Review Article

Developmental Origins of Non-Communicable Diseases

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The burden of chronic non-communicable diseases (NCDs) such as diabetes, obesity and cardiovascular diseases is rising rapidly in low- and middle-income countries (LMICs). LMICs also have a high occurrence of undernutrition and infectious diseases which together form the 'double burden' of diseases. The connection between undernutrition and NCDs is puzzling but explained by David Barker's idea of intrauterine programming. This idea [now expanded into 'Developmental Origins of Health and Disease' (DOHaD)] focuses on maternal health and nutrition as major determinants of the offspring's health. India offers a unique opportunity to study these links. Studies in India have shown that Indian babies are thin but fat (more adipose tissue) compared to European babies, and maternal nutrition during pregnancy is a determinant of offspring's size and body composition. Both, maternal-foetal undernutrition and overnutrition are associated with increased adiposity and insulin resistance in the children. Micronutrients like vitamin B_{12}, folate and vitamin D may be particularly important. Improving nutritional status of the young generation (especially that of young women in reproductive age) offers a potential for intergenerational prevention of NCDs.

Keywords: **Foetal Programming; Maternal Nutrition; Non-Communicable Diseases; Diabetes; Epigenetics; DOHaD**

Introduction

The chronic non-communicable diseases (NCDs) like type 2 diabetes (T2D) and cardiovascular disease (CVD) are leading causes of morbidity and premature death worldwide. They were thought of as problems of old age and of affluent countries, but now affect large numbers of relatively young people in low- and middle-income countries (LMICs) like India. LMICs have a 'double burden' of illness, with high NCD rates alongside long-standing and persisting problems of under-development and malnutrition, causing low birth weight, stunted childhood growth and impaired cognitive development and infections. Current approaches to preventing cardio-metabolic disease focus mainly on treating risk factors like obesity, hypertension, lipid abnormalities and glucose intolerance in adults. This can, at best, be called secondary prevention at 'end-stage' of the problem. It usually targets post-reproductive adults. It will improve the outlook for those individuals, but will do little to stem the predicted NCD epidemic.

Previous Work

Early work in this field of research dates to the 1970s when Forsdahl demonstrated a significant positive correlation between arteriosclerotic heart disease in middle-aged adults and the prevailing infant mortality rates during their childhood in twenty counties in Norway (Forsdahl, 1977). He attributed this finding to poor living conditions in childhood followed by affluence in later life. Subsequently, Barker and colleagues in Southampton showed a positive correlation between coronary heart disease (CHD) mortality rates in England and Wales in the 1970s and corresponding infant mortality rates in the earlier part of the century (Barker and Osmond, 1986).

In 1991, Hales and Barker reported that small size at birth was a strong risk factor for T2D and proposed that intrauterine undernutrition was the predisposing factor ('thrifty phenotype' hypothesis) (Barker *et al.*, 1989; Hales *et al.*, 1992).These findings have subsequently been replicated in several

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parts of the world including developing countries (Fall *et al*., 1998, Mi *et al*., 2000; Eriksson *et al*., 2001; Victora *et al*., 2008). These findings were supported by the finding of higher risk of NCDs (T2D, hypertension, mental disorders etc.) in individuals who were exposed to 'intrauterine famine' during the Dutch winter hunger towards the end of the Second World War (Heijmans *et al*., 2008). It was soon apparent that both low and high birth weights were associated with later T2D indicating a U-shaped relationship (Whincup *et al*., 2008). This suggests that both undernutrition and overnutrition of the foetus are associated with future risk. Studies also showed an association between different size measurements at birth (weight, length, head, abdominal circumference and placenta) and risk of NCDs (Fall, 2013).

Foetal Size, Body Composition and Metabolic-endocrine Axes

Humans have the highest adiposity (body fat %) at birth (~15%), much higher than a pig (2%) or a sea lion (5%). In humans, the relationship between weight and body composition varies between populations. Many developing populations have higher adiposity for a given body mass index (BMI), this disparity is very striking in Indians and has led to the description of a 'thin-fat' Indian. This is not related to the adult lifestyle, but is present even at birth: the smaller Indian new-borns have a comparable or higher adiposity compared to European babies (Yajnik *et al*., 2002, Yajnik *et al*., 2003). It would appear that multigenerational undernourished populations have developed this phenotype. The adiposity of the foetus reflects in the circulating levels of metabolites and hormones which have been measured in the cord blood. For example, Indian and Pakistani babies have higher concentrations of leptin in their cord blood compared to those in the European babies, and Indian babies also have higher insulin and lower adiponectin concentrations (West *et al*., 2014, Yajnik *et al*., 2002). This profile of hormones in the cord blood is strongly suggestive of higher risk of diabetes in future.

Plasticity, Programming and Epigenetics

A developing foetus has the ability to develop in many possible ways depending on the environment *in utero*. This is referred to as 'plasticity'. Environmental factors may restrict foetal development and interfere with its attempt to 'reach the potential'. Restriction of the plasticity is referred to as 'programming', and in clinical medicine this is usually related to increased disease susceptibility. It may manifest as structural (size, proportion, body composition) or functional (endocrine-metabolic) alterations. Many of these persist and are therefore demonstrable in later life. Waddington referred to the 'evolution of phenotype' from the genotype as an 'epigenetic' phenomenon (Waddington CH, 1968). The basic idea is that gene expression changes despite the same sequence of bases in the genome, producing a different phenotype. This is the basis of development and differentiation. Recent research has demonstrated molecular mechanisms underlying this phenomena. DNA methylation, histone modifications, and miRNAs are the currently understood 'epigenetic' mechanisms of programming.

A number of environmental factors influence epigenetic modifications. Some of the well-known factors include: maternal nutrition, metabolism, infections, stress, pollutants and endocrine disruptors. Nutrition is one of the frequently investigated exposure for epigenetic changes and foetal programming.

Nutrition and Foetal Programming

The concept of nutritional foetal programming has contributed to a paradigm shift in thinking in the field of nutrition, with a focus on intergenerational health. An exciting discovery was the demonstration that nutrients influence gene function by interacting with nuclear receptors (vitamins A and D) and also through epigenetic mechanisms (folate, vitamin B_{12}, etc.) (Anderson *et al*., 2012). Both mechanisms modulate gene expression. The resulting impact on multiple cellular processes, particularly during critical times in foetal development, could influence the structure and function of organs and systems, and contribute to long term effects on health and disease susceptibility (programming) (Lucas, 1991). Although pregnancy and infancy are important, the periconception period may be the most important (Regine *et al*., 2013). Together these ideas have led to the concept of 'first 1000 days' as an important window of opportunity to improve human health. It is relevant that majority of pregnancies are unplanned and therefore, improvement of periconception environment strongly depends on preconception lifestyle.

Animal models of foetal programming frequently investigate the effect of maternal protein deficiency (Langley-Evans, 2006, Snoeck A *et al*., 1990), but human data is sparse. A protein deficit leads to a shortage of amino acids, the building blocks of proteins, and can also disturb one-carbon metabolism and DNA methylation, resulting in widespread downstream effects. Protein supplementation during pregnancy showed variable effects on human foetal growth (Kramer and Kakuma, 2003), indicating the need for further research. No specific role has been ascribed to carbohydrates and fats as macronutrients in foetal programming, but both are a major energy source and contribute to macrosomia ('big baby', birth weight >4 kg in the developed world) in a diabetic pregnancy. In addition, fatty acids (especially n3 fatty acids) may have a special role in foetal development (Huffman *et al*., 2011).

Micronutrients have emerged as important contributors to foetal programming. Methyl donors (folate, methionine, choline, and betaine, and co-factors vitamin B_{12}, B_6 and B_2) are of special interest because of their potential to influence DNA methylation (Kalhan, 2013). Both maternal folate and vitamin B_{12} concentrations are associated with foetal growth (Rao *et al*., 2001; Muthayya *et al*., 2006). Higher folate is also associated with offspring adiposity and insulin resistance, especially if vitamin B_{12} is low (Yajnik *et al*., 2008; Krishnaveni *et al*., 2014). This suggests a need for balanced nutrition. These two vitamins also influence the offspring neurodevelopment (Veena *et al*., 2010; Godbole *et al*., 2009; Bhate *et al*., 2008). Higher maternal homocysteine concentration (caused by vitamin B_{12} and folate deficiency) is associated with foetal growth restriction. A maternal methyle netetrahydrofolate reductase (MTHFR) gene polymorphism that raises homocysteine concentrations also predicts foetal growth restriction, suggesting a causal role of homocysteine (Yajnik *et al*., 2014). Studies in The Gambia demonstrated a seasonal pattern in methyl donor availability in the maternal bloodstream, which was reflected in different levels of DNA methylation in the cord blood (Dominguez-Salas *et al*., 2014). Similarly, folic acid supplementation trials in pregnancy also influenced cord blood methylation (Khulan *et al*., 2012). These human findings suggest a possible role for methyl donors in epigenetic foetal programming and later occurrence of NCDs. Animal models support such a

role (Kumar *et al*., 2013), but there is an urgent need for more human studies. Other micronutrients of interest in foetal programming include vitamin D, vitamin A, calcium, zinc, and omega-3 fatty acids.

The role of macronutrients (glucose, lipids) is more obvious in clinical situations. A macrosomic baby in a diabetic pregnancy is a classic example. Pedersen proposed (Pedersen, 1971) and Freinkel developed (Freinkel, 1980) this idea into 'fuel mediated teratogenesis' concept. The role of maternal obesity is also becoming more obvious in recent years. Even in undernourished non-diabetic Indian pregnancies, maternal lipids play a significant role in foetal development, size and body composition (Kulkarni *et al*., 2013). Gestational diabetes is becoming more common worldwide (IDF, 2015) especially in India (Seshiah *et al*., 2004).

We have proposed a "dual teratogenesis" construct to explain the U-shaped association between birth weight and T2D (Fig. 1) (Yajnik, 2009). Maternal-fetal undernutrition produces thin (poor lean mass) children with high adipose percentage and insulin resistance ("nutrient-mediated teratogenesis"). If these children continue to live in a deprived situation, they propagate this phenotype without overt diabetes. However, if they face overnutrition in postnatal life (urban migration), they are likely to develop obesity and hyperglycaemia at a young age, and gestational diabetes in girls that propagates the obesity and diabetes phenotype ("fuel-mediated teratogenesis") (Freinkel, 1980). Rapidly transitioning countries like India seem to have both cycles running simultaneously, feeding into an explosive epidemic of NCDs (Silverman *et al*., 1995; Yajnik, 2009; Krishnaveni *et al*., 2010).

It has now been accepted at policy level that optimal nutrition during the 'first 1,000 days' (intra-uterine life and the first 2 postnatal years) would bring significant benefits for childhood growth and capacity. Rapid postnatal growth on the background of poor early growth is associated with risk of T2D. Adults with the highest risk of T2D are those who had low birth weight but high current BMI and a progressively increasing body size across childhood and adolescence (Eriksson *et al*., 2001; Eriksson *et al*., 2003; Bhargava *et al*., 2004). Thus, the foetal programming hypothesis was expanded to include postnatal factors and is now
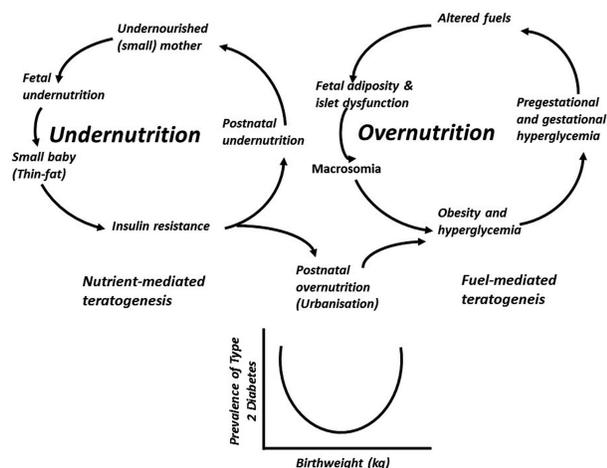
Fig. 1: In undernourished populations, foetal undernutrition results in "thin-fat" insulin resistant babies. If postnatal nutrition is also low, they continue with the phenotype, and females can transmit this phenotype intergenerationally. If there is postnatal overnutrition, it can result in obesity and hyperglycemia, which can cause pre-gestational and gestational diabetes, promoting foetal macrosomia, and setting up an intergenerational cycle of obesity and hyperglycemia. In rapidly developing countries such as India, the two cycles operate simultaneously. This construct provides an explanation for the U-shaped association (bottom graph) between birth weight (X-axis) and type 2 diabetes (T2D) (Y-axis). [Based on data in Yajnik, 2009]
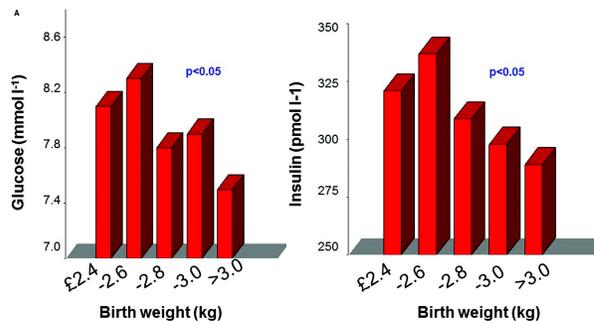

Fig. 2: The Pune Children Study showed that glucose and insulin concentrations (Y-axes) at 4 years of age were inversely associated with birthweight (X-axis). The p-value refers to this trend. [Based on data in Yajnik *et al*., 1995]

referred to as the "Developmental (or early) Origins of Health and Disease" hypothesis (DOHaD).

The DOHaD idea is sometimes interpreted as a 'two hit' phenomenon; the intrauterine 'programming' influences 'susceptibility' and later lifestyle act as 'precipitating' factors. The novelty of the concept lies in the fact that susceptibility is now considered modifiable, unlike the purely 'genetic' model where it was considered to be non-modifiable.

DOHaD Work in India

The DOHaD work in India was sparked off by the visit of David Barker and Caroline Fall in the early 1990s. They established a collaboration with KEM Hospital Pune (Dr. Yajnik and Dr. Anand Pandit) to set up a study of diabetes risk factors in young children and their relationship with birth weight. Subsequently, they contacted approximately 300 hospitals across India and set up studies in Mysore, Delhi and Vellore.

These collaborations led to the formation of the Society for the Natal Effects of Health in Adults (SNEHA) which has grown in strength and is currently the longest established "fetal origins" society (the 19th annual meeting was held in 2015). Growing work in this field across the world led to the formation of the International DOHaD Society with a world congress organized every two years (website: https://dohadsoc.org/).

The first study (Pune Children's Study) showed that children with lower birth weights had higher insulin and glucose levels at 4 years of age (Yajnik *et al*., 1995) (Fig. 2). A follow-up at 8 years of age showed that the children of lower birth weight who had the highest current weight also had higher insulin resistance and the worst cardiovascular risk profile (Bavdekar *et al*., 1999). In 40 to 60-year-old adults in Mysore, the risk of CHD was higher in those who had been small at birth as measured by birth weight, birth length and head circumference (Stein *et al*., 1996). In Mysore, however, the prevalence of T2D was higher in those with a greater ponderal index (a ratio of birth weight to birth length, similar to BMI heavier weight and shorter length leads to greater ponderal index) (Fall *et al*., 1998). This led to the suggestion that these heavier babies may have been the offspring of diabetic mothers, and that further work was necessary to look beyond birth size measurements. Younger adult cohorts (25 to 30 years) with detailed childhood growth data were retraced in Delhi and Vellore. These studies demonstrated that lower birth weight, poor growth in the first 2 years of life, followed by higher weight gain across childhood and
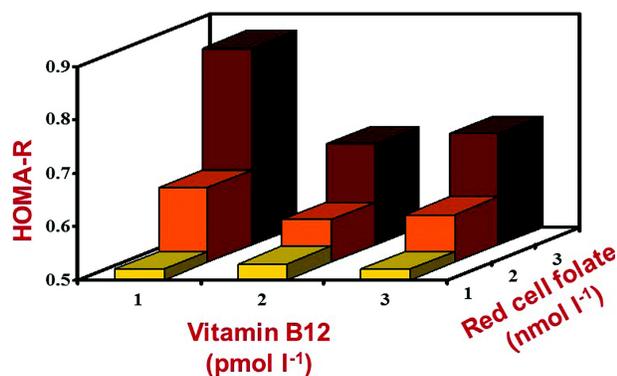
Fig. 3: In the Pune Maternal Nutrition Study, higher maternal folate (Z-axis) and lower vitamin B12 concentrations (X-axis) were associated with higher insulin resistance (HOMA-R) (Y-axis) in the children at 6 years of age. [Based on data inYajnik *et al*., 2008]

adolescence were associated with adult insulin resistance and glucose intolerance (Bhargava *et al*., 2004; Raghupathy *et al*., 2010). In Delhi, early adiposity rebound (the age after infancy when body mass starts to rise) increased risk of glucose intolerance in adult age. It is important to note that none of these subjects were obese at the age of 12 years despite an accelerated gain in BMI in childhood (Bhargava *et al*., 2004). Analysis in the Delhi cohort also showed that faster linear growth throughout childhood and faster relative weight gain in the first 6 postnatal months were associated more strongly with adult lean mass (Sachdev *et al*., 2005), while faster relative weight gain after 11 years was associated more strongly with adult fat mass, and with a higher risk of impaired glucose tolerance and T2D. The studies in India therefore confirmed western findings that lower birth and infant weight, followed by progressive weight gain in childhood and adolescence are associated with adiposity, insulin resistance and glucose intolerance in later life (Eriksson *et al*., 2003).

All these initial studies were retrospective, based on obstetric birth records. These were followed by setting up of new prospective cohorts in Pune and Mysore, collecting detailed maternal data during pregnancy, detailed size measurements at birth, and serial follow-up into childhood, adolescence and adult age. The Pune Maternal Nutrition Study (PMNS) was the first ever study in the developing world to prospectively study the effects of maternal nutrition (even before conception) on the cardiovascular risk

of the offspring. The study recruited married non-pregnant women in six villages near Pune consisting mostly of subsistence farming communities in a drought-prone area. Anthropometry (height, weight, circumferences and skinfold) was recorded every 3 months. Women missing two successive periods underwent an ultrasound examination to confirm pregnancy and assess gestational age.

During pregnancy, information was collected on socioeconomic status, dietary intake and physical activity. Anthropometry, glucose and insulin, and lipids were also measured. At birth, new-born measurements included birth weight, length, head circumference and skinfolds. These children have subsequently been followed every 6 months for anthropometry and detailed cardio-metabolic measurements at 6, 12 and 18 years; the study is ongoing.

The study demonstrated that the "thin-fat" Indian phenotype was present at birth and not a consequence of postnatal life as previously presumed (Yajnik *et al*., 2003). When the Pune babies were compared to babies born in UK, the Indian babies were 700 g lighter but had comparable subscapular skinfold than the English babies. Further studies using whole body magnetic resonance imaging (MRI) in the new-borns confirmed that Indian babies have higher abdominal fat at birth compared to the English babies (Modi *et al*., 2009), and also that they have higher insulin and leptin levels in the cord blood (Yajnik *et al*., 2002).

This study, for the first time, demonstrated a link between maternal intake of micronutrient rich foods and offspring size. Mothers who had the most frequent intake of green leafy vegetables and who had the highest red cell folate levels gave birth to the heaviest babies (Rao *et al*., 2001). Interestingly, there were no relationships with total energy or protein intake. Follow-up demonstrated a direct association between maternal micronutrient levels and risk factors for diabetes in the children. Higher folate concentrations in the mothers during pregnancy predicted higher adiposity in the offspring at 6 years (Yajnik *et al*., 2008). Vitamin B_{12} deficiency in the pregnant mothers was associated with increased insulin resistance in their children at 6 years of age; the highest insulin resistance was in children born to

mothers with low vitamin B_{12} and high folate during pregnancy (Fig. 3). These findings raise a number of interesting issues about current clinical and public health practices in India. Only a few pregnancies are planned and therefore amenable for preconceptional nutritional and other lifestyle modifications. This suggests a need for public education starting from school and adolescent age to promote the intake of healthy foods. Vitamin B_{12} is present only in animal origin foods and therefore it is difficult to achieve adequate intake in vegetarians. Adequate milk intake and supplements will help improve vitamin B_{12} status. Obstetricians in India have long prescribed high doses of folic acid (the most commonly used tablet is 'Folvite' which contains 5 mg i.e. 5000 mcg of folic acid which is more than 12 times the recommended dose of 400 mcg) to prevent first time neural tube defect. In a B_{12} deficient population, this can create an iatrogenic imbalance between B_{12} and folate and could add to the risk of diabesity in the offspring. A simple solution will be to avoid use of high dose folic acid (the safe upper limit for folic acid in Europe is 1 mg per day), and includes vitamin B_{12} in adequate doses along with recommended doses of folic acid during pregnancy. This can also be applied to the IFA (Iron:100mg +

Folic Acid: 500mcg) tablets used in Indian Government's adolescent and pregnancy programs, by including vitamin B_{12} (at least 2 mcg) in the tablet, keeping in mind that it is intended for use only once in a week.

The 1-C metabolism cycle, a network of inter-related biochemical reactions that involve the transfer of 1-carbon groups from one compound to another, is crucial for cell growth and differentiation. Both, vitamin B_{12} and folate (along with other B-group vitamins) influence several of these reactions. Insufficiency or imbalance of these micronutrients can cause an increase in homocysteine levels which has been linked to higher CHD risk (Refsum *et al.*, 1998). In the Pune study, lower vitamin B_{12} and folate were also associated with higher homocysteine, which predicted lower birth weight (Yajnik *et al.*, 2005) (Yajnik *et al.*, 2014) (Fig. 4). These findings were particularly significant in a context where the population was folate sufficient (0.2% deficiency) but vitamin B_{12} deficient (~70% deficiency).

Foetal overnutrition (due to maternal obesity and hyperglycaemia) may also program the offspring for T2D. These babies are born "larger", and develop
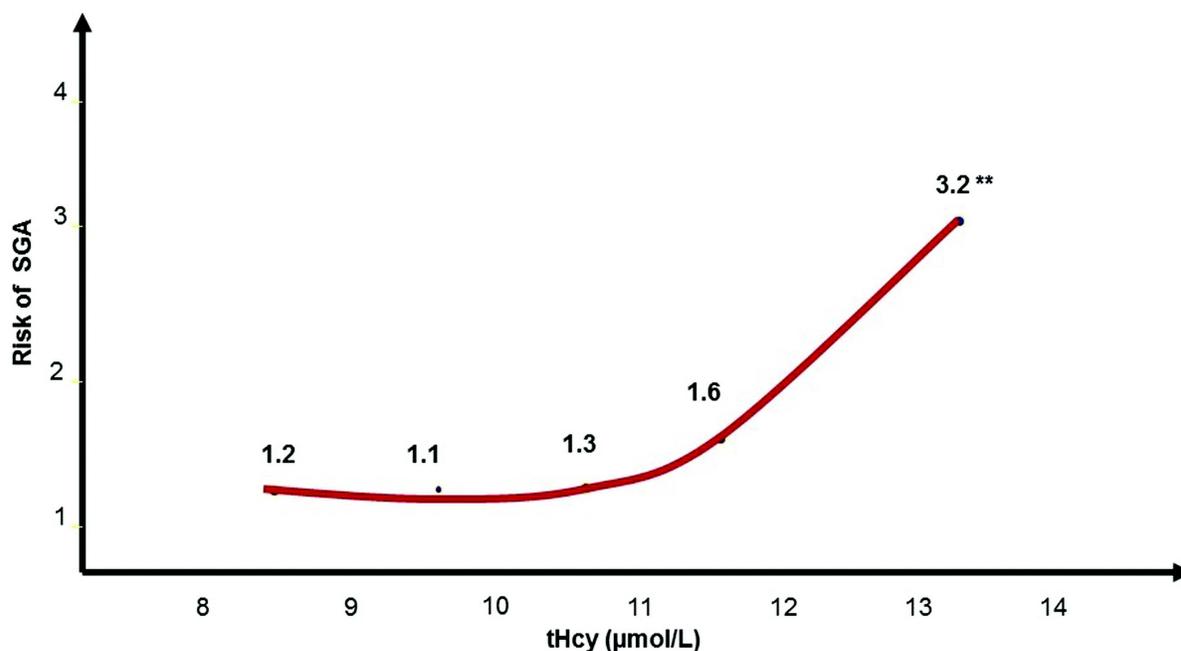

Fig. 4: In the Pune Maternal Nutrition Study, high maternal plasma homocysteine concentration in pregnancy (X-axis) was associated with lower birth weight. Babies born to mothers in the highest quintile of homocysteine concentrations had over three times higher risk of being small for gestational age (SGA) (Y-axis). The homocysteine concentrations were attributable to vitamin B12 deficiency. [Based on data in Yajnik *et al.*, 2014]

obesity, central obesity, insulin resistance, impaired glucose tolerance and T2D at a young age. This sequence of events was first described in the Pima Indian population in the USA (Pettitt, 1983). Pedersen proposed that the transfer of excess maternal glucose stimulates foetal islets to produce hyperinsulinaemia which leads to macrosomia (Pedersen, 1954). Freinkel (1980) suggested that a "mixture" of maternal nutrients (glucose, lipids and amino acids) affects not only foetal growth but also affects the risk of future obesity, diabetes and neurocognitive impairment (fuel-mediated teratogenesis). He suggested that this was due to an irreversible effect on gene expression rather than a genetic transmission of susceptibility. Research in Pima Indians has provided proof for 'non-genetic' transmission of the risk of obesity in the offspring of diabetic pregnancies. Thus, children born after the mother developed diabetes were more obese than those born when the mother was non-diabetic (Pettit, 1983; Dabelea *et al.*, 2000), despite similar genetic inheritance. Though GDM is associated with certain genetic markers which explain a small proportion of the risk; and for T2D where almost 100 genetic markers are described, they show less than 15% of susceptibility. Current thinking is that a substantial part of the remaining susceptibility may be 'epigenetic'. It is intriguing that foetal undernutrition also acts similarly to increase future risks of diabetes.

The Mysore Parthenon Study was specifically set up to study the long-term effects of GDM on the offspring. The study recruited women during pregnancy in a charitable hospital which attracts patients from all strata of society. Measurements during pregnancy included anthropometry, and serum glucose, insulin and lipids. This study showed that the children born to GDM mothers were larger at birth and had higher subcutaneous adiposity compared to the new-borns of non-GDM mothers (Hill *et al.*, 2005). The difference in adiposity continued to increase throughout childhood. At 9 years of age, the children of GDM mothers had higher glucose and insulin concentrations and higher insulin resistance compared to the children of non-GDM mothers (Krishnaveni *et al.*, 2010). Interestingly, maternal vitamin B_{12} deficiency was associated with an increased risk of GDM in the mothers, and B_{12}-deficient GDM mothers had a higher risk of being diabetic 5 years after delivery compared to those GDM who were not B_{12}-deficient in pregnancy (Krishnaveni *et al.*, 2009).

Thus, the prospective cohorts in India have made an important contribution in the studies of nutritional and metabolic factors in the mothers that predict foetal growth and future risk of NCDs.

Foetal Programming: Epigenetics - Concepts and Evidence

Epigenetic studies applied to DOHaD in humans are limited but maternal nutrition has been shown to influence gene-specific differences in DNA methylation at birth and in later life (Khulan *et al.*, 2012; Relton *et al.*, 2012). Higher DNA methylation of RXRA chr9:136355885+ at birth has been related to later adiposity (Godfrey *et al.*, 2011). Nutritional interventions in pregnant mothers (mostly using methyl donors in 1-C metabolism such as vitamin B_{12}, folic acid, betaine and choline) induced permanent changes in DNA methylation, gene expression, and later phenotype in the offspring. Children conceived during the Dutch winter hunger had less DNA methylation of the imprinted IGF-2 gene compared with their unexposed, same-sex siblings when studied six decades later. Maternal folic acid supplementation in the periconceptional period was associated with increased methylation of IGF-2 in the offspring (Steegers-Theunissen *et al.*, 2009). Waterland *et al.* (2010) studied the establishment of metastable epialleles (alleles that are variably expressed in genetically identical individuals due to epigenetic modifications established during early development and are thought to be particularly vulnerable to environmental influences) in early life development by season of conception.

At the five loci investigated, conception during the rainy season resulted in significantly more methylation of DNA compared to those who conceived in dry season. Studying the influence of early environmental exposures on metastable epialleles and imprinted genes could offer insight into the mechanisms affecting the foetal epigenome and subsequent disease susceptibility.

Lipogenesis and Insulin Resistance

There is some evidence that high maternal folate during pregnancy is associated with higher adiposity in the child (Yajnik 2008, Krishnavenni 2014) and that low maternal B_{12} status is associated with increased insulin resistance in the child (Yajnik *et al.*, 2008, Stewart *et al.*, 2011). This could be due to epigenetic

programming or due to altered mitochondrial handling of fatty acids, promoting lipogenesis in the adipose tissue. Increased adiposity and accumulation of triglycerides in myocytes has been associated with insulin resistance (Kelley *et al*., 2002). These mechanisms could be involved in the evolution of the thin-fat Indian phenotype (Rush *et al*., 2009; van Steijn *et al*., 2009; Yajnik *et al*., 2003). Indians are traditionally vegetarian and have a poor intake of animal products rich in vitamin B_{12} (Yajnik *et al*., 2006; Refsum *et al*., 2001) and proteins (Rao *et al*., 2009). The reason for this common dietary pattern includes religious and personal beliefs, cultural practices and poverty (Yajnik *et al*., 2006; Refsum *et al*., 2001; Misra *et al*., 2002; Antony, 2003).

Implications for Prevention and Policy

Current recommendations to reduce the burden of diabetes do not address the impact on the next generation. It is vital that measures to combat diabetes focus on the health and nutrition of young women before they become pregnant so that the incidence of diabetes in future generations will be minimized. The only sustainable way of tackling the epidemic is to protect the young and stop the intergenerational transmission of susceptibility to diabetes. The DOHaD hypothesis suggests several potential ways to break this intergenerational transmission in a 'lifecourse' approach. Some possibilities include: improving the health of adolescent girls through nutrition, treatment of infections, optimizing the age of marriage, stress management and others. This is the only reliable way of improving the periconceptional environment for the foetus at a community level. Supplementation of pregnant mothers and improving their health, and optimizing breastfeeding practices will add to the benefits. Observational evidence suggests an undesirable effect of postnatal rapid growth on risk of obesity and NCDs in low birth weight children. This is somewhat contrary to the current practices which aim to 'normalise' postnatal growth of these children. Trial evidence is needed to settle this dilemma. All efforts to reduce childhood obesity will help in the reduction in risk of NCDs.

The current DOHaD evidence in humans is largely limited to observational studies, using birth weight or extreme nutritional situations (e.g. famine) as exposures, with little information on maternal nutrition. Some recent studies have followed up children whose mothers took part in nutritional supplementation trials. Indian adolescents whose mothers were supplemented with energy and protein during pregnancy had lower insulin resistance and arterial stiffness at 12 years of age (Frery *et al*., 1992), which was not seen in the later follow-up (Kinra *et al*., 2008). Nepali children whose mothers were supplemented during pregnancy with multiple micronutrients had lower blood pressure (Lindblad *et al*., 2005). In contrast, supplementation of Gambian women with energy and protein had no effect on the children's body composition or blood pressure (Muthayya *et al*., 2006). However, these trials all started in mid-pregnancy, after the important early-gestation period when epigenetic programming, placentation, and foetal organogenesis occurs. Hence, there is an urgent need for pre-conceptional trials.

Indian DOHaD research has now moved to the next step through two intervention studies in Mumbai and Pune. The trial in Mumbai involved food based micronutrient supplementation of women before and during pregnancy. This resulted in a ~50 g increase in birthweight (Potdar *et al*., 2014) and interestingly in a reduction in the incidence of GDM by half (Sahariah *et al.,* 2016). The children born in the trial are being followed up to study cardiometabolic risk factors in later life. The Pune trial involves preconceptional vitamin B_{12} supplementation with or without other micronutrients and milk powder (to provide extra proteins) to adolescents (CTRI number: CTRI/2012/12/003212, protocol available at: http://www.ctri.nic.in/Clinicaltrials/pmaindet2.php?trialid=5174). The trial offers a unique opportunity to investigate not only the effects of maternal supplementation on offspring health, but to also examine epigenetic changes in them. The results of these trials will have significant implications for the public health policy in India. If successful, they will offer, for the first time, a primordial prevention approach to reduce and halt the epidemic of T2D in India.

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