” effect and altered metabolism in their offspring. Rodent models of maternal protein restriction yielded pups with decreased pancreatic β cell mass at birth and reduced insulin secretion in later life due to reduction in proliferation rate and increased apoptosis of β cells [54]. Besides, gestational protein restriction results in an offspring with a rise in hepatic triglycerides and hepatic expression of lipogenic enzymes favoring fat synthesis and excessive fat accumulation, ultimately leading to obesity and insulin resistance [55, 56]. Protein restriction is also shown to be involved in programming of food preferences as observed that exposure to low levels of protein in pregnant rats establishes a preference for high fat foods [57].

While effects of macronutrient deficiency were known for quite some time, recent years have witnessed the consequences of subclinical micronutrient deficiency, especially with regards to vitamin B12 (B12), folic acid, vitamin B6

(B6), vitamin D, selenium, iron, chromium, zinc, etc [58]. In rural Gambia, alternation of a rainy season with a long dry season had an enormous impact on nutrition patterns. High incidence of micronutrient deficiency is reported in pregnant women during the rainy season and the resultant offspring display high frequencies of low birth weight, and childhood morbidity and mortality [59]. In developing countries like India, subclinical micronutrient deficiency is highly prevalent especially in women of reproductive ages. In contrast to Europeans who are folate deficient, Indians are vitamin B12 deficient primarily due to vegetarianism [60]. A low vitamin B12 level (<150 pM) in Indians has been associated with low birth weight, neural tube defects, adiposity, insulin resistance, cardiovascular disease, and poor cognitive performance [61–63]. Hyperhomocysteinemia, a biochemical marker of B12 deficiency, is associated with cardiovascular morbidity, dementia, and osteoporosis [64–66]. Results from the Pune Maternal Nutrition Study (PMNS) showed that around 70 % of rural mothers had low B12 concentrations, and newborns were lighter, shorter, and thinner but fat (more abdominal and subcutaneous adipose tissue) compared to UK newborns supporting the “Thin-Fat Indian” notion [67, 68]. While paternal size chiefly influenced skeletal measurements, maternal micronutrient intake strongly determined fetal size and baby’s adiposity. Follow-up of these children revealed that higher maternal folate during pregnancy predicted higher adiposity and insulin resistance as early as 6 years of age [60, 68, 69]. In addition, hyperhomocysteinemia during pregnancy was associated with low birth weight. Most insulin-resistant children were born to mothers who were B12 deficient and had high folate concentrations reinforcing the importance of balance between B12 and folate levels [69]. Using a Wistar rat model, we have replicated the phenotype of lower birth weight, increased visceral adiposity, and altered lipid and glucose metabolism in the offspring born to dams deficient in B12 [70]. These offsprings also showed imbalance between pro- and anti-inflammatory cytokines, high levels of cortisol and leptin, and low adiponectin levels, and several of these changes were reversed by rehabilitation of B12-restricted dams from conception [71]. Food-based periconceptional supplementation including multiple micronutrients has been reported to be associated with positive fetal outcomes and improved child health at 2 years in mothers with higher BMI (>20 kg/m²) compared to those having a lower BMI (<20 kg/m²) [72]. These results put forward an important role for maternal one carbon metabolism (OCM) in fetal growth and programming in view of its role in nucleic acids synthesis, genomic stability, maintenance of methylation potential of cells, and consequent epigenetic regulation of gene function.

In the OCM cycle, dietary folate is converted in the body to 5-methyl tetrahydrofolate by methylenetetrahydrofolate reductase (*MTHFR*). Using B12 as a co-factor, methylated folate then provides methyl groups to convert homocysteine to methionine,

which is a universal methyl group donor for all methylation reactions in the body. Thus, imbalance in folate and B12 levels is associated with hyperhomocysteinemia and changes in methylation potential, which affects DNA methylation, an important epigenetic mechanism by which various aspects of embryogenesis and fetal development are regulated [73]. Owing to the importance of OCM in fetal programming, it is important to establish a causal relationship between maternal homocysteine levels and offspring birth weight and insulin resistance. This is also important since the biochemical parameters are influenced by reverse causality. In one such attempt to establish a causal relationship between maternal intermediate traits and fetal outcome, we have used the Mendelian randomization approach, which employs functionally proven genetic markers as a proxy of intermediate traits that associate with a phenotypic outcome. *MTHFR* variants such as 677C>T encodes a thermolabile enzyme with reduced enzyme activity and are strong predictors of homocysteine levels. Using this *MTHFR* variant as the instrument, we showed that elevated homocysteine levels due to the 677C>T variant decreased birth weight of the offspring by 61 g after adjustment of gender, gestational age, and offspring genotype and thus established a causal relationship of maternal homocysteine level during pregnancy with low birth weight in children in PMNS and Parthenon birth cohort studies [40••].

Another important player in the OCM is essential omega-3 polyunsaturated fatty acids (n-3 PUFAs) like docosahexaenoic acid (DHA), whose metabolism is interdependent on folic acid/B12 levels [74]. Recent studies have demonstrated that DHA availability during perinatal period is associated with long-term cognitive and visual development [75]. Using a rat model of maternal micronutrient imbalance, it has been shown that excess folate and less B12 levels influence n-3 PUFAs metabolism, decrease plasma and placental DHA levels, and increase placental pro-inflammatory cytokines levels [74]. A role has also been demonstrated for DHA in prevention of insulin resistance in animal models [76, 77]. In view of its critical role in OCM, it is proposed that altered DHA levels could possibly result in excess methyl group availability for DNA and histone methylation leading to chromatin remodeling and altered gene expression [78].

Fuel-Mediated Teratogenesis

While maternal undernutrition during pregnancy and its long-term effects on the offspring health have received a lot of attention recently, problems due to overnutrition have been viewed as risk factors for future development of cardio-metabolic disorders for a long time and are now again on the rise [79, 80]. Thus, fuel-mediated teratogenesis focuses on the relationship between prenatal exposure to increased levels of “mixed nutrients,” especially glucose and fatty acids, and long-lasting deleterious consequences in the offspring [81].

In line with rising incidence of “diabesity” in the adult population worldwide, various epidemiological studies have reported increasing prevalence of maternal obesity, GDM, and T2D [82, 83]. Increase in maternal obesity is paralleled by an increase in large for gestational age babies and early onset and increased rate of obesity in the offspring as neonates, children, and as young adults [84, 85]. Maternal obesity is shown to affect fetal leptin and adiponectin levels, and offsprings born out of pregnancies with maternal obesity and/or excessive gestational weight gain exhibit neonatal insulin resistance as a result of intrauterine activation of the endoplasmic reticulum stress response/unfolded protein response and increased levels of pro-inflammatory mediators [86–88]. The above results from clinical studies are substantiated by several rodent experiments, thus confirming the causal relationship of maternal obesity/overnutrition with fetal programming of obesity and glucose intolerance. Further, this tendency is augmented when offsprings are exposed to high-fat diet following weaning [89].

Similar to maternal obesity, it is well established that infants exposed to maternal hyperglycemia are susceptible to obesity, T2D, and cardio-metabolic complications [90–92]. Fetal macrosomia in neonates exposed to maternal hyperglycemia is largely due to growth-promoting effects of fetal insulin in response to high levels of maternal glucose, and the preliminary evidence came from the Pima Indian Study. The offspring of Pima Indian women with preexistent T2D and GDM were heavier at every age and exhibited higher body fat, higher incidence of childhood obesity, abnormal glucose tolerance, and diabetes compared to the offspring of non-diabetic or pre-diabetic women. Importantly, these differences in risk persist even after adjustment for the diabetic status of the father, age at onset of diabetes in either parent, and obesity in the offspring [93–95]. Furthermore, an increasing body of evidence now shows that intrauterine programming is more related to the maternal hyperglycemia than to the type of diabetes, such as T1D, T2D, or GDM [96–98]. The offspring of mildly diabetic mothers display normal mass of the endocrine pancreas with normal glucose tolerance at birth but show impaired glucose tolerance in adulthood under high glucose challenge. These observations are substantiated by streptozotocin-induced animal models of GDM, where severe intrauterine hyperglycemia in rats induces fetal islet hypertrophy and overstimulation of fetal beta cells.

In addition to the role of micronutrients such as folate and B12, observations from PMNS underline the importance of maternal levels of fuels such as lipids and glucose in modulating fetal growth. Rise in maternal total cholesterol and triglycerides levels at 18 and 28 weeks and rise in fasting glucose levels at 28 weeks were associated with birth weight of the offspring. These results indicate an impact of both micronutrients and macronutrients on neonatal size and possible risk of future cardio-metabolic disease [99].

Dual-Mediated Teratogenesis

The notions of nutrient- and fuel-mediated teratogenesis manifest opposite ends of effects of changes in the intrauterine environment mediated by maternal nutrition and metabolism. Maternal undernutrition produces thin-fat, insulin-resistant babies who remain in the same state in the absence of postnatal overnutrition. Upon reaching reproductive age, they become undernourished mothers and result in fetal undernutrition. However, postnatal overnutrition, especially in the presence of micronutrient deficiency, increases obesity and hyperglycemia in the offspring resulting in obese and insulin-resistant mothers who promote fetal adiposity and beta cell dysfunction. This forms the vicious cycle of dual-mediated teratogenesis where transition from undernutrition to overnutrition contributes to the epidemic of “Diabesity” through propagation of intergenerational insulin resistance-diabetes cycle (Fig. 1). In the Parthenon Birth Cohort Study, it has been demonstrated that maternal B12 deficiency is associated with increased adiposity and consequently with increased risk of insulin resistance and GDM which amplifies in the presence of excess folate [69]. Under such conditions, the developing fetus is exposed to collective programming effects leading to excess adiposity, beta cell dysfunction, and risk of incidence of diabetes and cardiovascular diseases at a younger age. These results indicate that disturbances in OCM could provide a plausible explanation to both fetal growth restriction due to nutrient-mediated teratogenesis and fetal macrosomia due to fuel-mediated teratogenesis.

Mechanisms of Intrauterine Programming

It is now evident that both maternal undernutrition and overnutrition during critical ontogenic periods induce persistent physiological alterations by programming effects. Of the several mechanisms by which maternal nutritional insults may mediate fetal programming of obesity, an impaired glucose metabolism, oxidative stress, and resetting of the hypothalamic-pituitary-adrenal (HPA) axis are supported by experimental evidence. Undernutrition due to protein deficiency leads to a pro-oxidative state due to less availability of amino acids required for synthesis of antioxidants like glutathione and albumin [100]. On similar lines, exposure to hyperglycemia also leads to increased oxidative stress by generating reactive oxygen species (ROS) in the developing fetus, which also influences mitochondrial functioning. Additionally, deficiency of micronutrients like B12, folate, etc. is linked to the rise in oxidative stress [101]. Excessive ROS is known to modulate gene expression and/or cause direct damage to cell membranes and other molecules at critical developmental windows. High sensitivity of pancreatic beta cells to ROS results in beta cell apoptosis and blunted insulin secretion [102]. On the other hand, hypothalamic circuitry which plays a critical role in regulation of satiety and body composition via HPA axis is known to undergo long-term changes as a manifestation of programming due to nutritional imbalance during pregnancy. This leads to differential expression, localization, and action of anorexigenic neuropeptide pro-opiomelanocortin (POMC) and orexigenic peptide neuropeptide Y (NPY) in such offsprings,

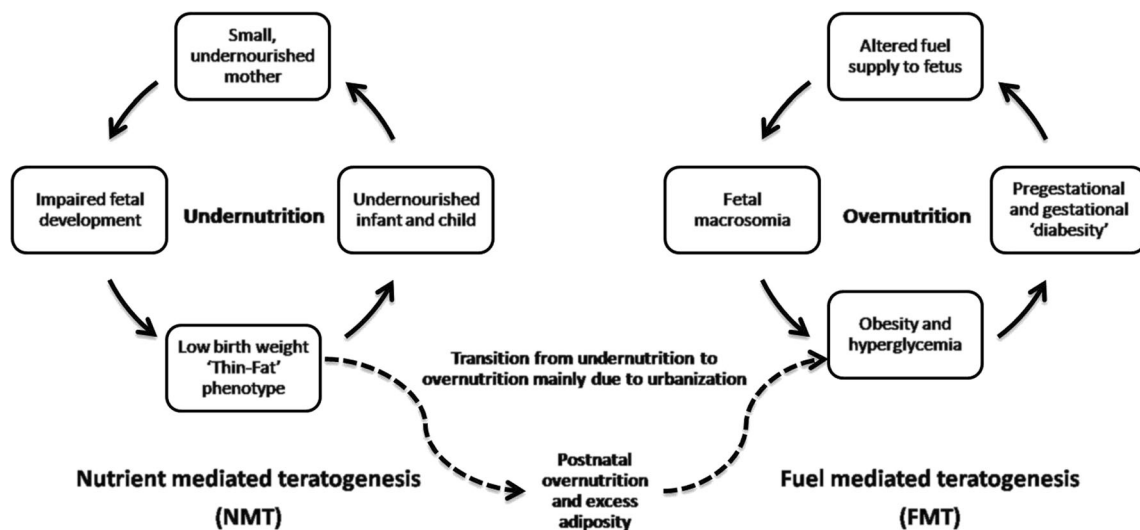


Fig. 1 Vicious cycle of dual mediated teratogenesis. This figure is a diagrammatic representation of the life cycles of babies born to undernourished and overnourished mothers. Babies of undernourished mothers suffer intrauterine undernourishment and are born with a “thin-fat” phenotype and are more insulin resistant. If postnatal nutrition levels are low, they remain thin-fat but “normal” adults and propagate the cycle.

If these babies are overfed (as witnessed by urbanization), they become more adipose. Such mothers can develop gestational diabetes, which leads to fetal macrosomia. Such babies are at higher risk of diabetes later in life. (Adapted from: Yajnik CS. Nutrient-mediated teratogenesis and fuel-mediated teratogenesis: two pathways of intrauterine programming of diabetes. *Int J Gynaecol Obstet.* 2009;104:S27-S31) [118]

primarily due to dysregulated leptin function. Reduced leptin availability or hypothalamic leptin resistance is suggested as a common mechanism by which low or high perinatal nutrition permanently alters hypothalamic circuits [103].

Epigenetic Perspective

As mentioned above, several common pathways are identified which are responsible for intrauterine programming of “diabesity”; however, increasing focus is now laid in understanding the epigenetic mechanisms that lead to their dysregulation. A number of animal and human studies have shown that maternal nutritional factors can modify the epigenome of the developing offspring. For example, pregnant rats fed on a protein-restricted diet exhibited altered DNA methylation and expression patterns of the glucocorticoid receptor (*NR3C1*) and peroxisome proliferator activated receptor alpha (*PPAR α*) genes in the liver and heart of the offspring, which was restored to that of controls with maternal folate supplementation. Low protein diet during pregnancy in sheep resulted in sex-dependent methylation differences in the promoter region of mtDNA-encoded mitochondrial oxidative phosphorylation (*OXPHOS*) genes and consequent imbalance in energy homeostasis [104]. Using a sheep model of periconceptional folic acid and B12 deficiency, another study has demonstrated widespread DNA methylation changes and long-term effects on the metabolic status of the offspring [105]. Comparison of individuals exposed to undernutrition during early gestation and their unexposed siblings born during the Dutch famine period showed differential methylation of loci like insulin-like growth factor 2 (*IGF2*) and leptin (*LEP*) which are associated with regulation of body growth and glucose metabolism [106, 107]. Furthermore, differential methylation at several identified loci is shown to depend on sex of the exposed individual and gestational timing of the exposure [106]. Similar results have been observed from studies on mothers in rural Gambia where altered methyl donor intake during the periconceptional period due to seasonal variations influenced maternal status of substrates and cofactors required for methyl donor pathways and DNA methylation at metastable epialleles in the offspring. Periconceptional supplementation with micronutrients in these women identified large, gender-specific effects on whole genome methylation patterns in their offspring [108, 109••].

On the same note, several studies have reported epigenetic changes in offsprings exposed to maternal obesity and hyperglycemia. In rodent models, maternal high fat intake during conception has been linked to offspring hypermethylation of the fatty acid desaturase 2 (*FADS2*) gene which encodes a rate-limiting enzyme in the synthesis of polyunsaturated fatty acids associated with reduction in T2D risk [110]. Further, maternal high-fat intake during lactation is shown to induce dopamine- and opioid-related gene-specific promoter

DNA hypomethylation in the brains of offsprings, resulting in potential means of programming of appetite and energy-rich food preference in postnatal life [111]. In addition to DNA methylation changes, maternal high fat intake is shown to be associated with sex-specific histone modifications leading to altered hepatic metabolism and oxidative stress and differential expression of hepatic miRNAs with targets mediating epigenetic regulation [112]. Studies on human placental samples of offsprings exposed to maternal hyperglycemia revealed a deregulated methylation profile of two important adipokines, *LEP*, and adiponectin (*ADIPOQ*) [113–115]. In a similar study, maternally imprinted mesoderm-specific transcript (*MEST*) and *NRC31* genes showed significant hypomethylation in placental and cord blood samples from offsprings exposed to intrauterine hyperglycemia [116].

The above evidence from epigenetic studies provides support for the “thrifty epigenotype” hypothesis, which addresses limitations and unifies merits of the former two thrifty hypotheses. The hypothesis suggests that an individual acquires a metabolic phenotype established during a time of overnutrition or malnutrition and the phenotype and underlying epigenetic settings are firmly established during adult life. However, upon encountering different environmental conditions from those existed during the programming phase, established phenotype fails to adjust, which increases disease risk. Furthermore, it is hinted that a particular epigenotype can be transmitted to the offspring, provided the epigenetic information is not erased in germ cells, or persistence of the environmental conditions would either reinforce or reintroduce a particular epigenotype in the parental germ line resulting in transgenerational or intergenerational inheritance and predisposition to obesity and T2D [117].

Conclusions and Future Perspectives

Recent advances encompassing epidemiological, animal, and human studies have proven the potential of early life determinants in playing a substantial role in programming of “diabesity.” With evidences from epigenetic studies, molecular mechanisms by which altered intrauterine and postnatal environments can mediate fetal programming are now being understood. Apart from macronutrients like glucose, fats, and proteins, appreciating the roles of micronutrients like folate, B12, DHA, B6, chromium, magnesium, and potassium and understanding their interdependence would help in exploring the combinatorial effects of multiple micronutrient supplementations. As the role of the adverse intrauterine environment and maternal factors is well established, increasing attention is now paid towards the role of paternal factors in fetal programming with evidence emerging from both animal and human studies. Paternal lifestyle and metabolic factors like smoking, nutritional status, BMI, and insulin resistance are identified as important factors in determining fetal growth

and cardiovascular disease in later life. In addition, there is emerging support for the notion that an environmental exposure in one generation could affect the phenotype in subsequent generations. This transfer of information from one generation to the other in response to environmental exposures, mainly due to germ line epigenetic mechanisms referred to as transgenerational epigenetic inheritance, has attracted huge scientific attention. Study of maternal and paternal exposures and interplay between their influences and their impact on the offspring's phenotype could possibly help in identifying critical windows and characterize epigenetic marks by which information from one generation passes to the other and result in undesirable programming. Together, a holistic approach embracing knowledge from effects of exposures to adverse maternal and paternal environments, identification of crucial windows for programming, assessment of nutritional status of men and women of reproductive age, and identification of ranges of subclinical and clinical deficiencies of various nutrients, which may vary from population to population, can provide momentum to design appropriate lifestyle interventions and multiple micronutrient supplementation to reduce risk of fetal programming and pave a path to decrease the rising epidemic of "diabesity."

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Conflict of Interest Ashutosh Singh Tomar, Divya Sri Priyanka Tallapragada, Suraj Singh Nongmaithem, Smeeta Shrestha, Chittaranjan S Yajnik, and Giriraj Ratan Chandak declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- Of importance
- Of major importance

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