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The small Indian baby: some balanced food for thought?

Anura V Kurpad and Mario J Soares

Introduction

Prevention of intrauterine growth retardation (IUGR), which affects nearly 30% of infants born in India, is a public health priority[1]. The term low birth weight (LBW) includes infants born prematurely or with IUGR. Since the majority of LBW infants in India and most developing countries are a result of IUGR, studies that explore the etiology of IUGR and interventions aimed at preventing foetal growth retardation are urgently needed. This review focuses on the energy and protein requirements during pregnancy, and on the potential gap that could exist between protein or energy intake and the requirements that occur during pregnancy. Further, it looks at the protein gap more critically, and assesses broader implications of protein deficiency. Clearly, this potential gap has the greatest implication during the time when tissue deposition in the growing foetus is at its maximum. It is also clear that the cause of LBW is multi- factorial, and that there are many more nutrients than what are reviewed below, that have critical roles in contributing to an optimal birth outcome.

Maternal energy and protein deficiencies are known to be associated with IUGR[1,2], and a systematic review of 6 available trials on balanced energy and protein supplementation in pregnancy reported a 32% decrease in small-for-gestation age (SGA) babies, although this was of borderline significance[2]. The macronutrient requirements also depend on what might be considered to be an optimal intermediary outcome of pregnancy, such as gestational weight gain (GWG). The recent 2004 FAO/WHO/UNU Consultation on energy requirements[3] reviewed the recent data on maternal weight gain and foetal body weight and decided that a GWG of 10 to 14 Kg, with an average of 12 Kg, and full-term birth weight of 3.1 - 3.6 Kg with an optimum infant weight of 3.3 Kg was desirable. Data from developed countries indicate that the optimal pregnancy outcome in terms of birth weight and infant growth and survival is seen when GWG is about 12-14 Kg. However, these outcomes may need to be interpreted in a more nuanced fashion, in terms of the smaller Indian woman with smaller GWG of 8-10 Kg, and in terms of the practicality of the derived requirement.

The energy requirement of pregnancy

For the energy requirement of pregnancy, the estimated additional energy required can be deduced by a factorial method in two ways: either by assuming an increase in the total energy expenditure during pregnancy or in the basal metabolic rate (BMR). To this are added the costs of protein and fat deposition, adjusted for the efficiency of energy utilization. Both methods give estimates that are similar and average at about 77000 Kcal, which is about 4% lower than the earlier estimate of 80,000 Kcal made by the WHO/FAO/UNU in 1985[4].

On the basis of the reference Indian woman weighing 55 Kg (pre-pregnant) and GWG of 12 Kg, the additional average energy requirement during the 2nd and 3rd trimesters is 375 Kcal/day[5]. If the GWG were 10 Kg, the average requirement would be 310 Kcal/day during the 2nd and 3rd trimesters. One could round off these values to about 350 kcal/day for a GWG of 10-12 Kg. However, for a lower GWG of 8 Kg, the requirement would theoretically be in the region of 300 Kcal/day, but it is difficult to advise that figure even if one considers a small woman with a potentially low GWG. In addition to the GWG, it is also important to consider the total daily energy requirement in pregnant women in terms of their body weight and physical activity. In situations where women have a low pre-pregnancy weight (of say 40 Kg), using a unitary (reference woman based) requirement may lead to the overestimation of the inadequacy of energy intake.

How do pregnant women meet these requirements? One way is by increasing their food intake, which in turn depends on their food security. Another way is by reducing requirements through a reduction in physical activity. The reduction in physical activity, which has long been postulated to be the most important, and in some cases the only, mechanism by which undernourished women are able to reduce their energy expenditure during pregnancy and thereby complete pregnancy with only a small increment in food intake. However, several studies in different societies have shown that there is no evidence of any reduced activities during pregnancy, although global literature suggests that women do less arduous tasks towards the end of pregnancy. Some studies in India have also shown that there is a shift towards more sedentary activity even among families whose usual occupation

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involves manual labour[6]. This seems to be the case in the Philippines as well as the Gambia[7,8]. However, in Thailand[9], women spent less time on childcare, housework and walking and more on agricultural tasks in late pregnancy. Clearly, conclusive data on exactly how much energy is saved by changing activity patterns during pregnancy is not known.

The question remains as to whether pregnant women meet their increased energy requirements successfully through an increased food intake. While there are several Indian studies on nutrient intake during pregnancy over the last 50 years, only recently reported studies are reviewed here, even though they report data that might be older. A study on a small sample of rural women from Tamil Nadu showed that women weighing only about 50 Kg in the 3rd trimester of pregnancy (assuming a GWG of about 7-8 Kg, this would mean a pre-pregnancy weight of about 42-43 Kg) had an energy intake of about 2200 Kcal/day[10]. Even with a moderate activity level, at this body weight, the women might be construed to have been eating adequate calories. However, this was not an optimal situation, since the diet was also of poor quality and low micronutrient content. Four relatively recent reports are also available from northern and western India. In a small rural study near Delhi[11], pregnant women in the 2nd trimester had energy intakes of about 1550 Kcal/day. However, details of anthropometry or GWG were not available in the manuscript, but even so, the energy intakes seemed extremely low. In perspective, this intake would only satisfy the need of a *non-pregnant* sedentary woman weighing just 45 Kg, and certainly could not support any GWG. Therefore, one must consider if these intakes were accurate. Similarly, in a small rural study in Haryana, energy intakes were very low, at about 1650 Kcal/day in women who weighed 49 Kg in the 3rd trimester[12]. These women worked (were physically active) for about 7.5 hours/day, and the energy intakes were extraordinarily low, at about 80% of their weight-adjusted requirement. Once again, these intakes are so low that the support of any GWG is questionable.

However, in another study in 4 districts of Rajasthan, mean energy intakes were about 1850 Kcal/day in the 2nd trimester[13]. The weight of the women was not reported and this makes it difficult to assess the adequacy of the energy intake; but if the women were reasonably similar in anthropometry to those in rural Haryana, one might expect that their diets were close to adequate in terms of energy, but that is an assumption. In a recent report of a study conducted 15 years ago in rural Pune, energy intakes in the 2nd trimester varied with the season, and was near 1900 kcal/day in winter, but closer to 1600 Kcal/day in the summer. Birth weight was dependent on this variation and it was interesting to note that in these moderately active women, whose pre-pregnant weight was about 42 Kg and whose 2nd trimester weight gain was 5 Kg, it might seem that their weight adjusted energy needs (about 2000 kcal/day) were indeed close to their intake in winter[14]. Overall, it might seem that while there is concern that there are low energy intakes in rural areas, this is coupled with low pre-pregnancy weight and GWG, and there is also some doubt about the size of the studies, the accuracy of the energy intakes, and the detail of their reportage.

In contrast, in an ongoing cohort study in urban Bangalore, a study of 550 pregnant women from poor to middle income groups, weighing 62 Kg on average in the 3rd trimester (ie, with a full term GWG of about 10 Kg), had energy intakes that met their additional pregnancy related energy requirement completely (Kurpad et al, unpublished). In another urban study in Pune[15], mean maternal energy intake was 2160 Kcal/day in affluent probably sedentary women who, at the end of the 2nd trimester, had gained 8 Kg and weighed 64 Kg, and this energy intake was close to the required weight specific intake.

The available data at present point out that while there may be differences in pregnancy related energy security between rural and urban India, there are also many qualitative differences between rural and urban diets, without great differences in live birth outcomes, especially if one is looking at the poor to middle class groups. It is possible that there are adaptations in energy economy that are as yet unmeasured, or that the pregnant woman uses her own energy stores to partly make up the deficit. There is, therefore, a need for contemporary, large, well-performed and explicit studies, with good measures of dietary intake and nutritional status, along with measures of birth outcomes. However, one must also look elsewhere within the diet for cause.

The protein requirement of pregnancy

The calculation of the daily requirement for protein during pregnancy is based on a factorial approach because of difficulties with direct measurements of nitrogen balance. The factorial approach uses values of the protein deposited (adjusted for the efficiency of utilization of dietary protein) and the maintenance costs of protein intake associated with increased body weight. In these calculations, there are several key questions. For example, what is the magnitude of the GWG for the average Indian woman? This has been discussed above, and figures are presented based on a GWG of 8, 10 and 12 Kg. Further, does the factorial method fully capture the possible adaptations that could occur during pregnancy? The efficiency of protein utilization is thought to be in the region of 42%[16], although it cannot be said with certainty that there is no increase in the efficiency of urea recycling in the low BMI pregnant woman. Finally, how relevant are maternal protein stores (muscle mass) in meeting some of the protein requirement of pregnancy? It is not clear how the undernourished Indian woman copes in this regard, but detailed measurements of the fat free mass (FFM), based on accurate measurements of total body potassium and nitrogen in well nourished women during and after pregnancy, showed that there was no net accretion or loss of protein during pregnancy[17], suggesting that the protein deposition during pregnancy was only into feto-placental tissue.

Based on the accurate methods referred to above, it has been found that 1.9 g/day of protein are deposited in the 2nd trimester and 7.4 g/day in the 3rd trimester, in well nourished women with a mean GWG of 13.8 Kg[16]. It can be reasonably assumed that there is relatively no protein deposited in the 1st trimester. One could make the further assumption that there is a linear relationship between GWG and protein deposition from these figures. Then, one could make a guess of the protein deposition rate for different GWG; for example, with a GWG of 12 Kg, mean protein deposited would be 1.6 and 6.5 g/day in the 2nd and 3rd trimester respectively. The daily dietary requirement of protein to meet this deposition need is based on the assumed efficiency of utilization of dietary protein, which is thought to be about 42%. The maintenance costs of the additional weight also need to be added, and these are based on product of the mid-trimester body weight (an assumption, based on different GWG) of the pregnant woman and the maintenance requirement value of protein of 0.66 g/Kg/day. Finally, the safe level of increased intake by trimester can be derived based on the coefficient of variation of this estimate. Based on these calculations, the extra protein requirement at safe levels of intake, are given in the Table, to support different GWG of 8, 10 and 12 Kg.

Table: Additional safe protein requirements g/d for different gestational weight gain (GWG)			
Trimester	GWG (Kg)		
	8	10	12
1st	0.4	0.5	0.6
2nd	6	7	8
3rd	18	23	27

At first sight, these extra protein allowances, particularly during the 3rd trimester may appear high, and may suggest that protein supplements are required. However, this is not the case when one considers the total dietary intake of protein. For example, if one considers a sedentary pregnant woman, whose pre-pregnant weight was 55 Kg, with a GWG of 10 Kg, an energy allowance of 350 kcal/day, and an extra allowance of about 20 g protein/day, the calculated protein:energy (PE) ratio of the required diet would be about 13%. This PE ratio would decrease to about 12% if the woman were moderately active. Therefore, the PE ratio (requirement) of the diet does not increase dramatically inspite of the higher protein requirement. In addition, in view of the possible adverse events that may follow the use of supplements that are very high (greater than 34% PE ratio) in protein[18], it is important that the higher intake of protein recommended during pregnancy should come from a normal varied diet, containing pulses, milk, eggs or meat, and not from commercial high-protein supplements. For example, increasing the pulse:cereal ratio, or adding milk to the intake are options. A final consideration is the habitual activity of the pregnant woman. Clearly, a sedentary lifestyle will need a higher PE ratio in the diet (about 13%). In a sedentary lifestyle, with a solely vegetarian diet, it is difficult to reach a PE ratio of 13%, unless milk intake (particularly toned milk) is substantial (about 600 gm/day), pulse:cereal intakes are about 1:5, and root vegetables and visible fat are reduced. Non-vegetarian foods can help fill the requirement for high quality protein.

As with energy, the question arises if pregnant women can meet this demand through an increased dietary intake. If women were to eat only a fraction of the required protein allowance, then adaptations to this low intake must be present so that a successful pregnancy outcome is obtained. One possibility is the mobilization of protein stores from the mother, but this has not been measured in these populations. Another is the reduction of protein oxidation through a reduction in urea synthesis rates[19], or the possible recycling of urea nitrogen through the involvement of the gut flora, but this too has not been measured in India. Based on data in other countries, the range of such adaptations encountered are unlikely to offset the total gap between intake and requirement, which can often be in excess of 50% for the extra protein allowance.

Therefore, the question of how much additional protein is eaten by pregnant women, particularly during late pregnancy must be addressed. In general, for a woman with a pregnant weight of about 40 Kg, and a GWG of 8 Kg, the total daily protein requirement in the 3rd trimester would be 58 g/day (40+18 g/day). Expressed as the total daily protein requirement per Kg bodyweight (for a body weight of 48 Kg), this requirement would be about 1.2 g protein/Kg/day. The subjects of the rural studies referred to above had observed daily protein intakes in the range of about 1 g/Kg/day in the 3rd trimester. A similar situation was observed in the urban studies in Bangalore and Pune, where the protein intakes in the 3rd trimester were well below the required amount: in the Bangalore study, for example, the observed extra protein intake was about 13 g/day versus the GWG based requirement of 23 g/day. This meant that these women had protein intakes that were only slightly higher than 1 g/Kg/day, which is again about 20% below the daily total protein requirement. Since the rural women ate predominantly cereal-based diets, while the urban women had more variety in their intake, it is likely that even though the protein intakes were suboptimal in both groups, the quality of protein intake may have been better in the urban women. The PE ratios of the intake in all these studies were very variable, ranging from about 8 to 12% (notwithstanding doubts about the accuracy of the measured intake), while the requirement PE ratio in sedentary women is about 13%.

In effect, it would appear that the energy intake of rural women is below normal, but that of urban women is reasonably normal in late pregnancy. Given that the rate of IUGR is high in urban poor and middle class women, these considerations argue for protein inadequacy rather than an energy deficit as central to the problem. The key element is the protein quality and content of a predominantly cereal-based diet; simply increasing the intake of a cereal-based diet during pregnancy ensures energy, but not protein, intake. Dietary selections, favoring more protein rich foods, along with the economics of such selections, are important considerations.

A broader look at protein intake during pregnancy

The relation of pregnancy protein intake to birth weight

Studies relating protein intake to birth weight outcomes are difficult when the protein intakes are closely clustered, and since protein intakes are so closely related to energy intakes in predominantly cereal eating populations. This particularly bedevils studies in rural areas, where protein intakes are generally low, along with energy intakes. Therefore, to assess protein effects on pregnancy, one needs populations in whom the dependence on cereal protein is not so high, and where other food groups that are rich in protein are also consumed. For example, in a study on urban affluent women in Pune[15], energy adjusted protein intake, and particularly milk intake was related to birth weight outcome, and this might have been related to the larger range of intakes that are possible in this group. In urban Bangalore, a similar finding of a higher birth weight at the higher energy adjusted protein intake during late pregnancy was observed (Kurpad et al, unpublished).

It is then worth considering what foods are likely to provide the extra protein requirement without raising the energy intake too much. With nuts, which are a rich source of protein, the accompanying increased energy intake through the nut oils, is high. Pulses, which are a rich source of protein, also contain dietary fiber, which in large amounts can cause bloating and discomfort, due to colonic fermentation. This leads to avoidance. Milk, which is thought to be abundantly available through the aptly named 'white revolution', contains about 335 Kcal and 16 g protein in 500 ml. It might appear therefore, that milk (or milk-based products) may be an ideal way to deliver the increased requirements of protein during late pregnancy, without too much additional energy. Toned milk may even be more appropriate to reduce fat intake, and the intake of an extra 500 ml of milk in the form of 4-5 servings of milk, curd and milk-based products may be an ideal solution to providing extra energy and protein during pregnancy. This needs to be tested appropriately.

Milk intake and birth weight

Are there any indications that milk intake specifically, is related to birth weight? In one study on 538 normal BMI mothers, protein intake during late pregnancy was related to placental and birth weight[20]. More specifically, dairy and meat protein intake was specifically related to birth weight. However, it is important to point out that the protein intake in these normal body mass index (BMI) mothers was in a range of 72-99 g/day in late pregnancy, which is a good intake. In a much larger epidemiological study of 50,117 normal BMI women, Olsen et al[21] found an association between intakes of protein and fat from dairy products (excluding cheese and ice cream) and birth weight. This estimate was adjusted for several confounders including the energy intake, but as in other Western studies, it is relevant to point out that the range of milk intake was large, with the highest intakes being in excess of 6 glasses per day. Pulses on the other hand did not show this effect[21]. It is also relevant to point out that milk could also contain substances other than protein that could stimulate growth. In another smaller study that found a positive relation between milk intake and birth weight[22], Vitamin D intake was also found to play a positive role (but not calcium or riboflavin). In Indian studies, both rural and urban Pune[15] and urban Bangalore studies have shown a relationship between milk intake and birth size. This was particularly marked in the rural women in Pune and urban women in Bangalore (perhaps because the sample size was adequate for the purpose). To take these findings forward into practice, carefully controlled intervention trials are needed, and there are no satisfactory data in the literature. Nevertheless, if one considers the amount of milk required, this could be calculated as the amount meeting the requirement in mid to late pregnancy, which is approximately 500 ml/day. If the intake of pulses is also improved, by increasing the pulse cereal ratio, the milk requirement could be reduced, although it remains to be seen how best (and economically) this could be done.

Vitamin B_{12}, protein intake and epigenetics

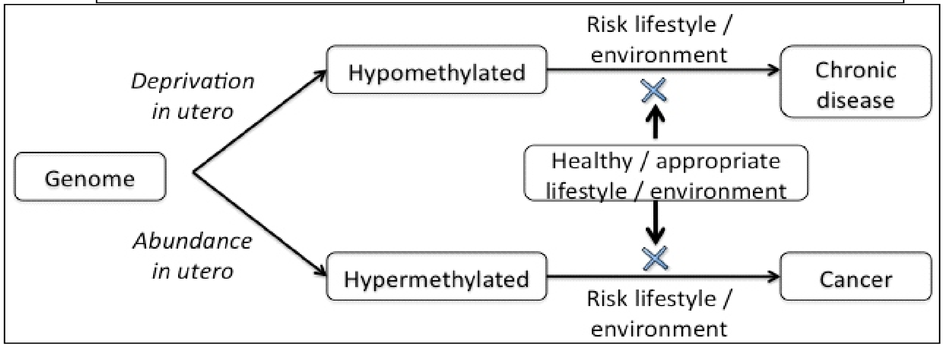
There is a lot of vitamin B_{12} deficiency in India. This has been attributed to vegetarianism, but could also be due to poor absorption. The deficiency of Vitamin B_{12} has clearly been shown to be associated with low birth weight[23], and there is interest in understanding the effect of intervention studies on this problem. While there are several questions around this issue, this review will briefly focus on the question of the effect of vitamin B_{12}/protein on epigenetic phenomena. The methylation of the foetal genome is linked to the availability of methyl groups and the activity of the enzyme DNA methyltransferase. There is a complex interplay between the need for sulphur and nitrogen for tissue deposition with the need for methyl group transfer linked to genomic methylation. The methionine cycle generates S-adenosyl methionine that transfers methyl groups, along with homocysteine. A diet that is low in protein, for example, in a pregnant rat fed 50% of its requirement, will be associated with relative sulphur - containing amino acid deficiency[24]. In consequence, the need for these amino acids for protein deposition is not met, and one way of saving methionine is through the remethylation of homocysteine. This would in turn, use methyl resources, and affect methylation processes leading to hypomethylation for specific genes resulting in stable changes to the expression of some genes involved in energy balance and nutrient homeostasis. Interestingly, it is also relevant to point out that an abundant nutrient environment *in utero* could lead to hypermethylation patterns that favor cancer development in later life[24]. These extremes are evocative of an ancient doggerel:

Ashes to ashes; dust to dust;
If the heart attacks don't get you; then cancer must.

However, this is an overly pessimistic and overtly deterministic assessment, since the effect of methylation of the genome in determining risk for later life disease is likely to be greatly tempered by contemporary lifestyles. While it is difficult to predict the optimal methylation pattern with present knowledge, it is equally difficult to consider just how much these foetal considerations add to the risk of disease evolution (whether cardiovascular disease or cancer) in later life, when it is more than likely that contemporary environmental and dietary exposures will play important confounding roles (Figure). It may be safe to say that a life of moderation and dietary caution can obviate risks of later disease that are linked to birth weight, such that the influence of foetal programming could be marginal at best. In this regard, a wider debate is relevant, on the wisdom of enthusiastically advocating increased birth weight through nutritional intervention without regard to what targets need to be achieved. Clearly, nutritional policies need to be developed that are rational, inclusive of different approaches, holistic, and that do not swing the pendulum all the way to the other side.

Glycine and serine are 2 other amino acids that serve as methyl sources, and a reduction in the levels of these amino acids could also result in poor birth outcomes. For example, increasing the availability of glycine and folic acid to pregnant rats consuming a protein-restricted diet prevented induction of hypertension and vascular dysfunction in the offspring[25]. However, the Indian pregnant woman is likely to have a deficiency of 20% in protein in contrast to the more extreme 50% protein deprived rat, and therefore mechanistic findings in the rat cannot be easily transferred to the human context. Nevertheless, they may exist in more subtle forms, and studies are required in this regard. Further, in contrast to humans, birth weight in rodents is generally unaffected by nutritional constraints during pregnancy. Birth weight must therefore be considered as surrogate for the effects of the intra-uterine environment on the development of the foetus rather than a causal link between environmental exposures *in utero* to later risk of disease. In addition, the remethylation of homocysteine is dependent on vitamin B_{12}, and the key strategy may be towards discovering whether a combined deficiency of vitamin B_{12} and protein could amplify the effect of low sulphur amino acid status. In addition, non-

Figure: Methylation, the genome, and later risk of disease (adapted from ref 24)



compliance with folate supplementation will further add to the problem of remethylation of homocysteine. It is not intended to explore these issues in detail, but it is evident that the complex interplay between protein, specifically sulphur-containing amino acids and glycine/serine, along with vitamin B_{12}, folate and pyridoxine status needs to be carefully evaluated. There are two points to end this section of the review: first, simply adding any one of these components to the diet will likely not solve the problem. For example, the ingestion of methionine in excess is harmful, and actually will deplete the glycine pool as the methionine is detoxified. Rational interventions are therefore very important. Second, it may be that moderate amounts of milk, in a balanced diet containing fruits and vegetables, may provide all the requirements in this complex interplay.

Summary

The macronutrient considerations reviewed here should not divert from the fact that the cause of the small Indian baby is multifactorial, and no single solution exists. For example, maternal illiteracy and low socio-economic status (SES) are known to be major risk factors for IUGR[26-28]. Factors that are related to SES, relating to the care of women, maternal infections, environmental hygiene and sanitation, household food security, and poverty are all likely to operate simultaneously in the etiology of IUGR. Low maternal weight at baseline and poor GWG are also important predictors of IUGR. Among the micronutrients, vitamin B_{12} status has been associated with the risk for IUGR, and the intake of green leafy vegetables has also been associated with better pregnancy outcome in terms of birth weight, particularly in rural mothers[15].

Clearly, policies that address the different facets of the problem in a holistic manner are the need of the day. Food-based strategies that select for more protein are likely to be more holistic in their approach, but also demand that the woman is empowered to make the correct choices for her own health and that of her baby. The choices tend to be in the realm of common sense, and apply across the life cycle. In principle, they are simple enough, but we must not discount the especially vulnerable state of the pregnant woman. These enhanced needs require more intervention, including those with publicly distributed, but possibly value-added foods. Tapping into success stories in food production and procurement, for example, related to milk production, may be the key.

The article is based on C Ramachandran Memorial Lecture delivered by Dr Kurpad on 25th November 2009. Dr Kurpad is at St John's Research Institute, Bangalore and Dr Soares is at Curtin Health Innovation Research Institute, Programme of Nutrition, School of Public Health Curtin University, Perth, Australia.

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