Pune Experience

Influence of early life environment on risk of non-nommunicable diseases (NCDs) in Indians

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Background

India is one of the capitals of the world for diabetes and coronary artery disease.[1] At the KEM Hospital Diabetes Unit in Pune, India, we have been actively involved in studying the susceptibility of Indians to these disorders. Our early research described the characteristics of Indian type 2 diabetic patients: younger age and lower BMI but higher waist-hip ratio and higher insulin resistance compared with European patients.[2,3,4] This was the beginning of the 'thin-fat' Indian concept which provided an explanation for higher susceptibility of Indians to diabetes compared to Europeans despite a lower BMI.[5]

Over the past 25 years, we have progressed to define life-course evolution of the Indian phenotype through a number of prospective studies and tested the contribution of genetics, epigenetics, nutrition and other environmental factors to this phenotype (**Figure 1**).

Birth weight and rise of non-communicable diseases (NCDs)

In our earlier studies, we studied characteristics of malnutrition-related diabetes mellitus (MRDM) in Indians. David Barker in 1991 persuaded us to test his low birth weight hypothesis in India. After all, India is the world's capital of low birth weight (LBW), contributing 30% to the world's LBW babies every year. Studies in the UK showed an inverse association between birth weight and later risk of NCDs, notably diabetes and cardiovascular disease.[6]

Our first "developmental origin of health and disease (DOHaD)" study (Pune Children's Study) confirmed that low birth weight children were more insulin-resistant at four years of age.[7] At eight years, we found that children born with low birth weight who had become heavier during childhood had the highest level of cardiovascular disease (CVD) risk factors[8] (**Figures 2 and 3**). These findings highlighted that an imbalance between intrauterine and childhood nutrition could be an important contributor to the risk of NCDs.

Maternal nutrition and fetal growth

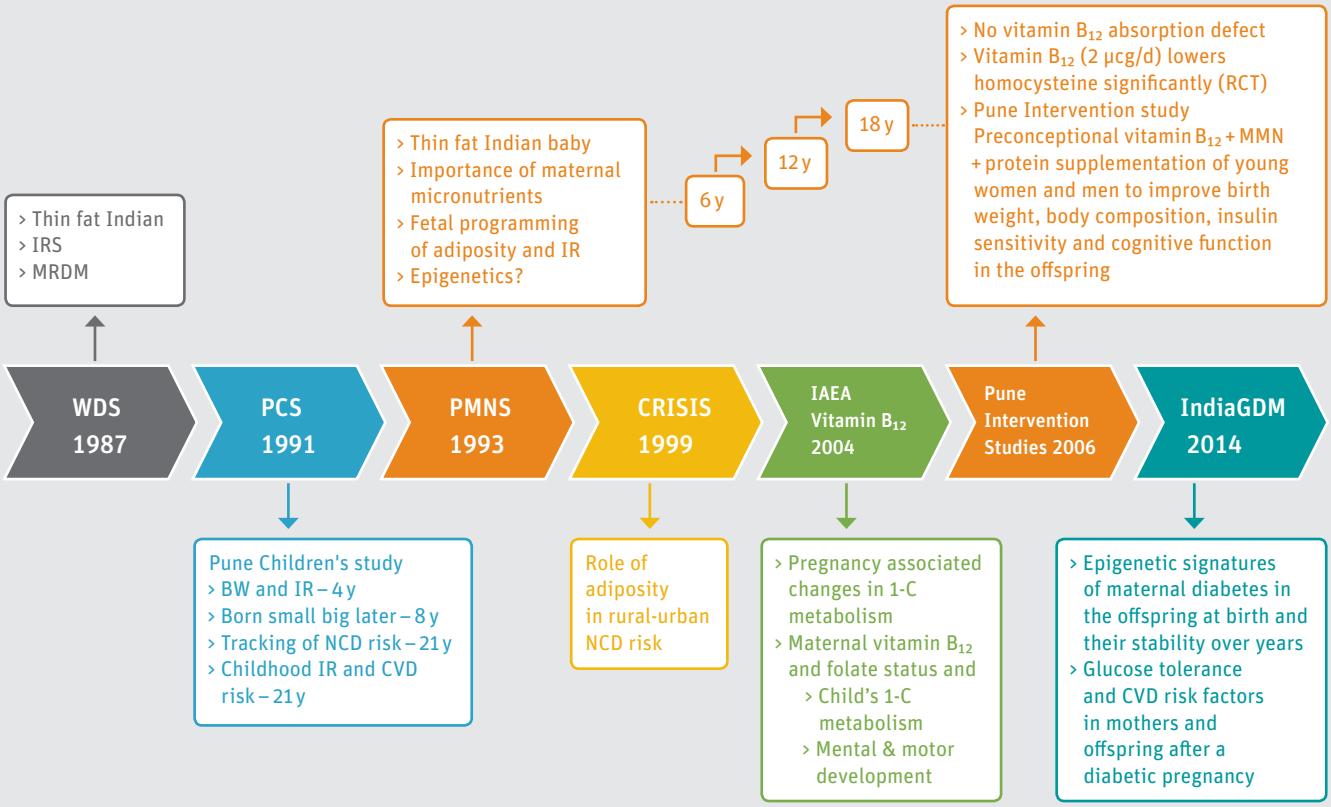
Having established a role for poor fetal growth in diabetes and CVD susceptibility, we investigated the factors contributing to poor intrauterine growth of Indian babies (PMNS, 1993).[9] In more than 800 pregnancies in 6 villages near Pune, we studied mother's nutrition, physical activity, metabolism and fetal growth by ultra-sonography. Babies were measured in detail at birth and were followed up every 6 months for anthropometry and at 6, 12 and 18 years for diabetes, cardiovascular and neurocognitive risk measurements (**Figure 4**).

The mothers were on average 42 kg (BMI 18.1 kg/m^2) at the start of the pregnancy and belonged to a farming community. Babies weighed on average 2.7 kg and were 'thin' by ponderal index (24.1 kg/cm^3) compared to European babies (weight 3.5 kg, ponderal index 28.2 kg/cm^3). Subscapular skin fold thickness of Indian babies was similar and the MRI of abdominal fat (subcutaneous and visceral) higher in Indian babies. Rather than the macronutrients, micronutrients in mother's diet influenced baby's size.[9] Higher frequency of intake of green leafy vegetables, fruit and milk, and higher red cell folate levels were associated with larger birth size. This made us focus on maternal micronutrient status as a determinant of offspring growth and development.

> "Rather than the macronutrients, micronutrients in mother's diet influenced baby's size"

Pregnant PMNS mothers had high prevalence (30%) of hyperhomocysteinemia (> 10 µmol/L), due to vitamin B_{12} rather than folate deficiency. Seventy percent of mothers had vitamin B_{12} insufficiency (< 150 pmol/L) and 31% were severely deficient (< 100 pmol/L). In contrast, fewer than 1% had low erythrocyte folate status (< 283 nmol/L). Ninety percent of these women had high methyl malonic acid (MMA) (> 0.26 µmol/L), which is a specific indicator of vitamin B_{12} deficiency. Higher maternal total homocysteine (tHcy) predicted lower birth weight for gestational age.[10] These findings suggested a role for one-carbon metabolism in fetal growth.

FIGURE 1 | OVERVIEW OF PUNE STUDIES: The figure summarizes studies in the Diabetes Unit, KEM Hospital, Pune over the last 25 years which generated the concept of the 'thin-fat Indian' and intergenerational transmission of the risk of non-communicable diseases by maternal micronutrient nutrition disturbances.



WDS: Wellcome Diabetes Study
PCS: Pune Children's Study
PMNS: Pune Maternal Children Study
CRISIS: Coronary Risk of Insulin Sensitivity in Indian Subjects
IAEA: International Atomic Energy Agency; Observational study for Vitamin B_{12} deficiency in pregnant women
IndiaGDM: Gestational Diabetes Study in Indians under Indo Danish-collaboration
IRS: Insulin Resistance Syndrome
MRDM: Malnutrition Related Diabetes Mellitus
IR: Insulin Resistance

FIGURE 2: Relationship between birth weight and glucose and insulin in 4-year-old children in Pune Children's Study[7]

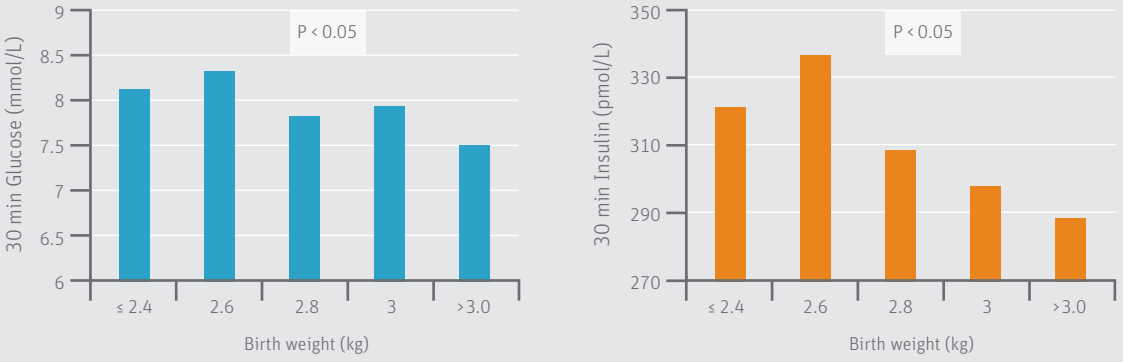
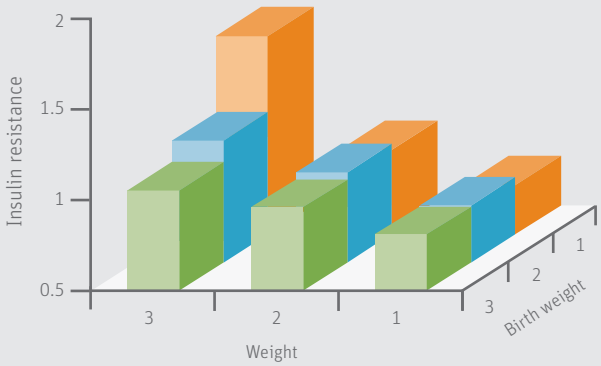


FIGURE 3: Relationship between birth weight (terciles), weight (at 8 years) and insulin resistance in 8-year-old children in Pune Children's Study.[8] Adiposity, glucose, blood pressure and lipids showed a similar association (not shown)



Maternal one-carbon metabolism and offspring health

One-carbon (1-C) metabolism refers to a network of interrelated biochemical pathways that donate and regenerate 1-C units, including the methyl group (**Figure 5**).[11,12,13] Across the life course, the dietary supply of the methyl donors folate, vitamin B_{12}, betaine, methionine and choline is essential for normal growth, development and physiological functions. Maternal diet is the primary source of nutrient availability to the conceptus.[14] Optimal organogenesis, growth and development of the fetus is dependent on the maternal diet and supply of nutrients. **Figure 5** shows possible molecular mechanisms contributing these effects.

Data from the Pune Maternal Nutrition Study highlighted that a relationship exists between maternal methyl donor vitamin nutrition and these also predict neurocognitive function, body composition and insulin resistance during childhood,[15,16] suggesting a critical role of 1-C metabolism in long-term health of the offspring.

> "Data from the Pune Maternal Nutrition Study suggest a critical role of 1-C metabolism in long-term health of the offspring"

Use of genetic markers to establish causality (Mendelian Randomization)

Proving causality from observational research is not easy. Conventionally this is done by interventional research, which is difficult to execute, takes a long time, and is expensive. A recently proposed alternative method is the use of relevant genetic markers which are reliably related to the nutritional exposure of interest. Because the genetic polymorphisms are randomly distributed at conception, they are not confounded by subsequent exposures or lifestyle. Demonstrating a significant association between a genetic marker and the outcome of interest therefore suggests causality.[17] Maternal 5,10-methylene-tetrahydrofolate reductase (MTHFR) genotype (C677T, A1298C) was tested in two Indian birth cohorts (PMNS, n=702 and Mysore Parthenon Study, n=526). Maternal MTHFR T677T predicted high plasma tHcy concentrations in the mother and lower birth weight of the offspring, independent of maternal BMI, socioeconomic status, gestational age and offspring MTHFR genotype. Higher maternal folate concentrations overcame the effect of maternal MTHFR 677TT genotype on birth weight. This suggests that maternal homocysteine status influences birth weight, and that improving maternal vitamin B_{12} and folate status may reduce intrauterine growth retardation (IUGR) and its long-term consequences.[18]

Intervention studies

Micronutrients and growth

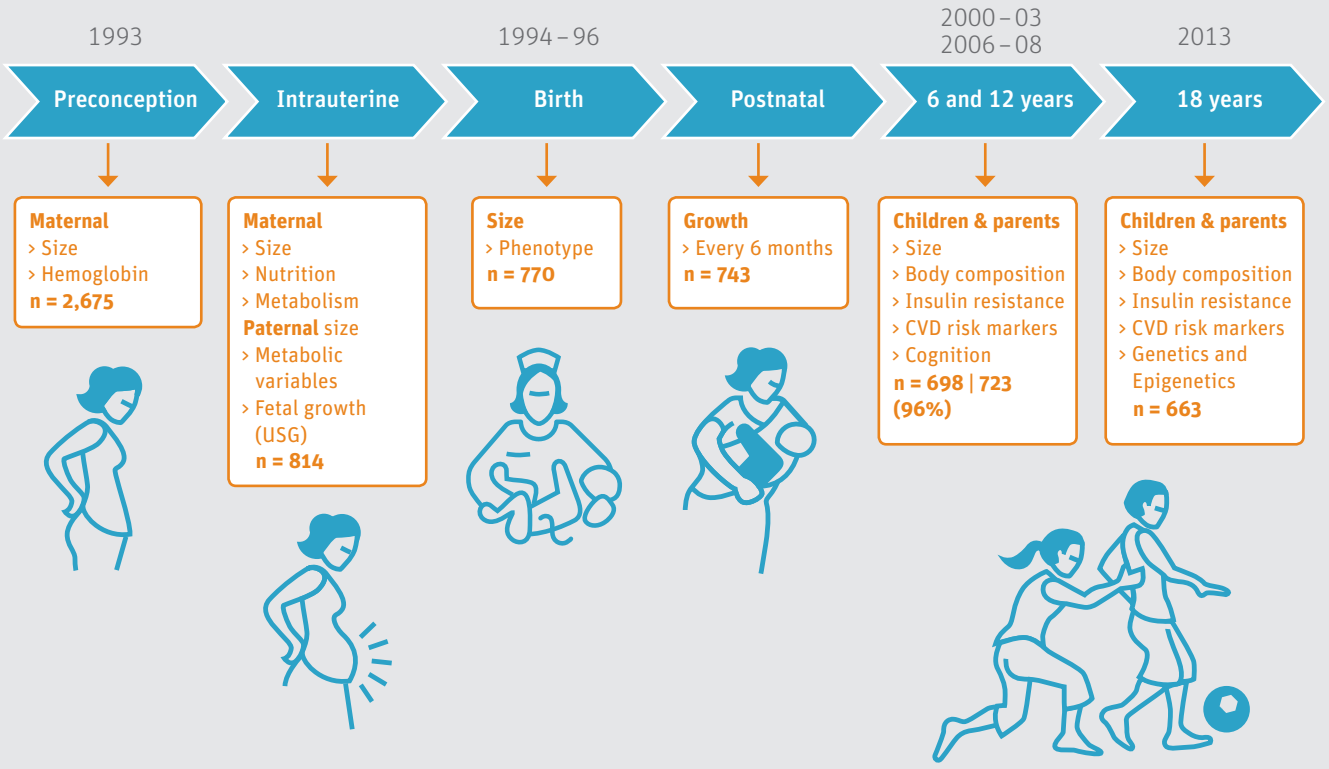
In observational studies and randomized control trials, micronutrients generated inconsistent results. The majority of the trials were in developed countries. A Cochrane review[19] concluded that despite a significant reduction in maternal anemia with micronutrient supplementation, there was only a small and non-significant effect on the incidence of LBW (relative risk [RR] 0.73; 95% CI: 0.47–1.13). However, these trials have to be interpreted in light of the nutritional status of the population: in undernourished populations (India and South Africa), there was a substantial increase in birth weight.[20,21]

Multiple micronutrients in fetal growth and pregnancy complications

Meta-analyses of the effects of antenatal multiple micronutrient supplements (MMS) in 12 randomized controlled trials (RCTs)[22,23] revealed a small but significant increase in birth weight (22.4 g, 95% CI 8.3, 36.4) and an 11% reduction in LBW (CI 3, 19). There were no significant effects on preterm births or prenatal mortality.

A recent Cochrane review[24] shows that MMS during pregnancy significantly decreased the number of LBW infants by 14% and small-for-gestational-age (SGA) by 13%. This review also further indicated that MMS compared with iron and folate supplementation resulted in a significant 11% decrease in the number of LBW and 13% decrease in SGA babies. The impact on pre-term birth, miscarriage, pre-eclampsia, maternal mortality and perinatal mortality were statistically non-significant.

FIGURE 4: Pune Maternal Nutrition Study



Recently married women were enrolled in study before conception. Both parents were characterized during the index pregnancy. Children born to these women are serially followed for growth every 6 months and for body composition, glucose tolerance and CVD risk factors every 6 years

Folic acid and neural tube defects

Prevention of neural tube defects (NTD) by periconceptional folic acid supplementation is considered a major achievement in public health nutrition. This was based on the landmark trials in the UK and Hungary and was supported by Chinese trials. This led to mandatory folic acid fortification of flour in many countries. It is of note that the trials were predominantly in non-vegetarian populations.

We investigated the role of maternal nutritional and genetic markers related to 1-C metabolism in the etiology of NTD in India. Mothers of NTD fetuses had higher plasma tHcy and lower holo-transcobalamin (TC) concentrations but similar folate and vitamin B_{12} concentrations to those in the mothers who delivered normal babies. The commonly associated maternal polymorphism C677T in the MTHFR gene did not predict risk of NTD in the offspring, but C776G polymorphism in transcobalamin II gene (TCN2) was strongly predictive of NTD in the offspring. This study has for the first time demonstrated a possible role for maternal vitamin B_{12} deficiency in the etiology of NTD in India over and above the well-established role of folate deficiency.[25] Policy-makers need to take these facts into account.

Evidences from animal models

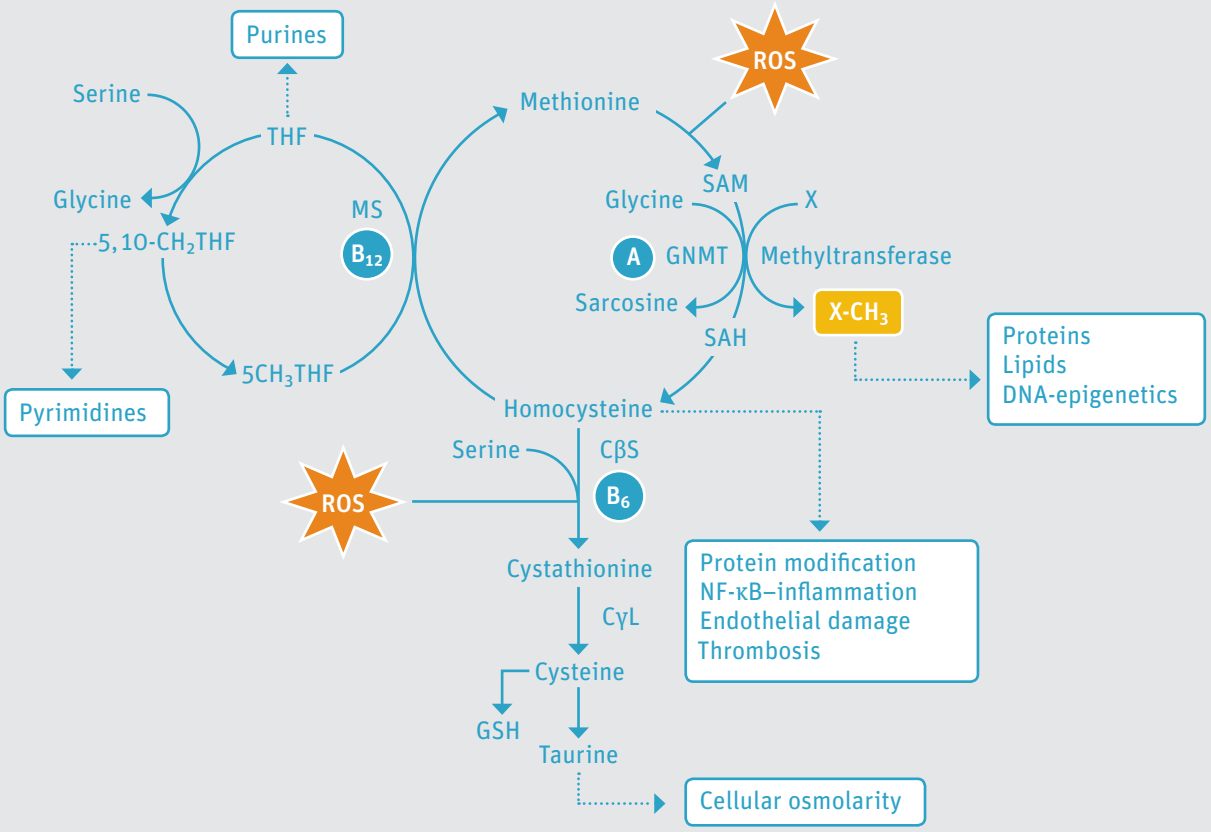
Animal studies have highlighted the role for maternal methyl donor intake and her 1-C metabolism in influencing fetal growth, body composition and programming of risk of blood pressure and diabetes. This has been done in Agouti mice,[26] sheep[27] and Wistar rats.[28] Molecular studies have demonstrated a possible role of DNA methylation in influencing fetal phenotypes.

Intervention studies in India

Pune Intervention Study

We found that vitamin B_{12} deficiency in our population is mainly caused by low dietary intakes, not malabsorption, and can be treated with physiological oral doses (2 µg daily) over 6–12 months.[29,30] We therefore commenced an intervention study as the next logical step to investigate whether improving maternal vitamin B_{12} status improves fetal growth and potentially interrupts the intergenerational transmission of diabetes risk. Previous intervention studies of maternal supplementation started in mid-pregnancy and would have missed the processes that occur around conception and early gestation such as epigenetic programming, placentation, and fetal organogenesis. We decided to start the intervention pre-conceptionally to influence these pro-

FIGURE 5: Vitamin B_{12}, folate and one-carbon metabolism



1-C metabolism is important in many processes involved in fetal growth and development. These include nucleic acid synthesis, DNA methylation and epigenetic regulation, generation of ROS, methylation of proteins and lipids which effect various cellular processes and functions. 1-C metabolism is governed by a number of dietary nutrients including vitamins B_{12}, B_6, B_2, folate.

cesses. Recent evidence suggests that the nutritional status of fathers influences the epigenetic processes in their offspring,[31] and we therefore included boys as well as girls in the trial.

The adolescents are individually randomized to receive a daily supplement for at least 3 years or until their first delivery (whichever is earlier) containing: **1)** vitamin B_{12}, 2 µg; or **2)** vitamin B_{12}, 2 µg + multiple micronutrients (MMN) + 5 g milk protein; or **3)** a placebo. The MMN composition is guided by the UNIMAPP formulation,[32] providing approximately 1 RDA of 15 vitamins and minerals, but with 2 µg vitamin B_{12} instead of 1 µg. Iron and folic acid is prescribed separately for all participants according to Indian guidelines.[33]

The results of this trial will have significant public health implications in a setting with widespread vitamin B_{12} deficiency but relative folate sufficiency. Moreover such "primordial" prevention offers a hope of curtailing the escalating diabetes epidemic in future generations in contrast to current prevention strategies. This trial will test the basic tenet of the DOHaD hypothesis. The RCT design allows us confidence that our findings will be causally relevant. The extended program will create an unparalleled repository of precious biological samples for future "omics" studies.

Mumbai Maternal Nutrition Project

The Mumbai Maternal Nutrition Project[34] was a randomized controlled trial of micronutrient-rich foods before and throughout pregnancy among women living in urban slums based on the studies in Pune. A snack made from green leafy vegetables, fruit and milk was provided each day as an addition to the normal diet. Women in the control group received similar snacks made from vegetables of low micronutrient content.

Six thousand five hundred and thirteen pre-pregnant women were recruited, and 2,067 babies were born. Overall, the intervention increased birth weight by 26 g (not statistically significant, p=0.20) but with a slightly larger effect (48 g, p=0.05) if women started the supplement >3 months before conception. There was a striking interaction with maternal BMI; the supplement had no effect on newborn weight among mothers in the

lowest BMI group (< 18.6 kg/m2, 7 g, p=0.84), but had an effect among women in the middle (18.6–21.8 kg/m2, 79 g, p=0.07) and highest BMI groups (> 21.8 kg/m2, 113 g, p=0.008). The intervention also reduced the prevalence of gestational diabetes (~7% vs 13%, p=0.01). The babies are being followed up for growth and cardiovascular risk factors.

Role of genetics and epigenetics

Fetal growth and development are influenced by an interaction between genetic factors and the intrauterine environment. The size of the newborn is influenced not only by inheritance of genes but also by maternal size, nutrition and metabolism. Hattersley et al[35] showed this through the interaction between the glucokinase gene and maternal hyperglycemia. It is increasingly appreciated that epigenetic changes, which refer to heritable modifications in the genome not associated with a change in the base sequence,[36] are at the center of programming. These changes may be mediated by methylation of DNA, acetylation of histones, and through the role of micro RNAs, all of which modify gene expression. These could potentially result in the production of different phenotypes from the same genotype by altering gene expression and increasing or decreasing the amount of encoded protein.

Our studies will provide relevant information in the near future.

> "The size of the newborn is influenced not only by inheritance of genes but also by maternal size, nutrition and metabolism"

Conclusion

In the past thirty years, we have defined the characteristic phenotype of Indians which explains their high susceptibility to diabetes and related disorders. In addition to genetic factors, we have additionally described a role for intergenerational fetal programming. This is influenced by modifiable environmental factors such as maternal nutrition, which influences the epigenome of the developing fetus. Our finding of the importance of maternal methyl donor nutrition and 1-C metabolism in fetal growth is exciting because of the potential influence it may have on DNA methylation. We are in the process of gathering this data. Our research has highlighted a role for improving the nutrition of young girls so as to curtail the epidemic of NCDs. Early-life interventions may be more cost-effective in preventing the NCD epidemic than controlling the lifestyle factors in later life.

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