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Influence of Maternal Vitamin B_{12} and Folate on Growth and Insulin Resistance in the Offspring

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Abstract

The burden of chronic noncommunicable diseases (NCDs) such as diabetes, obesity and cardiovascular disease is shifting rapidly to low- and middle-income countries. It calls for a review of the classic 'dogma' of genetic predisposition, precipitated by adult lifestyle. The paradigm of early life origins of chronic disease has focused attention on maternal health and nutrition as major determinants of the health of the offspring. India has high burden of maternal ill health and also of diabetes and cardiovascular disease, offering unique opportunities to study the links between the two. Pune studies showed that the Indian babies were thin but fat (more adipose) compared to European babies, and that maternal micronutrient status during pregnancy was a determinant of offspring size and body composition. Two thirds of the mothers had low vitamin B_{12} concentrations, while folate deficiency was rare. Higher circulating concentrations of homocysteine predicted smaller baby size. Follow-up studies revealed that higher maternal folate in pregnancy predicted higher adiposity and insulin resistance in the child at 6 years of age, and that low maternal vitamin B_{12} exaggerated the risk of insulin resistance. Low maternal vitamin B_{12} status is also associated with increased risk of neural tube defects and poor offspring cognitive functions. Our results suggest an important role for maternal one-carbon metabolism in offspring growth and programming of NCD risk. These ideas are supported by animal studies. Improvement of adolescent nutrition could effect intergenerational prevention of chronic diseases.

Change in Epidemiology of Diabetes

Diabetes is recognized as one of the most challenging health problems in the 21st century. The number of people with type 2 diabetes is increasing: a total of 366 million in 2011, to a projected number of 552 million in 2030! The epidemic of

diabetes is shifting to the young and the poor, and the low- and middle-income countries face the greatest burden. The focus on prevention must anticipate this changing epidemiology. India, with more than 61 million diabetic patients is the second highest in the world, and this number is expected to rise to 101.2 million in 2030 [1].

The traditional model of type 2 diabetes proposes genetic susceptibility ('thrifty genes') and precipitation by current lifestyle factors. The current idea of diabetes prevention revolves around changing the lifestyle in adults (postreproductive population) who are obese and impaired glucose tolerant, supported by many trial results. However, it has proved quite difficult to implement in free-living populations. Moreover, such a strategy applied to postreproductive population is unlikely to stem the tide of epidemic in the young and the poor.

Developmental Origins of Health and Disease

In 1992, Nick Hales and David Barker proposed that poor intrauterine nutrition of the fetus increased its susceptibility to type 2 diabetes and cardiovascular disease in later life [2]. The idea arose from their finding that lower birthweight is associated with higher risk of type 2 diabetes and the metabolic syndrome. They proposed that small size at birth represented a 'thrifty phenotype', an intrauterine adaptation to reduced nutrition, which compromised the responses in later life when food supply is adequate, manifesting as an increased susceptibility to disease. Later research showed that slower growth in infancy and rapid growth in childhood also predict risk of type 2 diabetes. Thus, the original concept of 'fetal' origins is now expanded to 'developmental' origins of health and disease (DOHaD). An international council has been set up to promote DOHaD ideas (http://www.mrc.soton.ac.uk/dohad/index.asp). Longitudinal birth cohort studies have been established in developed and developing world to investigate these ideas.

Maternal Nutrition and Offspring Growth and Development: Role of Vitamin B_{12}

A woman's nutritional status before conception and during pregnancy has a vital role in influencing fetal development and outcome of pregnancy. Research in India has made a major contribution towards understanding the role of maternal nutrition in early life antecedents of diabetes and related metabolic disorders in the developing populations.

The Pune Maternal Nutrition Study (PMNS) was started in 1993 in villages around Pune, to investigate influence of maternal nutrition on fetal growth and risk of chronic disease. The PMNS has provided several lines of important evidence of how maternal nutrition influences programming of the offspring for risk of diabetes.

We investigated over 800 pregnancies in these rural women. Mothers were 21 years old, weighed 42 kg (BMI 18.1) before pregnancy, and the babies weighed 2.7 kg (ponderal index 24.1) at birth [3]. Twenty-eight percent of babies were low birthweight (LBW). Despite the LBW and apparent thinness, the babies had comparable subscapular skinfold thickness as English babies which weighed on average 3.5 kg. This led to the definition of 'thin-fat' phenotype of the Indian babies [4].

In these rural women, a higher frequency of green leafy vegetable, fruit, and milk (foods rich in micronutrients) intake predicted a larger newborn size, whereas macronutrient intake (calories and proteins) was not predictive [3]. This finding highlighted the importance of micronutrients in fetal growth. Approximately 70% of mothers had low vitamin B_{12} concentrations, but folate deficiency was infrequent. Also, ~30% of mothers had high total homocysteine (tHcy) concentrations, and >90% had high methyl malonic acid concentrations, both attributable to the deficiency of vitamin B_{12} [5].

We then investigated the relationship between maternal circulating concentrations of tHcy, vitamin B_{12} and folate and offspring size at birth in a nested case-control study. Mothers of full-term small-for-gestational-age babies (SGA; gestation- and sex-specific birthweight <10th centile) and mothers of appropriate-for-gestational-age babies (AGA, >10th centile) were compared for their body size, plasma tHcy, vitamin B_{12} and red cell folate concentration at 28 weeks of gestation. Mothers of SGA babies were lighter and shorter than those of AGA babies and had higher plasma tHcy concentration ($p < 0.01$). tHcy concentrations were inversely related to plasma vitamin B_{12} and red cell folate concentrations ($p < 0.01$, both). The association of maternal plasma tHcy concentration with lower offspring birthweight was independent of maternal height, weight, gestation at delivery and baby's gender [6].

These results were substantiated by a cohort study in Bangalore, India, where 486 women were studied during pregnancy for sociodemographic and nutritional status in order to determine the association of these parameters with fetal growth. Women in the lowest tertile of serum vitamin B_{12} concentration during each of the three trimesters of pregnancy had significantly higher risk of IUGR [7].

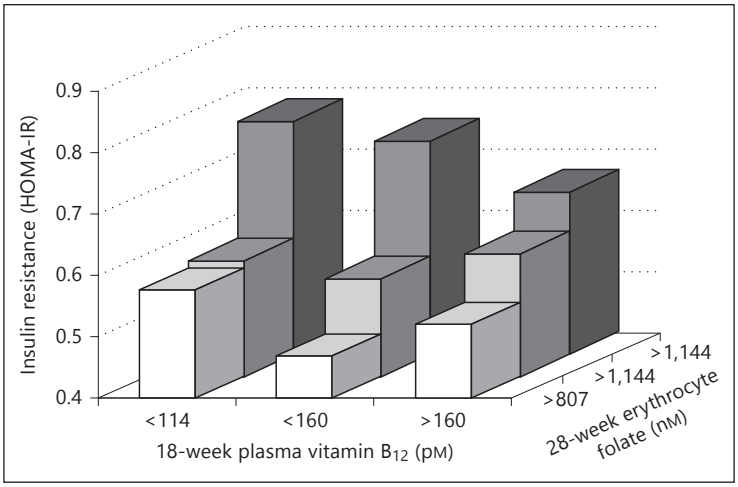

Fig. 1. PMNS: Insulin resistance in the children at 6 years in relation to maternal pregnancy vitamin B_{12} (18 weeks) and erythrocyte folate (28 weeks) [5] with permission.

Childhood Growth, Body Composition and Risk of Diabesity

The children born in the PMNS are followed up every 6 months at home for their body size, and every 6 years for detailed body size, body composition and cardiometabolic risk. At 6 years of age, the children's adiposity was predicted by maternal frequency of intake of green leafy vegetables and milk and by maternal erythrocyte folate concentrations during pregnancy. Low maternal vitamin B_{12} concentrations and high folate concentrations predicted higher insulin resistance in the child, and the offspring of mothers who had the lowest vitamin B_{12} and highest folate concentrations were the most insulin resistant at 6 years (fig. 1) [5]. This suggested a possible role of low maternal vitamin B_{12} and high folate status contributing to the epidemic of adiposity and type 2 diabetes in India.

Neurocognitive Development

Vitamin B_{12} is important for nervous system development. In the PMNS, we also investigated the relationship between maternal plasma vitamin B_{12} status during pregnancy and the child's cognitive function at 9 years of age. Children of mothers with low plasma vitamin B_{12} (lowest decile, <77 pM) concentration at 28 weeks of gestation performed lower on tests of sustained attention and short-term memory as compared to the children of mothers with high plasma vitamin B_{12} (highest decile, >224 pM) [8].

Gestational Diabetes

In a cohort of 785 women attending an antenatal clinic in Mysore, India, low plasma vitamin B_{12} concentrations (<150 pM) were observed in 43% of women and low plasma folate concentrations (<7 nM) in 4%. Vitamin B_{12}-deficient women had higher BMI, higher sum of skinfold thicknesses ($p < 0.01$), higher insulin resistance ($p = 0.02$), and a higher incidence of GDM (8.7 vs. 4.6%; odds ratio 2.1, $p = 0.02$) compared to vitamin B_{12}-sufficient women. Among vitamin B_{12}-deficient women, the incidence of GDM increased with increasing folate concentration (5.4, 10.5, 10.9% from lowest to highest tertile, $p = 0.04$). Vitamin B_{12} deficiency during pregnancy in the GDM mothers predicted higher insulin resistance ($p < 0.05$) and higher prevalence of permanent diabetes ($p = 0.008$, adjusted for BMI) 5 years after the delivery. This suggested that maternal vitamin B_{12} deficiency may be an important factor underlying the high risk of 'diabesity' in Asian Indian women [9].

Neural Tube Defects

In a multicenter case-control study, we investigated the role of maternal nutritional and genetic markers in the etiology of NTDs in India. We measured maternal plasma folate, vitamin B_{12}, tHcy and holo-transcobalamin (holo-TC) concentrations, and polymorphisms in methylenetetrahydrofolate reductase (MTHFR, 677C>T) and transcobalamin (TCN2, 776C>G) genes, in mothers of 318 cases of NTDs and 702 controls. Mothers of NTD fetuses had higher plasma tHcy and lower holo-TC concentrations ($p = 0.003$) but similar folate and vitamin B_{12} concentrations. The maternal polymorphism 677C>T in the MTHFR gene which is commonly associated with NTDs in European populations did not predict risk of NTD, but 776C>G polymorphism in TCN2 was strongly predictive ($p = 0.006$) [10]. This study has demonstrated for the first time in India, a possible role for maternal vitamin B_{12} deficiency in the etiology of NTD, over and above the well-established role of folate deficiency.

Thus, the Indian studies have demonstrated a relationship between maternal one-carbon (1C) metabolism in pregnancy and programming of body composition, neurocognitive function and cardiometabolic risk in the offspring, in addition to the risk of NTD. Vitamin B_{12} and folate are important vitamins in cellular 1C metabolism. The 1Cs are required for the de novo synthesis of purines and pyrimidines and for the remethylation of Hcy to methionine. The subsequent reactions involve protein and polyamine synthesis, and numerous methylation reactions including the methylation of proteins (including histones), cytosine bases on DNA, neurotransmitters, phospholipids, and other small molecules (fig. 2).

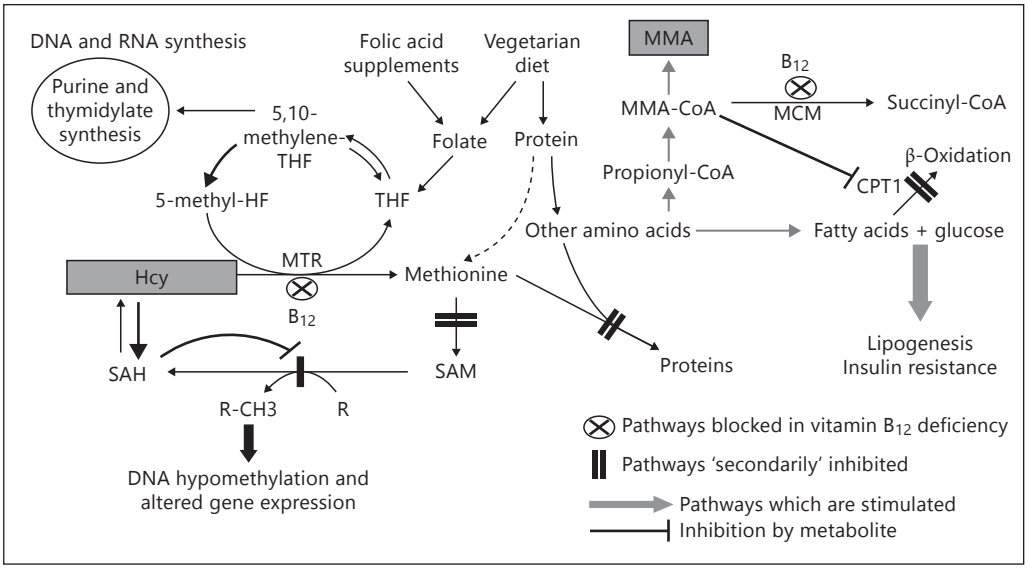

Fig. 2. Suggested metabolic mechanisms for adiposity, insulin resistance and altered gene expression in a situation of dietary vitamin B_{12} deficiency combined with adequate folate status. Vitamin B_{12} deficiency will trap folate as 5-methyltetrahydrofolate, prevent the generation of methionine from Hcy and therefore reduce protein synthesis and lean tissue deposition. Elevated methylmalonyl-CoA could contribute to increased lipogenesis by inhibiting carnitine palmitoyltransferase and thereby inhibit β-oxidation [5] with permission. CPT1 = Carnitine palmitoyltransferase; MCM = methylmalonyl-CoA mutase; MMA-CoA = methylmalonyl-CoA; MTR = methionine synthase; R = methyl acceptor; R-CH3 = methylated compound; SAH = *S*-adenosylhomocysteine; SAM = *S*-adenosylmethionine; THF = tetrahydrofolate.

Maternal Nutrition and Genetics-Epigenetics of Offspring Growth and Development

Fetal growth, birth size and subsequent phenotype are influenced by an interaction between the intrauterine environment and genetic factors. Hattersley et al. [11] showed this through the interaction between glucokinase gene and maternal hyperglycemia. The in utero environment depends mainly on the maternal size, her nutrition and metabolism. It is important to understand that observational associations may not be causal, and could be explained by reverse causation or confounding. Mendelian randomization studies use genetic variants as proxies of non-genetic risk factors to assess whether a risk factor is causally related to an outcome. We investigated the effect of genetic polymorphisms affecting the 1C metabolism on birthweight. Maternal *MTHFR* genotype (677C-T) was tested in two Indian birth cohorts (PMNS, n = 702, and Mysore Parthenon

Study, n = 526). Maternal *MTHFR 677TT* predicted high plasma tHcy concentrations and lower birthweight, independent of maternal BMI, socioeconomic status, gestational age and offspring MTHFR genotype [12]. This suggests that maternal 1C metabolism influences fetal growth, and improving the balance of maternal vitamin B_{12} and folate status may improve birth size, reduce IUGR and its long-term consequences.

It is increasingly appreciated (from human and animal studies) that epigenetic changes, which refer to heritable modifications in the genome not associated with a change in the base sequence, are at the center of fetal programming [13]. These changes are either mediated by methylation of DNA or histone acetylation or through miRNA, all of which modify gene expression. Thus, the same genotype can express a different phenotype by altering gene expression.

The role of DNA methylation in influencing the offspring phenotype at birth and postnatally has been well demonstrated in animal models. Waterland and Jirtle [14] fed genetically obese Agouti mice with a 'methylating cocktail' (B_{12}, folic acid, choline and betaine) and showed that the offspring had a different coat color and were less obese, despite inheriting the Agouti mutation. This was related to methylation status of the promoter region of the Agouti gene. Lillycrop et al. [15] demonstrated that the folate rescue in the rat model of maternal protein deficiency was related to methylation in some of the genetic sequences. Sinclair et al. [16] produced preconceptional methionine deficiency in female sheep (by dietary restriction of methionine, B_{12} and folate). Ova from these sheep were fertilized in vitro, and the blastocysts were transferred to surrogate mothers with normal methionine status. The offspring, especially males, were obese and insulin resistant, and demonstrated differential methylation at a number of sites in the genome. These animal models highlight the importance of maternal periconceptional 1C metabolism in fetal programming.

Vitamin B_{12} Deficiency in India, Nutrition Transition and Dual Teratogenesis

In Indians, hyperhomocysteinemia and vitamin B_{12} deficiency are common. Hyperhomocysteinemia in Indians is predominantly contributed by vitamin B_{12} deficiency rather than folate deficiency [17]. Vitamin B_{12} deficiency is related to vegetarian food habits which originated over 2,000 years ago, and are strongly influenced by religious, cultural, and socioeconomic factors. The ultimate source of vitamin B_{12} in nature is microbes, and this perhaps explains why vitamin B_{12} deficiency is associated with higher income and better hygiene [17]. Our finding of a disturbance in 1C metabolism in the rural undernourished as well as in the

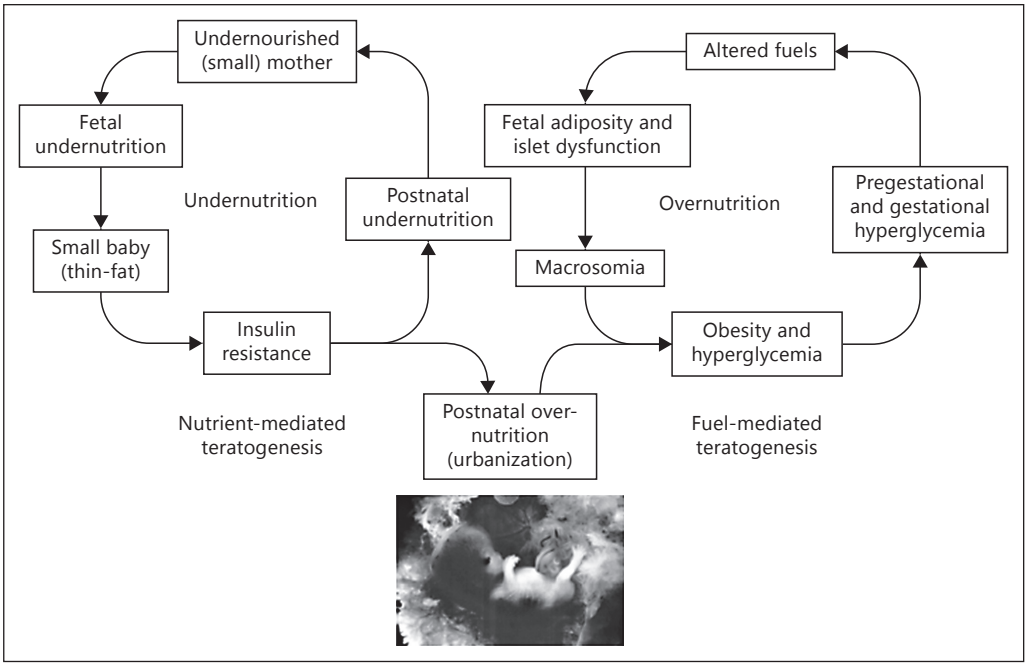

Fig. 3. Interrelationship of two major maternal factors (undernutrition and overnutrition) in fetal programming. An undernourished mother produces a small (thin-fat) insulin-resistant baby. If this baby remains undernourished in postnatal life, the cycle is propagated. If the thin-fat insulin-resistant baby is overnourished, it becomes obese and hyperglycemic. An obese and hyperglycemic mother produces a 'macrosomic' baby at higher risk of obesity and hyperglycemia. Thus, the intergenerational insulin resistance-diabetes cycle is propagated through a girl child. Rapid transition shifts the balance from undernutrition to overnutrition, and contributes to escalation of the diabetes epidemic. Improving health of a girl child is of paramount importance in controlling the diabetes epidemic [18] with permission.

urban overnourished, glucose-intolerant mothers provides an opportunity to reduce the problem of fetal growth restriction (nutrient-mediated teratogenesis) and fetal macrosomia (fuel-mediated teratogenesis), which we have conceptualized in our 'dual teratogenesis' model (fig. 3) [18].

Conclusion

The theory of early life programming offers a unique explanation for the rising burden of noncommunicable diseases (NCDs) in developing as well as developed countries. Our concept of 'dual teratogenesis' needs to be further investigated and acted upon, both by mechanistic investigations as well as by

interventional research to improve 1C metabolism in adolescent girls from before conception. Improving early life environment may be more cost-effective in preventing the NCD epidemic than controlling the lifestyle factors in later life.

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Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the content of the chapter.

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Discussion

Dr. Kurpad: Your work in the thin yet fat Indian is pioneering. In terms of programming, the business of looking at fetal growth versus mortality outcomes refers to very robust data. To some extent, looking at levels of a metabolite or hormone in pregnancy as a programming exposure is so susceptible to variable hemodilution during pregnancy that you often wonder whether the findings are true or false. I would refer to my own data with vitamin B_{12} where we found a very good correlation with birth outcomes [1], but I have repeated this analysis on a subsequent dataset and found no correlation. My worry with vitamin B_{12}, folate and all these plasma levels during pregnancy is that there is a big variable of hemodilution that we cannot measure easily, and it will add variability to this kind of analysis. I also ask myself if vitamin B_{12} is likely to work as a single nutrient in increasing the risk for low birthweight, and I agree with Dr. Kalhan that it is probably a mixture of exposures that is at work. I am sure Dr. Yajnik also agrees that there is a mixture of things happening during pregnancy in India, with a very high folate intake being probably the biggest culprit and vitamin B_{12} being a smaller culprit. The imbalance between the statuses of these two nutrients is worth looking at. I would also say that if you consider vitamin B_{12} deficiency to be widespread, then it is likely that riboflavin deficiency is even more widespread. Dr. Kalhan pointed out that riboflavin is linked to MTHFR, and we have to look at the status of all the B vitamins. If you consider that increased methylmalonic acid will stop substrates from getting into the mitochondria, it is also possible that a decreased riboflavin status will impair mitochondrial oxida-

tion. So, these effects are interrelated and widespread, but with a common outcome. Therefore, to put the blame for low birthweight onto one particular nutrient is difficult for me.

Dr. Bhutta: These were very interesting and provocative presentations. My question to Dr. Yajnik is the whole issue of folic acid sufficiency at a population level. I am very intrigued by the virtually negligible prevalence of folic acid deficiency in the cohort that you studied, and I am trying to reconcile that with large-scale population studies measuring folic acid. To give you an example, despite the fact that it is not a vegetarian population and socioeconomic data are broadly comparable, the overall population level prevalence of folic acid deficiency in women of reproductive age in Pakistan is around 36% and vitamin B_{12} deficiency in the same population is higher, at 47%. But it's not negligible. I am trying to reconcile the data on iron and folic acid intake from India from the national family health survey [2]. If you look at the poorest quintiles, as well as rural populations, the overall intake data from antenatal care visits are around 50% at a maximum, and then you also have the anemia prevalence data. So, what I am trying to reconcile is really, this hypothesis, which I think is very intriguing with the population level data. My suggestion would be that we do need, in addition to the kind of case control studies that you have, really robust micronutrient assessments to settle this issue because the implications are huge. I think to disassemble existing programs either of iron and folic acid intake, or potential fortification, would require a lot more robust population level information on prevalence based on standardized assessment methods.

Dr. Yajnik: I am the first to admit that it's not a single vitamin story, though you have to get motivated by something to do the research and raise the grants. If I say it's whole food that is responsible, then what am I going to do next, increase the intake of everyone? So I entirely agree, and that is why the second arm in our study is providing vitamin B_{12} with multi-micronutrients, which include all the vitamins mentioned earlier, and increasing protein intake with a relatively higher biological value protein from milk. We have built that sort of pragmatic approach in our trial in addition to testing the isolated effect of vitamin B_{12} supplementation. The folate deficiency statistics of course obviously need to be taken into account, but the limited data which are available from different parts of the country by the new micronutrient laboratory set up in Delhi again shows that vitamin B_{12} deficiency is much more common than folate. The original tablet for antenatal supplementation, which was devised in the 1960s, included vitamin B_{12} along with iron and folic acid, but because vitamin B_{12} is the costliest of the three, it was somehow dropped later, while persisting with folic acid. ICMR did a trial of folic acid supplementation in 1980s, and it was a trial of prevention of recurrence of neural tube defects (NTD). The dose for prevent-

ing the recurrence of neural tube defects in the original MRC trial was 4 mg per day [3], so that is what ICMR used. The ICMR trial was stopped halfway through, because the UK results were published and they thought it was unethical to continue. Everyone forgot that the ICMR trial was a trial of prevention of recurrence; it became a trial of prevention of NTD, and then obstetricians started using this dose of folate, believing that the ICMR trial had shown an effect of this dose of folate in preventing NTD, though the trial was stopped half way. So, there has been confusion at various levels about what was intended and what the interpretation of an average obstetrician was, so I think they certainly need to correct that. Dr. Kurpad raised the point about multi-micronutrient and protein, which I of course agree with, and he has repeated his study on vitamin B_{12} and low birthweight, and did not find an association. I have to accept that; we did not have any association of vitamin B_{12} with fetal size, and he found it earlier, so I reported it. I have decided to do the intervention study I spoke of only after the genetic analysis became available that gave us the extra confidence we needed over and above the epidemiological associations. Now at least we have some knowledge that the genetic polymorphisms which predict the nutritional status are also associated with the outcomes in the right way, so that's how we decided to do the trial, and as Dr. Fall said yesterday, it is difficult to do a trial, it's also very difficult to justify it and you might not see any results, and they also take a long time to do. So, with all these difficulties, unless we improve the level of evidence, we will not be able to influence the policy makers, and that is why we have decided to do it.

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