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MOTHERS, BABIES AND DISEASES IN LATER LIFE

CAROLINE FALL

Introduction

I would like to thank the Nutrition Society of India for awarding me the Dr C Gopalan oration and giving me an opportunity share with you all, the fascinating story of Developmental Origins of Health and Disease, (DOHaD) and the work that I have carried out with our Indian collaborators. I will start by paying tribute to Professor David Barker who was the father of DOHaD. DOHaD is the concept that the environment in which an individual develops as a fetus (the mother) and young child permanently influences their metabolism, physique and health, and sets their lifelong resilience or vulnerability to chronic non-communicable diseases, which are currently major public health problems. Prof Barker was the first person to propose that the health and nutritional status of mothers could explain the current epidemics of diseases like coronary heart disease and diabetes. He inspired me both as a physician and researcher and gave me the opportunity to work with our Indian collaborators for three decades.

Origin of the DOHaD studies

Prof Barker was initially led to the DOHaD concept by his interest in social inequalities in health in Britain. He created maps which showed that historically poorer regions of the UK suffered from more coronary heart disease (CHD). This was surprising at that time, because CHD was generally thought of as a disease of affluence. His 1987 study of the three towns Nelson, Colne and Burnley in Lancashire[1] was an example of his ability to see patterns in data that others had passed over. These towns are situated closely together on the cold damp slopes of the Pennine Hills, and hardly differ in present day climate, socio-economic characteristics, smoking rates or medical services. But they have very different health profiles, with Burnley and Colne having about 20% higher death rates from CHD (Fig 1). By studying archived records made by medical officers and nurses 70-100 years ago, he pieced together a story of major differences in the past between these towns in the health of mothers and infants, attributable to differences in food security, housing, sanitation, infection rates, and working conditions. His startling conclusion was that "explanations for current geographical differences in CHD mortality in Britain may be found in maternal characteristics and the environmental influences that determined past differences in child development". He hypothesized that optimal maternal health and nutrition, and optimal fetal and childhood growth led to lifelong resilience, while a poor maternal and infant environment could blight cardiovascular and metabolic health for a lifetime.

Hertfordshire birth cohort

Prof Barker expanded the staff of his research unit in Southampton, not as he might do today with molecular biologists and bioinformaticians, but with historians, to scour the country for old pregnancy and birth records. They discovered among others the Hertfordshire birth records, and using the National Health Service computer system, he went on to show that lower birth weight and weight at the age of one year, markers of a poorer fetal and infant environment, were associated with greater adult mortality from CHD, chronic lung and kidney disease, and poor mental health[2,3]. This supported his idea that a poor environment and nutrition in early life was a cause of later cardiovascular and other chronic diseases, and launched the science of DOHaD.

I joined David's group as a research fellow in the same year that the first Hertfordshire results were published, and was sent to run the Hertfordshire cohort study, tasked with tracking down people in their 60's and 70's whose birth records were now computerized in Southampton.

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Fig 1 Inequalities in health in Britain; the story of Nelson, Colne and Burnley

In 1900, the towns differed.

The main work for men and women in all three towns was in cotton mills.

In Nelson:
Women were recent migrants from nutritionally rich rural agricultural villages and were 'healthier and sturdier'. They breastfed infants for longer.

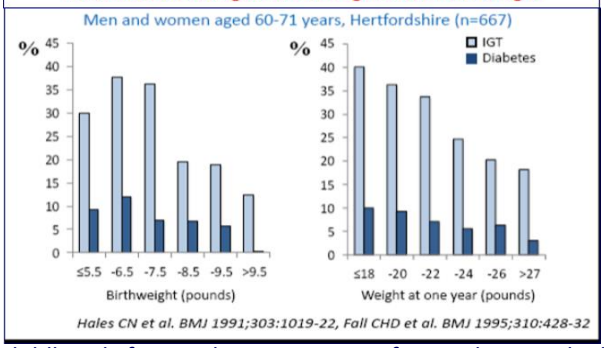
Housing was newer, less damp and more spacious.

Waste disposal pits were covered and disinfected regularly.

		NELSON	COLNE	BURNLEY
Infant mortality/1000	1896	154	170	197
	1911	87	130	177
Living in slum housing (%)	1911	0.6	17.0	9.9

Barker DJP and Osmond C. BMJ 1987;294:749-52

Fig 2 Prevalence (%) of impaired glucose tolerance (IGT) and type 2 diabetes according to birth weight and infant weight



Hales CN et al. BMJ 1991;303:1019-22, Fall CHD et al. BMJ 1995;310:428-32

My contribution was to show that men and women who had a lower birth weight and weight at age one year were not only more likely to die of heart disease, but were at increased risk of type 2 diabetes (Fig 2). The data also showed that the highest risk was in men and women who were the lightest at birth or one year but had gone on to become the heaviest adults[4,5]. There was a statistical interaction between birth weight and adult weight; the adverse effect of a high adult BMI on diabetes risk was stronger in those who had been smaller newborns and considerably less strong in those with normal birth weight (Fig 3). This is such an old and consistent finding that you could be forgiven for missing the powerful message, that the impact of a lifestyle-induced risk factor in adult life depends on what has gone before. In this case the consequences of the metabolic stress of adult obesity (risk of diabetes) varied according to intra-uterine and/or infant nutrition. Other studies have shown that the same applies to other adult risk factors such as smoking, poor diet and lack of exercise[6]; these are stronger risk factors for diabetes in people of lower birth weight.

Helsinki birth Cohort

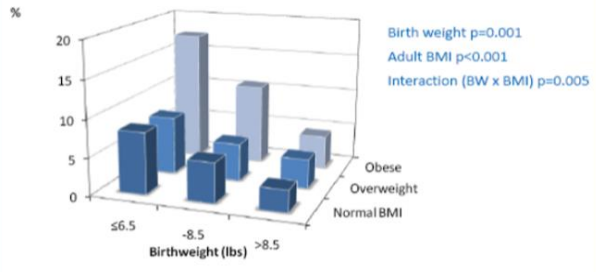
In a collaboration with Johan Eriksson in Finland, we found similar results in the Helsinki Birth Cohort[7,8], but the detailed childhood records for this cohort were able to fill in data for the intervening years between birth and adulthood. The men and women who developed CHD or type 2 diabetes were small at birth, thin as infants, and heavy as adults. Their weight gain started in mid-childhood; from a lower BMI in infancy, they gradually overtook the rest of the cohort at about the age of 10 years, becoming considerably heavier as adults. In terms of later chronic disease risk, weight gain during infancy (the first one or two post-natal years) was neutral in terms of later chronic disease risk, while weight gain after that and at any time during adolescence or adult life added to risk.

As people became fascinated by these findings and tried to replicate them, the association of low birth weight with later cardiac disease and its risk factors like diabetes proved to be highly consistent[9]. However, there was a 'twist in the tale' because, in addition, there emerged a clear adverse effect of very *high* birth weight (Fig 4). This was related to maternal diabetes in pregnancy which causes macrosomia (high birth weight) and is now also known to put the offspring at greater risk of developing later type 2 diabetes. Among the Pima Indians, a native American population with a high prevalence of gestational diabetes, the association between birth weight and later type 2 diabetes is U-shaped[10]. Similarly, in the US Nurses' Health Study, there was a kick upwards in adult type 2 diabetes risk at high birth weights, attributable to gestational diabetes[11].

The Barker Hypothesis

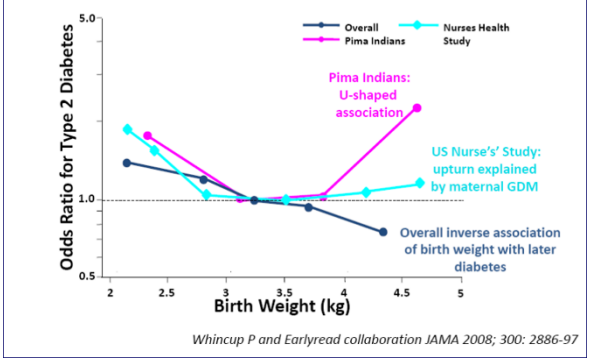
The Barker hypothesis, as it became known, proposed that fetal life and early post-natal development were critical periods in which any environmental factor that disrupted the ordered and orchestrated process of development

Fig 3 Prevalence (%) of IGT or type 2 diabetes in relation to birth weight and adult BMI Hertfordshire (n=667)



Hales CN et al. BMJ 1991;303:1019-22, Fall CHD et al. BMJ 1995;310:428-32

Fig 4 Risk of type 2 diabetes in relation to birth weight



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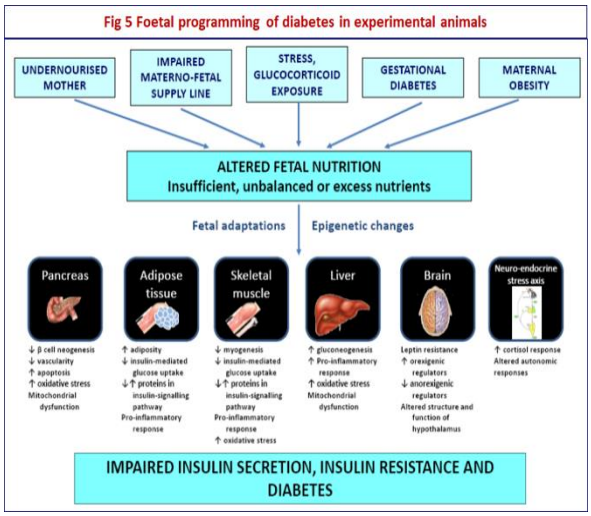
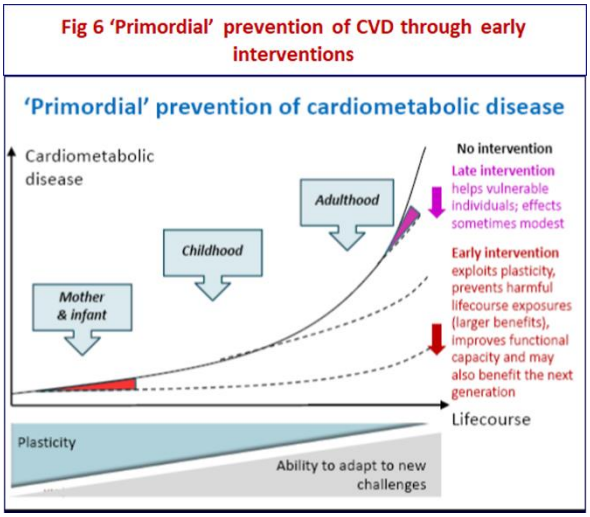




could cause permanent damage to the structure and function of tissues and systems (Prof Barker described this as 'programming') leading to later disease. It would especially damage tissues and systems that lose their flexibility (or plasticity) for cell division or repair beyond the early developmental period, such as the insulin-producing pancreatic beta cells or renal nephrons. The original hypothesis was based mainly on the low birth weight associations and therefore focused on fetal undernutrition, arising either because of maternal undernutrition or failure of the fetal supply line/placenta. This was supported by studies in animal models showing that nutritional deficits in pregnant animals produced hypertension and diabetes in the adult offspring. As evidence accumulated, it became clear that it was not only fetal undernutrition that could cause permanent developmental changes. In the case of type 2 diabetes, animal studies have shown that a wide range of environmental factors, including fetal exposure to maternal obesity, diabetes and high-fat feeding, and to maternal stress and glucocorticoids can re-programme the offspring and increase the risk of later diabetes, acting through increased insulin resistance or reduced insulin secretion, or both (Fig 5)[12]. To that list I would now add maternal inflammation and exposure to environmental toxins, pollutants and hormone disruptors[12].

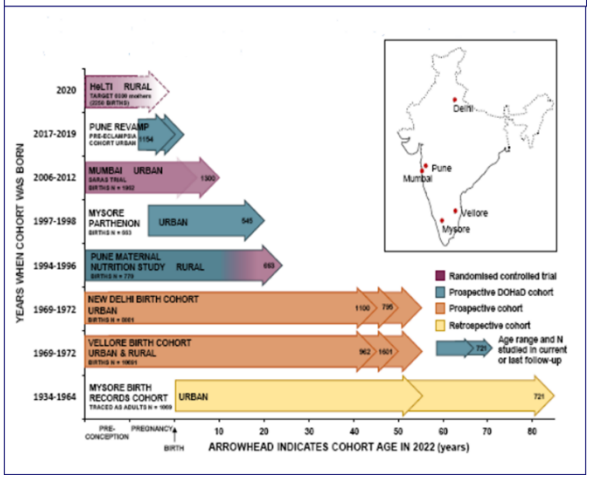
In some ways this appears a fatalistic view of disease; the idea that things are fixed in early life could play down the importance of healthy lifestyle behaviors in later life. This would be a wrong interpretation of the data, which clearly show that adult lifestyle is extremely important for health, just especially so in those who had a poor start in life. Looked at from another perspective the DOHaD concept opens up the tantalizing prospect that it may be possible to prevent disease, long before it starts ('primordial' prevention) by understanding fetal and early life development, promoting the conditions that favour healthy early development and thus promoting more resilient babies that grow into healthier adults. Currently, diseases like CHD and type 2 diabetes are mainly diagnosed and treated when they present in adult life, with established secondary effects and tissue damage. This of course benefits those individuals. However, by improving early development more holistic benefits could be achieved, improving physical and cognitive capital and even the health of future generations, as well as preventing disease (Fig 6).

Cohort studies in India

Early in the evolution of DOHaD research, Prof Barker wanted to explore these ideas beyond Britain and Europe. We knew that CHD and type 2 diabetes were rising rapidly in low- and middle-income countries that were undergoing rapid economic transition, with concomitant urbanization and rising obesity. Cardiovascular disease (CVD) mortality in India increased by 34% from 1990 to 2016[13]. The age-standardized CVD death rate in India was 282 per 100,000 population in 2017, accounting for 24% of total deaths; the mortality rates in India are higher than the global average (233 per 100,000) and that for high-income countries like the USA (151 per 100,000) and UK (122 per 100,000)[14]. The prevalence of type 2 diabetes in India is not the highest in the world, but is well above that in the UK and USA, despite a higher prevalence of obesity in UK and USA. The International Diabetes Federation (IDF) estimated that around 74 million adults in India have diabetes (mainly type 2)[15]; the IDF has projected an increase of 70% over the next 20 years. The figures for gestational diabetes are perhaps even more worrying; the current prevalence is around 20% of all pregnancies, and this is likely to further ratchet up diabetes in the next generation.

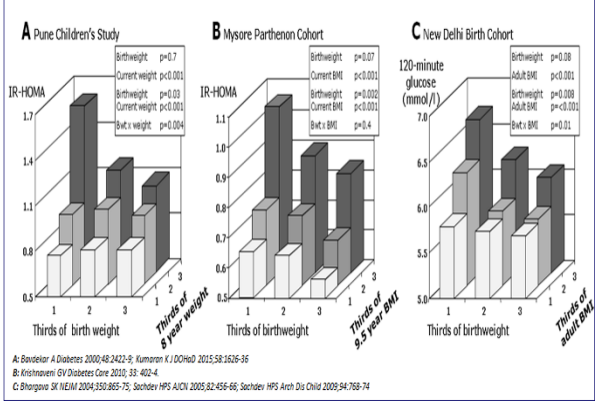
Prof Barker chose me to start building partnerships with Indian researchers; my relationship with India began in 1991 and DOHaD studies in India have enriched me and my research for the last 30 years. The objective of our research studies in India was to look for evidence of adverse consequences of problems in early life on adult chronic disease, especially diabetes, and to find ways of

Fig 7 Indian cohort studies on early life origins of cardiometabolic disease



mitigating them. These partnerships, which started in Pune and Mysore, had expanded over the years into the "Sneha" network of cohort studies and trials (Fig 7) from which I will take data to illustrate particular points during this lecture, using diabetes as my main outcome. The oldest cohort, whose participants are now in their 80's, is the Mysore Birth Records cohort, based on tracing adults from their obstetric records at the Holdsworth Memorial Hospital; apart from maternal and newborn size there was very little early life data available in this cohort. The New Delhi and Vellore birth cohorts were set up 1960's, long before DOHaD was thought of, for an entirely different reason, to investigate infant mortality and childhood growth. These cohorts have serial data on childhood weight and height in addition to birth size and the participants are now in their 50's, the age when heart disease and diabetes become manifest. The Pune Maternal Nutrition Study and Mysore Parthenon Study began later; informed by the DOHaD hypothesis, these studies collected detailed data on the pregnant mothers and their children. The REVAMP cohort in Pune is specifically studying the impact of pre-eclampsia on the fetus. Recently, intervention trials aiming to improve maternal health and nutrition and track any effects on later health have been initiated; these studies are either ongoing or the children are very young.

Fig 8 interaction between birth weight, later BMI and risk of diabetes: multiple cohorts



A: Bavdekar A Diabetes 2000;48:2422-9; Kumaran K J DOHaD 2015;58:1626-36
B: Krishnaveni GV Diabetes Care 2010; 33: 402-4.
C: Bhargava SK NEJM 2004;350:865-75; Sachdev HPS AJCN 2005;82:456-66; Sachdev HPS Arch Dis Child 2009;94:768-74

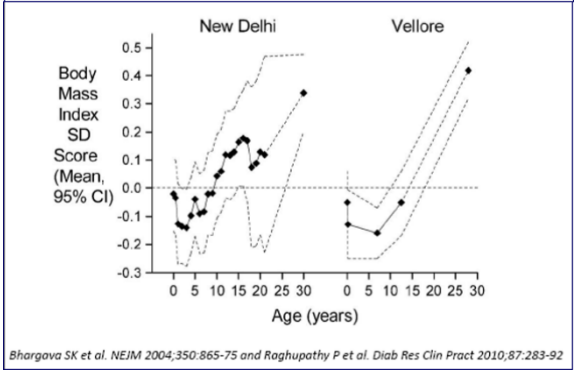
Low birth weight

Despite considerable improvements in the nutritional status of young women, India has a high prevalence of maternal underweight, nutritional deficiencies and low birth weight, mainly due to smallness for gestational age. One of our first collaborations was with Anand Pandit and Chittaranjan Yajnik at the KEM Hospital in Pune, on a study of healthy children born in the hospital, whose birth weight was recorded (Pune Children's Study)[16]. Risk factors for later cardiovascular disease and type 2 diabetes, including high blood pressure, plasma triglyceride and cholesterol levels, and insulin resistance were higher in children of lower birth weight and higher current weight ("born small, becoming big"). None of these children were overweight, in fact they were thinner than average European children; but for the small newborn, the transition to becoming relatively heavy *within each cohort* was associated with higher risk factors for non-communicable diseases. This was consistent with the UK findings but was documented in children for the first time. This finding was important because it showed that it may be possible to detect fetal programming at a young age. This cohort has now been followed up for 21 years and we know that insulin resistance tracks from childhood[17] and that the diabetes risk remains highest in those who were the smallest babies and became the heaviest adults[18]. As we studied the different Indian cohorts, the same pattern seen in the Pune children emerged everywhere (Fig 8); alongside insulin resistance among children in the Mysore Parthenon Cohort, and glucose concentrations in the New Delhi Birth Cohort. The message is that for people born with a low birth weight, gaining excess body weight in later life, as children, adolescents or adults, is a strong risk factor for type 2 diabetes.

Childhood growth

The New Delhi and Vellore birth cohorts enabled us to gain insights into child growth and later diabetes. These cohorts were set up in 1969, by Drs Shanti Ghosh and Dr Santosh Bhargava in New Delhi[19] and Drs Sundar Rao, Inbaraj and Richards in Vellore, supported by the Indian Council of Medical Research[20]. The investigators collected

Fig 9 Childhood BMI gain and adult diabetes: New Delhi and Vellore cohorts



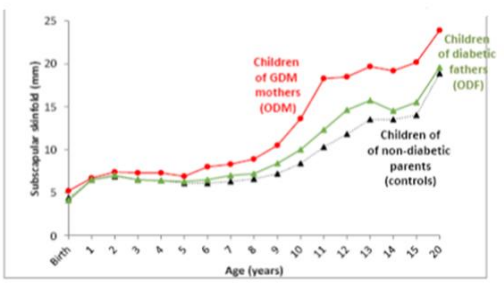
Bhargava SK et al. NEJM 2004;350:865-75 and Raghupathy P et al. Diab Res Clin Pract 2010;87:283-92

serial height and weight data on thousands of children from birth to the late teens or early twenties in delineated areas of South Delhi and urban and rural Vellore. For us as DOHaD researchers, these cohorts were precious and rare resources and it was wonderful working in the 1990's with some of the original investigators to re-trace the cohorts in mid-life for a DOHaD study of diabetes and cardiovascular disease. Fig 9 shows our data for body mass index (BMI) from birth onwards in relation to diabetes at age ~30 years. The solid lines show the BMI of children who developed pre-diabetes or type 2 diabetes as adults relative to the rest of the cohort, which is set at zero throughout the graph. In both cohorts, the men and women who developed type 2 diabetes were lighter and thinner at birth and during infancy compared to the rest of the cohort, but from around the age of 3-5 years started to increase in BMI, crossing the average for the rest of the cohort at 10-15 years, and continuing upwards until as adults they were of considerably higher BMI than the others. One message is that we need to take childhood obesity and its inexorable rise very seriously. The other important message, which may not be immediately obvious, is that none of these Indian children were heavy enough back then to be defined as obese; the ones at risk started life thinner than everyone else, and were thinner on average than the rest of their cohort throughout early childhood, *but were moving upwards and, crossing BMI centiles*. Picking up such children and finding a way to limit their BMI gain is, I believe, an important step in preventing diabetes later. The point I want to reiterate is that picking up obese children, though important, is not adequate because this approach would miss the majority of at-risk children in India. The focus should be on detecting children 'starting thin and becoming obese relative to themselves'. Many years ago, we published growth charts based on the New Delhi cohort data with Drs Sachdev and Osmond so that it was possible to detect Indian children growing like this[21], but it is one of our least-cited papers. The reason being, I think, that everyone shies away from either monitoring or tackling weight gain and overweight in children – it is an unpopular, even 'toxic', subject among children and parents the world over.

Gestational diabetes

Birth cohort studies in the USA had shown that *high* birth weight is associated with an increased risk of type 2 diabetes in later life, if that high birth weight was because of maternal gestational diabetes. The short-term effects of gestational diabetes (GDM) on the baby, including the macrosomia (elevated birth weight), caused by excessive glucose and other fuels passing across the placenta, leading to increased fetal insulin secretion and adiposity (so-called 'fuel-mediated teratogenesis') have been known for a long time. The concomitant short-term effects for the baby include a risk of traumatic delivery and neonatal hypoglycaemia. It was shown in the 1980's that Pima Indian children of GDM mothers developed more

Fig 10 Subscapular skinfold thickness in children of GDM mothers: Mysore Parthenon Cohort



Krishnaveni GV Diabetes Care 2005;28:2919-25, Diabetes Care 2010;33:402-4, Kumaran K et al. Global Trends in Diabetes, 2020

obesity as children and more diabetes as young adults[22]. The risk was higher than among older siblings born before the mother developed diabetes[23], pinpointing this as a direct result of fetal exposure to a diabetic intra-uterine environment, not solely to a genetic pre-disposition. The serious implications of this were perhaps under-estimated because in most western populations, GDM affecting only 1-2% of pregnant women, was not much of a problem at that time.

India was one of the first countries to set up a cohort specifically to study the long-term impact of GDM on the fetus: the Mysore Parthenon Cohort, led by Dr GV Krishnaveni has recently completed its 21st -year follow-up with over 90% of the cohort intact[24]. It was created by recruiting women in early/mid-pregnancy attending the antenatal clinic at the Holdsworth Memorial Hospital in Mysore. They had an oral glucose tolerance test at 28-32 weeks gestation, the children were measured at birth, and then followed up with annual anthropometry and detailed measurements of glucose-insulin metabolism at 5, 9, 13 and 21 years. The data confirmed that children of mothers who developed GDM were more adipose and hyperinsulinaemic than controls from birth and throughout childhood (Fig 10)[25]. At 21 years 50% were obese compared with 18% of controls. Only two members of the cohort had frank diabetes at age 21 years (both children of GDM mothers) but the glucose and insulin profiles of the children of GDM mothers put them clearly on a pathway to developing earlier type 2 diabetes. This has now been replicated on a grand scale in the huge multi-centre HAPO study[26-28]. Dr Krishnaveni has carried out detailed studies of stress responses in the cohort, and has shown that offspring of GDM mothers have greater cardiovascular responses, including cardiac output, to an experimental stressful stimulus[29]; the significance of this finding for later cardiovascular health is not yet known.

Not enough is currently being done to prevent gestational diabetes. The adverse effects of intra-uterine exposure to maternal diabetes are the most clearcut of any of our research findings and one of the strongest indications for public health messages and clinical action. When Dr Krishnaveni started the Parthenon cohort, the prevalence of GDM in that hospital in Mysore was 6%. Now it is 15%,

and all India figure is 20%. In a recent study in Bangalore by one of our PhD students, the GDM prevalence was 22%[30]. With GDM as common as that, the knock-on effects down the line, as their children develop obesity and diabetes, and perpetuate the problem into the next generation, are serious.

Trial data shows that by good control of maternal glucose concentrations during pregnancy, the short-term fetal adverse consequences of GDM, namely macrosomia, operative delivery and newborn hypoglycaemia can be reduced. However, these studies showed no reduction in the children's adiposity[31], suggesting they are still at risk of later diabetes. Data from the Pune Maternal Nutrition Study cohort, which now had crossed two generations, have shown that maternal hyperglycaemia during pregnancy is a risk factor for pre-diabetes in the young adult offspring[32]. In this rural, and generally non-obese, indeed thin, rural cohort, 30% of the young adults had pre-diabetes. Young women, who became pregnant and developed GDM had higher circulating glucose concentrations not only before pregnancy but even as children. This means that their developing ova were exposed to excess glucose, and when they became pregnant their fertilized ova and embryos were exposed to high glucose. Currently it has become clinical practice to diagnose and treat gestational diabetes early in pregnancy, but dealing with hyperglycaemia after the pregnancy is diagnosed is probably too late to prevent the inter-generational effects and the increased risk of diabetes in children. This is one of the strongest arguments for developing pre-conceptional care, for helping women get into the best metabolic shape possible before pregnancy, and preventing GDM as much as possible. But this ideal practice seems to be a long way off in most parts of the world.

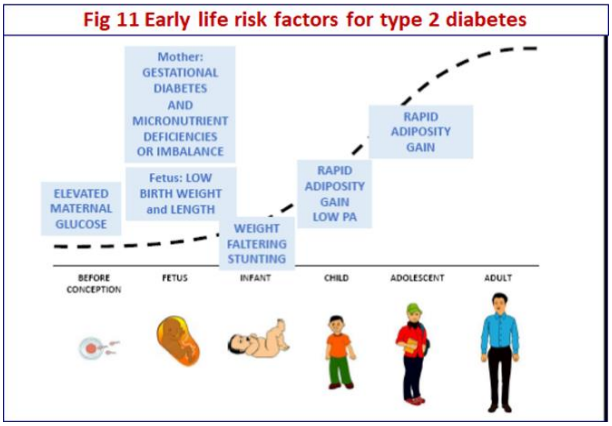
Maternal and fetal under-nutrition

So far, I have not said much about the causes of low birth weight, or about maternal nutrition and diet. The Pune Maternal Nutrition Study or PMNS, the cohort that Dr Yajnik, Dr Shobha Rao and I designed 25 years ago was aimed at studying nutritional determinants of birth size in an under-nourished rural population in Maharashtra. Married, non-pregnant women were recruited from 6 villages and followed up as they became pregnant[33]. During pregnancy, their diet was assessed using a food frequency questionnaire (FFQ) and 24-hour recall, and iron, folate and vitamin B12 levels in blood were measured. Gestational age was assessed using a combination of last menstrual period dates and ultrasound measurements of the fetus. They had glucose and insulin measurements twice during pregnancy. It was and remains a ground-breaking study. The mothers, fathers and children were followed and the children are now in their twenties. Over 80% of the cohort are still being followed-up, which is a remarkable achievement.

The mothers as a group were predominantly under-nourished at recruitment, with a mean BMI of 18.1 kg/m^2 (over 50% were underweight). Many were short (mean height 151.9cm). Mean birth weight was 2.7 kg, and 28% of newborns had low birth weight. Maternal height and BMI were strong determinants of birth weight[34]. Women with a higher workload had smaller babies, especially those doing strenuous farming work[35]. Among the elements of maternal diet associated with higher birth weight was the frequency with which women ate green leafy vegetables, fruit and milk/dairy products[33]. There were no associations between birthweight and calorie or protein intakes or with meat intake. Meat and energy intakes were almost universally low in this population, and this could conceal the impact if any of these. In this cohort, 65% of women had low B12 levels (<150pM), while folate deficiency was rare; higher vitamin B12 and folate status both predicted bigger birth measurements. Maternal food intakes in pregnancy, did not relate to diabetes outcomes (glucose and insulin) in childhood but a combination of lower maternal B12 status and higher folate status in pregnancy was associated with higher insulin resistance in the children[36]. The first adult follow-up of the Pune Maternal Nutrition Study children has shown that low maternal BMI, short birth length, elevated glucose concentrations in childhood and high maternal glucose concentrations in pregnancy, all predicted adult glucose intolerance[32].

In summary, the PMNS showed that the mother's childhood growth (reflected in her adult height), her current nutritional status (reflected in her BMI, diet quality and micronutrient status) and her physical workload are related to the growth of the fetus. Data from follow-up in childhood showed that lower maternal B12 status and higher folate concentrations in pregnancy were related to insulin resistance in the child. This was a first indication that nutrients which play a role in the 1-carbon cycle may be important for cardiometabolic programming. These nutrients play a role in DNA methylation and thus in epigenetic marks, one of the mechanisms proposed to link fetal nutrition with later metabolism and health. In adult follow-up, the study showed that lower maternal BMI, higher maternal glucose

Fig 11 Early life risk factors for type 2 diabetes



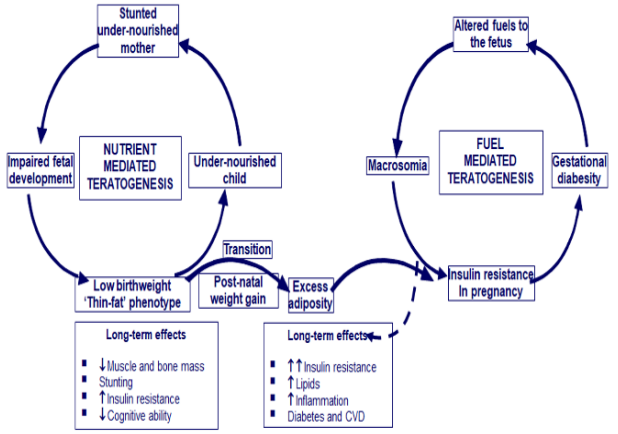
concentrations before and during pregnancy, smaller newborn size, and greater adult adiposity are all early life risk factors for type 2 diabetes.

Bringing together the findings from cohort studies

Bringing together the results from the Indian cohort studies with other DOHaD studies around the world gives us a picture of multiple early life factors associated with an increased risk of type 2 diabetes (Fig 11). The factors include maternal undernutrition (perhaps best illustrated by famine studies like the Dutch Hunger Winter Study); maternal micronutrient deficiencies such as vitamin B12 deficiency, maternal gestational diabetes, small size at birth, growth faltering in infancy, and adiposity gain in childhood, adolescence, and adult life. The Indian studies have given insight into how transitions within a population with a recent and inter-generational history of under-nutrition could result in a high prevalence of type 2 diabetes (Fig 12)[37]. The left-hand circle represents the situation in under-nourished communities in India, in which low birth weight and infant stunting remain common; this situation is associated with impaired neuro-development and reduced adult human capital. These children are programmed for higher risk of cardiovascular disease and type 2 diabetes because their metabolic tissues have also been hit by under-nutrition, but in the absence of catch-up weight gain in childhood they do not develop much cardiometabolic disease. The cycle can continue, but transition to some degree is happening everywhere, and enables better childhood nutrition, and an increase in adiposity. On a background of fetal programming, this relative over-nutrition leads to increased insulin resistance, hypertension, other cardio-metabolic risk markers, and the emergence of type 2 diabetes (left to right arrows between the two circles). In India it appears to take very little transition, and only a subtle increase in childhood adiposity to produce these metabolic changes. This in turn leads to problems in the next generation (right-hand circle). Among women, the combination of early life undernutrition and later adiposity increases the risk of gestational diabetes, which exposes the fetus to excess glucose and other fuels, leading to fetal hyperinsulinaemia, macrosomia, and increased insulin resistance and yet more diabetes in later life (dashed line).

The data that I have shown so far are mainly observational and associations rather than direct proof of causation. Intervention studies, ideally randomized controlled trials (RCTs), are needed, to determine causation and quantify the potential of the DOHaD concept to improve lifelong health. Interventions could be used to try and break these cycles at various points, for example by improving maternal nutrition, fetal development, and infant nutrition (to avoid adverse metabolic programming and reduce vulnerability to later NCDs) or by preventing childhood BMI gain (to avoid placing excessive strain on individuals programmed to have impaired metabolic

Fig 12 Nutritional transitions causing inter-generational disease



capacity). The former approach seemed the most logical strategy, to target the root cause, and nutritional trials in pregnancy have been our recent focus. I will describe three trials in India that I have contributed to.

Intervention trials targeting maternal under-nutrition

Most previous nutritional trials in pregnancy have tended to start in the mid- or late first trimester, thus missing the chance to influence fetal organogenesis (and thereby the opportunity to enhance the development of key metabolic tissues), placental development (and thereby the opportunity to enhance fetal nutrition throughout pregnancy), and peri-conceptional epigenetic changes, which are thought to play a mediating role in fetal programing[38,39]. From a DOHaD perspective, pre-conceptional interventions, though more challenging, seem the way forward.

Mumbai Maternal Nutrition Project or Project SARAS

Our first such RCT was the SARAS trial, which took place in 2006-2012 and was a non-blinded individually randomized controlled trial among women living in slums in Mumbai, in collaboration with Dr Ramesh Potdar[40]. The intervention was a daily snack (samosas, fritters, patties) made from locally sourced micronutrient-rich foods (green leafy vegetables, fruit and milk) and provided from before conception until delivery in addition to the women's normal diet. This intervention was based on the Pune Maternal Nutrition Study results, and aimed to test a potentially affordable and sustainable dietary change. The snacks were made with a variety of recipes and different green vegetables and fruits, aiming to improve women's diet quality and diversity. They contained on average 10-23% of the WHO Recommended Nutrient Intake (RNI) of β-carotene, vitamins B2 and B12, folate, calcium and iron, 0.7 MJ energy, and 6 g protein. Control snacks contained lower micronutrient vegetables (0-7% RNI for the micronutrients, 0.4 MJ energy and 2 g protein). Over 6,000 non-pregnant women were recruited, of whom around 2,000 became pregnant. The intervention increased maternal plasma β-carotene[41] and erythrocyte

ω-3 fatty acid concentrations[42]. It increased mean birth weight, with most of this effect seen among women of normal or high BMI[41]; there was no increase in birth weight among the thinnest mothers. The intervention also reduced the prevalence of GDM (7.3% v 12.4%; OR 0.56 [95%CI 0.36, 0.86], p=0.008)[43].

We recently completed measurements of body composition (DXA), cardiometabolic risk factors and cognitive function in the SARAS children at the age of 5-8 years (the SARAS KIDS study)[44]. Disappointingly, there were no or only minimal differences in outcomes between the control and intervention groups (Fig 13)[44]. While LDL-cholesterol and pulse rate were lower among children in the intervention group (changes in the hypothesized direction) these differences were small and no more in number than expected by chance. There was one potentially important difference: in sub-group analyses: we found greater adiposity among girls in the intervention group than among controls (approximately 7% higher fat percentage) and this was consistent across all adiposity measures[44]. We do not know the implications of this yet, namely whether the slightly increased adiposity is a healthy or unhealthy change in this under-nourished population. We looked for epigenetic impact of the maternal intervention in the children, by studying genome-wide DNA methylation in blood samples from the children, and again there was no evidence of differential methylation resulting from the intervention, though we did find evidence of epigenetic changes in children from another pre-conceptional intervention (multiple micronutrient supplements) in the Gambia[45].

In summary the SARAS intervention did not show evidence of a beneficial effect on cardio-metabolic risk markers in the children. Because of the reduction in GDM, and the increase in adiposity in girls, it is important that follow-up continues in these children. However, our conclusion, for now, is that the SARAS intervention was not enough, either because it did not provide enough additional nutrition for these women, or because additional nutrition alone is not sufficient to improve fetal development and programming in a setting where women experience many other challenges in addition to poor nutrition.

Fig 13 Anthropometry, body composition & cardio-metabolic risk markers: SARAS trial, Mumbai

Outcome	Control group N=657	Intervention group N=619	p
Height (cm)	**110.5** (4.9)	**110.4** (5.1)	0.8
Body mass index (kg/m²)	**13.3** (12.6, 14.0)	**13.3** (12.7, 14.1)	0.4
Lean body mass (kg)†	**13.0** (1.5)	**13.0** (1.5)	1.0
Body fat percentage (%)	**14.8** (12.0, 18.0)	**15.0** (12.3, 18.2)	0.4
Systolic BP (mm Hg)	**92.9** (8.4)	**92.8** (8.9)	0.7
Pulse Rate (bpm)	**97.7** (11.5)	**96.3** (11.3)	0.03
LDL-cholesterol (mmol/l)	**2.4** (0.7)	**2.3** (0.6)	0.05
Fasting glucose (mmol/l)	**4.68** (0.52)	**4.67** (0.58)	0.5
120-min glucose (mmol/l)	**4.66** (0.88)	**4.65** (1.04)	0.9
HOMA-S	**223** (142, 387)	**223** (137, 376)	0.6
Insulogenic index	**1.63** (1.11)	**1.50** (1.16)	0.06
Composite cognitive score (SD)	**3.7** (0.9)	**3.6** (0.9)	0.07
Bone mineral density (g/cm²)	**0.58** (0.04)	**0.58** (0.04)	0.4

All adjusted for child's age and sex

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Vitamin B12 trial ("PRIYA")

The intervention in Pune vitamin B12 trial also started pre-conceptionally, but it aimed to test the effect of providing a single nutrient. The data from the earlier study provided evidence that maternal vitamin B12 deficiency may be important in the fetal programming of type two diabetes in India. Maternal B12 deficiency was common in all the cohorts we looked at (70% among Pune Maternal Nutrition Study mothers [PMNS], 40% among Mysore Parthenon Study mothers [MPS]) while folate deficiency was rare (1-3%). In the PMNS, lower maternal B12 status in pregnancy was associated with lower birth weight and increased insulin resistance in the children[36,46]. It was also associated with a higher risk of the mother developing GDM[47], a finding that has been replicated in mothers in the UK, with a stronger association among those of South Asian ethnicity[48].

After much discussion, because it was a big step to interrupt a prospective cohort study with an intervention trial, Dr Yajnik in Pune carried out a double-blind RCT of B12 supplementation in the young men and women (N=690) from the PMNS cohort, with the aim of not only improving their own health but also that of the next generation when they themselves became parents[49]. In pilot work for the trial Dr Yajnik showed that low B12 levels in this population were due to low intakes and not malabsorption; B12 status could be normalized by oral supplementation with relatively small physiological doses (2 micrograms) of the vitamin B12[50]. In severely B12 deficient adolescents, supplementation with just 2 micrograms daily also improved peripheral nerve conduction[51]. In the PRIYA trial, PMNS cohort members, who were willing to participate and with plasma B12 at least 100 pmol/l (those who were B12 deficient were excluded from the trial and treated with B12) were randomized into three arms: 1) B12 alone (2µg/day), 2) B12 2µg + multiple micronutrients + 5 g protein as milk powder, and 3) standard care (Fig 14). All the women received iron and folate tablets before and during pregnancy. The primary outcome was newborn B12 status, with secondary outcomes body composition, gene expression and DNA methylation in cord blood, infant neurocognitive development, and (longer-term) glucose/insulin metabolism and other cardio-metabolic endpoints in the children. The trial took longer than anticipated, because of a striking inter-generational rise in the age of marriage and first child-bearing in the study villages. Of 266 PMNS women randomized in 2012 at the age of 17-18 years, 182 became pregnant over the ensuing five years highlighting the huge challenge of carrying out adequately powered pre-conceptional interventions to look for effects on childhood outcomes.

Maternal vitamin B12 and holo-transcobalamin (holo-TC) concentrations showed a marked increase in both intervention groups (B12 alone and vitamin B12 with multiple micronutrients and milk powder) both pre-

conceptionally and in pregnancy, and maternal homocysteine concentrations fell[52,53]. Cord blood holo-transcobalamin was higher in the B12 intervention groups but there were no differences in birth weight between the three trial groups. The children have not yet been studied for cardiometabolic outcomes, but there are data on cognitive function at the age of 2 years, tested using Bayley's Scale of Infant Development (BSID-III). Children whose mothers received B12 alone scored 5-7% higher in cognitive and language scores than children of mothers in the placebo group[52]. Cognitive function was not significantly different from the placebo group in children of mothers who received B12 plus multiple micronutrients and protein. The reasons for these findings are not known, although it has been suggested that combinations of micronutrients can interfere with each other's function. The trial showed differences in gene expression in cord blood mononuclear cells[53]; genes affected were related to mitotic processes (phase transition, chromosomal segregation and nuclear division). The pattern of genes affected was similar in both intervention groups, but more marked in the B12 plus micronutrients and milk group. The PRIYA data provide strong support for adding vitamin B12 to the existing national supplementation programme for young women. However, as recently discussed in a Lancet commentary, evidence that maternal vitamin B12 supplementation improves neurodevelopmental outcomes in children is mixed, and further work will be needed[54]. Additional data that will come from the PRIYA trial, including MRI brain scan data, and cardiometabolic outcomes, will be important in this debate.

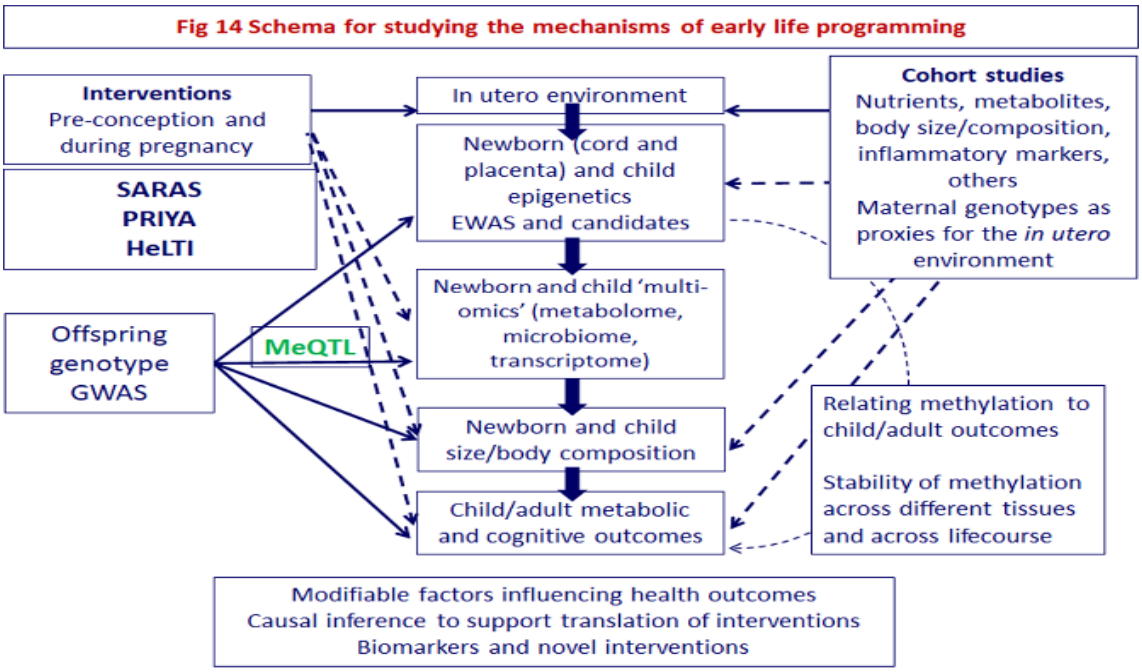
The HeLTI trials

The Healthy Early Life Trajectories Initiative (HeLTI trials) is currently underway in India, South Africa, China, and Canada. HeLTI is a multi-country set of RCTs using protocols that have been harmonized across widely different settings to deliver a multi-faceted intervention covering several domains including maternal health and mental health, nutrition, social and parenting support, and avoidance of infection and pollution exposure[55]. HeLTI plans to deliver a holistic 'package' of improvements for mothers, families, and children, starting before conception and continuing through pregnancy and in early childhood. It is a partnership between the World Health Organization, the Canadian Institutes of Health Research and government-led research funders in all the participating countries. It is visionary in its long-term view: the investigators are interested in pregnancy outcomes but the primary outcomes are downstream in the child, including cognitive development, body composition/obesity and cardiometabolic health (assessed by blood glucose and blood pressure) at the age of 5 years. It is also visionary in including extensive banking of biospecimens throughout the various stages in order to study mechanisms of any effects. There was extensive formative work by the research teams collectively in each of the community settings prior to starting the trials. In India, the HeLTI trial is called EINSTEIN[56] (Early Interventions to Support Trajectories for Healthy Life in India) and is taking place in a rural population near Mysore in Karnataka, led by Dr K Kumaran, and funded by the Department of Biotechnology (DBT) (Fig 14). EINSTEIN was delayed by the COVID pandemic, but started in 2021.

EINSTEIN is a cluster randomized trial, with individual villages as the clusters, and there are three arms: controls who receive routine care throughout, and two intervention arms starting the intervention either pre-conceptionally or once pregnancy is confirmed. Interventions are delivered by Community Health Workers (CHWs) trained in techniques to help parents make changes in their lifestyle and behavior. CHWs engage with the women, husbands, other key family members and the whole community in a programme of group and individual sessions. Nutritionally, women will be offered daily multiple micronutrient supplements, along with support to achieve more diverse diets, a normal body weight and healthy levels of physical activity before and throughout pregnancy and breastfeeding. They have access to support and advice on avoiding exposure to environmental toxins such as indoor cooking smoke, passive cigarette/beedi smoke and pesticides/fertilizers, and help to apply for the government scheme to access liquid petroleum gas stoves. Women are educated about hygiene and safe water, supported to get their infants fully vaccinated and to adopt hand washing after using the toilet, changing nappies, and before preparing food and feeding infants. Starting antenatally and continuing after pregnancy, the CHW sessions will address maternal depression, and support nurturing parental care to optimize neuro-development, using tried and tested methods (Thinking Healthy and Learning through play Plus). CHWs will provide support for exclusive breastfeeding for 6 months and diverse and nutritious complementary foods for 6-12 month infants. It will be several years before the results from HeLTI are published.

WINGS trial

It is important here to mention the contribution of the WINGS trial (Women and infants Integrated Interventions for Growth Study) recently carried out by Nita Bhandari's research group in Delhi[57]. WINGS trial also took a holistic approach, intervening to improve maternal health, nutrition, sanitation, psychosocial care and support, and covering preconception, pregnancy and early childhood periods. Early results show that women in the groups that included pre-conception intervention had fewer reproductive tract infections and less anaemia, and women in the groups receiving pregnancy interventions had better gestational weight gain and a lower risk of pre-eclampsia. The intervention delivered during preconception and pregnancy reduced the risk of low



birth weight by 24% and that delivered during preconception, pregnancy, and early childhood reduced the risk of stunting at two years of age by 51%. These are exciting results, and the investigators are currently scaling up the intervention package in various settings. These studies, along with HeLTI's work on mechanisms and long-term impacts on the children will be extremely important for nutrition policies for Indian mothers and children in the next decade.

Where are we after 30 years and what is the way forward?

Thirty years after David Barker suggested that fetal and child malnutrition were primary factors in causing the 20th-century epidemic of chronic non-communicable disease, I have summarised the contribution of research studies in the field of DOHaD to interventions for improving nutrition and health status. I think that the importance of early development for later capacity and health is now well-accepted by the scientific community, and that the message has reached governments, both national and global. This has led to a greater focus on provision of care for girls and mothers, newborns and young children. However, it is still too easy for these provisions to get neglected or cut when resources are short. The benefits for cognitive capacity have really hit home, but the benefits for adult chronic disease less so, until better evidence is available. The 'common woman' has largely still not heard about DOHaD and we have more work to do there; when she understands both the vulnerability and potential of the developing fetus, everything will change, because every mother wants the best future for her child.

We still need to know more about how best to advise women preparing for pregnancy with regard to their diet and lifestyle. We all know about adequate, balanced and diverse diets, high in fiber, and full of fresh fruit and vegetables, wholegrains and pulses; and it is important to recommend these. We can say with confidence that macro- and micro-nutrient deficiencies, which tend to be specific to different populations, should be addressed. But there is much still to be learned about the specific nutrients required for optimal fetal development. This goes hand in hand with understanding the mechanisms underlying early life programming, which may have specific nutritional implications. I have deliberately said very little about molecular-level mechanisms, which is not my expertise. But there is growing evidence, currently mainly in animals, that epigenetic modifications are programmed by early life nutrition and can permanently alter gene expression, and thus influence later health. All our studies include the collection of biospecimens from various stages of the life-course, with genetic and epigenetic studies carried out in collaboration with Giriraj Chandak at the Centre for Cellular and Molecular Biology in Hyderabad. Biobanks have been created for future analysis, because such 'omic' technologies are evolving rapidly. It will be impossible to carry out RCTs of every nutritional intervention, and an understanding of mechanisms will give us pointers to better focus the trials that we choose to do in future.

There clearly needs to be more emphasis on pre-conceptional preparation for pregnancy, for it to become routine for women to have access to assessment and advice on their nutrition and metabolic fitness before they start their families. This still seems a long way off, the world over, for the women who really need it. We know much about the risky patterns of fetal and childhood growth and how to identify them, and we have useful biomarkers: I would like to see more involvement from clinicians in trying to reverse or mitigate the risk of

diabetes and heart disease in individuals who have already been adversely programmed in early life.

I would like to express my heartfelt thanks to my research colleagues in India, Southampton and elsewhere, the funders that have supported this research, and the women and families who have participated in these research studies. David Barker used to say "There is a simple, but wrong, answer to every question". The nutrition of early human development and its link to later health is complex, and an army of researchers in multiple disciplines is needed to work it all out. After a long career trying to do just that, I can strongly recommend it to young researchers, because India's network of pregnancy/birth cohorts is second to none in the world and their research findings can contribute immensely India's programmes to improve the health and nutritional status of its citizens.

Dr Caroline Fall is Emeritus Professor of International Paediatric Epidemiology, University of Southampton, UK. This manuscript is based on the Dr C Gopalan Oration that she delivered in the 54th Annual International Conference of NSI held in December, 2022 at ICMR-National Institute of Nutrition, Hyderabad

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