Maternal and Child Nutrition: The First 1,000 Days
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Preface

Contemporary data from numerous clinical and scientific studies have underscored the critical significance of healthy growth and development during the first 1,000 days of a child’s life (9 months in the mother’s womb and first 2 years after birth) in relation to both immediate survival and morbidity as well as to the development of noncommunicable disorders such as obesity, type 2 diabetes, hypertension and heart disease in adult life. The fact that optimal growth and development during this period are also critical to the development of brain and cognition is now well recognized and is the principal basis for targeting nutrition interventions within this critical period. The fact that in some parts of the world close to a third of all births are low birthweight (either premature or growth retarded or both) also indicates that growth and nutrition in fetal life are important determinants of development in early childhood. Undernutrition and micronutrient deficiencies among children under 2 years of age significantly increase the likelihood of serious infectious morbidities such as pneumonia, diarrhea and malaria and are associated with almost a third of all deaths during the first 5 years of life. Each year, close to 2.6 million children under 5 years of age die as a consequence of various forms of undernutrition, and many more are affected by high burden of disease and disability. At the other end of the spectrum, there has been a rapid increase in the incidence of diabetes, obesity and heart disease across the world, more so in the developing world in association with the arrival of industrialization and affluence, and has been related to growth during the first 1,000 days. The combination of the immediate and long-term consequences of impaired growth and development during the critical first 1,000 days contributes enormously to the health care cost and to the impairment of the economic growth of the society. As stated by the United States Secretary of State Hillary Clinton, ‘improving nutrition for mothers and children is one of the most cost-effective and impactful tool we have for poverty alleviation and sustainable development’.

The first 1,000 days of an infant’s life offer a unique opportunity for optimizing health and nutrition outcomes. Optimal nutrition and health care of the
mother and infant during this period are closely linked to growth, learning potential and neurodevelopment, and to long-term outcomes. A child with poor brain development is at high risk for cognitive developmental disorders leading to poor school performance, early school dropout, low-skilled employment and falls into the vicious cycle of intergenerational sharing of nutritional deficiencies and poverty. Given the importance of nutrition across the life cycle, many hold the view that the most optimal opportunities for addressing the problem may actually necessitate addressing issues in the preconception period. In addition, the understanding of the mechanism of nutrient-related programming of the metabolism during this period via epigenetic and other mechanisms and development of innovative approaches for intervention are critical if we are to make an impact on the rapidly spreading epidemic of noncommunicable diseases in developing societies. Careful observation of the immediate metabolic consequences of intrauterine growth restriction and low birthweight is important to develop and evaluate strategies to improve survival and promote postnatal growth. Such interventions in the immediate neonatal period and during the 2 years after birth are aimed at achieving optimal growth, favorable neurocognitive outcome, reducing infant mortality and morbidity and reducing the burden of noncommunicable diseases in adult life. It was in this context that the 74th workshop of the Nestlé Nutrition Institute was organized in Goa, India. Renowned experts in the field from across the world discussed the critical importance of nutrition and environment during the first 1,000 days, between conception and the child’s 2nd birthday, in determining the health and development of the baby. Recognition of nutrient and environmental influences and appropriate intervention strategies can have profound impact on the child’s growth and development, on long-term consequences and can impact society’s health and economic prosperity. We very much appreciate the enthusiastic participation of the speakers and the invited clinical and non-clinical scientists and caregivers that resulted in a healthy, informative and scientific discussion. This monograph presents the state-of-the-art knowledge gained in this workshop and possible future areas of research. We are thankful to Nestlé Nutrition Institute for the support of this workshop and specifically Prof. Ferdinand Haschke, Dr. Natalia Wagemans, Dr. Sanjeev Ganguli, and Christine Stillhart for organizing an outstanding and stimulating workshop. We are hopeful that you will find these proceedings both informative and stimulating.

Jatinder Bhatia
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Satish C. Kalhan
The 74th Nestlé Nutrition Institute Workshop titled ‘Maternal and Child Nutrition: The First 1,000 Days’ looked at two important nutritional issues that can affect a child’s growth and development.

The first area of focus was the prevention of low birthweight (LBW), starting with the health of adolescent girls, through the pre-pregnancy and pregnancy stages and ending with lactation.

The second was the nutritional follow-up and feeding opportunities in relation to dietary requirements of children with an LBW. Our nutritional interventions must make the best possible short- and long-term outcomes possible. The importance of these issues for South and South East Asia brought this workshop to India.

The rate of LBW is still unacceptably high in some high-risk countries; in the South Asia region, for example, it stands at 28% of annual births [UNICEF, State of the World’s Children, Childinfo, and Demographic and Health Surveys by Macro International]. This significant number does not only include premature babies, but also those with intrauterine growth restrictions who consequently have a very high risk of developing metabolic syndrome in the future. That is why epidemiology, epigenetic programming, the correct nutrition strategy and monitoring of outcomes were chosen as the subjects of the scientific discussions for this workshop.

Preventing even one case of LBW brings considerable benefits, making this a valuable workshop for health care professionals globally. Reducing rates of LBW can bring economic value, through lower treatment costs, benefiting both families and public health systems, and result in better cognitive development for the child.

We wish to warmly thank the three chairpersons of this workshop – Prof. Zulfiqar A. Bhutta, Prof. Satish C. Kalhan and Prof. Jatinder Bhatia for establishing an excellent scientific workshop program. We are also indebted to the renowned speakers who have furthered the debate and understanding of this
important topic through their presentations and participation. We thank the many experts who came from across the globe to review and discuss the importance of the maternal and child nutrition during the first 1,000 days.

Finally, we wish to thank and congratulate Dr. Sanjeev Ganguly and his team from Nestlé Nutrition Institute, India, for their excellent logistical support that allowed us all to enjoy the scientific program and experience the wonderful cultural spirit of Goa.

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In this paper, I review the epidemiology of low birthweight (LBW). I begin by defining LBW and emphasizing the distinction between infants who are born early (preterm) and those who are born small for their gestational age (SGA). I then review data on the global burden of preterm birth and SGA and the evidence bearing on whether ‘one size fits all’, i.e. whether a single birthweight (or birthweight for gestational age) cutoff is appropriate for different regions and population subgroups. I summarize what is known about the etiologic determinants of preterm and SGA birth, how they differ among countries of varying socioeconomic development and prevalence of risk factors, and how they are changing over time. I conclude with a critical appraisal of LBW prevention as a public health priority and argue that reducing fetal and infant mortality is a more important and achievable target for intervention.

Definitions

The World Health Organization (WHO) defines low birthweight (LBW) as a weight at birth <2,500 g. But birthweight is known to be determined by two separate, if not entirely independent, processes: (1) the duration of gestation and (2) the rate of fetal growth [1]. Thus, LBW can arise through one or both of two mechanisms. Infants who are born ‘too soon’ are referred to as preterm, which WHO defines as a gestational age at birth <37 completed weeks [2]. Infants can also be LBW because they are born ‘too small’ for their age (small for gestational age, SGA), which has several definitions, the most common of which is a birthweight below the 10th percentile of gestational age based on an appropriate reference [3].
Figure 1 shows the relationship among LBW, SGA birth, and preterm birth (PTB). As the figure illustrates, all LBW infants are preterm, SGA, or both. But the figure also makes it clear that infants can be born preterm without having LBW. In the Canadian birthweight for gestational age reference [4], for example, many infants born between 34 and 36 completed weeks have a birthweight ≥2,500 g. Similarly, infants can be SGA without having LBW; the 10th percentile for Canadian infants born at 40 completed weeks (i.e. at term) is about 3,000 g (3,079 g for boys, 2,955 g for girls), while the majority of SGA infants born at 38 weeks or later weigh ≥2,500 g [4].

The importance of distinguishing preterm birth from SGA is evident from the contrast in temporal trends between preterm birth and LBW, as illustrated by figure 2, which is based on data from Canada over a quarter of a century. After slight declines in the rates of preterm birth and LBW in the early 1980s, LBW remained stable or decreased slightly over the next 20 years, while preterm birth increased steadily. This reflects an increase in weight for gestational age [5], along with the increased incidence of preterm birth in the population [6, 7].
Since about 2000, however, rates of LBW and preterm birth have risen in parallel and, in the most recent years, have also fallen in parallel, reflecting the end of the temporal trend towards increasing birthweight for gestational age and an apparent true decrease in preterm birth.

In addition to these differences in prevalence and temporal trend, preterm and SGA infants have very different prognoses for survival, morbidity, and development [8]. Preterm birth is the world’s leading cause of infant mortality [9]. SGA infants are at increased risk of stillbirth and infant mortality [10], but much recent literature has focused on long-term associations with chronic diseases of adulthood, including hypertension, type 2 diabetes, and coronary heart disease [11].

These considerations underline why it is important to distinguish between preterm birth and SGA as causes of LBW and why it is inappropriate to use LBW as a population health indicator to compare geographic differences in countries or regions, or trends over time. Of course, the ability to distinguish between SGA and preterm birth depends on reasonably valid estimates of gestational age at birth, and of weight at or soon after birth. In many poor rural areas of the world, particularly in South Asia and sub-Saharan Africa, many women are not seen regularly during the course of pregnancy, and births occur in the home. Thus, reasonably accurate measures of gestational duration and size at birth are not available for a very large fraction of births in those regions.

The Global Burden of LBW

The limited value of LBW prevalence as an indicator of maternal and child population health has been recognized by the World Health Organization. For example, in WHO’s World Health Report of 2005 [12], which focused on maternal and child health, tabulated indicators included numbers of births, cesarean delivery, maternal mortality, stillbirth, and neonatal mortality, but not LBW. This is in line with WHO’s emphasis on the Millennium Development Goals (MDGs), particularly MDG 4 and 5, which bear on reducing young child and maternal mortality, respectively.

In its 2009 The State of the World’s Children [13], UNICEF estimated the world birth prevalence of LBW from 2000 to 2007 based on the most recent year’s data available in each country and region. The region-specific birth prevalence ranged from 6% in the East Asia and Pacific region, and 7% in industrialized countries to 27% in South Asia. To my knowledge, the last time WHO estimated the international SGA birth prevalence was in 1998 [3], based on the threshold <10th percentile from the Williams et al. [14] (California) reference curve, as occurring in 30 million newborns per year, or 20.8% of all births. Of
the total number of SGA infants, 75% were estimated to be born in Asia, with another 20% in Africa and 5% in Latin America. Figure 3 is taken from the WHO report [3] and shows the prevalence of newborn infants born between 1985 and 1995 who were both SGA and LBW.

Because preterm birth is a far more important cause of neonatal and infant mortality than intrauterine growth restriction [1], both WHO and the March of Dimes have focused more recently on preterm birth, despite the difficulty in ascertaining gestational age at birth in many low- and middle-income countries [9, 15]. Figure 4 is a world map showing estimated country-specific prevalences of preterm birth in 2005; 12.9 million births, or 9.6% of all births worldwide, were estimated as being preterm, 85% of whom were concentrated in Africa and Asia [9]. The lowest estimated rates were seen in Europe and Australia.

Does One Size Fit All?

One question that frequently emerges in public health circles when discussing international comparisons of birthweight distributions is whether the prevalence of LBW (or the prevalence of SGA) should be based on a single birthweight cutoff or a single birthweight for gestational age reference, or whether different
cutoffs and references should be applied in different regions or countries. The need for sex-specific birthweight for gestational age standards is generally accepted because in every region in the world, newborn girls weigh less than newborn boys [16]. These sex differences have also been noted as early as the first trimester in ultrasound measurements obtained in nonselected populations. Despite their smaller size in utero and at birth, girls have lower stillbirth and infant mortality rates than do boys, and thus it is reasonable to infer that their ‘smallness’ relative to boys is physiologic, rather than pathologic.

But other widely recognized differences in birthweight, or birthweight for gestational age, are not so clearly classified as physiologic (‘normal’) or pathologic. For example, birthweights are higher and LBW rates lower in rich countries than in low- and middle-income countries [10]. Within countries, robust differences have been reported according to ethnicity, parity, maternal height, maternal prepregnancy BMI, and plurality (singleton vs. multiple gestation) [1]. Despite these robust differences, there is little agreement as to which of these differences are physiologic versus pathologic.

We carried out a study related to ethnic differences in birthweight for gestational age in the Canadian province of British Columbia [17]. We examined live and stillbirths in 1981–2000 and compared 4 ethnic groups of infants with substan-
tial prevalence in the British Columbia population: Chinese, South Asian, First Nations (‘American Indians’), and other (predominantly Caucasian). We found a substantially higher prevalence of SGA (based on a single, internal standard for the province) among South Asians at all gestational ages. For the Chinese population, SGA rates were not different from those of First Nations or others until term when they resembled rates among the South Asian population. SGA rates were actually lowest at term in the First Nations populations, who are well-known to have higher birthweights for gestational age [18, 19]. Yet despite these differences, gestational age-specific perinatal mortality rates were lowest among the Chinese, next lowest among the South Asian population, and highest among First Nations. Results for the Chinese and South Asian groups are likely to be affected by the ‘healthy migrant’ bias, but such a bias does not seem to protect them against higher SGA rates. It is difficult to understand how that bias would simultaneously lead to higher SGA rates and lower perinatal mortality rates. We therefore inferred that the lower weights of South Asian and Chinese infants were likely to be ‘physiologic’ [17].

In a more recent study based on the Swedish National Birth Register (which includes data on maternal height), we observed higher perinatal mortality rates in women of short stature and in primiparous (vs. multiparous) women [20]. Using marginal structural models to avoid the collider stratification bias that can occur in regression models including a causal intermediate, those higher mortality rates were ‘explained’ by the lower birthweight for gestational age among short and primiparous women. In other words, the smaller size seemed to be largely responsible for the higher perinatal mortality rates, suggesting that the smaller size of infants born to short or primiparous women can indeed be considered ‘pathologic’ [20].

It is also important to emphasize that at preterm gestational ages, all fetal growth references based on birthweight for gestational age are biased, because infants born at these pathologic (preterm) gestational ages tend to be significantly undergrown compared with the population of normal fetuses who remain in utero at those gestational ages [21]. Thus, increasingly, fetal growth at birth is evaluated based on ultrasound-derived estimates of fetal weight [22]. Two large international studies are currently under way to develop new reference curves of fetal weight for gestational age based on repeated ultrasound measurements in pregnancy.

**Etiology of LBW**

The major known causes of preterm birth probably do not appear to vary much among rich, middle-income, and low-income countries [1]. The leading identified causes of spontaneous (i.e. not induced or delivered by prelabor ce-
sarean section) include multiple birth, genitourinary tract infection, pregnancy-induced hypertension/preeclampsia, low prepregnancy BMI, incompetent cervix, cigarette smoking, prior history of preterm birth, abruptio placentae, prolonged standing and lifting at work, and cocaine use in pregnancy [1]. Most cases of spontaneous preterm birth remained unexplained, however, but nonspontaneous preterm births (those resulting from labor induction or prelabor cesarean delivery) are becoming increasingly large contributors to preterm birth, particularly in high-income countries [6, 23, 24]. In the United States, for example, preterm birth rates have increased to about 11–12%, with much of the increase over the last 20 years attributable to increased obstetric intervention [24].

Unlike the situation for preterm birth, the causes of SGA birth differ considerably among countries and regions [1]. Cigarette smoking is probably accountable for the largest fraction of SGA births in those countries where a sizeable fraction of women smoke during pregnancy. Other causes include low gestational weight gain, low prepregnancy BMI, primiparity, short stature, pregnancy-induced hypertension/preeclampsia, congenital anomalies, other genetic factors, and alcohol/drug use. In low-income countries where smoking during pregnancy is rare or nonexistent, nutritional factors (low gestational weight gain, low prepregnancy BMI, and short stature) account for a much larger fraction of the prevalence of SGA birth [1]. In endemic areas, malaria is another important cause of SGA birth, particularly among primiparous women [1]. Maternal morbidity due to common respiratory and gastrointestinal infections is also likely to contribute to SGA occurrence in such settings [1].

Few interventions have been shown to reduce the risk of preterm birth. Based on the most recent Cochrane reviews, effective interventions include intensive counseling to reduce cigarette smoking [25] and progesterone treatment of women with short cervix or prior history of preterm birth [26]. Reduced risk of SGA birth has been demonstrated with balanced energy/protein supplementation [27], intensive counseling to reduce smoking [25], and malaria prophylaxis [28, 29].

**Prevention of LBW: A Public Health Priority?**

LBW is highly associated with infant (and especially neonatal) mortality in both rich and poor countries [8, 30]. Moreover, countries with the highest infant mortality rates are also those with the highest apparent rates of LBW [10]. Because many developing countries cannot afford the technologies (e.g. neonatal
intensive care units) required to reduce mortality among LBW infants, the conventional ‘wisdom’ is that poor countries should emphasize prevention of LBW as a public health priority [31–33].

We carried out a study comparing temporal trends in infant mortality and LBW in several countries in the Americas (the United States, Canada, Argentina, Uruguay, and Chile) between the late 1980s and the late 1990s [34]. With the exception of Chile, which reported a slight reduction in their LBW rate, all of the other study countries reported increases in LBW, yet substantial reductions in infant mortality, over the decade. The countries’ infant mortality rates were highly correlated with their LBW rates in the late 1980s (Spearman r = +0.80) but much less closely correlated in the late 1990s (r = +0.25). The risk of infant mortality was negatively associated with the proportion of infant deaths occurring among LBW infants. The RR per SD increase in that proportion was 0.68 (95% confidence interval 0.67–0.68) in the late 1980s and diminished even further to 0.47 (95% CI 0.46–0.47) in the late 1990s.

Infant, and particularly neonatal, mortality is affected far more by preterm birth than by SGA birth [1]. Moreover, modifiable determinants such as maternal nutrition, smoking, and malaria affect SGA birth to a much larger degree than preterm birth [1]. To my knowledge, no country has successfully reduced its infant (or neonatal) mortality rate by preventing LBW. Yet many countries have succeeded in substantially lowering their infant and neonatal mortality without reducing their rates of LBW or preterm birth [7, 35–37]. In fact, because of increased obstetric intervention, preterm birth rates are rising in many high- and middle-income countries [6, 23, 24, 38]. Thus, prevention of LBW, and especially preterm birth, are elusive goals with limited success thus far. In my opinion, the major public health priorities should focus on the MDG goals of reducing maternal and young child (especially neonatal) mortality and on reducing stillbirth. Prevention of LBW has proved difficult and is not necessary to reduce neonatal mortality or stillbirth; though a laudable long-term public health goal, far more research is required to achieve it.

Disclosure Statement

The author declares that no financial or other conflict of interest exists in relation to the content of the chapter.
References

Fetal Malnutrition and Long-Term Outcomes

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Abstract

Epidemiological studies have shown that lower birthweight is associated with a wide range of adverse outcomes in later life, including poorer ‘human capital’ (shorter stature, lower cognitive performance), increased risk factors for later disease (higher blood pressure and reduced glucose tolerance, and lung, kidney and immune function), clinical disease (diabetes, coronary heart disease, chronic lung and kidney disease), and increased all-cause and cardiovascular mortality. Higher birthweight is associated with an increased risk of cancer and (if caused by gestational diabetes) obesity and diabetes. The ‘developmental origins of health and disease’ hypothesis proposes that fetal nutrition has permanent effects on growth, structure and metabolism (‘programming’). This is supported by studies in animals showing that maternal under- and overnutrition during pregnancy can produce similar abnormalities in the adult offspring. Common chronic diseases could potentially be prevented by achieving optimal fetal nutrition, and this could have additional benefits for survival and human capital. Recent follow-up of children born after randomized nutritional interventions in pregnancy provides weak evidence of beneficial effects on growth, vascular function, lipid concentrations, glucose tolerance and insulin resistance. Animal studies indicate that epigenetic phenomena may be an important mechanism underlying programming, and that nutritional interventions may need to start preconceptionally.

Fetal Undernutrition and Long-Term Outcomes

The first convincing evidence that fetal undernutrition could have a long-term influence on human health came from the follow-up of adults who were in utero during the Dutch Famine (‘Hunger Winter’) of 1944–1945. Young men whose mothers lived in famine-affected areas of the Netherlands during early pregnancy had an increased risk of obesity compared to men whose mothers
lived in non-famine areas (2.7 vs. 1.5%) [1]. Men whose mothers were exposed to famine in late pregnancy or early postnatal life had lower rates of obesity. The authors speculated that these findings reflected permanent effects of nutritional deprivation on fetal hypothalamic centers, causing lifelong changes in food intake and growth.

The science of ‘developmental origins of health and disease’ (DOHaD) started to attract intense interest some 20 years later, when Barker and Osmond linked birthweight (collected by health visitors in the UK from 1911 to 1930) with death certificate data and discovered that men and women who had a lower birthweight were at increased risk of death from cardiovascular and chronic lung disease [2]. Following on from this, a large number of birth cohort studies have now linked lower birthweight to other adverse outcomes in later life. These include reduced ‘human capital’ (shorter stature, lower lean body mass, and poorer cognition, educational achievement, work capacity, income and reproductive performance) [3], increased risk factors for later disease (higher blood pressure [3], central adiposity [4], insulin resistance [3] and stress responses [5], and reduced glucose tolerance [6], lung function [7], glomerular filtration rate [8] and immune function [9]), increased clinical disease (type 2 diabetes, coronary heart disease, chronic renal disease and chronic lung disease) [3, 6, 7], and increased all-cause and cardiovascular mortality [10]. The associations with risk factors for disease have been shown in children as well as adults. The associations extend across the range of birthweight, and are not limited to low birthweight (<2,500 g), although in some studies an upturn in risk is observed at high birthweights for some outcomes (see below, fetal ‘over-nutrition’). Most studies have been carried out in predominantly full-term births, suggesting that the phenomenon is linked to low birthweight for gestational age (interpreted as an indicator of fetal nutrition) rather than low birthweight due to preterm birth. However, there is some evidence that preterm birth is also a risk factor for cardio-metabolic outcomes like hypertension, insulin resistance and the metabolic syndrome [11].

Research in animals had already shown that transient environmental conditions, including nutrition, in early life could permanently alter or ‘program’ the body’s structure and function [12]. Subsequent work in animal models has shown that fetal undernutrition, achieved either by undernourishing the mother during pregnancy, or by impairing the fetal supply line (uterine artery ligation, placental reduction or gene knockout models that impair placental growth), produces permanent effects on a wide range of tissues and systems [13–15]. For example, there are several maternal undernutrition models in animals that produce obesity, insulin resistance and diabetes in the offspring, who show changes at whole animal level (e.g. sedentary behavior), tissue level (e.g. altered arrangement of cell types
in hepatic lobules, reduced cell numbers and vascularization of pancreatic islets), and molecular level (e.g. altered expression of genes in the insulin signaling pathway). This evidence from animal studies suggests that the associations between birthweight and later health in humans are likely to reflect the programming of a variety of tissues by intrauterine nutrition (fig. 1). The associations with birthweight occur because the same factors that perturb/program metabolic function
can also reduce fetal growth; animal studies have shown that programming can occur in the absence of reductions in birth size [13–15].

A consistent feature of the human studies is that the highest risk of cardiometabolic disease and its risk factors is in children or adults who had a low birthweight but became relatively heavy (fig. 2). This ‘small becoming big’ pattern is also seen in animal models, in which postnatal high-energy or high-fat feeding amplifies the adverse cardiometabolic effects of prenatal undernutrition [13–15]. It fits with the concept that the fetus programmed by undernutrition develops metabolic traits (higher blood pressure, insulin resistance, central adiposity) that make them vulnerable to disease when exposed to additional stressors in later life such as inadequate exercise, excess energy intake.

![Graphs showing IR-HOMA and glucose levels](image)

**Fig. 2.** Lower birthweight followed by the development of above-average weight or BMI in childhood or adulthood is associated with increased insulin resistance and impaired glucose tolerance (data from 3 Indian birth cohorts).
and obesity. It could also explain the recent rise in cardiometabolic disease in low-income countries undergoing rapid economic transition. This has raised the question of whether pediatricians should try and limit postnatal weight gain in lower birthweight infants. Even if this were possible, there is no evidence to support such a strategy in infancy; current evidence from low- and middle-income countries suggests that greater infant weight gain is associated with benefits for survival and human capital, and is neutral in terms of later cardiometabolic risk [3].

**Nutritional Interventions in Undernourished Pregnant Women**

The DOHaD concept has stimulated enormous scientific interest, but has had little impact on how the ‘common women’ think about nutrition in pregnancy, or on national policies on maternal nutrition. Human evidence for fetal programming is still largely based on observational data and on birth measurements, which are only crude indicators of fetal nutrition. However, the DOHaD hypothesis is beginning to be tested by studying children of undernourished women who took part in nutrition supplementation trials in pregnancy (table 1). If maternal undernutrition is an important cause of fetal undernutrition, better outcomes would be expected in children whose mothers were supplemented. The published trials include a variety of interventions and ages at follow-up, but fall into three groups (mainly protein-energy supplementation [16–18], multiple micronutrient, MMN, supplementation [19–21], or both [22]); all started in pregnancy, usually in the second or third trimester, and the offspring outcomes most frequently studied were growth and cardiometabolic risk factors.

In the INCAP trial in Guatemala, villages were randomized to receive either a protein-energy drink (Atole) or a low-energy drink (Fresco) which were supplied daily to pregnant women and children <7 years of age [16]. There was no significant difference between Atole and Fresco villages in birthweight. This is the oldest trial and the only one with adult follow-up data. It showed lower triglyceride and higher HDL cholesterol concentrations in men and women who received Atole before the age of 24 months (either given to the mother in pregnancy or to the children themselves postnatally). There were no significant effects on adult blood pressure or fasting glucose. In the Gambian trial, women received a daily high-energy biscuit, from 20 weeks of pregnancy (intervention group) or during lactation only (controls). The intervention certainly influenced fetal nutrition, increasing birthweight by a mean 136 g and halving perinatal mortality. In the adolescent offspring (11–17 years), there was a small reduction
### Table 1. Studies of growth and cardiometabolic risk factors in offspring of mothers who took part in nutritional supplementation trials during pregnancy

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Intervention in mothers</th>
<th>Effect of intervention on birth-weight</th>
<th>Sample size in offspring follow-up study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stein et al. [16]</td>
<td>Guatemala</td>
<td>Intervention: High-protein (6.4 g) and high-energy (3.8 MJ) drink (Atole) daily. Control: Low-energy (1.35 MJ) drink (Fresco) daily. Cluster randomized trial in 4 villages. Pregnant women and children &lt;7 years.</td>
<td>No</td>
<td>Group who received supplementation during fetal life (via the pregnant mother) and during the first 2 postnatal years ranged from n = 257 to 332.</td>
</tr>
<tr>
<td>Hawkesworth et al. [17]</td>
<td>The Gambia</td>
<td>Intervention: High-energy biscuit (1,015 kcal, 22 g protein) daily from 20 weeks' gestation to delivery. Control: Same high-energy biscuit daily for 20 weeks after delivery. Cluster randomized trial in 28 villages.</td>
<td>Yes</td>
<td>n = 1,317</td>
</tr>
<tr>
<td>Kinra et al. [18]</td>
<td>India</td>
<td>Intervention: Local food preparation made from corn-soya flour and soybean oil (2.5 MJ, 20 g protein) daily + health education and anemia control. Control: Health education and anemia control only. Cluster randomized trial in 29 villages. Women from diagnosis of pregnancy to delivery.</td>
<td>Yes</td>
<td>n = 1,165</td>
</tr>
<tr>
<td>Vaidya et al. [19]</td>
<td>Nepal</td>
<td>Intervention: Multiple micronutrients (UNIMAPP) daily. Control: Iron (60 mg) and folic acid (400 μg) daily. Individually randomized. Pregnant women from ~12 weeks' gestation to delivery.</td>
<td>Yes</td>
<td>n = 917</td>
</tr>
<tr>
<td>Stewart et al. [20]</td>
<td>Nepal</td>
<td>Intervention: Vitamin A + (1) Folic acid (400 μg). (2) Folic acid + iron (60 mg). (3) Folic acid + iron + zinc (30 mg). (4) Multiple micronutrients. Control: Vitamin A alone. Cluster randomized trial in 426 sectors. From early pregnancy to 3 months postpartum.</td>
<td>Yes</td>
<td>n = 3,524</td>
</tr>
</tbody>
</table>

**Group 2:** +37 g  
**Group 4:** +64 g
<table>
<thead>
<tr>
<th>Age of offspring at follow-up mean (SD)</th>
<th>Results</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>32 (4)</td>
<td>Atole group had lower triglycerides (−27 mg/dl, 95% CI: 5–49) and higher HDL cholesterol (+2.7 mg/dl, 95% CI: 0.3–5.0). No effects on glucose, blood pressure, total cholesterol or metabolic syndrome.</td>
<td>Only trial with adult follow-up. Small sample size. Unable to distinguish prenatal supplementation from supplementation in first 2 postnatal years.</td>
</tr>
<tr>
<td>14 (2)</td>
<td>Intervention group had lower fasting glucose (−0.05 mM, 95% CI: −0.10 to −0.001). No effects on body composition, blood pressure, lipid and insulin concentrations.</td>
<td>The original trial showed a large effect on birthweight. Age at follow-up (adolescence) may have masked effects.</td>
</tr>
<tr>
<td>16 (1)</td>
<td>Intervention group had lower augmentation index (arterial stiffness; –3.16, 95% CI: −5.51 to −0.8), lower insulin resistance (HOMA; −0.18, 95% CI: −0.32 to −0.04), lower lean body mass (−0.29 kg, 95% CI: −0.57 to −0.01) and taller height (+10 mm, 95% CI: 1.4–18.7). No effects on blood pressure, adiposity, lipids and glucose.</td>
<td>Age at follow-up (adolescence) may have masked effects.</td>
</tr>
<tr>
<td>2.5 years</td>
<td>Intervention group were heavier (+0.19 kg, 95% CI: 0.04–0.35) and had larger head (+0.23 cm), chest (+0.30 cm) and MUAC (+0.24 cm), but lower systolic blood pressure (−2.5 mm Hg, 95% CI: −4.6 to −0.5). No other risk factors were measured.</td>
<td>–</td>
</tr>
<tr>
<td>6.2–8.5 years</td>
<td>Triglycerides were lower in group 3 (0.98 mM) than in controls (1.06 mM). Metabolic syndrome was reduced in group 1 (8%) compared with controls (12%). Height was increased (+0.6 cm) and triceps (−0.25 mm) and subscapular (−0.20 mm) skinfolds were reduced in group 3 relative to controls. No effects on blood pressure, other lipids, glucose or insulin.</td>
<td>–</td>
</tr>
</tbody>
</table>
in fasting plasma glucose concentrations (mean $-0.05 \text{ mM}$) but no differences in adiposity, blood pressure, insulin concentrations or serum lipids [17]. In India, pregnant mothers and children under 6 years of age in intervention villages received food-based energy and protein supplements. At 16 years, the children had lower insulin resistance and arterial stiffness compared to children born in control villages [18]. There were no differences in their blood pressure or lipid concentrations. In both the Guatemala and Indian trials, offspring of intervention mothers were taller, and in Guatemala an increase in height was also observed in the next generation.

Two of the MMN trials (both in Nepal) showed a small increase in birthweight in the MMN groups [19, 20]. Two-year-old children whose mothers received MMN supplements during pregnancy were heavier and had larger head, chest and mid-arm circumferences and larger skinfold thickness, but lower systolic blood pressure, than children of control women, who received only iron and folic acid [19]. In the other trial, 7-year-old children whose mothers received vitamin A, iron, folic acid and zinc were taller and less adipose than children of control mothers (vitamin A alone) and had lower triglyceride concentrations, while children whose mothers received folic acid had a lower prevalence of metabolic syndrome than controls [20]. There were no effects on blood pressure,
other lipids, glucose or insulin. In a trial in Peru, infants of mothers who were supplemented with iron, folic acid and zinc were heavier and had larger chest circumference and calf muscle area than those of women who received iron and folic acid without zinc [21].

The Bangladesh ‘Minimat’ trial combined protein-energy and MMN interventions; women were randomized to receive food supplements ‘early’ (≈9 weeks’ gestation) or at the usual time (≈14 weeks), with (in a factorial design) either additional MMN or iron + folic acid. This is the only trial that attempted to correct both macronutrient and micronutrient deficiencies in the mother, and started in the first trimester of pregnancy, but it is so far published only in abstract form [22]. Early food supplements were associated with less stunting and lower LDL cholesterol concentrations in the children. MMN supplementation was associated with lower insulin concentrations, and, interestingly, more stunting.

These studies provide some evidence that improving the nutrient intake of undernourished human mothers in pregnancy has benefits for growth and cardiometabolic risk in the children, but it cannot be called strong evidence. All took place in low-income populations, where levels of cardiometabolic risk factors are still relatively low and where the opportunity for ‘becoming big’

<table>
<thead>
<tr>
<th>Age of offspring at follow-up</th>
<th>Results</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 year</td>
<td>Intervention group were heavier (+0.58 kg), and had larger calf muscle area (+36 mm²). No differences in adiposity (skinfolds). No other risk factors were measured.</td>
<td>–</td>
</tr>
<tr>
<td>5 years</td>
<td>Early food supplementation resulted in less stunting and a more favorable lipid profile. Multiple micronutrients resulted in more stunting, and lower IGF-1 and insulin concentrations.</td>
<td>So far published only in conference abstract form.</td>
</tr>
</tbody>
</table>
postnatally is low compared with high-income settings. This would tend to reduce any differences between children from intervention and control groups. If early pregnancy is a critical time for nutritional programming of adiposity, as suggested by animal studies [23] and the Dutch Famine studies [24], effects of supplements started later in pregnancy may be limited. The only study with adult follow-up (Guatemala) had a very small sample size, and the age at follow-up in the other trials (either in young children or adolescents) was not ideal for examining programming effects. Longer follow-up of these trials is needed, and data are required from studies in other populations and of other interventions, including preconceptional trials, before conclusions can be reached.

**Fetal ‘Overnutrition’ and Long-Term Outcomes**

Maternal diabetes during pregnancy exposes the fetus to an excess of nutrients. Diabetic mothers are not only hyperglycemic, but also have elevated circulating lipids and amino acids. The fetal pancreas and liver are stimulated to secrete increased insulin and insulin-like growth factors, resulting in the macrosomic infant of the diabetic mother. Freinkel [25] suggested 30 years ago that this could cause obesity and diabetes in later life (‘fuel-mediated teratogenesis’), and it is now established that gestational diabetes is a risk factor for later diabetes in the offspring [4]. There are therefore problems at both extremes of birthweight. In most populations, the predominant association of birthweight with later diabetes risk is linear and inverse, except in very large studies such as the US Nurses’ Study, in which gestational diabetes produces a small upturn in risk at high birthweights [6]. In populations with a very high prevalence of gestational diabetes, such as the Pima Indians, the curve becomes U shaped [6].

Lesser degrees of maternal glucose intolerance may also be associated with increased adiposity in the children. In a US study of 9,439 women routinely screened for gestational diabetes, there was a positive association, even in the non-diabetic group, between maternal glucose concentrations and overweight in the children [26]. Maternal insulin resistance and glycemia form part of the normal process of fetal nutrition, and we do not yet know the optimal levels of glucose and other fuels and nutrients in the mother. There is also increasing interest in whether maternal obesity, in the absence of diabetes, causes fuel-mediated teratogenesis and programs cardiometabolic risk in the children. In animal models, maternal obesity, or high-fat feeding during pregnancy, causes obesity, insulin resistance and diabetes in the offspring [15]. Like the diabetic mother, an
obese mother has increased circulating glucose, insulin, lipids and proinflammatory factors. Maternal obesity is associated with an increased risk of obesity and metabolic syndrome in the children [27], and children born to obese mothers after they underwent biliopancreatic bypass surgery have a lower risk of obesity than siblings born before surgery [28], but better evidence of a causal intrauterine effect is awaited.

Another important disease to mention in relation to higher birthweight (and thus possibly to a fetal ‘overnutrition’ effect) is cancer. There is consistent evidence that breast cancer and leukemia are more common among people of higher birthweight, and cancer mortality is higher in men of higher birthweight [10].

As mothers become heavier in almost all populations, the incidence of diabetes in pregnancy is increasing, and this is likely to make an increasing contribution to the burden of obesity and diabetes in future generations. This may be a particularly important phenomenon in transitioning populations, in which mothers who themselves had a low birthweight are at increased risk of developing gestational diabetes and thus exposing their offspring to fuel-mediated teratogenesis (fig. 3). Improved management of diabetes in pregnancy reduces fetal macrosomia, but it is not yet known whether this prevents later effects in the children.

**Fig. 3.** Intergenerational effects of fetal nutrition on diabetes risk. Intergenerational undernutrition results in low birthweight and a number of adverse later outcomes, including impaired human capital (left). Rapid childhood or adult weight gain on a background of low birthweight is associated with an increased risk of cardiometabolic disease (left to right arrow), and (in women) with gestational diabetes, which exposes her fetus to excess fuel-mediated teratogenesis, another route to increased diabetes risk (right).
Possible mechanisms for the long-term programming of health and disease by fetal nutrition have been extensively reviewed [15, 29, 30]. The simplest mechanism is inadequate growth and/or remodeling of tissues due to inadequate substrates at critical periods of development. Fetal tissues develop during specific times and in a specific order, and inadequate nutrients at these times could lead to permanently reduced cell numbers, and/or altered structure due to selection of more ‘robust’ alternative cell types. There is good evidence for this in the kidney; protein deprivation in pregnant animals during fetal nephrogenesis results in smaller offspring nephron number, and later hypertension [30]. There is also evidence from animal studies of tissue remodeling in the pancreas, liver and hypothalamus in response to fetal undernutrition.

Remodeling of tissues that regulate endocrine and metabolic pathways could have wide-ranging effects. For example, the hypothalamus is the main brain center regulating appetite and feeding behavior, and monitors and responds to signals about nutritional state (the presence of food in the gut, circulating fuels, stored fat and glycogen). Different populations of hypothalamic cells stimulate or suppress food intake via projections to other brain areas, for example altering feeding behavior. Animal research shows that the cell proliferation, migration, differentiation, growth and apoptosis required to make these projections can be altered by the nutritional environment during fetal development [31]. Leptin and insulin, which are important fetal growth hormones and respond to fetal nutrition, are thought to regulate this hypothalamic development.

The above mechanisms would have to involve changes in gene expression, and there is great interest currently in effects of fetal nutrition on epigenetic phenomena, such as DNA methylation, that have a role in switching genes on and off. Patterns of DNA methylation are substantially established during embryogenesis and fetal development, and are sensitive to the nutritional environment. For example, maternal protein restriction during pregnancy, which causes hypertension and increased adiposity in rat offspring, appears to act, at least partially, through altered methylation and expression of specific genes involved in energy and lipid metabolism [32]. Both the altered methylation and the later abnormalities are prevented by supplementing the maternal diet with folic acid. Human data are limited, but epigenetic changes have been shown in newborns whose mothers took part in a randomized controlled trial of preconceptional MMN supplements, in Gambian children conceived in the ‘hungry’ versus the ‘harvest’ season, and in adult offspring.
of women exposed to the Dutch famine [33]. Epigenetic variation in umbilical cord tissue has also been related to later outcomes such as childhood adiposity [34].

Epigenetic patterns can be inherited, which could explain how transient alterations in fetal nutrition can alter body composition and metabolic parameters across more than one generation [29]. Epigenetic perturbations in a limited number of key metabolic genes could also explain the striking phenomenon in animal models whereby widely differing nutritional interventions in the mother (from global nutrient restriction to high-fat feeding) apparently result in the same ‘metabolic syndrome’ phenotype in the offspring [30]. Nutritional effects on epigenetic characteristics could produce the ‘plasticity’ of phenotype in early life that is inherent to the concept of fetal programming. Furthermore, this type of plasticity means that the programming of long-term outcomes may not require major nutritional deficits during organogenesis and differentiation, but could result from short-lived and subtle changes in the nutritional environment at stages of development when nutrient demands for growth are still quite small, such as during the periconceptional period and early embryogenesis [23]. A further intriguing aspect of epigenetic programming is that it could act through paternal as well as maternal nutrition. Offspring of male mice fed a low-protein diet from weaning until sexual maturity had increased hepatic expression of genes involved in lipid and cholesterol biosynthesis [35]. In future, we may need to consider the nutrition of fathers as much as that of mothers among the intergenerational determinants of health and disease.

Disclosure Statement

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

References


Comments by Discussant

The workshop audience listened to two excellent expert presentations by Dr. Kramer and Dr. Fall on low birthweight (LBW) caused by either preterm birth or intrauterine growth retardation. One important point when looking at the global figures on the prevalence of both conditions is the fact that very few reliable data are available from Sub-Saharan Africa and parts of Latin America and South Asia. Therefore, our knowledge is mostly derived from affluent populations.

Twinning is a contributing factor in LBW, and besides the natural occurrence of this phenomenon, there are iatrogenic increases in the number of preterm deliveries by a rising number of early caesarean sections and by extracorporeal fertilization; the common practice of implanting more than one embryo adds to the number of multiple-fetus pregnancies in affluent populations.

The prognosis of LBW babies depends not just on pathophysiological causes but essentially on the environment the child is born in: socioeconomic, cultural, and physical conditions determine the risk of LBW [1–3] as well as the associated perinatal, neonatal, and infant mortality. Intergenerational undernutrition affects the mother and child dyad, requiring a broader approach to prevention. When do the 1,000 days start? Increasingly, the prepregnancy nutritional status of the mother gets identified as a determining condition of the pregnancy outcome.
Dr. Kramer states that few interventions have been shown to reduce the risk of preterm birth. He refers to the Cochrane review on the impact of cigarette smoking [4] and on progesterone treatment [5]. When he finds that ‘reduced risk of SGA birth has been demonstrated with balanced energy/protein supplementation [6] and malaria prophylaxis’ [7, 8], the observation of an effect of preventing undernutrition and malaria during pregnancy clearly relates to the context. Causes of preterm delivery in industrialized countries are difficult to address by preventive measures, but preterm delivery in resource-poor countries and those with a high number of poor families are essentially socioeconomic, e.g. heavy workload on pregnant women, partly infectious due to a high prevalence of sexually transmitted infections and placental malaria, and partly nutritional.

Definitely, wherever the management of preterm babies and SGA neonates has improved, perinatal and neonatal mortality has gone down. Many programs focusing on improved neonatal resuscitation and care try to expand this effect – but it must be questioned if this aspect justifies Dr. Kramer’s conclusion: don’t focus on prevention but on better management of LBW. The truth probably lies in the context and not in the problem to be solved: improving the health and nutritional status of women living in poverty is a promising intervention for preventing LBW [9]. But, observations from Brazil also support Dr. Kramer’s conclusion [10].

The second question raised was, does one size fit all? Here, Dr. Kramer made very clear that there are differences in birthweight of girls and boys, but lower birthweight in girls is not a disadvantage regarding health. Boys have higher morbidity and mortality rates. Therefore, interventions will continue to focus on fetal growth independent of sex. The WHO Multicenter Growth Study has shown that the differences between infants and preschool children from Asia, Europe, North and South America and Africa are not relevant if the mothers had a normal health and nutritional status [11].

An interpretation of birthweight data trends in the sense of ‘small is beautiful’ does not fully satisfy if we adopt a critical approach: an increased birthweight might be caused by gestational diabetes mellitus, and as the prevalence of this condition is increasing worldwide, it may be necessary to carefully check for cofounders when a simple message is derived from the observed trends.

When Dr. Kramer states that ‘prevention of LBW has proved difficult and is not necessary to reduce neonatal mortality and stillbirth’ and takes this further to the point ‘the major public health priorities should focus on the Millennium Development Goals of reducing maternal and young child (especially neonatal) mortality and on reducing stillbirth’, it is debatable if this is not dependent on
the environment. In affluent societies, the conclusion might be justified; in populations living in poor conditions, prevention of LBW has its place in reducing LBW and postnatal mortality [12].

**Discussion**

Dr. Kalhan mentions the secular trend as responsible for lower SGA rates; for the Arab Gulf countries, a secular trend is well documented; the main driving forces are the reduction of smoking during pregnancy and an increase in gestational diabetes mellitus. The environment cannot be changed quickly.

Pulmonary tuberculosis is regarded as a cause of a larger than expected fraction of LBW in developing countries [13].

Dr. Koletzko raises the question if ethnic reference standards for child growth are valid when it comes to cutoff levels for pathologic conditions like obesity or LBW. When a different nutritional status has no adverse health effects, Dr. Kramer pleads for ethno-specific references for birthweight.

Dr. De Curtis mentions the large variability of LBW prevalence rates in European countries; this directs towards national references.

Regarding birthweight references, the WHO sticks to a Californian standard of the early 1990s; fetal growth standards are not very good; studies on international references for term babies are ongoing.

Dr. Ramakrishnan asks why we are interested in preventing LBW when LBW is associated with lower infant mortality rate. She expresses the need for more information on normal fetal growth. From the WHO-MCGS, we learned that ethnic differences in early child growth are small. This implies the question if prenatal growth is different from postnatal growth? There is little variation in fetal growth – besides the effects of smoking – but a great variation in child growth.

Dr. Bloomfield points to the fact that the definition of small for gestational age is based on birthweight, not on fetal growth. The observed higher rate of pregnancies in women of short stature indicates that variables beyond physiological and medical conditions need to be considered. Finally, it is to be taken into account that gestational age is poorly measured in many – if not most – circumstances. Therefore, the figures for LBW may be largely underestimating the number of premature-born babies.

The discussion somehow concluded that LBW is a highly composite variable.

Dr. Fall in her presentation refers to the offspring of famine-exposed mothers in the Netherlands and the observation of David Barker and others that LBW is associated with higher risk of diabetes mellitus type 2 and coronary heart disease
in later life. Data from Hertfordshire show that low weight at age 1 year is determined by LBW as well.

Pathophysiologic changes in LBW babies have a number of long-term consequences, e.g. reduced human capital – an extremely composite variable. Parental obesity also confers the risk of noncommunicable diseases in later life of LBW babies [14]. But, interventions restricting food intake in LBW babies are not recommended because of the immediate and long-term benefits undernourished children have from food supplements and catch-up growth. Type 2 diabetes mellitus is also triggered by gestational diabetes.

Dr. Fall concludes that specific nutritional requirements during pregnancy may be low but sensitive for programming. The role of paternal nutrition (biological aspects) and the time of exposure during pregnancy impact on programming effects are largely not yet understood. In the Gambia supplementation during pregnancy trial, lower fasting glucose levels were found in the intervention group at age 11–17 years. Provision of iron, folate and zinc resulted in less metabolic syndrome, but multiple-micronutrient supplementation trials did not lead to changes in glucose metabolism of the offspring. In a study from Bangladesh, lower glucose, LDL cholesterol and stunting rates have been reported after food supplementation in pregnancy [15]. In general, there is no strong evidence for nutritional interventions during pregnancy. The variation in study designs is great. In addition, preconceptional trials are needed, preferably food-based preconceptional interventions.

Dr. Uauy raised the question how to measure catch-up growth properly. Prenatal weight gain is rather due to the gain in lean body mass. There is a need for further research in the underlying process of pre- and postnatal weight gain. In addition, Dr. Barclay mentioned that there are no data on associations between the fat content of the maternal diet and the pregnancy outcome.

Dr. Kalhan states that the best evidence for an effect of nutrient intervention during pregnancy comes from folate supplementation. He also underlines the need for more studies in humans to better understand the impact of dietary interventions during pregnancy.

Dr. Makrides asks if we may be overestimating data from animal and epidemiological observations regarding fetal growth, and urges to design animal experiments in a way to make them more similar to human situations.

Dr. Vaidya points to the fact that catch-up weight gain in the first 2 years of life is beneficial, whilst weight gain later in childhood is associated with higher risks.

Dr. Ziegler argues that even if there is some evidence of accelerated growth in preterms being associated with a higher risk of metabolic syndrome later in life, the immediate and short-term benefits of catch-up growth outweigh these risks.
Dr. Gibson points to ethical concerns with intervention studies during pregnancy inferring short-term or long-term adverse effects on the offspring.

The first session of the workshop raised the awareness of the participants on how little is known about the mechanisms of nutrition and nutrition interventions in fetal and metabolic programming. In addition to the immediate aspects of nutrients, water and food energy, the impact of bioactive components in the diet on programming is not addressed by any known research activity.

Michael B. Krawinkel

References

Abstract

The need to prevent low birthweight (LBW) defined as a birthweight ≤2,500 g is presently well recognized, not only because of the immediate consequences increasing the risk of neonatal death and burden of disease but also in terms of the impact of being LBW on lifelong health and well-being. Children are born LBW (<2,500 g) either because they were born too early (true preterm LBW infants) or alternatively they failed to grow adequately despite a normal duration of gestation (intrauterine growth retardation IUGR). In this later case, the weight may be over 2,500 g, but the infant is lighter than expected for his/her gestational age. In fact, many preterm infants are to some degree growth retarded. Despite the differences in origin, all LBW categories are considered at increased risk of neonatal death and later morbidity.

Preventive actions are more likely to succeed if we consider the nutritional interventions as part of a package that addresses in a holistic manner the full spectrum of needs of women from before conception as well as during pregnancy. We have gained sufficient experience with single nutrient and/or ‘magic bullet’ approaches to learn from this and avoid them in the future. New fetal growth standards (INTERGROWTH 2012) represent major progress in terms of evaluating the effect of early life events on later growth, health and well-being. Thus, for the first time, clinicians and researchers will have sequential longitudinal data that will serve to characterize whole body as well as brain, liver, and long bone growth, relating this indirectly to placental blood flow and transfer function, neonatal health, morbidity and mortality.
Introduction

The need to prevent low birthweight (LBW) defined as birthweight ≤2,500 g is presently well recognized, not only because of the immediate consequences increasing the risk of neonatal death and burden of disease but also in terms of the impact of being LBW on lifelong health and well-being. Children are born LBW (<2,500 g) either because they were born too early (true preterm LBW infants) or alternatively they failed to grow adequately despite the duration of gestation (intrauterine growth retardation, IUGR). In this later case, the weight may be over 2,500 g, but the infant is lighter than expected for his/her gestational age. So, we may have LBW due to preterm birth or IUGR; in addition, most preterm infants are to some degree growth retarded [1]. Despite the potential differences determined by the suggested groupings, all LBW categories are considered at increased risk of neonatal death and later morbidity. In addition, among the LBW, there might be true IUGR and constitutional small for gestational age (SGA; i.e. children that are small but are growing according to all their potential) [2].

The relative contribution of each of these conditions to the prevalence of LBW will differ by country depending on prevalence of infections, undernutrition, sanitary conditions, access and quality of the health care system among several factors [3]. In order to dissect the contribution of maternal nutrition to the burden of LBW, there is a need to define as accurately as possible the relative contribution of each of these conditions to the problem [4]. Defining effective preventive strategies requires that we have the best possible estimates of how these factors operate in isolation and in synergy with each other. Ideally, LBW prevention should be cause specific in order to enhance the opportunity for effective action (see fig. 1).

The implications of this model to LBW prevention – the topic of this paper – are multiple. Firstly, it may serve to guide what is the most effective single intervention based on the estimated population-specific attributable risk or what are the best combined interventions in a given setting considering the additive or synergistic interactions between the selected actions [5]. Unfortunately, few nutritional intervention studies have been designed with a broad conceptual model in mind; in most cases, single nutrient interventions are defined by the priorities established by the funding agency or the investigator’s interest. The model may also serve to explain why in most cases effective actions to prevent LBW require that we affect multiple factors amongst which nutrition is only one of them. It may also serve to explain why a given intervention may be very successful in one setting while in others show moderate or null impact. Preventive actions are more likely to succeed if we consider the nutritional interventions as part of a package that addresses in a holistic manner the full spectrum of needs of women from before conception as well as during pregnancy. We have gained sufficient experi-
ence with single nutrient and/or ‘magic bullets’ approaches to learn from this and avoid them in the future. We must examine the causal web for LBW in a context-specific manner before we define what we need to do to prevent it; there are clearly no solutions that we can think of that will work under all conditions. Unfortunately, establishing the relative contribution of each primary factor and underlying conditions causing LBW is very difficult, particularly in poor countries where this differentiation is most critical, given the high prevalence of LBW [6].

In most of developing countries, even something as basic as establishing the timing of conception is a difficult task, early ultrasound is rarely available except in the larger cities, and even there it will be available only to those that can afford it. Even if it were available, it might not be used early enough, since most women seek medical care well advanced into the pregnancy. An alternative would be to use an indirect measurement of gestational age such as date of last menstrual

**Fig. 1.** Conceptual model to assess fetal growth and root causes of LBW. Key factors are duration of gestation and fetal nutrient balance. Fetal nutrient balance is affected by maternal factors such as maternal height (linked to early growth of the mother), prepregnancy BMI, associated with maternal energy reserves (fat stores), placental blood flow and function; all are key to supply the fetus with energy, and the essential nutrients required for normal growth (glucose, essential amino acids, AA, and fatty acids) provided through the placenta are key determinants of fetal anabolic hormonal responses leading to normal or abnormal growth and tissue deposition. Maternal stress due to infection or other factors can compromise fetal growth.
period; however, in most places the first visit might be at a late stage in pregnancy when most of women do not recall this date. Even if we are able to establish that the fetus is a true IUGR rather than a constitutional SGA, we might not be able to really confirm this diagnosis (fig. 2) since the predictive models based on clinical information perform rather poorly and the variables considered are not readily available in a developing country setting [7].
Intervention Strategies for Preventing LBW in Developing Countries

Appropriate intrauterine growth is key to ensure survival in the first days of life; adequate liver glycogen stores are vital to prevent hypoglycemia and avoid its consequences. The maturation of intestinal function is of vital importance since appropriate carbohydrate and protein supply is vital to secure early recuperation of the depletion typical of infants born IUGR. The early phase of recovery of IUGR is an important factor in determining later linear growth during infancy and final adult stature. Further evidence suggests that there are transgenerational effects of being born IUGR, thus affecting the growth of the next generations (fig. 4). The IUGR condition has been associated with poor school performance and low adult productivity; thus, optimizing fetal and early postnatal growth becomes a key factor in human capital formation, especially in developing countries. More recently, birthweight has also been associated with a myriad of outcomes that impact adult mortality and morbidity such as cardiovascular disease, some forms of cancer,
diabetes, obesity and stroke [8, 9]. In fact, these observations support the concept of the early origin of adult diseases hypothesis, recently coined as Developmental Origins of Health and Disease. However, there is great heterogeneity in the prevalence of these later outcomes; not all LBW children will develop diabetes or will have decreased cognitive performance; the short- and long-term outcomes of LBW will be also dependent on the underlying cause leading to LBW.

There is now a better understanding of the mechanisms that link early life events to later health. The proposed hypothesis is that events taking place in the intrauterine period and the first months of life induce epigenetic changes (DNA methylation and/or histone modifications that affect gene expression without changes in DNA sequence) [10, 11]; in some cases, the changes are associated with an advantage to withstand the detrimental conditions that induced them, and thus may have a clear short-term benefit. However, this adaptation may render a disadvantage under environmental conditions in later life. For example, acquiring peripheral insulin resistance may be of relevance to the IUGR fetus in order to support fuel supply for the brain; however, maintaining this condition would be deleterious in later life since it will favor central obesity and increase the risk of diabetes and hypertension [11].

Epigenetic modifications induced by malnutrition or by specific nutritional deficits such as, folate, vitamin B12 and low-protein diets have been described. These observations suggest that epigenetics may not only provide the biological explanation to link LBW to long-term health, but also it might provide us with the opportunity to test the efficacy of nutritional supplementation to revert the adverse long-term outcomes [12]. The interpretation of the observations leading to Developmental Origins of Health and Disease, particularly in developing countries, may also be confounded by the presence of socioeconomic and other environmental conditions that may impose additional risk for chronic diseases in adult life as well as limiting fetal growth. In fact, these structural causes are probably most important in explaining differences in LBW prevalence between countries; but they do not explain variation within a given population. We recognize the importance of taking the necessary actions to decrease income inequalities and access to food and health by addressing the economic and political structural factors that condition LBW; however, this is not the focus of this paper.

**Genetic Influences on Fetal Growth**

This is perhaps most clearly demonstrated by the consequences of an abnormal number of chromosomes (aneuploidy). Turner’s syndrome (45 X0), trisomy 21, trisomy 13 and 18, triploidy and polyploidy are all associated with poor fetal
growth [13]; experimental studies have shown slow cell division in trisomic or triploid cell lines. Aberrant fetal growth also accompanies non-aneuploid disorders. Hereditary gigantism (Sotos syndrome) and the genetic forms of dwarfism represent extremes. Uniparental disomy (abnormal parent-of-origin genetic expression) of chromosome 7 is associated with poor growth in Silver-Russell syndrome [14–16], while a similar abnormality of chromosome 11 leads to fetal overgrowth in Beckwith-Wiedemann syndrome (BWS) [16, 17]. The effect of the environment on the early development of the embryos transferred after in vitro fertilization (IVF) characteristically incubated under high glucose concentrations offers further insights into this phenomenon; the distribution of birthweights of infants born from IVF is displaced to the right (towards higher birthweights); moreover, there is an increased incidence of BWS in babies born after IVF procedures [18]. In summary, genetic makeup has a profound influence on fetal growth. Multiple genes influence the variability in birthweight observed among different ethnic groups, ranging from a mean birthweight of 2,400 g in pygmies to a mean of 3,500 g or greater in affluent populations of industrialized countries. The effect of the genotype is also evident in the greater birthweight among males, averaging 150 g greater than in females at term.

Uterine Milieu and Placental Influences

Environmental influences on fetal growth include the placental structure and function, uterine and placental blood flow, and local umbilical circulation [19, 20]. Taken together, these flows determine substrate and oxygen flux available to the growing fetus as well as the clearance of excretion products and carbon dioxide [21]. In general, the placenta and baby grow proportionately; large babies have large placentas and small babies, smaller placentas; normally, the placenta weighs about 20% of the baby’s weight. Conditions that compromise placental localization, size and function such as abnormal uterine anatomy, ectopic placental implantation, placental abruption or infarction, placental hemangioma or arteriovenous shunts, congenital infections, and abnormal cord insertion may adversely affect fetal growth [22]. Increased placental vascular resistance measured as high resistance to blood flow at the umbilical artery documented by Doppler ultrasound and imaging has served to document the key role of blood flow, and hence substrate flux, in IUGR associated with tobacco smoking, preeclampsia and/or long-standing hypertension, maternal gestational diabetes with vascular compromise [21, 23]. The placenta also influences fetal growth by secreting placental hormones and growth factors in addition to regulating blood flow; the placental surface area defines maternal-fetal
exchange. The placenta also affects fetal growth indirectly through modification of blood and substrate flow or directly by regulation of cell replication and differentiation [24].

**Maternal Nutrition**

Maternal overall nutrition is critical for fetal growth, although the mother tends to buffer the effect of adverse environmental conditions on the fetus. Both pre-gestational weight and weight gain during pregnancy are positively correlated with infant birthweight. The classic studies of the Dutch famine during World War II showed a mean birthweight reduction of 300 g among infants whose mothers suffered severe caloric deprivation during the last trimester of gestation [25]. In previously well-nourished mothers, caloric deprivation must be quite extreme before fetal growth is compromised. However, in women from developing countries, where malnutrition is entrenched over generations, a moderate energy deficit may have an adverse effect. In this regard, studies have shown that maternal height, partly the result of early nutritional influences, has a positive association with birthweight [26–29]. After 2 years of age, the growth of the infant correlates better with mean parental height rather than maternal height alone; genetic factors contributed by both parents are important in determining final size while early maternal nutrition, reflected in maternal height, is the major determinant of fetal growth. The effects of maternal size may be multigenerational. Mothers who were SGA at birth are at greater risk of having an SGA or preterm baby [30]. These effects may be mediated through the size of the uterus and its capacity to hypertrophy and increase blood flow in response to pregnancy. First-born infants on average weigh less than subsequent infants. Mothers <15 and >35 years of age have a higher incidence of LBW babies, only partially explained by parity and socioeconomic risk factors. Maternal nutrition, especially in adolescents, and uterine and placental factors are thought to play a role. The practical consequence of these findings is that for research purposes and even in clinical use the evaluation of the adequacy of fetal growth and birthweight should take into consideration infant sex, maternal height and birth order in addition to gestational age. In addition, access to adequate food by women may be limited by social, cultural and/or economic factors; in many societies, women are given lowest priority in access to quality foods and thus are most affected by malnutrition, especially micronutrient deficits. This is often missed in studies of household food security. Unless these factors are considered, we will miss undernutrition in our assessment of the relative importance of maternal malnutrition. Discrimination of women on religious or cultural grounds is
also often a cause of maternal malnutrition; this is particularly the case in low-income countries where young adolescent women are often exploited and have to work under conditions of near slavery.

**Micronutrients**

Micronutrient minerals and vitamins are increasingly recognized as agents that affect embryogenesis and the incidence of congenital malformations. Folate intake before and during early embryogenesis (fig. 3) alters the incidence of neural tube defects [31]. The involvement of a genetic component for neural tube defects is evident in the high rate of recurrence in families and individual mothers. Neural tube defects are also more frequent in certain ethnic groups [32]. If neural tube defects were solely genetic, prevalence should not vary over time, yet neural tube defects are more frequent in periods of nutritional deprivation, such as during the 1944–1945 Dutch Famine [33]. These observations led to the discovery of the striking benefit of maternal administration of doses of 400 μg of folic acid preconceptionally [34]. Folic acid from food sources must be reduced to tetrahydrofolate before it is metabolically active. This is genetically determined by the activity of methylenetetrahydrofolate reductase, 35–50% of mothers bearing children with neural tube defects have been found to have low enzymatic activity, thus accounting for the protective effect of folate supplementation [35]. Heterogeneity in receptor-mediated folate transport may explain additional susceptibility to neural tube defects unrelated to reductase activity [36]. Retinoic acid, derived from retinol (vitamin A), is a regulator of gene expression and a teratogen early in embryonic development. Of interest, retinoic acid decreases the risk of spina bifida in animals. The folate receptor gene is a target for retinoic acid transcriptional regulation, providing a possible explanation for folate-retinol interaction. Regulation of folate receptors may explain the occurrence of neural tube defects in association with low vitamin A intake. Maternal zinc deficiency has also been implicated in abnormal fetal growth and enhanced susceptibility to such teratogens as alcohol, valproic acid and arsenic. There have been a number of interventions that provide single or multiple micronutrients to pregnant women with the objective of decreasing LBW. These studies have shown that the direction and magnitude of the results depends on the baseline nutritional status and possibly the genetic background of the population; thus, the specific combination of micronutrients needed should be defined based on the assessment of micronutrient status of the particular population to be intervened. Several participants in this workshop have addressed and discussed in detail specific micronutrient interventions and where the evidence stands regarding their efficacy and effectiveness.
Gene-Nutrient Interactions

The beneficial effect of folic acid is only one example of the relationship between genetic makeup and diet. The interaction of genes and the early diet not only determines brain development, growth and body composition but also the later prevalence of nutrition-related chronic disease and some types of cancers. Genes are differentially expressed depending on the exposure to the epigenetic nutrients and toxicants. Thus, a similar genotype may define multiple phenotypes. Regulation of gene expression can occur at multiple levels. Nutrients can bind to specific or non-specific ligands that interact with response elements in DNA. Nutrients may change the phosphorylation status of a protein and thus its activity. At the posttranscriptional level, nutrients may modify native RNA processing, mRNA transport and stability, and breakdown rates. Nutrients may modify the rate of mRNA translation. Finally, nutrients can modify the turnover rates of enzymes and other proteins, thus affecting their activity level. Pregnancy is undoubtedly one of the periods of life in which genes can be regulated by epigenetic changes. Several studies have already shown how caloric restriction or exposure to particular micronutrients before and during gestation induces lasting epigenetic modification in the offspring; it is thus important to consider the future impact that these changes might have on lifelong health; based on present evidence, it is plausible that they will define the future predisposition or amelioration of risk to develop chronic diseases.

Maternal Medical Disorders

Preeclampsia, chronic hypertension, collagen vascular disease and renal disease all affect fetal growth by compromising maternal nutritional status and interfering with uterine and placental perfusion. Severe maternal anemia and diminished cardiac output secondary to heart disease and/or cyanotic congenital heart disease may affect fetal growth by decreasing oxygen availability to the maternal uterine compartment. Early abnormalities in embryonic fuel metabolism in pre-diabetic or diabetic mothers may play a role in determining abnormal fetal growth and may also be teratogenic; later in gestation, maternal glucose elevation induces fetal hyperglycemia leading to hyperinsulinism and macrosomia with enhanced growth of peripheral adipose tissue, muscle hypertrophy and increased liver glycogen stores. The goal of preventive health care and nutrition before and during pregnancy must be to avoid both IUGR and macrosomia. Maternal obesity and its associated metabolic complications are increasingly being observed even in low-income population settings worldwide. Pregnancy is itself a state of metabolic stress; thus, the combination of these factors may lead an increased risk
of abnormal fetal growth. This is a very challenging field since ideally preventive actions need to be taken preconceptionally. Only if the latter were not possible should we carefully attempt to control the disease condition during pregnancy in order to avoid disarrangements that might affect the fetus or the pregnancy.

**Intrauterine Infections**

Several maternal infections including rubella and cytomegalovirus infections can impair fetal growth. Toxoplasmosis, syphilis and herpes infections, although less frequent during the first trimester, may affect fetal growth by arresting cell replication during critical stages of development, causing typical patterns of malformations and severely compromised growth. Similarly to what was mentioned with respect to other maternal factors, in order to decrease intrauterine infections it is important to address this issue in a comprehensive way, examining women’s societal role. In the case of maternal infections, potential interventions to decrease the risk to the offspring need to consider early screening and treatment.

**Other Environmental Influences on Fetal Growth**

Altitude is associated with diminished fetal growth due to lower ambient oxygen tension. However, in human populations there are multiple confounders that may obscure the relationship; for example, general populations living at higher altitude have been displaced from the more fertile lower valleys by the colonization process, so they have less access to quality foods, or are food insecure; they usually have more children, are of lower income and are less likely to have adequate sanitation. Exposure to ionizing radiation (X-rays) has been associated with microcephaly and abnormal fetal growth. Organic solvents and heavy metals, especially mercury and cadmium, have been associated with malformation and compromised fetal growth. Smoking, especially in the last trimester of pregnancy, reduces birthweight and length; the effect is proportional to the number of cigarettes smoked. Pre- and postnatal growth failure and microcephaly characterize the fetal alcohol syndrome. Growth restriction occurs in infants born to mothers addicted to heroin, cocaine or methadone. Other drugs with adverse effects include anticonvulsants (dilantin, phenobarbital and tegretol), antifolates (methotrexate), coumadin and prednisone. Some of these exposures may be not modifiable or at least not in an easy way (i.e. altitude); however, other factors such as exposure to pollutants or the consumption of toxic deleterious substances during pregnancy may be targeted through campaigns and directed follow-up of the pregnant women.
Interaction of Nutrients and Hormones during Perinatal Growth

Fetal growth also is affected by overall nutrition. Glucose supply, a key energy substrate, is also a mediator of insulin secretion; in turn, the action of insulin and insulin-like growth factors (IGF-1 and -2) affects fetal growth. Insulin regulates fetal lipogenic activity and has a permissive role in protein synthesis and hepatic glycogen deposition. Fetal hyperinsulinism results in increased adiposity in human infants of diabetic mothers. Conversely, fetuses with insulin deficiency secondary to pancreatic agenesis or with a defective insulin receptor have marked IUGR with decreased adipose tissue and little weight gain during the last trimester of pregnancy (Leprechaun syndrome).

Protein feeding as well administration of several essential and non-essential amino acids stimulates insulin secretion in the fetus and neonate. Increasing arginine levels during parenteral infusion has also been shown to increase serum insulin levels, lowering glucose and promoting growth hormone release by the anterior pituitary gland. The correlation of urinary excretion of the insulin precursor C-peptide with weight gain suggests that insulin behaves like a growth-promoting factor for infants on high-protein diets. Preliminary evidence from controlled clinical studies in extremely small preterm infants has shown increased tolerance to glucose and higher weight gain in infants infused with insulin during their initial postnatal days.

Other peptides such as insulin-like growth factors (IGF-1 and -2) act as growth factors influencing fetal growth and maturation. In the fetus, these act independently of growth hormone. IGF-1 influences terminal differentiation of a number of tissues, including brain astrocytes, neural outgrowth and myogenesis, and even though the influences of IGF-1 appears to be local; serum concentrations of IGF-1 correlate with birthweight. Both IGF-1 and -2 are complexed to binding proteins that modulate their biological activity. After birth, higher levels of IGF-1 are observed in IUGR infants during catch-up growth, especially in association with length gain. Epidermal growth factor and TGF-α also influence growth and differentiation of epithelial cells in lung and gut. Receptors for EGF are present throughout development and are increased in a number in placenta and lung in fetuses with growth restriction induced by uterine artery ligation, suggesting a role for EGF in fetal growth retardation. Maternal leptin, a circulating polypeptide hormone expressed by adipocytes and placenta, is positively related to fetal leptin concentrations affecting fetal weight. Additionally, thyroxin and glucocorticosteroids have important influences on specific organ development and functional and metabolic adaptation, but relatively little influence on fetal somatic growth. However, excess glucocorticoids as well as other hormones have been shown
to promote cell differentiation and maturation but arresting cell division, thus potentially compromising organ growth and development.

**Placental Nutrient Metabolism and Transport**

The placenta transfers metabolic substrates, oxygen and other nutrients from the mother to the rapidly growing fetus. In addition, the placenta allows for the excretion of fetal waste products and performs important metabolic and hormonal functions. The human fetal villi are directly bathed by maternal blood; therefore, the fetal capillary circulation is separated from maternal blood by placental vascular endothelium and connective tissue and the placental epithelium composed of the cytotrophoblast and the syncytiotrophoblast [37]. A clear understanding of placental ultrastructure is necessary in order to discuss the functional correlates.

The placenta grows at a very rapid rate during the initial stages of pregnancy. Placental growth is characterized by both increased numbers and branching of villi and microvilli and proliferation of the fetal capillary vessels in these villi. In this way, the surface area available for maternal-fetal exchange is greatly enhanced [38, 39]. Nutrients in maternal blood must cross the trophoblast cell layer and the basal membrane to reach the loose connective tissue surrounding the fetal capillaries. The uppermost layer of fetal tissues, the syncytiotrophoblast, is in direct contact with maternal blood. Microvilli increase the surface area necessary for nutrient transport [40]. Syncytial vacuoles are responsible for the transport of macromolecules and may be specifically targeted by cell surface receptors. The extensive endoplasmic reticulum and the high density of mitochondria provide the anatomical basis for both synthetic activities and transport through the cytoplasm of the syncytiotrophoblast. The multi-nucleated syncytiotrophoblast is the most important placental cell type in the second half of pregnancy and derives from the actively replicating cytotrophoblast. The syncytiotrophoblast represents the only uninterrupted cell layer interposed between the fetal capillary and maternal circulation.

**Placental Transfer**

Nutrient transfer occurs by simple diffusion, facilitated diffusion, active transport and receptor-mediated endocytosis, and is dependent on the surface area for exchange. Diffusion is determined by the concentration gradient of nutrients between fetal and maternal blood [41]. Most small molecules appear to be transferred by simple diffusion; these include water, sodium, urea, oxygen and carbon dioxide. A special case is the transport of glucose, the major energy substrate of
the fetus [42]. Glucose diffusion is selective and facilitated. Glucose transporter proteins have been described in the microvilli of the trophoblast facing the maternal decidua and the fetal capillary [43]. The transporters are not responsive to insulin [44]; they bind specifically to hexoses, have the highest affinity for glucose and are responsible for its active transport (requires energy and occurs against a concentration gradient). Similarly calcium [45], magnesium [46] and L-amino acids [47] have specific transport proteins. The transfer of intact proteins or other hydrophobic macromolecules is mostly mediated by pinocytosis or, more specifically, receptor-mediated endocytosis. The latter process requires a specific cell surface receptor and was initially described for the low-density lipoproteins. It has now been characterized for iron, folate, vitamin B₁₂, insulin and other macromolecules. This mechanism probably accounts for the transfer of IgG during the latter half of pregnancy. Transplacental transfer of IgG is highly specific and occurs at a faster rate than the transport of smaller proteins [48].

The fetus is totally dependent on placental circulation for the provision of substrates and other specific nutrients [49]. Later in gestation, fetal swallowing of amniotic fluid represents a non-significant additional transfer mechanism. As mentioned, glucose is transferred by selective facilitated diffusion. Of interest, the placenta metabolizes large amounts of glucose to lactate, and lactate, rather than glucose is likely the major precursor for fetal hepatic glycogen and fatty acid synthesis. Thus, the major sources of energy for the fetus, glucose and lactate, are transferred by diffusion. Gas exchange and fetal urea excretion occur by the same mechanism. In contrast, transfer of amino acids occurs against a concentration gradient and is energy dependent; circulating fetal amino acid levels are 30% higher than in the mother. In addition, L-amino acids are transferred more rapidly than the D isomers; except for IgG, intact proteins are not transferred across the placenta. Placental transport of lipids occurs mainly in the form of free fatty acids. Intact very low-density lipoproteins or low-density lipoproteins do not cross. During the last trimester of pregnancy, some of the increase in the fetal requirement for fatty acids is met by increased transport across the placenta. However, most fatty acid accretion is a product of fetal lipogenesis from non-lipid precursors. Essential fatty acid needs of the fetus are met solely by transplacental transport and reflect maternal dietary supply.

Considering the key role played by the placenta in fetal growth, careful examination of the placenta after birth may give us insights into the potential causes of IUGR as well as contribute in defining future risks of the baby. In some settings, the placenta may not be available for examination since it is used in rituals after the birth of the child; however, it should still be relatively easy to examine it immediately after the placenta is expelled. If we are to make use of the placenta as an indicator of normal fetal growth and future health of the baby,
there is a need to systematize its observations and define what measurements will be obtained beyond weight. It would be of potential benefit to this field to establish a common protocol to assess shape, key diameters, thickness, cord insertion and characteristics at the time of birth, including vessels, chorion and amnion on the fetal side, as well as structure and vascular indemnity of the cotyledons on the maternal side. This might prove of critical importance in defining the predictive value of the different findings in different categories of IUGR.

Since we have limited information on the mechanisms involved in fetal programming, the possibilities for treatment or intervention opportunities are for now limited. This is the case of interventions that could eventually lead to changes in placental structure, area or function. The association of IUGR and limited placental transport has been extensively described, and therefore enhancing placental transport in IUGR potentially could improve fetal growth and development. Several groups have shown benefits of maternal IGF-1 infusion on placental and fetal growth; the mechanisms shown in vitro suggest an important role for this key growth factor in trophoblast glucose and amino acid uptake. On the other hand, maternal IGF-2 infusion in guinea pig stimulates fetal growth by affecting the placental structure, rather than its transport capacity [50]. A limitation of these studies is that they were conducted in normal pregnant mammals, and not on animals affected by placental insufficiency or IUGR. Other possible but more speculative interventions focus on stimulating the placental L-amino acid transporter system responsible for transferring multiple essential amino acids through mTOR; administering low doses of atrial natriuretic peptide has been shown to selectively dilate placental circulation. Finally, the option of altering the methylation status of important placental genes such as 11-β-HSD-2 involved in oxidative/nitrative stress through maternal folate supplementation has been postulated.

Glucose is the major energy substrate for the fetus and the newborn, although the fetus is capable of utilizing lactate, free fatty acids or ketone bodies under special conditions. Glucose is able to induce its own non-energy-dependent transporters, appropriately named the GLUT1–7 family [42, 43]. The variation in GLUT isoform expression during development is time and tissue specific. GLUT1 is expressed in virtually every fetal cell except neurons; it accounts for most basal glucose uptake. GLUT3 is a high-affinity transporter found in the placenta. In situations of maternal glucose scarcity, this transporter may be capable of scavenging maternal glucose for use by the placenta and the fetus. The expression of insulin-sensitive GLUT4 appears late in gestation in cardiac and skeletal muscles, and in adipocytes. Glut 1 is expressed to a greater extent in fetal than adult lung. Normal insulin/cortisol ratios stimulate glucose transport while high insulin/cortisol ratios inhibit glucose uptake. This hormone balance delays lung maturation in infants of diabetic mothers that have a high circulat-
ing insulin and promotes lung maturation in IUGR infants that have lower insulin and higher cortisol levels.

The high glucose demand of the brain is successfully met by a combination of GLUT3 and GLUT1 and a hexokinase with a higher affinity for glucose than its counterpart in liver, muscle or kidney, thus favoring glucose uptake by the brain. During fetal life, GLUT1 constitutes the main brain glucose transporter, while GLUT3 appears after birth. Glucose transport may also be mediated by the Na-glucose-linked transporter family that actively transports glucose in polarized intestinal and renal epithelial cells. Glucose/Na cotransporters appear to be active prenatally; thus, the intestine is ready to absorb glucose with the first feed.

**Energy Stores**

Fetal liver progressively increases its glycogen concentration throughout gestation reaching a maximum of 10% of organ weight at the time of birth. However, in the full-term newborn, glycogen stores provide a reserve of ~100 kcal, barely sufficient to provide the basal energy needs of a full-term infant for 8–10 h. In extremely low-birthweight infants, glucose supply runs out in minutes. Preterm babies are therefore susceptible to hypoglycemia. Since liver, skeletal muscle and heart are able to oxidize free fatty acids derived from adipose tissue, adipose tissue represents the main energy reserve for the normal newborn. This is explained in part by the greater energy released by fat oxidation (1 g of fat yields 8–9 kcal) and also by the fact that triglycerides are stored in a water-free environment giving adipose tissue an energy density of around 8 kcal per gram, as opposed to 1 kcal per gram of liver. Only a third of the energy reserve from protein is available as an energy source; lean body mass losses in excess of 1/3 are associated with adverse functional consequences. This information allows for a quantitative assessment of energy reserves for the fetus and has been linked to the survival potential under conditions of semi-starvation such as the IUGR fetus. A full-term infant has enough energy reserve to support its needs for several weeks. An infant with a birthweight under 1,000 g has reserves for only a few days [49].

**Lipid Metabolism**

Most fetal triglycerides are derived from fatty acids produced in the fetal liver and placenta since fatty acid transport is meager and lipoprotein transfer from the mother is insignificant or nonexistent [51]. Of interest, the fetal brain and lung are also capable of lipogenesis to produce the unique lipids important for
their function. As fetal plasma fatty acids enter adipocytes and are re-esterified to triglycerides, adipose tissue exhibits dramatic growth during the last trimester of pregnancy. A 27-week fetus has only 1% of its bodyweight as fat, whereas the figure is 16% in the normal full-term infant. Substantial quantities of fetal body fat, especially that located in the vicinity of the major large vessels, are metabolically active. This fat is rich in heme-containing cytochromes and mitochondria and hence is brown in color. Its function is to produce heat. Lipid vesicles provide the fatty acids for oxidation, and the mitochondria possess a specific protein that uncouples aerobic fuel oxidation from ATP formation, producing heat. Activation of this non-shivering thermogenesis is triggered by sympathetic stimulation of lipolysis. With advancing postnatal age, shivering thermogenesis is established. Most brown fat involutes or becomes white fat.

The brain is unable to oxidize free fatty acids directly, and therefore relies mainly on glucose as a substrate. Fat can be oxidized indirectly, after conversion to ketone bodies if the concentration is high enough. The ability of the newborn liver to generate large amounts of ketone bodies is not present at birth and takes several days to develop. The maintenance of cerebral metabolism on an acute basis is therefore dependent on liver glycogen stores and gluconeogenesis in the liver and the kidney. Alanine and glutamine, generated from protein breakdown, and lactate and glycerol are the predominant gluconeogenic substrates. Ketone body oxidation and gluconeogenesis enable the newborn to maintain glucose homeostasis under the conditions of fasting or semi-starvation typical of early postnatal life. Early postnatal diets supplement and complement these responses. Human milk is uniquely suited for this purpose. It contains lactose and substantial amounts of easily absorbable medium-chain triglycerides. Even long-chain triglycerides in human milk have a special molecular configuration which makes them easier to digest. In addition, human milk provides the carnitine necessary for mitochondrial fatty acid transport and ketone body production, as evidenced by higher levels of plasma ketone bodies in babies fed human milk.

Metabolic regulation in the newborn period is based on substrate availability and the endocrine response induced by these substrates. During fetal life, after the 20th week of gestation, the fetal pancreas produces insulin. This hormone regulates the accumulation of glycogen in liver, muscle and lung. At the same time, it promotes lipogenesis and triglyceride storage within adipose tissue. Insulin also enhances protein synthesis in muscle. The action of insulin is modulated by glucocorticoids, which regulate gene expression and induce various enzymes related to glycogen and lipid synthesis. Steroid hormones are responsible for the induction of glycogen synthetase type I which is activated by insulin and responsible for glycogen synthesis. Fetal steroid hormone concentrations are
low throughout much of gestation. This explains why, despite detectable circulating insulin after 13 weeks of gestation, glycogen accumulation does not occur until the 27th week.

At the time of birth, the constant supply of maternal glucose is interrupted, and the infant’s blood glucose concentrations decrease. As a result, insulin levels fall and glucagon increases. The ratio between insulin and glucagon is essential in the regulation of gluconeogenesis and glycogenolysis. The hepatic intracellular signaling cascade induced by glucagon responds exponentially such that small changes in glucagon induce large changes in glycogenolysis and glucose availability. Glucagon and cortisol promote gluconeogenesis. Glucagon and catecholamines, in addition to activating glycogen breakdown pathways, promote lipolysis, generating glycerol, a gluconeogenic precursor, and free fatty acids. Free fatty acids directly and indirectly provide an alternative to glucose as oxidative fuel (see above). High glucagon and cortisol and low insulin favor protein catabolism, especially skeletal muscle protein breakdown, yielding amino acids for gluconeogenesis [52].

The postnatal changes in respiratory quotient, defined as the ratio between carbon dioxide production and oxygen consumption, reflect the postnatal fuel transition from glucose to a mixture of glucose and fat. A respiratory quotient of 1.0 corresponds to predominant use of glucose as a fuel for oxidation. A value of 0.7 corresponds to exclusive fatty acid oxidation.

Thyroxine and growth hormone promote lipolysis and further potentiate gluconeogenesis. Arginine vasopressin (AVP) has also been shown to induce a hyperglycemic response in the fetus. AVP is released during fetal stress and may represent a major modulator of fetal metabolism in addition to regulating cardiovascular responses.

In summary, the goal of neonatal glucose homeostasis is to provide the brain and other vital organs with sufficient glucose as a key energy source. Virtually all hormones except insulin will increase glucose, which is then taken up preferentially by the brain. The sequence described in this section constitutes the basis for perinatal glucose adaptation and can be verified in virtually all mammalian species.

How to Achieve Optimal Fetal Growth

The development of growth parameters to be used as a ‘gold standard’ is important not only for medical use in individual clinical monitoring; it is also critical because inadequate standards will have major unintended and possibly detrimental consequences on the health of future generations. Using the wrong growth charts to monitor fetal growth and size can, for example, result in an in-
crease in maternal antenatal hospitalization, unjustified intensive neonatal care, increase in induction of labor and cesarean sections which will impose risks on mothers and children, or at the very least on mothers, without benefiting either.

Recently, the International Fetal and Newborn Growth Consortium (INTERGROWTH 21st) have taken the responsibility of developing growth charts with a prescriptive approach [53, 54]. The study succeeded in its ambitious agenda and will provide us not only with well-timed fetal measures of gestational age confirmed by early US taken at 9–13 weeks, but will also provide us with 6 serial US assessments. The selected sites of US measurements were: (a) placental localization and fetal presentation, amniotic fluid volume index, (b) two head diameters (BPD and OFD) and head circumference, (c) transverse and anteroposterior abdominal diameters and circumference, (d) femur length obtained under strict quality controls to ensure the validity and precision of the data collected.

INTERGROWTH will provide clinicians and researchers with sequential longitudinal data that will serve to characterize whole body as well as brain, liver, and long bone growth, giving us the possibility to relate this to placental blood flow and transfer function, neonatal health, morbidity and mortality. The proposed normative fetal growth standards will represent a quantum leap in terms of evaluating the effect of early-life events on later growth, health and well-being. The INTERGROWTH study used a large, contemporary, representative, and multi-ethnic sample; a critical issue is to define ‘optimal’ environmental conditions. This is particularly difficult in the case of intrauterine growth standards since fetal nutrition depends not only on maternal conditions but also on the indemnity and functional state of the fetoplacental unit as we have already discussed. It is likely that in the future we will learn more about the true determinants of fetal growth and how these may condition long-term health and disease. In order to address this issue, the INTERGROWTH group defined a minimum set of sociodemographic (i.e. maternal education, assets, age, etc.), health (i.e. presence of gestational diabetes, history of stillbirths, etc.), nutritional (i.e. adequate weight status and diet), and environmental exposures (i.e. smoke, pollutants, etc.) as a prerequisite for all subjects entering the study. The adequacy of the prescriptive approach in developing the standard will be finally evaluated by linking the impact of the ‘new standard’ on short (i.e. neonatal morbidity and mortality, cognitive development) as well as long-term outcomes (i.e. disability, obesity, cardiovascular diseases).

The results of the INTERGROWTH prescriptive standards, soon to be reported, will serve to objectively define normal fetal growth; it represents great progress. It will not only give us well-timed fetal measures since gestational age was confirmed by an early US taken at 9–13 weeks, but will also provide serial US assessments of (a) placental localization and fetal presentation, amniotic fluid volume index, (b) two head diameters (BPD and OFD) and head circumference, (c) trans-
verse and anteroposterior abdominal diameters and circumference, (d) femur length that will be obtained under strict quality controls to ensure the validity and precision of the data collected. Thus, for the first time clinicians and researchers will have sequential longitudinal data that will serve to characterize whole body as well as brain, liver, and long bone growth relating this indirectly to placental blood flow and transfer function, neonatal health, morbidity and mortality. The proposed fetal growth standards will represent a quantum leap in terms of evaluating the effect of early life events on later growth, health and well-being.

The study used a large, contemporary, representative, and multi-ethnic sample. However, a critical issue in the generation of the INTERGROWTH standard is how to define ‘optimal’ environmental conditions. In the case of intrauterine growth, this is further complicated by the fact that fetal nutrition depends not only on maternal conditions but also on the indemnity and functional state of the fetoplacental unit. It is likely that in the future we will learn more on the determinants of fetal growth and how these may condition long-term health and disease. However, in the meantime it seems reasonable to consider a minimum set of sociodemographic (i.e. maternal education, assets, age, etc.), health (i.e. presence of gestational diabetes, history of stillbirths, etc.), nutritional (i.e. adequate weight status and diet), and environmental exposures (i.e. smoke, pollutants, etc.) in defining what it is ‘optimal’.

**Disclosure Statement**

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

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13 Hall JG: Review and hypothesis: syndromes with severe intrauterine growth restriction and very short stature – are they related to the epigenetic mechanism(s) of fetal survival involved in the developmental origins of adult health and disease? Am J Med Genet A 2010;152A:512–527.
Prevention of Low Birthweight, Epidemiology


Abstract

This review examines the effects of prenatal multiple micronutrient (MM) supplementation (≥5 micronutrients) on intrauterine growth. We identified publications from 16 randomized controlled trials through PubMed and EMBASE database searches. Meta-analyses were performed by pooling results, and sub-analyses by timing of intervention and amount of iron were also done. The primary outcome measures were birthweight, low birthweight (LBW; <2,500 g) and small for gestational age (SGA). Prenatal MM supplementation significantly reduced the incidence of LBW (risk ratio, RR: 0.86; 95% CI: 0.81–0.92) and SGA (RR: 0.83; 95% CI: 0.73–0.95) compared to iron-folate supplementation; mean birthweight was significantly higher by 55 g for MM with borderline increases in gestational age. MM supplementation was associated with larger decreases in the risk of LBW and SGA in the subgroup of trials that used supplements containing 60 mg of iron, but were not statistically significantly different from those for trials that used 30 mg iron. Prenatal MM supplementation improved intrauterine growth and can be recommended instead of prenatal IFA supplements in settings where micronutrient deficiencies are common.

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Introduction

Adverse pregnancy outcomes such as low birthweight (LBW), preterm birth (PTB), maternal and neonatal mortality continue to be significant public health problems in many resource-poor environments in spite of recommendations and efforts to improve the quality and timely access to reproductive health services such as family planning, prenatal health care and emergency obstetric care, prevention of malaria, and provision of iron and folic acid (IFA) supplements to all pregnant women [1]. Poor maternal nutrition before and during pregnancy has been associated with fetal growth, but measures to address women’s health and nutrition remain suboptimal. Diets of pregnant women in many developing countries are often low in energy and protein combined with inadequate intakes of nutrients such as iron, zinc and vitamin A that can affect fetal growth and development [2, 3]. Prenatal IFA supplementation has been shown to reduce the risk of LBW and maternal anemia under controlled conditions, but problems of distribution and compliance remain [4]. Several intervention trials have also demonstrated the benefits of providing food supplements during pregnancy especially to undernourished women, but this approach remains both logistically and economically challenging in resource-poor settings [5]. It is in this context that several studies were undertaken to examine the potential benefit of providing supplements containing several micronutrients in addition to IFA [6–8]. The objective of this paper is to conduct a systematic review of the evidence on the effects of prenatal multiple micronutrient (MM) supplements on intrauterine growth. Specific outcomes include birthweight, LBW and small for gestational age (SGA) along with gestational age, PTB, stillbirth, neonatal death, maternal anemia and mortality. We also examine dose-response relationships that relate to the amount of iron received by the intervention and control groups and time of initiation.

Methods

Search Strategy

We identified published studies through October 2011 from PubMed and EMBASE databases using search strategies that have been published elsewhere [6, 7]. Additional studies were identified through hand search of references from previous review articles and from personal communications. Inclusion criteria were (1) randomized, controlled intervention trial; (2) pregnant women (including non-symptomatic HIV-positive pregnant women); (3) studies that compared MM supplementation (≥5 micronutrients) with control (≤3 micronutrients including iron, folic acid and/or only one additional vitamin/mineral), and (4) reported findings for key outcomes of interest, namely LBW (birth-
weight <2,500 g), SGA and birthweight. Exclusion criteria were (1) animal studies, (2) review articles, cross-sectional, case-control, cohort studies, commentary letter, or editorial, (3) non-intervention studies, (4) non-healthy pregnant women (hospitalized patients, symptomatic HIV-positive women), (5) studies without MM interventions (<5 micronutrients, fortified food), (6) studies of preconceptual or periconceptual interventions, (7) non-accessible full texts.

Data Abstraction and Statistical Analyses

Details of all included studies were abstracted, and effect sizes were calculated for each study by dividing the difference between the mean values for the treatment (i.e. MM supplements) and control groups by the pooled SD. Pooled analyses were conducted where data were available from more than one study for an outcome, and the results are presented as risk ratios (RR) for dichotomous variables or weighted mean difference (WMD) for continuous variables with 95% confidence intervals (95% CIs). In the case of trials with more than one comparison that shared the same treatment or control group, we selected the most appropriate comparison; we also conducted sensitivity analysis in which we used pooled estimates that combined the results for more than one arm that shared the same control or treatment group [9]. The overall mean effect size and 95% CI across studies were estimated by a fixed or random effects model that used the weighted mean effect size for each study where the weight was the inverse of the intra-study variance. For all outcomes of interest, we conducted an overall comparison of all studies that provided MM (≥5 micronutrients) with control (≤3 micronutrients). To examine dose-response relationships, we carried out sub-analysis for the following comparisons: MM (including 30 mg iron) versus control (60 mg iron with or without folic acid); MM (including ≥60 mg iron) versus control (≥60 mg iron with or without folic acid). We also examined the effects by timing of initiation of the intervention by comparing estimates from studies that began the intervention in the (a) first trimester (<12 weeks gestation) and (b) second or third trimester (>12 weeks gestation). In all cases, we assessed heterogeneity of effect sizes using the χ² test of homogeneity; p < 0.05 was considered as evidence of heterogeneity and I² >50% were deemed as exhibiting substantial statistical heterogeneity. Estimates based on the random effects model were used in cases with significant heterogeneity. We evaluated the presence of publication bias using funnel plots [10] and the Begg’s rank correlation test for statistical testing of funnel plot symmetry [11]. All statistical analyses were conducted using Review Manager v5.0 (Cochrane Collaboration, Copenhagen, Denmark), and all tests were two sided, with p < 0.05 reported as significant.

Results

We identified a total of 827 titles of which 52 were initially considered for inclusion, and results from 46 eligible publications were included. The key characteristics of 16 RCTs trials including study location, sample size, nature of intervention and outcomes reported are shown in table 1. Nine of the included studies
were from Asia [12–20], six from sub-Saharan Africa [21–26], one from Europe [27], and one from Central America [28]. Only one trial was conducted among HIV-positive pregnant women in Tanzania in which women were assigned to one of 4 groups, namely MM with or without vitamin A, vitamin A only and placebo; all women received IFA [21, 22]. Supplementation began in the first or second trimester of pregnancy for most studies with a few that began only in the third trimester and sample sizes ranged from 100 in the study in France [27] to 31,290 in Indonesia [18]. The MM group received supplements containing 5 or

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Sample size</th>
<th>Intervention</th>
<th>Control</th>
<th>Outcome(s) reported</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LBW</td>
</tr>
<tr>
<td>Tanzania [25]</td>
<td>1998</td>
<td>1,075</td>
<td>MM ± vit. A¹</td>
<td>placebo¹</td>
<td>✓ ✓ ✓ ✓ ✓</td>
</tr>
<tr>
<td>Nepal [17, 18]</td>
<td>2003</td>
<td>4,926</td>
<td>MM¹</td>
<td>vit. A only; vit. A+ IFA¹, vit. A + Zn + IFA¹</td>
<td>✓ ✓ ✓ ✓ ✓ ✓</td>
</tr>
<tr>
<td>Mexico [32]</td>
<td>2003</td>
<td>873</td>
<td>MM¹</td>
<td>Fe only¹</td>
<td>✓ ✓ ✓</td>
</tr>
<tr>
<td>Zimbabwe [27]</td>
<td>2004</td>
<td>1,669</td>
<td>MM¹</td>
<td>placebo¹</td>
<td>✓ ✓</td>
</tr>
<tr>
<td>France [31]</td>
<td>2004</td>
<td>100</td>
<td>MM</td>
<td>placebo</td>
<td>✓</td>
</tr>
<tr>
<td>Guinea-Bissau [28]</td>
<td>2005</td>
<td>2,100</td>
<td>MM²</td>
<td>IFA¹</td>
<td>✓ ✓ ✓</td>
</tr>
<tr>
<td>Nepal [21]</td>
<td>2005</td>
<td>1,200</td>
<td>MM²</td>
<td>IFA¹</td>
<td>✓ ✓ ✓ ✓ ✓</td>
</tr>
<tr>
<td>India [20]</td>
<td>2007</td>
<td>200</td>
<td>MM¹</td>
<td>calcium¹</td>
<td>✓ ✓ ✓</td>
</tr>
<tr>
<td>Niger [29]</td>
<td>2007</td>
<td>2,550</td>
<td>MM²</td>
<td>IFA¹</td>
<td>✓</td>
</tr>
<tr>
<td>Tanzania [26]</td>
<td>2007</td>
<td>1,078</td>
<td>MM ± vit. A¹</td>
<td>placebo¹</td>
<td>✓ ✓</td>
</tr>
<tr>
<td>Burkina-Faso [30]</td>
<td>2008</td>
<td>1,426</td>
<td>MM²</td>
<td>IFA¹</td>
<td>✓ ✓ ✓ ✓ ✓</td>
</tr>
<tr>
<td>Indonesia [22]</td>
<td>2008</td>
<td>31,290</td>
<td>MM²</td>
<td>IFA²</td>
<td>✓ ✓</td>
</tr>
<tr>
<td>China [24]</td>
<td>2008</td>
<td>5,828</td>
<td>MM²</td>
<td>IFA¹</td>
<td>✓ ✓ ✓ ✓ ✓ ✓</td>
</tr>
<tr>
<td>Pakistan [16]</td>
<td>2009</td>
<td>2,378</td>
<td>MM²</td>
<td>IFA¹</td>
<td>✓ ✓ ✓ ✓</td>
</tr>
<tr>
<td>Indonesia [23]</td>
<td>2009</td>
<td>843</td>
<td>MM²</td>
<td>IFA¹</td>
<td>✓ ✓ ✓</td>
</tr>
<tr>
<td>Bangladesh [19]</td>
<td>2010</td>
<td>4,436</td>
<td>MM²</td>
<td>IFA¹²</td>
<td>✓</td>
</tr>
</tbody>
</table>

GA = Gestational age; SB = stillbirth; ND = neonatal death; MA = maternal anemia during third trimester.
All studies that reported LBW also reported mean birthweight except the earlier trial from Tanzania. All studies that reported MA also reported mean hemoglobin except the one from Burkina-Faso.
¹ Received supplements containing at least 60 mg iron.
² Supplements contained 30 mg iron.
more micronutrients including iron, and the control group received ≤3 micronutrients that typically included iron alone or iron plus folic acid (IFA). The amount of iron in the MM supplement was 30 mg for 9 trials, many of which were the UNIMMAP formulation containing 1–2 RDA of several micronutrients [2], while others provided supplements containing at least 60 mg iron to both intervention and control groups.

The overall pooled estimates and results for stratified analysis by the amount of iron for the key outcomes of interest are shown in tables 2 and 3, respectively. The impact on mean birthweight was reported for 14 trials that compared MM supplementation with iron alone or IFA supplementation [12, 13, 15–17, 19, 20, 22–28]. The effects on birthweight ranged from 4 g in Mexico [28] to 251 g in France [27], and the pooled analysis showed an overall increase of 55 g in the mean birthweight of the offspring of women who received prenatal MM supplements compared to iron or IFA (WMD: 54.5 g; 95% CI: 45.4–63.48). Few studies reported other measures of birth size such as birth length. The pooled analysis also showed a 14 and 16% reduction in the risk of LBW and SGA (p < 0.05 for both outcomes), respectively. The reduction in the risk of delivering LBW infants was greater in the subgroup of six trials [13, 14, 16, 21–23, 28] that compared MM supplements containing ≥60 mg iron with the control group receiving ≥60 mg iron with or without folic acid, but was not significantly different from the subgroup of eight trials [12, 15, 17, 19, 20, 24–26] that compared MM supplements containing 30 mg with the control group who received ≥60 mg iron with or without folic acid.

Table 2. Summary estimates of effects of MM supplements during pregnancy

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Studies</th>
<th>Sample size</th>
<th>Effect size (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MM</td>
<td>control</td>
</tr>
<tr>
<td>LBW</td>
<td>15</td>
<td>1,387/12,190</td>
<td>1,576/12,065</td>
</tr>
<tr>
<td>Birthweight, g</td>
<td>14</td>
<td>11,676</td>
<td>11,575</td>
</tr>
<tr>
<td>SGA</td>
<td>8</td>
<td>1,340/7,920</td>
<td>1,519/7,877</td>
</tr>
<tr>
<td>Gestational age, weeks</td>
<td>10</td>
<td>9,200</td>
<td>9,273</td>
</tr>
<tr>
<td>Preterm birth</td>
<td>9</td>
<td>4,973/23,139</td>
<td>4,903/22,770</td>
</tr>
<tr>
<td>Stillbirths</td>
<td>10</td>
<td>670/24,517</td>
<td>690/24,233</td>
</tr>
<tr>
<td>Neonatal death</td>
<td>9</td>
<td>599/23,190</td>
<td>607/22,553</td>
</tr>
<tr>
<td>Maternal anemia</td>
<td>7</td>
<td>654/1,873</td>
<td>645/1,820</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>5</td>
<td>1,546</td>
<td>1,594</td>
</tr>
</tbody>
</table>

Effect sizes were estimated by RR for all outcomes except birthweight, gestational age, and hemoglobin, which were estimated by weighted mean difference. Sample sizes are presented as number of events/total for categorical variables and total for continuous variables.

1 Third trimester.

2 Using random effects models.
iron with or without folic acid (table 3). There were also no differences in the estimates by amount of iron for SGA and by timing of initiation for LBW, SGA and mean birthweight.

Gestational age was reported in 10 trials [12, 16, 17, 19, 20, 22, 23, 26–28], and we found a borderline significant effect of increased gestational age for MM compared to those given iron or IFA (WMD = 0.07 weeks; 95% CI: 0.00–0.14 weeks). There were no differences by the amount of iron or timing of intervention. The pooled analysis revealed no significant differences in the overall risk of PTB, maternal anemia, stillbirth and neonatal death. Stratified analysis did not reveal any differences for these outcomes as well. We found no evidence of publication bias except for the studies of effect of MM on birthweight based on visual examination of funnel plots and by Egger’s weighted regression and Begg’s rank correlation methods.

## Discussion

Supplementation of women during pregnancy with five or more micronutrients significantly reduced the risk of delivering LBW and/or SGA infants when compared to supplementation with three or fewer micronutrients including IFA. The birthweight of infants whose mothers were given MM was on average 55 g greater than the controls who received IFA with borderline increases in mean gestational age. There were no significant differences in the risk of PTB, still-
birth, neonatal death and maternal anemia in the third trimester. As reported in earlier publications, the included trials were of high quality and the overall quality of evidence for our outcomes were from moderate to high [6, 7]. One major concern is the definition of the intervention and control group. Some of the trials included in the current review provided nutrients such as vitamins A or D or calcium to both the control group (in addition to the iron/or iron+folic acid) and the intervention group [13, 14, 16, 21, 22]. This resulted in the control group receiving 3 micronutrients (i.e. iron/or iron + folic acid and one additional vitamin/mineral), which has sometimes been used as a definition for MM interventions. Earlier reviews have defined the control group as receiving two or fewer micronutrients but we found that this is not necessarily the case. Another problem is that some trials had several arms and the control group received a placebo that contained ≤1 micronutrient, and iron or IFA supplements may have been provided separately to all women. However, we found similar results when we conducted a sensitivity analysis that used pooled estimates for trials that had more than one comparison but shared the same control or intervention arm, namely: (1) MM vs. IFA and placebo in the trial in Nepal by Christian et al. [13, 14], (2) MM vs. IFA with 30 mg Fe and IFA with 60 mg Fe in Bangladesh [15], (3) MM (1 RDA) and MM (2 RDA) vs. IFA in Guinea-Bissau [24], and (4) MM with vitamin A and MM without vitamin A vs. vitamin A and placebo in the Tanzanian trial among HIV-positive women [21].

Our findings confirm earlier conclusions that prenatal MM supplements are efficacious in reducing the incidence of LBW and SGA when compared to providing only IFA. The direction and magnitude of effect sizes for LBW, SGA, and birthweight are similar to those reported by Fall et al. [29] who included the trials that compared the UNIMMAP MM supplements with IFA, and that reported by Haider et al. [8] for SGA. Shah and Ohlsson [30] reported similar reductions in LBW but did not find significant differences in the risk of delivering SGA infants; we used similar methods but included findings from seven additional trials (n = 16,839). Most importantly, we have new findings on the effects of MM supplementation stratified by (a) the amount of iron in the supplements provided to the treatment and control groups and (b) the timing of initiation of supplementation. The reductions in the risk of LBW and/or SGA were greater for the subgroup of trials in which MM supplements contained at least 60 mg iron, but these estimates were not statistically different when compared to the subgroup of trials using MM supplements containing 30 mg iron, indicating that a lower amount of iron may be adequate. We did not find significant differences in the effect of MM interventions by timing of intervention for measures of intrauterine growth, PTB or neonatal death. However there were very few studies that began the intervention in the first trimester. The quality of evidence was graded ‘moderate’
for the estimates of the effect of MM after 12 weeks gestation on neonatal death but ‘low’ for the estimates based on studies that began the intervention in the first trimester. Similarly, the quality of evidence was ‘moderate’ and ‘low’ for the estimates of effects for these outcomes for the subanalysis of studies providing MM supplements containing 30 and 60 mg iron, respectively [6].

In conclusion, prenatal MM supplements improve intrauterine growth when compared to routine IFA under controlled conditions. The reductions in the risk of LBW (14%) are comparable to what has been shown for prenatal IFA (19%), suggesting greater overall benefit in settings where women do not receive any micronutrient supplements [4, 6]. The absence of significant differences in the risk of maternal anemia however suggest that the mechanism for improved growth may not be mediated via improvements in hemoglobin concentrations in contrast to prenatal IFA supplementation which significantly reduces the risk of maternal anemia and LBW [4]. Few studies, however, have examined the effects of MM supplements on biomarkers of iron status besides hemoglobin and/or other micronutrients. Further research and reviews should include data on baseline nutrient status and quality of prenatal and obstetric care that would help identify which target groups would benefit the most. Information on the acceptability of the MM supplements and compliance in programmatic settings would also be useful in light of the problems that have been reported with routine IFA supplementation in many developing country settings. In conclusion, our findings indicate that policy recommendations can be made to replace prenatal IFA with MM supplements especially in settings were the burden of low birth weight is high. However, investments to strengthen existing programs that provide IFA supplements and support for strategies that improve maternal nutrition before and during early gestation are needed to promote healthy growth and development for the next generation.

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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.
References


Importance of Intervening in the Preconception Period to Impact Pregnancy Outcomes

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Abstract
Preconception care that begins in adolescence and is provided before and between pregnancies has the potential to impact 136 million women who give birth each year and ensure that newborns receive the healthiest start possible. Providing simple interventions before pregnancy can prevent a significant proportion of maternal and neonatal mortality and morbidity. Interventions to promote adolescent health and prevent teenage pregnancies, encourage contraceptive use and appropriate birth spacing, optimize weight and micronutrient status, and screen for and manage chronic conditions have proven efficacy. These interventions must now be scaled up to maximize delivery. Women who receive preconception care are more likely to adopt healthy behaviors, and therefore have better pregnancy outcomes. Preconception care is particularly effective when men are involved and care is provided in the community setting. All healthcare providers can and should begin to provide preconception care to all adolescent girls, women and couples of reproductive age by asking them if they wish to become pregnant or are actively trying to prevent pregnancy.

Maternal and Newborn Health
Poor maternal, newborn and child health (MNCH) remains a significant problem. Approximately 273,500 women of reproductive age die annually due to complications of pregnancy and childbirth; over 15 million suffer long-term
illness and disability [1]. This scenario is much graver in developing nations in South Asia and Sub-Saharan Africa, where women lack access to reproductive health services and prenatal care, are chronically undernourished, and generally have multiple closely spaced pregnancies [2]. Pregnancy is also a period of risk for the child to be born. An estimated 2.9 million newborns die in their first month of life every year [1]. Maternal health complications contribute to 1.5 million of these deaths during the first week of life and 1.4 million stillbirths [3].

Innovative research and systematic reviews have drawn increasing global attention to the burden of maternal and neonatal deaths, as well as stillbirths [4–7]. More significantly, they provide evidence that most of these deaths are from preventable causes. Further, they demonstrate that improving the health of women and newborns has wider social and economic benefits since healthy children require less hospitalization and tend to remain healthier across their lifespan. A gap remains, however, in both research and healthcare across the life-cycle for women, many of whom receive no care after childhood until their first pregnancy. There is a strong scientific basis for interventions to improve maternal and child health during pregnancy and labor, and after birth. Yet more needs to be done to lower maternal and neonatal mortality and morbidity. Women and providers continue to be unaware of the fact that a woman’s health status before pregnancy influences the outcomes for both mother and child. A simple way to ensure that more women receive the timely care they need was proposed – preconception care.

What is Preconception Care?

Preconception care may be defined as ‘any intervention provided to women and couples of childbearing age, regardless of pregnancy status or desire, before pregnancy, to improve health outcomes for women, newborns and children’ [Bhutta et al., unpubl.]. It proposes a process of delivering direct or indirect health care interventions that have a potential to identify or modify the biomedical, behavioral and social risk factors attached to pre-pregnancy, pregnancy, intrapartum, neonatal and childhood mortality and morbidity. Preconception care encompasses broader initiatives such as women’s education and empowerment, and more targeted healthcare interventions such as vaccination and micronutrient supplementation. Preconception care may begin in adolescence, and also be provided between pregnancies, to allow the time necessary for positive behavioral changes to occur.
The Changing Paradigm: From Prenatal Care to Preconception Care

A number of health problems such as obesity, behaviors such as tobacco and alcohol use, and risk factors such as indoor air pollution, affecting both men and women, contribute to maternal and child morbidity and mortality. In some instances, these problems, behaviors or risk factors disproportionately affect women. They may also increase the risk for having babies that are born prematurely, with low birthweight or congenital problems. These babies are more prone to become sick, require more medical care, and account for an increasing proportion of neonatal deaths. Screening for and addressing these problems, behaviors and risk factors will improve the health of men, women and children. Since it is difficult to predict exactly when a pregnancy is conceived, and because the health of both parents influences the outcome of the pregnancy, it is recommended that preconception care be provided to all women and men of reproductive age. There are 75 million unplanned/unwanted pregnancies each year [8], and preconception care ensures that women are in the best health possible even when their pregnancy is unintended. Intervening before pregnancy recognizes that for many women and their babies, prenatal care even in the first trimester, occurs too late. Preconception care ensures that risk is minimized and that women are in the best health possible at the start of pregnancy, before the crucial time of fetal development and before health problems can lead to adverse maternal outcomes.

Systematic Review on Preconception Risks and Interventions

A systematic review was undertaken to collate the evidence on preconception risks and interventions [Bhatta et al., unpubl.] (table 1). Published and unpublished literature was considered relevant if it was specifically stated that the risk or intervention occurred before conception or in women of reproductive age who were not pregnant, and the outcome assessed impact on the health of the women, mothers (during pregnancy), newborns or children up to age 5 years. Although preference was given to randomized trials, since the area of preconception care is relatively new, quasi-randomized trials and observational studies (cohort, case-control) were also included. The advocacy articles were especially useful in understanding the development of preconception care, and the descriptive studies helped to frame the context and details of intervention packages or programs so that they might be replicated. Electronic databases were searched using MeSH terms and keywords relevant to preconception care overall and to specific risks and interventions. The articles found were cross-
referenced, and bibliographies of reviews were also reviewed to ensure that all relevant sources were identified. Titles and abstracts were screened, and data extracted by 2 independent researchers, and the quality of each study was assessed using standardized criteria. Meta-analyses of quantitative studies were conducted where possible using Review Manager (RevMan) software v5.1. In total, 57,036 studies were identified, of which 2,059 were initially deemed eligible and 516 were used in the meta-analyses. The findings of this review (table 2) were also presented at international meetings and shared with experts in the field of maternal and child health, which gave greater strength to the results.

### Table 1. Preconception risks and interventions reviewed

<table>
<thead>
<tr>
<th>Risk aversion</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced maternal age</td>
<td>Preconception counseling</td>
</tr>
<tr>
<td>Short (&lt;6 months) or long (&gt;60 months) interpregnancy intervals</td>
<td>Community empowerment to cease female genital mutilation/cutting</td>
</tr>
<tr>
<td>Maternal prepregnancy weight (body mass index)</td>
<td>Promoting adolescent health and preventing adolescent pregnancy</td>
</tr>
<tr>
<td>Other chronic maternal conditions and medication use</td>
<td>Postabortion care</td>
</tr>
<tr>
<td>Mental health disorders, depression</td>
<td>Genetic counseling and screening</td>
</tr>
<tr>
<td>Coerced sex and intimate partner violence</td>
<td>Nutrition, micronutrient supplementation</td>
</tr>
<tr>
<td>Substance use</td>
<td>Counseling and metabolic control for women with diabetes mellitus, phenylketonuria</td>
</tr>
<tr>
<td>Harmful environmental exposures</td>
<td>Screening and treatment for infectious diseases</td>
</tr>
<tr>
<td></td>
<td>Immunization</td>
</tr>
</tbody>
</table>

Preconception Intervention Leads to Improved Pregnancy Outcomes: The Evidence

Each year, 16 million births are to adolescent girls aged 15–19 years [9]. If preconception care is to really impact the health of women and children, it must begin in adolescence. Teenage girls are extremely vulnerable, and face many challenges to their health and well-being, but are not equipped with the education, skills or self-esteem needed to overcome these. Gender inequality means that violence against girls and women is common and tolerated. Ten percent (10%) of girls who have had intercourse before the age of 15 years report that it was forced; these young women are subsequently more likely to experience un-
intended pregnancies, sexually transmitted infections (STIs), and depression. School-based programs can successfully prevent dating violence and long-term psychological distress. Interventions that empower women and promote human rights and community development double the number of women who intend not to practice female genital mutilation on their daughters, and prevent horrific sequelae for women, including stillbirths.

Early marriage, risky sexual behaviors, and lack of access to contraception or safe abortion care mean that adolescent girls experience disproportionately high rates of intrapartum complications, stillbirths, neonatal deaths, prematurity and low birthweight. Personal development programs that incorporate skill building and include contraceptive provision prevent 15% (95% CI: 2–26%) of first adolescent pregnancies, and programs that teach parenting skills and enable teen mothers to complete their education decrease repeat adolescent pregnancies by 37% (95% CI: 18–51%).

### Table 2. Evidence from meta-analyses of preconception interventions

<table>
<thead>
<tr>
<th>Preconception intervention</th>
<th>Outcomes</th>
<th>Studies</th>
<th>Impact estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preconception counseling in community groups</td>
<td>neonatal mortality</td>
<td>5</td>
<td>RR 0.76 (0.66–0.88)</td>
</tr>
<tr>
<td></td>
<td>antenatal care</td>
<td>5</td>
<td>RR 1.39 (1.00–1.93)</td>
</tr>
<tr>
<td></td>
<td>use of clean delivery kit</td>
<td>4</td>
<td>RR 2.36 (1.55–3.60)</td>
</tr>
<tr>
<td></td>
<td>breastfeeding</td>
<td>4</td>
<td>RR 1.20 (1.07–1.36)</td>
</tr>
<tr>
<td>Preventing adolescent pregnancy</td>
<td>repeat pregnancy</td>
<td>16</td>
<td>OR 0.63 (0.49–0.82)</td>
</tr>
<tr>
<td></td>
<td>first pregnancy</td>
<td>24</td>
<td>RR 0.85 (0.74–0.98)</td>
</tr>
<tr>
<td>Multivitamin supplementation</td>
<td>preeclampsia</td>
<td>12</td>
<td>OR 2.28 (2.04–2.55)</td>
</tr>
<tr>
<td></td>
<td>gestational diabetes</td>
<td>8</td>
<td>OR 1.91 (1.58–2.32)</td>
</tr>
<tr>
<td></td>
<td>postpartum hemorrhage</td>
<td>4</td>
<td>OR 1.18 (1.15–1.21)</td>
</tr>
<tr>
<td></td>
<td>instrumental delivery</td>
<td>4</td>
<td>OR 1.10 (1.01–1.19)</td>
</tr>
<tr>
<td></td>
<td>stillbirths</td>
<td>6</td>
<td>OR 1.40 (1.05–1.85)</td>
</tr>
<tr>
<td></td>
<td>macrosomia</td>
<td>11</td>
<td>RR 1.77 (1.54–2.03)</td>
</tr>
<tr>
<td></td>
<td>large for gestational age</td>
<td>6</td>
<td>OR 1.65 (1.37–2.00)</td>
</tr>
<tr>
<td></td>
<td>CHDs</td>
<td>3</td>
<td>OR 1.14 (1.06–1.23)</td>
</tr>
<tr>
<td></td>
<td>preeclampsia</td>
<td>2</td>
<td>RR 0.73 (0.58–0.92)</td>
</tr>
<tr>
<td>Folic acid supplementation</td>
<td>recurrent NTDs</td>
<td>5</td>
<td>RR 0.43 (0.13–1.40)</td>
</tr>
<tr>
<td></td>
<td>occurrent NTDs</td>
<td>3</td>
<td>RR 0.47 (0.34–0.64)</td>
</tr>
<tr>
<td>Counseling and glycemic control in diabetic women</td>
<td>congenital malformations</td>
<td>21</td>
<td>RR 0.32 (0.23–0.43)</td>
</tr>
<tr>
<td></td>
<td>perinatal mortality</td>
<td>9</td>
<td>RR 0.31 (0.18–0.54)</td>
</tr>
<tr>
<td>STI identification and management</td>
<td>prevalence of STIs</td>
<td>3</td>
<td>OR 0.78 (0.68–0.89)</td>
</tr>
<tr>
<td></td>
<td>safer sexual behaviors</td>
<td>4</td>
<td>OR 1.26 (1.01, 1.56)</td>
</tr>
<tr>
<td></td>
<td>incidence of STIs</td>
<td>5</td>
<td>OR 0.65 (0.53, 0.80)</td>
</tr>
<tr>
<td>Preventing HIV transmission</td>
<td>consistent condom use</td>
<td>4</td>
<td>RR 0.15 (0.09–0.25)</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate 95% CIs.
Socioeconomic changes in the last decade have resulted in more women choosing to delay childbearing, with fertility rates for women aged 30–39 years having doubled. In addition to difficulty conceiving and chromosomal abnormalities, advanced maternal age (over 35 years) immensely increases the odds of antepartum hemorrhage, gestational diabetes and hypertension, caesarean delivery, stillbirths, perinatal deaths and low birthweight (fig. 1).

Like maternal age at conception, birth spacing also has a direct effect on maternal health and subsequent pregnancy outcomes. Short interpregnancy intervals (less than 6 months) result in a higher probability of maternal deaths (OR 66%, 95% CI: 19–133%) and stillbirths (OR 42%, 95% CI: 9–86%); long intervals (exceeding 60 months) increase the risk of preeclampsia (60–80%), while both long and short intervals raise the chances for neonates to be born preterm or low birthweight, and die within the first month of life. There is a desperate need to meet the need for family planning through effective contraceptive use and promoting exclusive breastfeeding, so that women ideally space their pregnancies 18–24 months apart.

Of the millions of women becoming pregnant each year, one third did not intend to become pregnant, and one fifth of pregnancies end in abortion [8]. Although promoting the use of consistent effective contraception may prevent many undesirable outcomes and is central to preconception care, unintended pregnancies will continue to occur. Safe postabortion care (whether the abortion was spontaneous or induced) saves women from death and disability (especial-
ly psychological distress) and prevents preterm low birthweight babies and ante- 
partum hemorrhage in subsequent pregnancies. Restructuring postabortion 
care and training providers drastically increases women’s uptake of and their 
partners’ support for contraception.

Another important consideration in reproductive planning is the risk of genetic disease. Couples who delay childbearing or in a consanguineous marriage 
are at higher risk, but all parents potentially risk having a child with a birth defect 
or hereditary abnormality. Premarital genetic screening has reduced the inci-
dence of thalassemia in Iran by 70% [10], but in other countries, positive atti-
tudes towards screening for various genetic diseases did not translate into in-
creased uptake of testing.

Maternal nutritional status is affected by numerous factors during adoles-
cence and childhood, but may be amended by interventions in the more imme-
diate preconception period. Maternal pre-pregnancy overweight and obesity in-
crease the propensity for gestational hypertension and diabetes, postpartum 
hemorrhage, caesarean delivery, and the risk of stillbirths and congenital heart 
defects 1.5–2 times. Underweight women have a higher incidence of stillbirths, 
preterm birth (OR 32%, 95% CI: 22–43%), low birthweight and small-for-gesta-
tional-age babies (OR 64%, 95% CI: 35–101%). The preconception micronutri-
ent status of women is crucial. Substantial evidence exists for the potential of 
preconception folic acid supplementation to more than halve the risk of neural 
tube defects. Multivitamin supplementation lowers the rates of preeclampsia, 
multiple congenital anomalies and limb reduction defects. Folic acid fortifica-
tion has been implemented in many countries to ensure that women of repro-
ductive age consume the recommended daily amount, and this has shown to be 
effective and safe. Intensive promotional campaigns with individual counseling 
could increase coverage of daily vitamin supplementation. Literature on the 
MNCH effects of other micronutrients in the preconception period is scarce. 
Iron deficiency anemia is significantly associated with a 5-fold rise in the risk of 
fetal growth restriction and 7-fold increase in risk of low birthweight, yet iron 
supplementation is generally begun during pregnancy, and studies only report 
biochemical outcomes.

In the United States alone, over 12% of women of reproductive age suffer 
from a chronic medical condition, especially diabetes and hypertension [11]. 
Preconception care for diabetic women that includes diet and exercise counsel-
ing, family planning and strict glycemic control leads to 70% reduction in the 
two most noted adverse fetal outcomes of diabetic pregnancies, congenital mal-
formations and perinatal mortality. Unfortunately, less than a third of women 
with preexisting diabetes receive preconception care. Thus, it is imperative that 
every health visit for women with chronic medical conditions be regarded as an
opportunity to provide preconception care. Conversely, as more women have children during their later reproductive years, screening for chronic diseases also becomes more important.

Depression, a leading mental health problem is twice as prevalent in women as in men. Adolescent depression may increase the risk of miscarriage by more than 2-fold and the risk of intimate partner violence (IPV) by more than 3-fold. Mental health problems in women are significantly interconnected with other risks, such as gender-based roles, and IPV. World Health Organization multi-country study estimates that 15–71% of women experience IPV at some point in their lives. Women suffering from IPV are twice as likely to have an unplanned pregnancy, and 50% more likely to experience fetal loss and gynecologic morbidity. Further, they are twice as likely to suffer from impaired physical health and mental health. Interventions such as group or couple’s behavioral therapy have shown significant reductions in postintervention aggression. Delivering solutions to prevent and respond to women’s psychological health problems are urgently needed to combat this leading cause of morbidity.

Certain infectious diseases also pose a real threat to mothers and the fetus in utero. STIs are a serious global reproductive health problem, the burden of which falls disproportionately on women, especially those who are younger or socioeconomically disadvantaged. While many interventions have been tested, they mostly look at end points other than MNCH outcomes like safer sexual behavior. Presumably, these would have an indirect effect on reducing exposure before and during pregnancy, and thus adverse pregnancy outcomes. Mass treatment of STIs with antibiotics was found to lead to a reduction in its prevalence by one fifth. Behavioral and counseling interventions also led to a decrease in STI prevalence. These interventions were shown to significantly increase (by 15%) safer sexual behaviors, especially condom use.

Nearly 16 million women are currently living with human immunodeficiency virus (HIV) [12]. Women, especially in adolescence, are infected even in stable sexual relationships since they are seldom equipped with the skills to refuse risky sexual behavior. Women who are seropositive have a high probability of transmitting the virus to their newborn during birth, and also put their newborns at greater risk in the perinatal period. Ongoing trials of preexposure prophylaxis may give women a new way to prevent HIV infection, and antiretroviral therapy may prove effective in prevention as well. Until then, it is imperative that men are involved in HIV prevention for women, since male circumcision halves the risk of infection (and thereby transmission), and the use of male condoms significantly reduces the risk of transmission by 85%. Female-dependent contraception and voluntary counseling and testing have not proven effective in reducing transmission or risk behaviors. Preconception
Intervening in the Preconception Period

Care should entail screening of all women of reproductive age, and provide women with skills to negotiate condom use, so that women are informed of their serostatus (and their partner’s) and take appropriate preventive measures. Screening and management of other STIs may also reduce the risk of HIV infection. As HIV continues to spread, there is a desperate need for behavioral interventions in women and adolescents to be replicated to show consistent evidence of effect, and for innovative interventions to be clearly evaluated.

Immunization against certain infectious diseases protects mothers and newborns from serious health consequences during a very vulnerable time. The tetanus toxoid vaccine reduces neonatal mortality and neonatal deaths due to tetanus by one third with a single dose, and over 70% with completion of the primary vaccination series. All women of reproductive age should also have their immunization status for rubella reviewed, and if women who are not immune should be vaccinated at least 3 months prior to pregnancy. Vaccination campaigns have been successful in increasing coverage, and show that immunization in the periconception period is safe and effective in preventing congenital rubella syndrome. The human papillomavirus (HPV) vaccine is also recommended in the early reproductive years to prevent cervical cancer. Synthesis of clinical trials show that the HPV vaccine is safe, but have not demonstrated a beneficial effect on pregnancy outcomes. Cytomegalovirus (CMV) is a leading cause of deafness and intellectual disability in infants. The available literature shows that women who acquire primary CMV infection preconceptionally are at less risk of transmitting the virus to their newborns than those who become infected during pregnancy. Stronger evidence of risk aversion with preconceptional immunity is needed to develop an effective vaccine, and until then women should be made aware of the risk, and ways to protect themselves from exposure such as avoiding contact with young children’s saliva and urine.

A shocking number of women in their reproductive years continue to consume caffeine, alcohol, tobacco or illicit drugs. For example, more than half of all pregnant women in the United States report alcohol use during pregnancy, and 40,000 babies are born with fetal alcohol spectrum disorder [13]. Preconception counseling and behavioral interventions to reduce alcohol use and provide contraception lead to a significant improvement in drinking behavior and fewer pregnancies affected by alcohol. Preconception cigarette smoking doubles the threat of babies being born prematurely. Preconception counseling led to an almost 3-fold increase in women quitting smoking before pregnancy. While it is distressing that women continue to put themselves at risk, it is inexcusable that healthcare providers generally do not intervene to ameliorate these
risks. Further, while there is some public awareness regarding alcohol use and its adverse effect on pregnancy, one can only speculate how great the risk might be from use of substances of whose consequences they are oblivious. For instance, we found that caffeine consumption exceeding 300 mg/day, which is nearly ubiquitous in some countries, increases the risk of early fetal loss by 31% (95% CI: 8–38%).

Increasingly, evidence is accumulating that harmful environmental exposures impact human health and reproduction. Occupational radiation or pesticide exposure in women before conception increases the risk of first-trimester miscarriage by approximately 30%. Paternal radiation exposure prior to pregnancy increases the chances of fetal growth restriction.

**Conclusions and Recommendations**

The literature clearly points towards the need to make preconception care part of the continuum of care so that women and couples are healthier before they become pregnant [14–21]. The provision of such care should begin in adolescence so that pregnancies at an early age, when the risk to mother and child is significantly greater, are prevented. Adolescent girls and women must be empowered with the skills needed to make decisions regarding their own health and access to care. Reproductive planning and birth spacing form an integral part of preconception care, and have the potential to prevent maternal deaths, stillbirths and newborn mortality. Nutritional status before pregnancy should be assessed, and women who are overweight should be encouraged to optimize their body mass to prevent maternal complications such as preeclampsia, gestational diabetes and postpartum hemorrhage. All women of reproductive age should take a multivitamin supplement with at least 400 μg of folic acid daily to prevent congenital anomalies especially neural tube defects. Women should also be screened for chronic diseases, and measures for glycemic control should be instituted for diabetic women to prevent perinatal mortality and birth defects. Preconception care is simply a logical extension of care during pregnancy, yet this small measure will make a major impact in ensuring that every pregnancy is planned and healthy, and that mother and babies do not continue to be at risk.

**Disclosure Statement**

None.
References


Commentary on Prevention of Low Birthweight in Low-Income Societies

Poor pregnancy outcomes remain a significant public health problem in many parts of the world with an estimated 18 million newborns born with low birthweight (LBW; weighing <2,500 g at birth) each year in the lower income countries [1]. More than half of these infants are born in South Asia, which has the highest incidence of LBW by far, at 27% [2]. Infants born with LBW either due to prematurity, or due to being born small for their gestational age, or both, are known to be at risk of increased mortality and morbidity throughout childhood [3]. LBW may also impact adult health: in follow-up studies of historic cohorts from transitional countries, associations of LBW and underweight and stunting (i.e. being short for age) in young children with later occurrence of central obesity, insulin resistance, type 2 diabetes, hypertension and cardiovascular disease have been reported [4]. Moreover, women who had been malnourished themselves during gestation and early childhood are more likely to give birth to LBW infants, thus contributing to the transgenerational cycle of malnutrition and poverty [4].

Prenatal Interventions

The determinants leading to LBW are complex and inter-related. Prevalence of infections, undernutrition, sanitary conditions, access and quality of the health care system have among others been associated with poor pregnancy outcomes. For effective interventions, it will be important to gain more insight into these determinants. Determinants as well as possible interventions are likely to be different depending on the context and whether the LBW was due to premature delivery, intrauterine growth retardation or both. Challenges in adequately measuring gestational length, particularly in developing country settings, make it
difficult to define the underlying nature of the LBW and may result in misclassifications and inappropriate interventions. As discussed by Dr. Uauy, the international fetal growth standards that are currently being developed will enable a more accurate definition of gestational duration, better designed interventions, and prevent unjustified neonatal care interventions.

Most antenatal studies and programs focus on single interventions, thereby ignoring the complex and inter-related risk factors leading to adverse pregnancy outcomes. Ideally, conceptual models are required which will enable us to determine the estimated population-specific, relative contribution of single and combined risk factors to the public health issue while taking synergy between risk factors into account. Establishing these conceptual models is clearly no easy task, and will require more research and resources than currently available.

Meanwhile, systematic reviews and meta-analyses of antenatal interventions are important tools to assess the relative contribution of single attributable risks, but these reviews do not always provide unequivocal answers. This is clearly demonstrated in the growing evidence base on prenatal multiple micronutrient interventions.

Antenatal multiple micronutrient supplement interventions for instance were found to improve intrauterine growth with an average of 54.5 g (95% CI: 45.4–63.5 g) compared to iron-folic acid supplements under controlled settings as was concluded in the systematic review presented by Dr. Ramakrishnan. However, the findings of this review also suggested that prenatal multiple micronutrient supplements may result in an increased risk of neonatal deaths in a subgroup of interventions starting after the first trimester. These findings therefore cannot immediately be translated into policy or program recommendations, particularly not in settings without adequate antenatal care.

Targeted or blanket food fortification may be a more effective and sustainable delivery channel to improve nutritional status of pregnant women and women of reproductive age, but has been relatively little studied. A recent review examined the impact of vitamin- and mineral-fortified products developed specifically for pregnant and lactating women on maternal nutritional status and growth, birth outcomes, and development of the offspring [5]. The review included findings from 16 studies in low- and high-income countries, and included interventions with micronutrient-fortified beverages with or without milk, fortified supplementary foods, and fortified high-fat supplements. The authors concluded that fortified supplementary foods during pregnancy, especially those containing milk and/or essential fatty acids have the potential to increase mean birthweight by around 60–73 g. Under certain circumstances, for example during times of food shortages in food-insecure regions of the Gambia, the impact
on increased birthweight was much larger, as high as 115–330 g [6, 7]. Both the macro- and micronutrients were thought to have contributed to these positive effects of fortified foods on birthweight.

In addition, the findings of these reviews suggest that we will have to be modest in our expectations on the size of effect with nutrition interventions alone. In general, even under controlled conditions, single nutrition interventions resulted in a 45–55 g increase in mean birthweight, and a 14–19% reduction in LBW. Integrated and holistic approaches are required that combine nutrition interventions with interventions to improve sanitation, hygiene, and antenatal care practices, and the impact of these integrated approaches on pregnancy outcomes will need to be evaluated in controlled and uncontrolled conditions, in research and program settings.

To further improve the effectiveness in programs of already established interventions with a proven benefit on pregnancy outcomes, more (operational) research is needed. Issues with compliance, costs, distribution and reach currently often limit the potential benefit in programs of interventions such as iron-folic acid supplementation.

There is growing evidence that the effects of antenatal micronutrient interventions may be extended into infancy with the infants of supplemented mothers experiencing better length and weight growth during the first year of infancy in Burkina Faso [8], and less morbidity from infectious diseases in the first year of life in Peru [9] and Bangladesh [10]. Data from birth cohort studies in Pune, India, and emerging evidence from intervention studies in Nepal associated maternal B12 and folic acid status and antenatal multiple micronutrient, including folate, supplementation, with benefits on markers of metabolic syndrome in children 6–8 years of age [11, 12]. This could be due to a role of folate in preventing epigenetic changes in developmental programming. Dietary restriction in methyl donors of folate, vitamin B12 and methionine around the time of conception may result in alterations in DNA methylation, increased adiposity, and promote insulin resistance [13]. Human data to determine the causality of these hypotheses are generally lacking, but these findings do provide further evidence for the importance of antenatal interventions for long-term health and emphasize the critical first trimester of pregnancy, during a period of rapid embryonic growth when the placental system and most of the organs are being developed. In this early stage of gestation, nutrients act not only as building blocks but also as gene regulators. For instance, data from animal models demonstrated that vitamin A plays a role in folic acid regulation, while supplementation with folate, vitamin B12, betaine and choline could also prevent epigenetic changes involved in developmental programming [13, 14].

Interventions early in pregnancy are therefore recommended but often not achievable in low-income countries, where women enter antenatal care only in the second trimester of pregnancy or even later. In addition, in societies such as
those in South Asia, many women enter pregnancy already in a disadvantaged health and nutritional status, limiting the potential effect size one can expect with antenatal interventions alone.

**Preconception Interventions**

This has shifted the attention from prenatal to preconception care to ensure that women are in the best health possible at the start of their pregnancy. In the systematic review on preconception risks and interventions presented by Dr. Bhutta, it was concluded that interventions that promote adolescent health and prevent teenage pregnancies, and that encourage contraceptive use and appropriate birth spacing, as well as optimize weight and micronutrient status, and ensure management of chronic conditions, especially preconception diabetic care, all have been shown to be efficacious in reducing maternal and neonatal mortality and morbidity.

Empowering women and adolescent girls can perhaps be considered as one of the most effective interventions to improve pregnancy outcomes. Successful interventions have been reported which enabled women to time and delay their first and subsequent pregnancies. As discussed by Dr. Bhutta, personal development programs that incorporate skill building and include contraceptive provision prevented 15% (95% CI: 2–26%) of first adolescent pregnancies, and programs that teach parenting skills and enable teen mothers to complete their education decreased repeat adolescent pregnancies by 37% (95% CI: 18–51%). The challenge is to ensure that these interventions are equally accessible at scale to all, to develop and measure impact of interventions that address social determinants and to target gaps in the continuum of care.

Changing societies and social structures is difficult and will take time. The rapid economic growth, accompanied with improvements in nutritional status of women and children in emerging economies such as India, are proof that these changes are achievable and should be aimed for in the long run. Systematic reviews as those that have been presented at this workshop are important tools in this context to help authorities and policymakers in defining priorities and moving into action.

However, meanwhile, efforts are required to improve the nutritional status of women before entering pregnancy, to improve delivery systems and scale up proven successful antenatal interventions in order to reduce the prevalence of LBW and its short- and long-term health consequences and thereby improve lives of current and future generations of women and children.

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References


Nutritional Regulation of Fetal Growth

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Abstract

Fetal growth is largely regulated by nutritional supply. The placenta is responsible for fetal nutrient supply for much of pregnancy, but in early pregnancy nutrition is histiotrophic. Both placental size and efficiency, and fetal growth, may be affected by maternal nutritional state before and during very early pregnancy. In contrast, manipulating maternal nutrition during later stages of pregnancy has a smaller than expected effect on fetal growth. Maternal nutrition before and during early pregnancy also has a greater effect on gestation length than maternal nutrition later in pregnancy, suggesting that nutritional status may regulate both fetal growth trajectory and gestation length and that these two outcomes may be linked. Thus, determination of the nutritional factors regulating fetal growth, and potentially postnatal growth and body phenotype, may lie with the maternal nutritional status even before conception.

Postnatal growth is largely determined by genetic factors if nutrition is sufficient. In contrast, fetal growth is largely determined by the maternal uterine environment. For example, experimental studies of either cross-breeding or embryo transfer between breeds of different maternal body size have demonstrated that fetal growth is determined largely by factors related to maternal body size [1, 2]. In humans, the paternal genome has a relatively small role in determining birthweight, with the intraclass correlation coefficient for birthweight for maternal half-sibs substantially greater than that for paternal half-sibs (0.58 vs. 0.10) [3]. The maternal influence on fetal growth is termed maternal constraint and is thought predominantly to reflect maternal uterine size and capacity to supply
nutrients to the developing fetus. Thus, this concept implies that fetal growth is largely determined by fetal nutritional status, a concept supported by the fact that the major hormonal mediators of fetal growth, the insulin-like growth factors, are also regulated by fetal nutrient supply [4, 5], again in contrast to their postnatal regulation. Although the fetus is dependent upon the mother for nutrient supply, fetal nutrition is quite distinct from maternal nutrition as will be discussed below. In addition, there also is increasing evidence for the role of nutrition in the very early pregnancy period in determining fetal growth, fetal developmental trajectory and the developmental origins of disease.

**Fetal Nutrition in Early and Late Gestation – Role of the Placenta**

For much of the first trimester, fetal nutrition is histiotrophic rather than hemotrophic, with the conceptus receiving nutrients secreted from uterine glands [6]. Nutrition of the early conceptus is much less well understood than transplacental nutrition and, although the nutrient requirements of the early embryo are tiny, alterations in this environment, probably largely regulated by hormonal signals, do affect fetal development [reviewed in 6]. Maternal placental blood flow, and therefore presumably hemotrophic nutrition, is present towards the end of the first trimester, initially in the periphery of the placenta and later centrally [7]. The placenta then forms the interface between maternal nutrition and fetal nutrition, and a variety of factors affect the transfer of maternal nutrients to the fetus, including placental blood supply on both maternal and fetal sides, placental size and morphology, and nutrient transporter abundance and function. Thus, fetal nutrition is determined not only by maternal nutrition but also by this supply line connecting the mother and fetus [8]. Maternal factors such as maternal disease (e.g. diabetes, pre-existing hypertension) or excessive exercise, and placental factors such as impaired placental development, may have a profound effect on this supply line and thus on fetal nutrient supply.

**Nutritional Determinants of Placental Development**

The placenta develops from the trophectoderm, which initially forms villi surrounding the whole of the chorionic sac. Villi over the superficial pole then regress, forming the discoid placenta [6]. Maternal undernutrition throughout pregnancy has been reported to impact on placental development [9]. When the nutritional deprivation is in the second half of pregnancy, both placental and fetal weight appear to be affected. In contrast, maternal undernutrition in early
pregnancy appears to have a greater effect on placental size and efficiency than on fetal growth, perhaps indicating an adaptive response of the placenta to nutritional signals. Practice in sheep farmers has long been to place ewes on poorer pasture in early pregnancy to increase placental size and, therefore, lamb birthweight [10]. Experimental manipulation of maternal nutrition in sheep, with a period of undernutrition from around the time of placental attachment to mid-pregnancy (28–77 days’ gestation), has been shown to result in increased placental size and placental:fetal weight ratio, indicating increased placental efficiency [11]. Elegant studies in rats have demonstrated that a maternal low-protein diet (9 vs. 18% casein) only in the peri-implantation period leads to reduced blastocyst cell number, due to decreased proliferation, and alterations in fetal growth [12]. This change in cell number was initially found in the inner cell mass (which forms the embryo) and then in the trophectoderm (which forms the extraembryonic tissues), and was associated with reduced $H19$ mRNA expression in male embryos [13]. Embryo transfer experiments between dams fed low-protein and normal protein diets confirmed that these changes are inherent to the blastocyst rather than the ongoing maternal environment, indicating that the altered maternal nutrition resulted in developmental changes in the embryo [14]. Further investigation of the visceral yolk sac endoderm (VYSE), which is responsible for histiotrophic nutrition of the rat embryo from before placental development but which also makes a nutritional contribution through to term, demonstrated increased VYSE endocytic capacity and upregulated megalin protein expression, a transmembrane protein involved in endocytosis of plasma proteins for fetal growth [14]. Taken together, these experiments suggest that in both small and large mammals, maternal nutritional status in early pregnancy influences placental development, efficiency and fetal growth.

Observational studies suggest that the same is probably true in humans. Women exposed to severe undernutrition in late gestation due to the Dutch Famine of 1944–1945 had babies and placentae of reduced weight, but the placental:birthweight ratio, a measure of placental efficiency, was unaltered [15]. In contrast, women exposed to famine in early pregnancy had increased placental weights without an effect on fetal size, suggesting increased placental efficiency and indicating an adaptation of the placenta in response to early nutritional signals to maintain nutritional supply in late gestation. Data from a cohort of women in India suggest that even longer term indicators of maternal nutrition may affect placental development [16]. In this study, relationships between placental morphometry and markers of various phases of maternal growth (mother’s own fetal/early infant growth represented by adult head circumference; mother’s own childhood growth represented by adult height, and mother’s current nutritional status represented by fat mass) were assessed. Maternal head
circumference and maternal fat mass, but not maternal height, were both re-
lated to placental size and efficiency (assessed by ratios of various placental mea-
urements to birthweight), suggesting that the mother’s nutritional experience
as a fetus and also her prepregnancy nutritional status affect placental develop-
ment [16]. The effects of mothers’ nutrition as fetuses may be explained by the
fact that a mother’s own oocytes develop in early gestation before entering a pe-
riod of quiescence until puberty implying an intergenerational effect of maternal
nutrition on grand-offspring fetal nutrition. Indeed, this appears to be the case,
with intergenerational studies from the Dutch Famine reporting effects on
birthweight of babies born to mothers who were in utero during the Famine; ef-
fects were greatest in women exposed as fetuses to famine in the first trimester
of pregnancy [17]. It has been estimated that maternal factors at the time of the
pregnancy account for not more than 15% of the variability in fetal growth,
whereas the parents’ own fetal growth can account for up to 50% in unadjusted
analyses and 33% in adjusted analyses [18].

**Maternal Nutritional Determinants of Fetal Growth**

The concept of the fetal nutrient supply line, the central role of the placenta
in fetal nutrient supply and the evidence suggesting that placental size and ef-
ficiency may be affected more by maternal nutritional status in early preg-
nancy help explain the relatively small effect that maternal nutritional status
during pregnancy appears to have on fetal size at birth. Meta-analyses of ma-
ternal nutritional supplementation during pregnancy have found only small
effects on size at birth [weighted mean difference (95% confidence intervals,
CI): +37.6 (–0.2 to 75.5) g], even when only trials involving supplementation
of undernourished women are considered [weighted mean difference +49.4
(–2.0 to 100.8) g] [19]. Similarly, although documented periods of significant
nutritional deprivation, such as the Dutch Famine [17] and seasonal famine
in the Gambia [20], do result in reduced birthweight, the effect size is rela-
tively modest.

In contrast to this small effect of maternal nutritional status during preg-
nancy, maternal prepregnancy weight and body mass index are associated with
impaired fetal growth. A recent large meta-analysis found a 50–60% increased
risk of low birthweight in women who were underweight before pregnancy (ad-
justed relative risk 1.64, 95% CI: 1.38–1.94) compared with normal weight
women [21]. Artificial reproductive technology (ART), which may involve al-
terred nutrition of the early embryo in the case of in vitro fertilization and, via
different hormonal and uterine environments, possibly in other modes of ART
also, is also associated with reduced size at birth in both singleton and twin pregnancies [22, 23].

Thus, it seems that maternal factors operating both well before and during very early pregnancy may be more important for determination of placental efficiency, fetal growth trajectory and birthweight than variations in maternal nutrition during pregnancy. If these factors operate solely through effects on the placenta, then one would expect effects on fetal growth only to become apparent after the onset of hemotrophic nutrition; that is, after the first trimester. Indeed, given consistent maternal phenotypes and ‘normal’ pregnancies, fetal growth does appear to be fairly uniform during the first trimester [24], forming the basis of the reliance on first trimester ultrasound dating of gestational age. However, observational data in humans and experimental data in animals indicate that fetal growth trajectory may be determined before hemotrophic nutrition is fully established. For example, a fetal size that is smaller than expected on first trimester ultrasonography in fetuses of known gestational age is linearly associated with reduced size at birth [25].

Intriguingly, all of the pre- and/or early-pregnancy factors discussed above (maternal prepregnancy weight/body mass index, discrepancy between observed and expected fetal size on first trimester ultrasonogram and ART) also increase the risk of preterm birth [26]. Babies born preterm are relatively growth restricted compared with fetuses of similar gestational age who go on to deliver at term [27], raising the intriguing possibility that the early factors leading to reduced fetal growth may also lead to preterm birth.

**Experimental Manipulation of Maternal Nutrition and Fetal Growth**

Although maternal nutrition during pregnancy in humans appears to have relatively small effects on fetal growth, in experimental animals it is relatively straightforward to induce fetal growth restriction via significant levels of maternal undernutrition throughout gestation or in late gestation in rats [28], guinea pigs [29] and sheep [30]. Direct fetal catheterization in late-gestation sheep has shown slowing of fetal growth with severe maternal undernutrition that is reversible only for a limited period of time; with ongoing undernutrition, the reduced fetal growth trajectory does not return to normal with refeeding [31].

In singleton sheep pregnancies, we also have shown that moderate maternal undernutrition only around the time of conception results in a reduced fetal growth trajectory in late gestation [32]. Intriguingly, although in this study the fetuses of periconceptionally undernourished ewes had slower growth rates in late gestation, they exhibited a lesser reduction in growth trajectory in response
to an additional, brief, maternal fast in late gestation than fetuses whose mothers had been well nourished throughout pregnancy [32, 33]. Thus, a fetus with a slower growth rate determined by a periconceptional exposure may be better able to tolerate nutritional restriction in late gestation, either due to reduced demand (although the birthweight of fetuses in the two groups was very similar) or due to altered metabolic regulation.

Twins are often considered to be growth restricted [34] and, indeed, are born smaller than singletons. The growth restriction in twins has long been considered to be due to constraints of intrauterine space and limitations of placental nutrient supply, although there are few data to support this. In sheep, twin fetuses also grow more slowly than singletons in mid-late gestation and are born smaller, yet have a greater slowing of growth in response to a maternal fast in late gestation than do singletons [35], presumably reflecting the fact that the reduced maternal nutrient supply is distributed between two fetuses. However, when twin-bearing ewes are also exposed to periconceptional undernutrition, the effect of the early pregnancy influence on late gestation responses to nutritional deprivation is again apparent, with the lighter twins of periconceptionally well-nourished ewes exhibiting a greater slowing of growth than the lighter twins of periconceptionally undernourished ewes [35]. These complex interactions between periconceptional events (maternal nutrition, twin conception) and late gestation fetal growth responses to nutritional stress suggest that the periconceptional stressors themselves determine fetal growth trajectory and, perhaps, also the nutritional regulation of fetal growth. We recently have demonstrated that this is indeed the case in twins in sheep. Twin-bearing ewes were randomly assigned to either fetal reduction of one twin at the end of the first trimester, converting the twin pregnancy to a singleton, or a sham procedure [36]. Fetuses conceived as twins, but which spent the majority of pregnancy as singletons (reductions), were of very similar size to twins at birth, particularly in measures of linear growth, indicating that constraint of fetal growth in twins is largely determined in early gestation. Gestation length was also the same in reductions and twins, again suggesting that fetal growth and gestation length in twins may be linked and determined in early pregnancy. Perhaps most intriguingly, it appears that twin conception also determines the regulation of postnatal growth to some degree. Reductions had accelerated postnatal growth between birth and weaning, growing faster than both singletons and twins, despite similar milk intakes. After weaning, twins demonstrated accelerated growth such that by 2 years of age (puberty is at approximately 7–9 months) animals in all three groups were of similar size (fig. 1). However, twins and reductions had greater fat mass than singletons, suggesting that adult fat mass in twins is determined by twin conception and not by fetal number in late gestation, birthweight
or postnatal growth trajectory [36]. We also have shown that maternal periconceptional undernutrition, with ewes undernourished from 60 days before to 30 days after mating (approximately the time of placental attachment in sheep) and fed normally thereafter, perturbs metabolic and endocrine regulation of postnatal growth (fig. 2) [37] and alters adult fat mass [Jaquiery, unpubl. data].

Taken together, these data indicate that the intrauterine environment, including the nutritional environment, during the very earliest stages of pregnancy may impact not only on fetal and placental growth, but also on postnatal growth and its regulation.

**Fetal Nutrition and Epigenetic Modifications**

The reprogramming of epigenetic marks in the zygote that occurs up to the blastocyst stage [38] makes epigenetic modifications one possible mechanism mediating the effect of the intrauterine environment on fetal development. Imprinted genes (resulting in parent of origin gene expression) are known to affect placental function and to be associated with patterns of fetal growth, with paternally expressed genes associated with promotion of fetal growth and maternally expressed genes associated with constraint of fetal growth. However, there is less evidence for an association between environmental factors known to affect fetal growth and epi-
genetic modifications in placental genes [38, 39]. Extraembryonic tissues, derived from the trophectoderm, are globally hypomethylated compared with embryonic tissues derived from the inner cell mass from as early as the blastocyst stage, and it has been proposed that this may be important for allocation or function of the lineages [38]. In contrast, a variety of environmental factors in pregnancy, including nutritional factors, have been shown to result in epigenetic modifications in the fetus. Recent data from human cohorts have correlated epigenetic marks at birth with childhood adiposity [40] and have described epigenetic changes in metastable epialleles in offspring conceived during periods of famine [41]. Experimental data in sheep have shown that periconceptional maternal undernutrition and twin conception result in similar epigenetic modifications in the pro-opiomelanocortin and glucocorticoid receptor genes in the appetite regulatory centers of the fetal ventral hypothalamus [42], and offspring have increased adult fat mass [43]. However, the signals that are responsible for transmitting intrauterine environmental information to the embryo remain unknown.

**Conclusion**

Fetal growth is regulated largely by nutrition and the intrauterine environment. Evidence increasingly is pointing towards nutritional factors in very early pregnancy, and even before pregnancy, playing key roles in determining fetal growth trajectory. Whether these nutritional factors act directly on the embryo or act via intermediary factors is not yet known. The observations that a variety of seemingly very different periconceptional factors (maternal nutrition, ART,
twin conception) can all affect fetal growth, gestation length and epigenetic marks in the fetus suggest that research into the mechanisms underlying these effects may have wide applicability to our understanding of fetal growth and, potentially, to ways of optimizing fetal growth in pregnancies where this may be at risk of being suboptimal.

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Disclosure Statement

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References

Abstract
Hormones are both growth stimulatory and growth inhibitory in utero. They act as envi-
ronmental and maturational signals in regulating tissue accretion and differentiation dur-
ing late gestation. They ensure that fetal development is appropriate for the nutrient sup-
ply and is optimal for neonatal survival. Growth-stimulatory hormones, such as insulin,
the insulin-like growth factors and the thyroid hormones, have anabolic effects on fetal
metabolism and increase cellular nutrient uptake and energy production for tissue accre-
tion. Thyroid hormones also have specific effects on tissue differentiation at key develop-
mental milestones. Similarly, leptin appears to affect development of specific fetal tissues
and may counterbalance the maturational actions of other hormones near term. Gluco-
corticoids inhibit growth in utero but are essential for prepartum tissue differentiation in
preparation for delivery. They also affect fetal bioavailability of most of the other growth-
regulatory hormones. In addition, many of these hormones alter the placental capacity to
supply nutrients for fetal growth. In producing a fetoplacental epigenome specific to the
prevailing intrauterine environment, hormones interact to produce phenotypical diver-
sity with potential health consequences long after birth.

Introduction
Hormones are both growth stimulatory and growth inhibitory in utero [1]. They
regulate tissue growth and development through actions on cell proliferation
and differentiation. They have anabolic and catabolic actions on fetal metabo-
lim and alter the phenotype of the placenta, the principal source of nutrients
for fetal growth [2]. They signal nutrient availability to the fetus and fetal nutrient demands for growth back to the placenta [1, 2]. They also act as maturational signals near term [3]. By modifying the fetal growth trajectory, hormones have a central role in programming development in utero and in ensuring survival both before and at birth [1, 4]. The main growth regulatory hormones during late gestation are insulin, the insulin-like growth factors (IGFs), the thyroid hormones, cortisol and possibly leptin [1]. Of these, some increase in concentration towards term while others maintain stable values throughout late gestation in normal conditions (fig. 1a). This review examines the role of hormones in controlling fetal growth with particular emphasis on the endocrine interactions involved during late gestation.

**Insulin**

Insulin is essential for normal fetal growth [1]. Its deficiency in utero lowers fetal bodyweight but has little apparent effect on placental weight or fetal tissue differentiation (table 1). In fetal sheep, surgical removal of the pancreas reduces fetal growth rate uniformly by 50–60% during late gestation (fig. 1b) and results in a symmetric type of intrauterine growth restriction (IUGR) [8]. These growth defects can be prevented by giving insulin replacement [8]. In contrast, induction of hyperinsulinemia has less consistent effects on fetal growth and is associated primarily with increased adiposity [4]. Weight gain in response to fetal hyperinsulinemia is, therefore, greater in species that have a high fat content at birth [3].

Insulin stimulates fetal growth, in part, by its anabolic actions on glucose and amino acid metabolism [17]. It increases the tissue uptake of these metabolites and enhances the rates of glucose utilization and protein synthesis by fetal sheep [4, 17]. Fetal glucose and amino acid concentrations, therefore, fall in response to fetal insulin administration. This increases the transplacental concentration gradient for glucose and its diffusion into the fetus [2]. Consequently, more glucose and amino acids are available for fetal growth and energy production in the presence of insulin. Although fetal insulin concentrations normally rise in parallel with fetal glucose concentrations, insulin is not primarily a glucoregulatory hormone in utero but, rather, acts to match the fetal rate of glucose utilization to the placental rate of glucose supply [1, 4]. Thus, fetal insulin concentrations are directly related to fetal rates of growth and glucose metabolism [1]. However, these actions of insulin may be mediated, in part, by the IGFs as plasma IGF-1 concentrations are low in pancreatectomized fetuses [6].
Fig. 1. Mean values of plasma concentrations of leptin, cortisol, T₃, insulin and IGF-1 in fetal sheep during normal conditions (a), and growth rate (±SEM) measured as crown rump (CRL) increment in control, sham-operated, pancreatectomized, adrenalectomized and cortisol-infused sheep fetuses with respect to days from term (assigned as 140 days of gestation; b). Columns with different letters are significantly different from each other (p < 0.02, one-way repeated measures ANOVA). * p < 0.05, significantly different from value in control fetuses (two-way repeated-measures ANOVA). Data from references [3–9].
Table 1. Effects of manipulating hormone concentrations on the growth, development and endocrine interactions of fetal sheep during late gestation

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Procedure</th>
<th>Growth (% normal at term)</th>
<th>Specific tissue defects</th>
<th>Endocrine interactions</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>bodyweight</td>
<td>CRL</td>
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<tr>
<td>Insulin</td>
<td>pancreatectomy</td>
<td>70</td>
<td>90</td>
<td>spleen, thymus, liver</td>
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<tr>
<td></td>
<td>administration</td>
<td>100–120</td>
<td>100</td>
<td>increased fat</td>
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<td>IGF-1</td>
<td>administration</td>
<td>100</td>
<td>100</td>
<td>liver, lung, heart, kidney, adrenal</td>
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<td>Thyroid hormones</td>
<td>thyroidectomy</td>
<td>70</td>
<td>90</td>
<td>wool follicles, lungs, skeletal muscle, liver, adrenal gland, bones, heart, SNS, CNS</td>
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<td>T₃ administration</td>
<td>100</td>
<td>100</td>
<td>liver</td>
<td>no Δplasma insulin, T₄, T₃, cortisol before term</td>
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<tr>
<td>Leptin</td>
<td>administration</td>
<td>100</td>
<td>100</td>
<td>liver, adipose tissue</td>
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<td>Cortisol</td>
<td>adrenalectomy</td>
<td>115</td>
<td>110</td>
<td>liver, lungs, gut, pituitary, heart</td>
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<tr>
<td></td>
<td>administration</td>
<td>80–85</td>
<td>90</td>
<td>liver, lungs, gut, brain, heart, skeletal muscle, placenta</td>
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Data from references [4–6, 10–16, 19]. SNS = Sympathetic nervous system; CNS = central nervous system.
Insulin-Like Growth Factors

Like insulin, IGFs stimulate fetal growth [6]. Deletion of either the Igf1 or Igf2 gene in mice reduces fetal bodyweight by 40% at term and leads to developmental abnormalities in a range of fetal tissues [18]. Deletion of both genes or of the Igf type 1 receptor through which the two IGFs act causes an even more severe form of IUGR, which is lethal at birth [18]. Defects in expression of the IGF1 or IGF1R gene also lead to severe IUGR in human infants [18]. Conversely, fetal overexposure to IGF-2 causes macrosomia and specific organomegaly in mice, cattle, sheep and human infants [2, 6]. In contrast, administration of IGF-1 has little effect on overall growth of normal fetal sheep, although it increases the weight of individual fetal tissues (table 1). However, in growth-restricted fetuses, both body and organ weights are improved by IGF-1 treatment [19].

The IGFs are mitogens which regulate cell proliferation and differentiation [6]. In particular, IGF-2 appears to control the balance between these two processes, especially close to term when fetal tissues are maturing in preparation for birth [1, 6]. IGF-1 also has anabolic effects on fetal metabolism [6, 19]. In fetal sheep, IGF-1 administration stimulates glucose utilization, although to a lesser extent than insulin [19]. It also reduces protein catabolism and oxidation of amino acids in utero with the net effect that fetal protein synthesis increases [6, 19]. In part, these actions of IGFs may be mediated indirectly by changes in placental development [2, 6]. IGF-2 overexpression causes placentomegaly, whereas deletion of the Igf2 gene restricts placental growth, in line with the changes in fetal weight. IGF-2 also influences the transport phenotype of the mouse placenta by altering its morphology and nutrient transporter abundance [18]. Similarly, IGF-1 has been shown to alter nutrient transfer across ovine placenta in vivo and human syncytiotrophoblast in vitro [19, 20].

Tissue expression and circulating concentrations of the IGFs are influenced by a number of nutritional and hormonal factors [6], although basal IGF-1 concentrations vary little during late gestation (fig. 1a). IGF-1 is more responsive to fetal glucose and oxygen levels than IGF-2, which indicates that IGF-2 may be the constitutive drive to fetal mass accumulation while IGF-1 acts as a nutrient sensor like insulin, ensuring that fetal growth is commensurate with the nutrient supply [6]. Expression of both IGFs is sensitive to thyroid and glucocorticoid hormones during late gestation, although the responses are tissue specific and dependent on gestational age (table 1). Indeed, in fetal ovine liver, the normal prepartum decline in IGF2 expression and upregulation of IGF1 transcript abundance depends on the coordinated actions of glucocorticoid and thyroid hormones [4]. In turn, the IGFs affect several other endocrine axes involved in
fetal growth including the pancreatic β-cells and adrenal cortex [1, 3, 6]. For instance, IGF-1 infusion lowers insulin concentrations in fetal sheep, which may explain their lack of bodyweight increment during treatment (table 1).

**Thyroid Hormones**

Before birth, thyroid hormones promote general body growth and the development of specific tissues, such as the brain and skeletal-muscular system [1]. Surgical removal of the fetal thyroid gland causes IUGR in species with little, if any, placental transfer of maternal thyroid hormones, such as sheep and pigs [1]. In fetal sheep, thyroidectomy prevents specific developmental events, such as wool follicle differentiation, at mid-gestation and reduces fetal growth more generally during late gestation with reductions in limb lengths and in body and organ weights by term (table 1). All these growth defects can be ameliorated by thyroxine (T₄) replacement [21]. In human and rodent species that have greater placental permeability to maternal thyroid hormones, deficient fetal T₄ production has less marked effects on fetal bodyweight but still has adverse consequences for brain development, particularly close to term [22, 23].

In thyroidectomized fetal sheep, IUGR is asymmetrical with more pronounced reductions in growth of the appendicular than axial skeleton and changes in metatarsal structure and mechanical properties, indicative of delayed bone development [10, 15]. These changes are also associated with a reduction in the circulating levels of osteocalcin, a marker of osteoblast activity, without any change in the plasma concentrations of total calcium or CTX, a marker of osteoclast activity. Fetal hypothyroidism, therefore, appears to lead to reduced bone deposition rather than any change in bone degradation or calcium homeostasis in utero [15]. Abnormal bone structure is also seen in human neonates with congenital hypothyroidism, despite some transplacental transfer of maternal thyroid hormones [23]. Taken together, these findings suggest that bone development is particularly sensitive to thyroid hormone levels in utero.

In several species, the fetal concentration of tri-iodothyronine (T₃) increases towards term (fig. 1a) due to maturational changes in tissue activity of the deiodinases responsible for converting T₄ to T₃ [24]. In turn, the rise in plasma T₃ initiates differentiation of key fetal tissues essential for neonatal survival (fig. 2). Thyroid hormones are also sensitive to nutrient and oxygen availability and, in association with insulin and IGF-1, have an important role in matching fetal growth to the nutrient supply [1]. Fetal T₄ and T₃ concentrations are suppressed by undernutrition and several other types of experimental IUGR [1, 25]. For example, in rats with uterine artery ligation, bodyweight and
plasma T₄ concentrations are positively correlated in the pups at 20–21 days of gestation and at birth [25]. In addition, thyroid hormone receptor binding in skeletal muscle is reduced in newborn runt compared to normal-sized piglets [26].

Thyroid hormones appear to regulate fetal growth directly through anabolic effects on fetal metabolism [21] and/or indirectly via interactions with other endocrine systems (table 1). They can also affect placental growth or transport function [10, 21]. In fetal sheep, thyroidectomy reduces fetal rates of oxygen consumption and glucose oxidation, which can be restored to normal values by T₄ replacement [21]. Overall, plasma fetal T₄ concentrations correlate with fetal oxygen consumption [21], possibly via changes in sodium-potassium ATPase expression and activity [4]. Thus, less energy will be derived from oxidative metabolism in hypothyroid than euthyroid fetuses, which may constrain growth, particularly of nonessential tissues. Fetal thyroidectomy has also been shown to alter gene expression for the growth hor-

**Fig. 2.** Schematic diagram showing the effects of hormones on tissue accretion and differentiation in fetal sheep during late gestation and the interactions between hormones in controlling these processes. Tissue accretion decreases while tissue differentiation increases towards birth. Stimulatory effects are shown with light grey arrows, while inhibitory effects are shown with dark grey arrows. Solid arrows indicate known effects, dotted arrow potential effects. Data from references [1–9, 12].
mone receptor (GHR), IGF-1 and IGF-2 in fetal liver and skeletal muscle (table 1), which will have consequences for development of these and other tissues, like the placenta, responsive to circulating IGFs [2, 6].

**Leptin**

Leptin is present in the circulation of human and ovine fetuses from mid-gestation, and the genes for leptin and its receptors are expressed widely in fetoplacental tissues [27, 28]. The role of leptin in the control of fetal growth, however, is controversial. In human neonates, umbilical leptin concentration correlates with several indices of intrauterine growth, such as placental and bodyweights, adiposity and bone mineral content [29]. However, there is little evidence to suggest that leptin deficiency affects birthweight or gross morphology in human and murine neonates [27, 28]. Furthermore, in fetal sheep during late gestation, administration of recombinant ovine leptin has no effect on the fetal growth rate or body or organ weights [11, 14]. In fetal rodents, leptin stimulates proliferation of pancreatic islet cells in vitro and is important for the normal development of neuronal and glial lineage cells in the cerebral cortex [27, 30]. It may also antagonize some of the maturational effects of glucocorticoids in the fetal liver near term [11, 13, 28]. Therefore, leptin may have tissue-specific developmental effects and/or act simply as an endocrine marker of fetal size and energy stores, rather than as a physiological regulator of fetal growth per se.

Leptin produced by the placenta may have an important role in controlling placental growth and function [2, 20, 31]. In pregnant mice heterozygous for a mutation in the leptin receptor, placental leptin concentration is elevated, and this is associated with an increase in fetal weight near term, both in mutant and wild-type pups [32]. In addition, leptin treatment of wild-type pregnant mice decreases placental leptin content and causes reductions in both placental and fetal weights [32]. In vitro studies using human trophoblast cells have shown that leptin stimulates proliferation and inhibits apoptotic processes, while molecular inhibition of placental leptin expression upregulates indicators of apoptosis and causes a reduction in cell division [31]. Leptin also increases amino acid transport in human placental villous fragments in vitro [20].

Leptin synthesis in adipose tissue and circulating concentrations in utero are influenced by hormones known to be involved in the control of fetal growth (table 1). Leptin availability is increased by insulin, thyroid and glucocorticoid hormones (fig. 2). Leptin concentrations, therefore, rise in parallel
with the prepartum cortisol surge in fetal sheep (fig. 1a). In human infants, umbilical leptin concentration is positively associated with insulin and IGF-1 concentrations [29]. Exogenous infusion of leptin in fetal sheep increases IGF type 1 receptor protein levels in perirenal adipose tissue and causes a shift in the relative proportions of unilocular and multilocular cells [11, 14]. However, the extent to which leptin is a marker of the activity of hormones like insulin and the IGFs and/or contributes to their growth-regulatory actions remains unclear.

**Glucocorticoids**

In several species, glucocorticoid administration to either the mother or fetus leads to IUGR [1, 3]. In sheep, cortisol infusion into preterm fetuses reduces their growth rate by 50% to values similar to those seen in older fetuses closer to term (fig. 1b). Similarly, maternal cortisol infusion for 10 days during late gestation reduces fetal growth rate by 30% in association with decreased fetal body and individual tissue weights [3, 33]. Conversely, preventing the normal prepartum cortisol surge towards term by fetal adrenalectomy prevents the normal prepartum decline in fetal growth rate and increases bodyweight at term (fig. 1b). Glucocorticoids are, therefore, responsible for the natural decrease in growth rate towards term (fig. 1) and probably also contribute to IUGR during adverse conditions which raise fetal glucocorticoid concentrations. In addition, glucocorticoids stimulate the morphological and functional differentiation of a wide range of fetal tissues (table 1). They alter cellular availability of receptors, enzymes, ion channels and transporters, which, in turn, activate many processes that have little or no function prenatally but are vital postnatally [1, 3]. Thus, glucocorticoids switch the fetus from tissue accretion to differentiation both at term and in adverse conditions earlier in gestation.

The growth-inhibitory actions of glucocorticoids are mediated indirectly by the placenta and directly by catabolic effects on fetal metabolism [2, 33]. In all species studied to date, administration of either natural or synthetic glucocorticoids reduces placental weight and compromises placental morphology [33]. These treatments also alter placental transport of glucose and amino acids as well as placental production and metabolism of hormones such as leptin, placental lactogens, eicosanoids and the thyroid hormones [2, 33]. Altogether, these placental changes alter bioavailability of key growth regulatory factors and the nutrient supply for fetal growth. In the fetus, glucocorticoids activate glucogenesis, proteolysis and oxidation of amino acids, which further restricts accretion of new tissue [3, 4]. The actions of glucocorticoids on differentiation

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are also direct via changes in gene expression and indirect through alterations in other endocrine systems such as the IGFs, leptin and thyroid hormones (fig. 2). Cortisol downregulates IGF2 gene expression in fetal ovine liver in association with upregulated expression of the GHR and adult transcript of the IGF1 genes [4]. It also activates hepatic and renal deiodinase type 1 which converts T₄ to T₃ and downregulates placental deiodinase type 3 which converts T₄ to biologically inactive reverse T₃ [24]. Indeed, since there appears to be no glucocorticoid response element on the relevant promoter of the ovine IGF2 gene [6], cortisol-induced suppression of fetal hepatic IGF2 may be dependent on the increased hepatic T₃ bioavailability. Thus, near term, glucocorticoids may be the master regulator of an endocrine cascade responsible for coordinating tissue growth and differentiation to maximize the chances of surviving at birth (fig. 2).

Conclusions

Hormones act as environmental and maturational signals in the regulation of tissue accretion and differentiation during late gestation (fig. 2). The different growth stimulatory and inhibitory hormones interact in regulating the fetoplacental transcriptome to ensure that fetal growth and development are matched to the nutrient supply and are optimal for immediate survival. However, by producing an epigenome specific to the prevailing intrauterine environment, hormones can modify the fetal growth trajectory with ensuing phenotypical consequences long after birth.

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Disclosure Statement

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References

Dr. Bloomfield and Dr. Fowden both gave stimulating talks that addressed the complex and multifactorial nature that nutrition and hormones contribute to the control of fetal growth. While energy and nutrients are necessary substrates at the simplest level, growth is not left to chance, and there is a complex web of hormonal regulation, with multiple redundancies, which orchestrate growth. Certain hormones, such as insulin, insulin-like growth factors and thyroid hormones, stimulate growth by their anabolic effects on fetal nutritional metabolism, while the glucocorticoids have catabolic effects on fetal nutritional metabolism. Furthermore, some hormones are regulators of tissue differentiation at specific developmental milestones. Dr. Fowden’s presentation and the subsequent discussion focused heavily on the importance of glucocorticoids and the complex interactions relative to organ growth and maturity. One of the issues debated related to growth vs. maturity and the role of glucocorticoids. Put simply, the timing of glucocorticoid concentration surges during the in utero period is important. Exposure to increasing glucocorticoid concentrations may create an early switch from tissue accretion to tissue differentiation and result in adverse changes in a variety of different organs that may have negative effects later in life. However, these observations, largely from animal studies are challenging to directly translate to the human situation.

The most relevant and clinically important discussion related to the use of antenatal glucocorticoids in pregnancy for women threatening to deliver a preterm infant and the desire to promote lung maturation so that the risk of respiratory distress is minimized for the preterm infant, but there are potential trade-offs to consider between lung maturation, growth and later neurodevelopment. While facilitating lung maturation for the preterm infant with antenatal gluco-
corticoids is a positive outcome, antenatal glucocorticoids are known to slow fetal growth, and some studies have raised concerns regarding negative consequences on later cognitive outcomes for children. However, there is evidence from randomized controlled trials that antenatal glucocorticoids have no adverse effects on longer term neurodevelopment of the offspring. The most recent systematic review of randomized trials, using the Cochrane methodology, evaluating the effectiveness and safety of one or more repeat doses given to women at risk of preterm birth included 10 trials involving over 4,730 women and 5,700 infants [1]. This review showed that repeat antenatal glucocorticoids versus a single-dose treatment reduced the risk of respiratory distress syndrome and serious neonatal morbidity, with no evidence of either significant benefit or harm at follow-up. The authors concluded that repeat doses of glucocorticoids should be considered in women at risk of preterm birth in view of the neonatal benefits [1].

One of the features of Dr. Bloomfield’s presentation was to highlight the difference between fetal nutrition and maternal nutrition. Fetal nutrition is largely governed by the placenta, and many of the experiments of placental restriction in animal models are not analogous to nutritional restriction in human pregnancy but more analogous to a placental insufficiency model such as preeclampsia. On the other hand, it is maternal nutrition and the maternal diet that is of core interest to health professionals because of the possibility of altering the maternal diet. Clearly from the previous discussion, the mother’s diet is not the only factor determining fetal growth, and Dr. Bloomfield did highlight that the best we can probably hope to achieve by supplementing the maternal diet is to increase birthweight of the order of about 50 g. Although this effect size of maternal nutrition on birthweight (as a surrogate for fetal growth) appears quite modest, it is important to provide a context and to bear in mind that women who are from truly nutritionally deprived environments from non-industrialized countries on average would have infants with birthweights 300–500 g lighter than women who may be from poor nutritional settings in industrialized countries. Dr. Bloomfield also argued that the fetal growth trajectory may be set very early in pregnancy, and it may be that to maximize any effect of maternal nutrition on fetal growth the dietary interventions may need to be in place early, and before pregnancy has started. Nevertheless, the cautionary tale from many randomized trials in the area of perinatal nutrition is that the actual effect size is often smaller than expected, and it would not be surprising if the results of planned or future early intervention strategies of diet and nutrition also demonstrate modest effects on fetal growth. In fact, as we learn more about factors controlling fetal growth, there are many protections and inbuilt mechanisms to buffer the fetus from various positive and negative stresses, some of which are obviously nutritional.
In summary, the maternal diet, rather than the fetal environment, can have relatively modest effects on fetal growth, and this is perhaps not unexpected. However, these modest changes may be important in the longer term, and from a public health perspective the basic message of maintaining good nutritional status across the life course remains.

Maria Makrides

Reference

Abstract

Epidemiological, clinical, physiological, cellular and molecular evidence suggests the origins of obesity and metabolic dysfunction can be traced back to intrauterine life and supports an important role for maternal nutrition prior to and during gestation in fetal programming. The elucidation of underlying mechanisms is an area of interest and intense investigation. We propose that in addition to maternal nutrition-related processes, it may be important to concurrently consider the potential role of intrauterine stress and stress biology. We frame our arguments in the larger context of an evolutionary-developmental perspective that supports roles for both nutrition and stress as key environmental conditions driving natural selection and developmental plasticity. We suggest that intrauterine stress exposure may interact with the nutritional milieu, and that stress biology may represent an underlying mechanism mediating the effects of diverse intrauterine perturbations, including but not limited to maternal nutritional insults (undernutrition and overnutrition), on brain and peripheral targets of programming of body composition, energy balance homeostasis and metabolic function. We discuss putative maternal-placental-fetal endocrine and immune/inflammatory candidate processes that may underlie the long-term effects of intrauterine stress.

Introduction

The origins of health and disease susceptibility for many of the complex, common disorders that confer the major, global burden of disease in developed societies as well as societies in rapid transition can be traced back to the intrauteri-
ine period of life (i.e. the concept of fetal or developmental programming of health and disease risk [1]). A large number of studies of fetal programming of obesity and metabolic dysfunction have focused on the critical role of maternal nutrition prior to or during gestation and have produced important findings and insights [reviewed in 2]. Questions currently under investigation include those related to mechanisms or pathways by which nutritional programming can exert life-long effects on the developing organism. Some major nutrition-related pathways relate to the effects of nutritional insults on maternal-placental-fetal glucose/insulin physiology and downstream effects on the developing fetal brain and peripheral systems. In this paper, we suggest it may be important to also simultaneously consider the potential role of intrauterine stress and stress biology for the following reasons: (a) From an evolutionary-developmental perspective, energy availability (i.e. nutrition) and challenges that have the potential to impact the structural or functional integrity and survival of the organism (i.e. stress) represent the most important environmental conditions underlying natural selection and developmental plasticity along all times scales. It is therefore likely and plausible that stress represents an important aspect of the intrauterine environment that would be expected to influence many, if not all, developmental outcomes. (b) Stress-related biological factors may exert direct effects on fetal targets of programming of body composition and metabolic function. (c) Many of the effects of nutritional insults (both undernutrition and overnutrition) may be mediated by common stress-related pathways involving the hypothalamic-pituitary-adrenal axis and inflammation. Hence, stress biology may represent a common underlying mechanism. (d) Stress and stress biology is known to alter nutrition at several levels, including caloric intake, selection of food types, and metabolic fate of energy. Conversely, nutritional status is also known to alter stress at multiple levels in the brain and periphery, including appraisals of potentially stressful circumstances, psychological and physiological stress responses, and feedback regulation. Hence, in natural in vivo settings it is likely that the effects of either nutrition or stress are modified by or conditioned upon the state of the other. This issue is particularly important in the human context, since nutritional insults and stress tend to co-occur in populations across the world.

For these reasons, we highlight below the effects of stress and stress biology on fetal programming of body composition, obesity and metabolic function. We review empirical evidence for interactive effects between stress and nutrition, describe findings from some of our own recent studies on prenatal stress and stress biology, and discuss putative maternal-placental-fetal endocrine and immune/inflammatory candidate mechanisms that may underlie and mediate short- and long-term effects of prenatal stress on the developing human fetus, with a specific focus on body composition, metabolic function, and obesity risk.
Rationale for Considering a Role for Stress in Fetal Programming

From conception onwards, the mother and her developing fetus both play an obligatory, active role in all aspects of development. Based on the consideration that environmental conditions that have shaped evolutionary selection and developmental plasticity include not only variation in energy substrate availability (i.e. nutrition) but also challenges that have the potential to impact the structural or functional integrity and survival of the organism (i.e. stress), it is likely and plausible that prenatal stress represents an important aspect of the intrauterine environment that would be expected to influence developmental outcomes [3]. Moreover, we suggest the application of a prenatal stress and stress biology framework offers an excellent model system for the study of intrauterine development and associated developmental, birth and subsequent health-related phenotypes because it is increasingly apparent that the developing fetus acquires and incorporates information about the nature of its environment in part via the same biological systems that in an already-developed individual mediate adaptation and central and peripheral responses to endogenous and exogenous stress (i.e. the neuroendocrine and immune systems [4]).

Another compelling rationale for considering a role for in utero stress as a contributor to subsequent risk of obesity and metabolic dysfunction derives from the effort to elucidate and better understand the underlying reason(s) for the well-documented, persistent and large socioeconomic and racial/ethnic disparities in the population distribution of these outcomes in the US and other developed nations. The search for explanations has led to the hypothesis that stress may, in part, independently, or in combination with other factors, account for these disparities, because the experience of social disadvantage and minority racial/ethnic status is characterized by higher levels of stress and lack of resources, and because stress and stress-related biological processes have been implicated in a wide array of adverse reproductive, developmental and other health outcomes [5, 6].

The Role of Context: Potential Interactive Effects between Stress and Nutrition

Maternal nutrition, assessed by indicators of body size (body mass index, BMI), nutritional intake or measures of nutritional biomarkers, is a well-established risk factor for childhood and adult obesity and metabolic dysfunction. Growing evidence supports the concept of a bidirectional interaction between nutrition and stress, such that the effects of nutrition on health may vary as a function of stress, or that the effects of stress on health may vary as a function of nutritional
status. For example, several experimental studies in animals have demonstrated that nutritional manipulations, particularly in the preconception or early pregnancy period, may produce their effects on maternal and fetal outcomes via alterations in stress biology [for example 7, 8]. Conversely, studies in animals and humans of stress induction (by exposure to laboratory-based stressors or endocrine stress analogues) have demonstrated effects on feeding behavior, food choice (high-calorie-dense food preference) and the metabolic fate of food in target tissues [9–13]. For example, chronic stress or cortisol administration motivates people to select high-fat food and to overeat [9, 12], and corticotropin-releasing hormone (CRH) infusion in healthy human adults also increases subsequent food intake [11]. In addition, cortisol increases insulin levels [14, 15]. Although insulin is anabolic and under normal basal conditions can increase both lean and fat mass, coelevation of insulin with cortisol preferentially increases abdominal fat stores [16, 17]. Further evidence of an interaction between stress and nutrition comes from a recent experimental study in humans demonstrating that under conditions of stress the brain’s energy need increases and it actively ‘demands’ energy from the periphery (a concept termed ‘brain-pull’ [10]).

We note that only a small number of studies have examined the relationship between maternal stress and diet or nutritional state in pregnancy. One study found that pregnant women who were more stressed in mid-pregnancy consumed more food (increased macronutrient intake) but concurrently decreased their intake of some micronutrients [18]. Another recent study demonstrated that the level of maternal stress during pregnancy was positively associated with pre-pregnancy BMI [19]. In an animal model, the interactive effects of maternal stress and nutrition on subsequent risk of offspring obesity were investigated [18], and the results suggested that prenatal stress and/or high-fat diet during the intrauterine environment affects offspring in a manner that increases their susceptibility to diet-induced obesity and leads to adverse metabolic consequences. Despite the plausibility of stress-nutrition interaction effects in the context of pregnancy, we are not aware of any human study to date that has examined these interactive effects during pregnancy on offspring body composition and metabolic function.

**Stress-Related Maternal-Placental-Fetal Endocrine and Immune/Inflammatory Processes as Potential Mediators of Fetal Programming of Health and Disease**

The fetal programming hypothesis has led to the search for underlying mechanisms by which disparate intrauterine insults exert a multitude of effects on different physiological systems in the offspring. A question of particular interest
relates to whether these biological mechanisms are exposure and/or outcome specific, or whether there may be some common mechanisms that mediate the effects of various exposures on a range of disparate outcomes. We suggest that stress-related maternal-placental-fetal endocrine and immune processes in gestation constitute an attractive underlying common candidate mechanism because they are responsive to many classes of intrauterine perturbations and they act on multiple targets of fetal programming [4]. Unlike exposure to toxins and teratogens, it is important to appreciate the fact that maternal-placental-fetal hormones and cytokines play an essential and obligatory role in orchestrating key events underlying cellular growth, replication and differentiation in the brain and peripheral tissues [4]. Thus, perturbations in the level and/or time of exposure of these biologic effectors are likely to produce alterations of normal structure and function. Furthermore, it also is important to appreciate that the state of pregnancy itself produces major and progressive alterations in the function of these systems, and that these changes may have important implications for altering the responsivity of these systems to exogenous or endogenous perturbations, and hence their downstream effects on fetal targets of programming.

**Stress Biology in Human Pregnancy**

Stress biology refers to the set of biological adaptations in response to challenges or demands that threaten or are perceived to have the potential to threaten the stability of the internal milieu of the organism. The nervous, endocrine, immune and vascular systems play a major role in adaptations to stress. There are no direct neural, vascular or other connections between the mother and her developing fetus – all communication between the maternal and fetal compartments is mediated via the placenta, an organ of fetal origin. Based on the physiology of stress, parturition and the evidence linking maternal stress to earlier delivery, we have previously proposed a biobehavioral framework of stress and adverse birth outcomes [4] that may also be applicable in the present context.

Pregnancy produces major alterations in neuroendocrine and immune function, including changes in hormone and cytokine levels and control mechanisms (feedback loops), that are crucial in providing a favorable environment within the uterus and fetal compartment for growth, differentiation and maturation and conveying signals when the fetus is ready for extrauterine life. Glucocorticoid physiology (cortisol in humans) has received extensive and well-placed consideration as a critical endocrine mediator of fetal programming, with an emphasis on not only hormone production but also hormone action mediated by tissue-specific glucocorticoid receptor expression, sensitivity and affinity,
and by maternal-fetal transfer mediated by the activity of the placental 11β-hydroxysteroid dehydrogenase enzyme system [20]. Less well recognized is the potential and perhaps equally important role of the peptide CRH. In primates, but not other mammals, the placenta synthesizes and releases CRH in large amounts into the fetal and maternal circulations, with actions on central and multiple peripheral target systems in both compartments [reviewed in 4].

With respect to the immune axis, a major endeavor of pregnancy-related alterations in immune function is to achieve and maintain the optimal balance between tolerating the fetal semi-allograft while not suppressing maternal immune responses to an extent that increases maternal or fetal susceptibility to infection. Thus, a generalized reduction of maternal immune responsiveness occurs during pregnancy, mediated by hormonal changes (e.g. increased levels of progesterone), trophoblast expression of key immunomodulatory molecules, and a progressive switch from a TH₁/TH₂ balance to a predominantly T-helper 2 type pattern of cytokines [21].

Prenatal Stress and Maternal-Placental-Fetal Endocrine and Immune Function

The well-demonstrated link between stress exposure and activation of the neuroendocrine system and exaggerated inflammatory responses cannot be assumed to also be present in the pregnant state because the above-described changes in endocrine and immune physiology have consequences for attenuating the responsivity of these systems to stress. However, we and others have shown that despite the large pregnancy-associated changes in maternal physiology, the system is responsive to maternal psychosocial states (such as high stress and low social support), that maternal psychophysiological stress responses are progressively attenuated with advancing gestation, and that after accounting for the effects of other established risk factors, the degree of attenuation is a significant predictor of shortened length of gestation and earlier delivery [reviewed in 3]. Studies by other groups have reported that elevated psychosocial stress and depressive symptoms in pregnant women are associated with changes in immune and inflammatory markers (in vivo and in vitro evidence, summarized in [3]).

In addition to psychosocial stress, substantial in vitro and in vivo evidence indicates that maternal-placental-fetal endocrine and immune processes during pregnancy respond to a variety of other maternal and intrauterine perturbations, including biological effectors of stress, obstetric risk conditions such as preeclampsia, pregnancy-induced hypertension, gestational diabetes, infection,
reduced uteroplacental blood flow, and behavioral factors such as the constituents of maternal diet, over- and undernutrition, and smoking [reviewed in 22]. Thus, based on these observations, it is apparent that measures of maternal-fetal endocrine and immune/inflammatory stress markers capture physiological responses to a wide range of intrauterine perturbations including, but not limited to, prenatal stress.

Long-Term Effects of Prenatal Stress Exposure on Human Adult Physiology and Health

The majority of human epidemiological studies of the fetal programming hypothesis have operationalized unfavorable intrauterine environments using indicators such as low birthweight. However, the long-term effects on child or adult disease-related phenotypes of interest may not necessarily be mediated by adverse birth outcomes. Only a very small number of studies have investigated this issue in humans. As a first step to addressing this question, we conducted a retrospective case-control study in a sample of healthy young adults born to mothers with healthy pregnancies and normal birth outcomes. One half of the study population of young adults was born to mothers who had experienced a major stressful life event during the index pregnancy (prenatal stress group), whereas the other half was a sociodemographically matched population with no history of maternal exposure to prenatal stress (comparison group). We selected a study population of younger as opposed to older adults in order to focus on pre-disease markers of physiological dysregulation of metabolic, endocrine and immune systems as early predictors of disease susceptibility. The potential effects of other established obstetric, newborn and childhood risk factors on adult health were controlled using a stringent set of exclusionary criteria.

Our results indicated that the young adults exposed during intrauterine life to maternal psychosocial stress consistently exhibited significant dysregulation in key physiological parameters, thereby placing them at increased risk for developing complex common disorders. Specifically, individuals in the prenatal stress group exhibited: higher BMI and percent body fat, primary insulin resistance, and a lipid profile consistent with the metabolic syndrome [23]; altered immune function with a TH2 shift in the TH1/TH2 balance (consistent with increased risk of asthma and autoimmune disorders [24]; altered endocrine function, with an increased ACTH and reduced cortisol levels during pharmacological and psychological stimulation paradigms; accelerated cellular aging (as indexed by shortened leukocyte telomere length that extrapolated to approxi-
mately a 3.5-year increase in the rate of cell aging [25]), and impaired prefrontal cortex-related cognitive performance (impairments in working memory performance after hydrocortisone administration) [26].

Taken together, our findings suggest that in utero exposure to prenatal psychosocial stress may confer increased long-term risk of a range of negative physiological and cognitive health outcomes in humans; these effects are independent from those of other established obstetric and childhood risk factors, and these long-term effects are not necessarily mediated by unfavorable birth outcomes such as low birthweight.

Fetal Programming of Body Composition, Metabolic Function and Obesity Risk

At the individual level, obesity (or, more precisely, adiposity) results when energy intake exceeds energy expenditure. However, there is wide variation among children or adults at identical levels of excess energy intake in their propensity to gain weight and accrue fat mass. This variation across individuals defines susceptibility for developing obesity/adiposity. Once an individual becomes obese, it is difficult to lose weight, and even more difficult to sustain weight loss, because of the remarkable efficiency of energy balance homeostasis mechanisms. For these reasons, it is important to gain a better understanding of the origins of individual differences in the propensity for weight and fat mass gain in order to predict obesity risk and develop strategies for primary prevention. Continuing with the theme of a common underlying biological mechanism, in this section we address the issue of the potential impact of intrauterine stress biology on multiple targets of fetal programming related to body composition, metabolic function and obesity risk.

Targets of Programming of Obesity: Potential Role of the Maternal-Placental-Fetal Endocrine and Immune/Inflammatory Pathway

It is well established that the primary targets of programming of body composition, metabolic function and obesity risk are the neural networks that regulate energy balance (appetite, feeding and basal energy expenditure) and peripheral organs and tissues involved in fat synthesis/breakdown, storage and metabolic function (adipocyte, liver, pancreas, muscle). We recently reviewed findings that pertain to the potential role of prenatal stress biology in programming these major targets of interest [see 22].
For example, we have reported that placental CRH concentrations in human pregnancy significantly predict the rate of fetal growth and size at birth [27], which, in turn, is a significant predictor of childhood and adult adiposity [28]. Other researchers have found a positive association between CRH levels in pregnancy and an increase in central adiposity [29] and alterations in adiponectin levels in 3-year-old children [30]. Yet others have reported a positive association between maternal levels of interleukin-6 in pregnancy and neonatal adiposity [31]. A recent, large epidemiological study in humans found an association between maternal bereavement from death of someone close during pregnancy and an increased risk of overweight in the offspring in later childhood [32]. Furthermore, many animal studies have demonstrated long-term effects of prenatal stress exposure on increased bodyweight in the offspring [18, 33].

Neural Circuits
A growing body of literature suggests that intrauterine perturbations can produce reorganization of these neural pathways that regulate energy intake and expenditure in ways that enhance the development of obesity. Several studies have convincingly demonstrated that biological (endocrine, immune) stress during gestation, triggered by a variety of nutritional, inflammatory, vascular, behavioral or psychosocial perturbations, can promote obesity in the offspring by reorganizing central neural pathways through programming of energy balance ‘set points’ [see 34 for recent review]. One key system involved in the regulation of energy balance is the hypothalamic (CRH)-pituitary (ACTH)-adrenal (cortisol) neuroendocrine stress axis, which forms a network of neuronal pathways capable of interacting with brain circuits controlling energy balance [35]. For instance, the adipogenic hormone leptin which is the afferent loop informing the hypothalamus about the states of fat stores, participates in the expression of hypothalamic CRH, interacts at the adrenal with ACTH, and is regulated by cortisol. Cortisol increases leptin secretion and limits CNS leptin-induced efferents [36].

Adipocytes
Obesity is impacted by increases in fat cell number, size, or both. Fetal adipose tissue development is regulated by the complex interaction of maternal, endocrine, and paracrine influences that initiate specific changes in angiogenesis, adipogenesis, and metabolism [37]. Adipogenesis, the process of adipocyte development from mesenchymal stem cell precursors, occurs primarily during late fetal and early postnatal life in humans, and the number of adipocytes is relatively fixed after young adulthood [37–39], supporting the notion that fetal and early postnatal periods are crucial windows in the development of adipose depots. Adipogenesis is highly sensitive to the intrauterine biological environment,
in particular to concentrations of insulin-like growth factors, glucose, insulin and glucocorticoids [37, 38]. In vitro studies could show that the differentiation of human adipocyte precursor cells in the presence of insulin is stimulated by cortisol in a dose-dependent manner and occurs at physiological concentrations [40, 41]. Furthermore, in vitro exposure of isolated human adipocytes to insulin and corticosteroids synergistically induces peroxisome proliferator-activated receptor-γ mRNA expression [42].

CRH seems to be an important regulator of adipocyte function, and CRH receptors are expressed in both white and brown adipocytes [43]. The role of cytokines as regulators of adipose tissue metabolism is well established. Proinflammatory cytokines are elevated in obese individuals, and they seem to modulate leptin secretion from adipocytes [44]. Prenatal exposure to proinflammatory cytokines or dexamethasone in animals has been shown to increase offspring fat depots [45].

Liver and Pancreas
The liver controls the production and fate of metabolic fuels through the action of hepatic enzymes. Phosphoenolpyruvate carboxykinase (PEPCK), a key enzyme in hepatic gluconeogenesis, is under potent glucocorticoid regulation. In animals, prenatal exposure to dexamethasone produces an increased expression of hepatic glucocorticoid receptors as well as increased levels and activity of PEPCK [46], thereby predisposing these animals to glucose intolerance later in life. Furthermore, manipulation of diet during pregnancy is associated with epigenetic changes in the promoter regions of the genes encoding peroxisome proliferator-activated receptor-α and the glucocorticoid receptors in the liver in offspring after birth, thereby altering their metabolic phenotype [47, 48]. Insulin is produced by the β-cells in the pancreas in response to elevated blood glucose levels. Increased glucocorticoid exposure and malnutrition during fetal development have the potential to permanently reduce the pancreatic β-cell mass and lower pancreatic insulin content, thereby increasing the risk for metabolic disease later in life [reviewed in 49]. For example, in humans prenatal exposure to glucocorticoids or stress was associated with higher insulin resistance in the adult offspring [23, 50].

Genes, Gene-Environment Interactions and Epigenetic Mechanisms
Although weight and body composition are highly heritable, known genes account for only a modest proportion of their variance. Genetic makeup alone cannot explain the rapid increase in obesity prevalence in the population because the genetic characteristics of the human population have not changed in the last three decades, but the prevalence of obesity has tripled during that time...
Estimates of maternal transmission of heritability are stronger than those for paternal transmission, which argues in favor of intrauterine effects and/or mitochondrial DNA effects. Moreover, the strongest genetic associations seem to vary as a function of the environment (e.g. effects are seen at specific times but not other times in the life cycle). These observations suggest gene-environment interactions are particularly relevant in the context of the obesity phenotype. Interestingly, a variant in the gene encoding the glucocorticoid receptor gene has been associated with increased body fatness in children [52], and we and others have described the association of the same variant with altered physiological stress responses [53]. Potential epigenetic mechanisms are areas of great interest in this context. A detailed review of epigenetics in the context of stress- and nutrition-related programming is beyond the scope of the current paper, and we and others have elaborated on this issue elsewhere [54–56].

Conclusions

Based on the conceptual framework and empirical findings presented here, we suggest in addition to maternal nutrition it may be important to also consider the potential role of intrauterine stress and stress biology in arriving at a better understanding of developmental programming of health and disease susceptibility. Moreover, we submit that stress-related maternal-placental-fetal endocrine and immune processes in human gestation represent a potentially attractive underlying candidate mechanism for elucidating the common biological basis (pathway) for mediating not only the long-term effects of prenatal stress but also those of a host of other intrauterine perturbations including maternal over- and undernutrition that have been implicated in this area. This framework and related empirical findings add further support and highlight the critical importance of the early developmental period (i.e. the first 1,000 days) in child and adult health outcomes.

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Disclosure Statement

The authors declare no conflict of interest.


Commentary on the Role of Prenatal Stress in Developmental Programming of Obesity and Metabolic Dysfunction

Research on developmental origins of adult disease has devoted considerable attention to exploring the critical role of nutrition and metabolism during pre- and postnatal life in early growth and development, and their long-term impact on health, performance and well-being [1, 2]. Dr. Entringer and Dr. Wadhwa add an innovative aspect to exploring the concept of early programming that might well turn out to be very important. They suggest that maternal psychosocial stress and stress biology have a considerable impact on fetal development, characteristics at birth, and subsequent developmental and health outcomes in the child. In particular, they propose that maternal psychosocial stress induces increased risks for adverse pregnancy and birth outcomes, preterm birth, low birthweight and small-for-gestational age infants [3]. Entringer et al. [4] present convincing arguments as to why one should include factors other than diet and nutrition in the exploration of developmental origins of long-term health. Furthermore, they propose that greater levels of maternal stress in disadvantaged populations with low socioeconomic status or from ethnic minorities might be one causal factor for the observed occurrence of higher rates of adverse health outcomes in the children from these population groups. Their findings achieved so far are rather impressive. For example, in a group of 26 young adults with a mean age of 25 years whose mothers had experienced a high level of psychosocial stress (negative life events during pregnancy), fasting glucose levels were similar as in controls, while they showed significantly higher glucose and c-peptide response after an oral glucose load [4]. Entringer and coworkers also found prenatal stress to be associated with shorter leukocyte telomere length [5] and altered cytokine production [6] in adult offspring.
Animal studies have provided evidence to support a considerable role of pre-natal stress on later outcome. High levels of biological stress markers in pregnancy were associated with offspring adiposity and related disorders [7, 8]. Enhanced cortisol exposure during pregnancy induced lowered offspring birthweight but increased adiposity in later life, along with enhanced insulin resistance, glucose intolerance and hypertension, and alterations in the hypothalamic-pituitary-adrenal axis [9–12].

Biological stress factors in pregnancy, such as hypoglycemia or hypoxia, induce an endocrine stress response affecting the key hormones which regulate fetal growth during late gestation. Hypoglycemia or hypoxia lead to increased secretion of ACTH, cortisol, noradrenaline and PGE2, along with decreased levels of insulin and IGF, which can markedly modify fetal growth and body composition particularly under prolonged or repeated stress exposure [13]. Psychosocial stress would be expected to induce a similar endocrine response and modulating effects on fetal growth and body composition, with relevance for long-term health and obesity risk. In animals, prenatal glucocorticoid exposure induced altered DNA methylation patterns in the offspring that not only persisted into adulthood, but were also transmitted to the next generation [14]. These observations are of interest given that some first associations of higher methylation of the RXRA gene locus 136355885+ on chromosome 9 with childhood adiposity at school were recently reported [15].

The intriguing hypothesis that prenatal psychosocial stress programs adverse long-term health effects in the offspring, such as adiposity and related disorders, deserves further detailed exploration. Adequately designed prospective human studies should assess stress levels and associated biomarkers, because currently available data do not yet allow firm conclusions. For example, a large school-based study in Denmark identified 459 children whose mothers were exposed to prenatal stress due to the death of a close family member from one year before pregnancy until birth of the child. The prevalence of overweight was higher in these children from 10 years of age and onwards, as compared to a control group [16]. However, the effect sizes on overweight were similar independent of the time of bereavement at 12–7 or 6–0 months before pregnancy, or during pregnancy. Therefore, one wonders which proportion of the effect is actually attributable to the biological response to prenatal stress and which proportion might be related to associated confounding factors. Clearly, psychosocial stress is not independent of nutritional factors because there is interaction between stress and dietary intake. Studies in non-pregnant subjects reported associations of stress with altered dietary patterns, preferential consumption of foods with higher energy density and higher glycemic load, and increased bodyweight [17–19]. Such effects of stress on dietary choices in pregnancy might contribute to
enhanced fetal growth and body fat deposition and hence increased long-term obesity risk of the child [20]. A high level of psychosocial stress may also be associated with more tobacco smoking in pregnancy, which was shown to markedly increase the child’s risk to become obese later [21–23].

The concept and hypotheses put forward by Entringer and coworkers focus on stress effects in pregnancy. They state that the origins of common disorders conferring the major global burden of disease can be traced to intrauterine life, and consequently they refer to the term ‘fetal developmental programming’. Indeed, the initial interest in developmental origins of lifelong health had been fuelled by observations relating to events in pregnancy, based on findings in experimental studies in animals and retrospective observational studies in human cohorts. However, over time strong evidence has accumulated to show that developmental origins of noncommunicable diseases occur during a much wider time period, ranging from before pregnancy to early childhood [1, 24]. For example, folate supply prior to conception is a very important predictor of red blood cell folate concentration at the time of neural tube closure, and hence the occurrence of neural tube defects [25, 26]. The Eden cohort study in 1,756 mother-child pairs in France showed that weight change prior to pregnancy, between the age of 20 years and pregnancy, was significantly related to infant birthweight which predicts child obesity risk, and the relation persisted after adjustment for a number of potential confounders including prepregnancy BMI, pregnancy weight gain, education, smoking habits, age, parity, gestational age, and infant gender [27]. With regard to early childhood, a large body of evidence indicates that postnatal nutrition and growth have strong programming effects on long-term health. A prime example is breastfeeding which induces ample long-term programming effects, as documented in numerous long-term observational studies and systematic reviews [28]. For example, previously breastfed subjects show in later life lower cholesterol levels, blood pressure, reduced risks of obesity, type 1 diabetes, type 2 diabetes and many other health risks, as compared to previously formula-fed infants [28]. Rapid weight gain in infancy and in the 2nd year of life predicts a 2- to 3-fold risk increase for obesity in childhood and adulthood [29, 30]. Conclusive evidence on the powerful programming effects of postnatal nutrition has also become available from first randomized controlled trials on infant feeding demonstrating, for example, a marked reduction of long-term obesity risk by lowering protein supply with infant formulas [31] or improved cognitive outcomes at school age following enhanced early nutrient intake in preterm infants [32, 33].

Therefore, not only maternal stress in pregnancy but also postnatal stress in the infant may well be a very important programming factor for child outcome. Smith et al. [34] recently found in a prospective cohort study in preterm infants born with a gestational age below 30 weeks’ gestation that higher neonatal infant stressor scale
scores were related to abnormalities in brain structure and motor behavior, which persisted after corrected for confounding variables. Similarly, a program to provide individualized developmental care to preterm infants that aims at reducing stress exposure was reported to significantly improve neurobehavior, electrophysiology and brain structure in premature babies with intrauterine growth restriction [35].

In conclusion, given the numerous indications for a potential role of early stress exposure in long-term outcome, it appears highly worthwhile to invest in detailed studies that explore stress markers and lifestyle factors in pregnancy and early childhood, along with diet, physical activity, growth and metabolism, as relevant predictors of offspring growth, body composition, metabolic response, and long-term health. The results of innovative, high-quality studies in this exciting area of research may well contribute to novel and improved interventions that reduce the burden of obesity and associated disorders through addressing maternal diet, physical activity and biological stress before and during pregnancy, and in early childhood. The EarlyNutrition project (www.project-earlynutrition.eu), currently the world’s largest research consortium in the early programming field that brings together leading researchers in this area from Europe, the USA and Australia [1], will also explore the relationship of maternal diet, physical activity and stress markers to offspring body composition in cohort studies in different countries and hopefully add to our understanding of the role of stress in modulating later health.

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**References**


Abstract
One-carbon metabolism, or methyl transfer, is critical for metabolism in all cells, is involved in the synthesis of purines, pyrimidines, in the methylation of numerous substrates, proteins, DNA and RNA, and in the expression of a number of genes. Serine is the primary endogenous methyl donor to the one carbon pool. Perturbations in methyl transfer due to nutrient and hormonal changes can have profound effect on cell function, growth and proliferation. It is postulated that at critical stages in development, nutrient and environmental influences by their effect on methyl transfer can impair fetal growth, reprogram metabolism and cause long-term morbidity in the offspring. The potential for their effects is underscored by the unique gestation-related changes in methyl transfer in healthy women, the late expression of transsulfuration cascade in the fetus and the unique metabolism of glycine and serine in the fetus. Dietary protein restriction in animal models and protein malnutrition in humans causes remarkable changes in the methyl transfer in vivo. Although the specific consequences of perturbation in maternal and fetal methyl transfer remain to be determined, a profound influence is suggested by the demonstrated relationship between maternal folate and B12 insufficiency and metabolic programming.

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Introduction
Epidemiological and observational data from studies in human and data from experimental animal models have now established the relationship between impaired fetal growth and long-term morbidity in the offspring [1–4]. These data suggest that nutritional and environmental influences at critical times during
development can lead to programming of the metabolism of the fetus, cause alterations in growth and the development of chronic noncommunicable diseases in adult life (the Developmental Origins of Health and Disease paradigm). Although much has been learnt regarding the molecular, metabolic and physiological associations and mechanisms of the changes in the offspring, the metabolic and physiological changes in the mother that mediate the observed changes in the developing organism have not been studied in detail. Methyl transfer or one-carbon metabolism is the key component of cellular metabolism, is involved in synthesis of purines, pyrimidines, and methylation of a number of substrates, proteins, DNA, RNA and indirectly, expression of a number of genes. The non-essential amino acid serine, folate and the essential amino acid methionine constitute the key components of methyl transfer. Since the methionine and folate cycles are ubiquitously present in every cell in the body and participate in key metabolic reactions, perturbation in their metabolism by nutrient deficiency, or by nutrient, hormonal and environmental interactions can have profound impact on the cell function, metabolism, growth and proliferation. Interest in the physiological changes in maternal-fetal and neonatal one-carbon metabolism, particularly in humans, has been primarily focused on the consequence of micronutrient deficiencies. Clinical, physiological and molecular data from studies in human and those from animal models suggest that alterations in methyl transfers may be critical contributors to the impaired fetal growth and to the re-programming of the metabolism of developing embryo and the fetus, leading to morbidity in the offspring. In this review, some of the data in support of such a concept are presented. Future studies will delineate the hormonal, nutritional and environmental contributors to such perturbations and allow us to develop targeted intervention strategies.

**One-Carbon Metabolism (Methyl Transfers) in vivo**

One-carbon pool refers to the pool of the methyl groups that are available for the methylation of a variety of compounds, including protein, DNA, RNA, etc. This process involves the methyl form of tetrahydrofolate (THF) and is carried out by s-adenosyl methionine (SAM). As shown in figure 1, the transfer of the methyl group involves both the folate cycle and the ubiquitous methionine cycle, also called the transmethylation cycle or the ‘activated’ methionine cycle. L-serine, a nutritionally non-essential amino acid, is the primary endogenous methyl donor. Stable isotope tracer studies in young healthy volunteers show that almost 100% of the methyl groups used for whole body remethylation of homocysteine are derived from serine under the conditions of their study [5]. The
latter is important, since the tracer studies were done after an overnight fast, however in a fed state, while the subjects were given a protein-free isocaloric diet. Previous data in isolated perfused liver preparation had also shown serine to be the major contributor to the methyl groups used for the transmethylation of homocysteine. During fasting, serine is released into the circulation by the kidney and is taken up by most organs, including the skeletal muscle and the liver, the latter being quantitatively the most important consumer of serine [6, 7]. As shown in figure 1, the methyl group (β-carbon) of serine is transferred to THF, catalyzed by serine hydroxymethyl transferase, resulting in the formation of glycine and N5,N10-methylene THF, which is then converted to 5-methyl THF, catalyzed by methylenetetrahydrofolate reductase. The methylation of homocysteine involves the transfer of the methyl group from 5-CH3THF by methio-

Fig. 1. One-carbon metabolism or methyl transfers in vivo; shown are folate cycle (black), methionine transmethylation cycle (italics), and the methionine transsulfuration cascade (bold). The flow of methyl groups from serine to methyltransferase is shown in light grey. 5CH3THF = 5-methyl tetrahydrofolate; MS = methionine synthase; SAH = s-adenosylhomocysteine; CβS = cystathionine β-synthase; CγL = cystathionine γ-lyase.
nine synthase. Vitamin B₁₂ is a cofactor for this reaction. The other contributor to the methyl groups for methylation of homocysteine, primarily in the liver, is betaine; however, its quantitative contribution has not been measured in vivo. SAM is the key intermediate of the activated methionine cycle, is the universal bioactive methyl donor and participates in numerous methyl transferase reactions resulting in methylated products (X-CH₃). SAM is also the primary allosteric regulator of the methionine metabolism in vivo. The catabolic pathway of methionine is the transsulfuration cascade. This pathway involves the condensation of homocysteine with serine to form cystathionine, which is then converted to cysteine, α-ketobutyrate and ammonia catalyzed by cystathionine γ-lyase. Cysteine is the precursor of taurine as well as a component of glutathione, the major intracellular antioxidant.

The metabolism of serine, folate and methionine has been studied extensively and discussed in several reviews. It is important to note that folate cobalamine (B₁₂) and pyridoxine (B₆) are directly involved in the metabolism of methionine. Insulin and glucagon exert their effect on methionine metabolism by (a) directly affecting the activity of transsulfuration cascade and methionine synthase and (b) indirectly by their effect on whole-body protein turnover and therefore effecting methionine flux. In addition, several reactions in the methionine metabolism respond to the change in the cellular redox state.

_Methionine and Serine Metabolism in Human Pregnancy_

The adaptive changes in methionine metabolism during pregnancy have been examined by us in healthy women studied early and late in gestation and compared with non-pregnant controls [8]. The data show that the fractional rate and the total rate of transsulfuration of methionine were significantly increased during early gestation with a decrease to the rate seen in non-pregnant subjects in the third trimester. In contrast, the rate of transmethylation, a measure of one-carbon transfers, was markedly higher in the third trimester of pregnancy. The high rates of transmethylation were speculated to be the consequence of higher methylation demands in the later part of pregnancy.

The rate of appearance of serine was quantified in healthy pregnant women by Kalhan et al. [9] using [2-¹⁵N₁³C]serine tracer. Plasma serine concentration and the rate of appearance of serine were lower in pregnant women in both early and in late gestation compared with healthy non-pregnant women. The rate of appearance of serine was significantly less in the 3rd trimester of pregnancy when compared with early pregnancy [serine Ra: early (n = 12) 123.7 ± 21.5, late (n = 8) 102.8 ± 18.2 μmol·kg⁻¹·h⁻¹, mean ± SD]. The isotopic tracer used in this
study would have recycled between serine and glycine and therefore would have resulted in an underestimation of serine flux. The lower estimation of serine flux would be proportional to the magnitude of tracer recycling. Assuming no significant change in actual serine turnover between early and late gestation, the higher rate of recycling is qualitatively consistent with the data in sheep fetus showing a higher rate of serine glycine flux in the fetal compartment (discussed below) and with the higher rate of transmethylation of methionine observed in healthy pregnant women [8].

**Serine and Glycine Metabolism in the Placenta and the Fetus**

The transport of serine and glycine from the mother to the fetus has been examined both in animal models and in humans. The data in humans have been obtained at term gestation. These data show a higher concentration of both serine and glycine in the fetal umbilical vein than in a simultaneously obtained maternal arterial sample [10, 11]. In addition, an infusion of amino acid mixture to the mother prior to caesarean section delivery resulted in a significant increase in all amino acids including serine and glycine in the fetus [12]. Bolus infusion studies using tracer isotopes of glycine, leucine and phenylalanine in human pregnancy show a much greater dilution or lower enrichment of glycine tracer in the fetal compartment as compared with leucine or phenylalanine [13]. The authors interpreted these data to suggest a slower transfer rate for glycine when compared with leucine or phenylalanine. However, as also suggested by them, these data could indicate a significant production of glycine by the fetus or by the placenta [13]. However, as shown by Lewis et al. [14], a low serine hydroxymethyltransferase activity in the human placenta (as compared with sheep) would suggest that placental production of glycine from serine may not be a significant source of fetal glycine. Other data in chronically catheterized sheep fetus show a uteroplacental uptake of serine from the mother, no net transport of serine from the mother to the fetus and a virtual equimolar (to serine uptake) release of glycine from the placenta into the fetal compartment [15, 16]. These data suggest that the ovine placenta converts large quantities of maternal serine into glycine and releases it into the fetus. Additionally, studies by Cetin et al. [17, 18] demonstrated production of serine from glycine by the fetal liver in a chronically catheterized sheep preparation. Thus, a unique inter-organ cycling of serine and glycine occurs in the sheep fetus and placenta, where glycine is released by the placenta into the fetal circulation and is then taken up by the fetal liver and converted to serine which in turn is taken up by the placenta in substantial quantities. Of significance were remarkably high turnover rates of serine and glycine, as compared with leu-
cine, in the fetus (glycine: \( \sim 720 \, \text{μmol·kg}^{-1}·\text{h}^{-1} \); serine: \( \sim 2,700 \, \text{μmol·kg}^{-1}·\text{h}^{-1} \); leucine: \( \sim 52 \, \text{μmol·kg}^{-1}·\text{h}^{-1} \)). Taken together, these studies suggest a high rate of serine and glycine interconversion, and therefore methyl (one-carbon) transfers in the placenta and the fetal liver with some quantitative differences between species. Such studies cannot be done in humans; however, these data are consistent with the higher rates of transmethylation measured in human pregnancy during the third trimester using stable isotopic tracers [8].

**One-Carbon Metabolism and the Fetus**

*Effect of Dietary Protein*

Isocaloric protein restriction in pregnancy in the rodents impairs fetal growth and causes programming of the metabolism in the offspring [reviewed in 3, 4]. Collectively, these data show that protein restriction during various periods of pregnancy results in fetal growth retardation and is associated with pancreatic dysfunction, impaired glucose homeostasis, hypertension, changes in the circadian rhythm and other metabolic dysfunctions in the offspring. The possible mechanisms of these changes in the fetus and the offspring have been reported and discussed in several excellent reviews [1–4]. However, there is paucity of data regarding changes in the maternal metabolism that are responsible for the observed responses in the fetus. Petrie et al. [19] reported high serum concentrations of homocysteine early in pregnancy (day 4) in rat placed on protein-restricted diet. Other changes in maternal metabolic and amino acid patterns have been reported. These data are difficult to interpret because of the differences in time of sampling in relation to gestation, the controls not being pair fed and significant differences in dietary regimens employed. It is also important to recognize the differences in the metabolic responses to isocaloric protein restriction as compared with simple protein restriction. Simple protein, energy restriction could be in part compensated by an increase in overall food consumption. In addition, isocaloric restriction results in suppression of adipose tissue and skeletal muscle responses seen with total energy restriction, i.e. mobilization of fatty acids and amino acids from lipolysis and proteolysis.

Parimi et al. [20] examined changes in plasma amino acids in pair-fed pregnant rats on a protein-restricted diet. They showed no change in total \( \alpha \)-amino nitrogen in the plasma in early gestation and an increase late in pregnancy. The increase in total \( \alpha \)-amino nitrogen was primarily due to increase in serine, glycine and glutamine concentration. Rees et al. [21] also reported a significant increase in the plasma glycine concentration in response to dietary protein restriction in
pregnant rat. Since serine and glycine are primary methyl donors, a change in their levels, along with higher homocysteine levels noted above, suggests change in one-carbon or methyl transfer in response to dietary protein restriction. Although such studies cannot be done in humans, an observational study in (non-pregnant) humans showed that subclinical protein malnutrition evidenced by lower transthyretin levels was associated with increase in plasma homocysteine levels, suggesting an impaired methionine-homocysteine metabolism [22]. The plasma concentration of most essential amino acids was lower in the malnourished group, while there was no significant change in the plasma methionine levels, suggesting an independent regulation of methionine levels possibly by down-regulating the transsulfuration cascade. The plasma concentrations of pyridoxal-5-phosphate (vitamin B₆) and folate of the malnourished group were not different from controls, while cobalamines (vitamin B₁₂) were lower only in the severely malnourished (state III) subjects [22]. Recently, the same investigators have reported hyperhomocysteinemia in a vegetarian, plant-eating population of Chad, who had a lower dietary intake of protein and sulfur amino acids [23]. Their data suggest a direct effect of lower protein intake on methionine-homocysteine and one-carbon metabolism. Our group has examined the effect of isocaloric protein restriction in the rat on methyl transfers and methionine metabolism [24]. The data show that dietary protein restriction, in this instance for 7–10 days, resulted in profound change in hepatic metabolism (fig. 2), was associated with differential expression of a number of genes involved in cell cycle, differentiation, transcription, transport, and other metabolic processes. Of importance, there was a marked increase in serine biosynthesis and methionine transmethylation and a decrease in the activity of transsulfuration cascade (fig. 2). All these data underscore the important effect of adequate protein intake on methyl transfers, an effect that is independent of the vitamin status. The observed fetal programming effects of maternal protein restriction could be mediated via changes in one-carbon metabolism in the mother. Such a hypothesis is supported by the data demonstrating an amelioration of the changes caused by protein restriction when the animals were supplemented with methyl donors like glycine and folate [25].

Folate and Vitamin B₁₂

The clinical evidence relating maternal folate and vitamin B₁₂ status during pregnancy and the long-term consequences for the offspring is discussed in the chapter by Deshmukh et al. [pp. 145–156]. As noted above, folate is an integral part of the transfer of methyl groups from serine or other methyl donors like betaine for the methylation of homocysteine to form methionine (fig. 1). Vi-
tamin B<sub>12</sub> is a cofactor for the enzyme methionine synthase (homocysteine methyl transferase). Insufficiency of either folate or B<sub>12</sub> will affect the methyl transfer by influencing methylation of homocysteine. Iatrogenically induced folate deficiency in healthy human subjects resulted in significant increase in plasma homocysteine levels and attenuation of the rate of methionine synthesis via remethylation of homocysteine [26]. Such kinetic studies have not been done in B<sub>12</sub>-deficient subjects; however, B<sub>12</sub> deficiency by decreasing the activity of methionine synthase will be expected to suppress methylation of homocysteine. These data indicate that both folate and B<sub>12</sub> deficiencies can influence maternal-fetal metabolism and cause long-term consequences by effecting methyl transfers in the mother and the fetus. A direct evidence of such a cause and effect relationship is not available as yet. Folate deficiency during pregnancy in the rat has been shown to effect methyl metabolism; however, it did

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**Fig. 2.** Effect of isocaloric protein restriction on methyl transfer in the rat. Rats were placed on a casein-based 6% protein diet for 7–10 days; controls were on a 24% protein diet and were pair fed. Dietary protein restriction resulted in a marked upregulation (dark grey boxes) of 3-phosphoglycerate dehydrogenase (3PGDH), phosphoserineaminotransferase (PSAT), glutamate-cysteine ligase (GCL), and cysteinesulfonic acid decarboxylase (CSAD). The activity of CβS and CγL was decreased (light grey boxes). Tracer dilution-measured rate of appearance of serine and the rate of transmethylation of methionine were increased (thick arrows). Figure reproduced from Kalhan et al. [24].
not impact global DNA methylation in the fetus [27]. Whether there were changes in the methylation pattern of specific genes was not determined in that study.

**Homocysteine**

Alterations in one-carbon metabolism as a result of nutrient or other influences result in an increase in plasma concentrations of homocysteine, the demethylated product of methionine and an intermediate amino acid in the methionine transmethylation cycle (fig. 1). Homocysteine does not participate in protein synthesis and an increase in plasma levels of homocysteine reflects altered intracellular homocysteine and one-carbon metabolism. The relationship between elevated homocysteine levels and vascular disease, thrombosis, vascular endothelial dysfunction has been studied extensively and discussed in excellent scientific reviews [28]. A number of studies have shown a pregnancy-related decrease in plasma homocysteine concentration in healthy women. Whether homocysteine directly causes impaired fetal growth or via its effect on placental growth and function via its vascular effects has not been delineated in studies in human. An association between elevated levels of homocysteine and pregnancy-related disorders such as preeclampsia, early pregnancy loss and abruptio placentae has been reported [reviewed in 29]. A recent systematic review and meta-analysis by Hogeveen et al. [30] concluded that higher maternal total homocysteine concentrations are associated with a small increased risk of small-for-gestational-age offspring. It corresponded to a decrease in birthweight of 31 g (95% CI: –13 to –51 g) for 1-SD increase in maternal total homocysteine. The authors concluded that the small birthweight difference might be of little clinical relevance, but may be of greater importance at the population levels. However, the long-term programming consequences of such impairment of growth have not been determined. It is likely that the attenuated fetal growth, as reflected in lower birthweight is only a crude reflection of the altered metabolic and epigenomic changes in the growing fetus that may lead to programming and morbidity in later life.

**Multinutrient Deficiencies**

As noted above, folate and B12 deficiencies cause a decrease in the transmethylation pathway be impacting the transfer of the methyl groups to homocysteine. In contrast, protein malnutrition in humans and in animal models causes an increase in transmethylation, in addition to suppressing the transsulfuration
cascade. On the other hand, deficiency of B6 did not appear to have any significant effect on activated methionine cycle. Thus the net effect of these opposing responses to multiple nutrient insufficiency, commonly seen in developing societies, is not known. It is speculated that the combined effect of multiple nutrient insufficiency on one carbon metabolism will be the sum of individual affects and could result in a spectrum of responses from low rate of transmethylation (dominant vitamin B12 and folate deficiency) to high rates of transmethylation (in a predominant protein deficiency state) and modified by the hormonal milieu and the physiological adaptation (as in healthy pregnancy) of the organism. Such effects cannot be discovered from single measurement of biomarkers such as measurement of plasma homocysteine, SAM/SAH ratio or by measurement of single micronutrient. These responses may also explain the lack of significant effect on for example birthweight when nutrient intervention involves a single micro- or macronutrient.

Conclusions

Methionine, an essential amino acid, along with folic acid, are key components of one-carbon metabolism in every cell in the body. Specific changes in the one-carbon metabolism have been identified in the mother during pregnancy, in the placenta and in the fetus during development. Methionine and one-carbon metabolism can easily be altered by nutrient and hormonal mediators and may cause specific changes in many organs in the mother, placenta and the fetus. We hypothesize that changes in one-carbon metabolism as a result of nutrient (vitamins and protein) insufficiency cause fetal growth retardation by nutrient-gene interaction and cause permanent changes leading to adult disease like diabetes, obesity and hypertension.

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Discussion on One-Carbon Metabolism, Fetal Growth and Long-Term Consequences

Comments by Discussant

Dr. Kalhan has given us a very detailed presentation of the methyl cycle, in which he showed us the first human pregnancy data on transmethylation and transsulfuration rates in American women. The key finding there was an increase in transmethylation and a decrease in transsulfuration rates as pregnancy progressed; we have measured these rates in India as well [1]. Unlike American women, the Indian women did not show an increase in transmethylation rates in the third trimester, while transsulfuration rates on the other hand actually increased. Transsulfuration is the oxidative pathway that also yields cysteine, and we do not know whether Indian pregnancy has a burden of infectious stresses that require a higher level of glutathione. But it’s still very interesting, and I think a lot more needs to be done in this area. His reflection on the quantitative approach to methyl transfers in terms of creatine and phosphatidyl choline is also revealing. If you were to quantify the number of CpG islands on the genome and make a very rough calculation of the number of methyl groups that you would require in pregnancy in total, it would be some 2.5 mmol, but the actual daily transmethylation flux is many times greater. It may be that the actual quantitative need of methyl groups is not an important issue. It is hard to believe that such a nicely conserved mechanism like genome methylation that is so important would be dependent on the availability of methyl groups, since the availability of methyl groups is far in excess of what you actually require to methylate the genome. It seems that the methylation process is linked to something that senses the uterine or fetal environment and changes methylation patterns, but it...
has nothing to do with the provision of methyl groups that is available in plenty. I think the other very important issue presented was the efflux of taurine from cells in the dietary protein restriction model. Taurine is one of the largest intra-cellular pools of amino acids, and this efflux may actually change cellular size and function. In this context, one needs to consider what is meant by a balanced energy protein supplement during pregnancy. I am not sure what that means, but when one talks of a balanced energy protein supplement, it usually means eating more of what is normally eaten, yet when we look at the WHO/FAO/UNU [2] requirements for energy and protein during pregnancy, the additional protein requirement is high compared to energy. Whether eating more of a normal diet during pregnancy then constitutes a protein-deficient supplement needs to be understood.

Anura Kurpad

Interactive Discussion

Dr. Bhatia: You made a comment about cysteine sufficiency, yet why then teleologically are all mammalian species at birth glutathione deficient or have small amounts of antioxidant capacity?

Dr. Kalhan: In terms of cysteine requirement, you need a very small amount of cysteine. If you look at the cysteine flux in adults, it is only about 30 μmol/kg per hour. The amount of new cysteine synthesized is very low, only about 3–4 μmol/kg per hour. Dr. Goudoever was telling me that in a very small baby it was not even measurable, and yet we do demonstrate transsulfuration. So, to answer the first part of your question, babies can synthesize cysteine, we don’t dispute that even though we can’t measure it, and we can’t measure it because it is too low. As to the next question, my bias is that glutathione measurements in the plasma are not reflective of cellular glutathione. Most glutathione in the plasma comes from the liver. Glutathione is very interesting, it comes out of the cell, is degraded on the cell surface and then breakdown products like cysteinylglycine are later recycled back into the cell. So, when we are talking about antioxidant capacity of the baby, I think that this is not a good measure. I have spent a lot of time looking at this, and it seems to me there is really not a good measure of antioxidant capacity, although there are a lot of kits and everything else to measure antioxidant capacity, and none of them is good.

Dr. Lentze: I have a methodological question. In the stable isotope studies like the ones you presented, how do you account for the changes in rates of oxidation and tissue deposition through pregnancy? I don’t know if the same animal was studied repeatedly, but I am sure there are going to be different rates
of tissue oxidation or isotope oxidation and also tissue deposition throughout pregnancy.

*Dr. Kalhan:* There are a couple of sets of data I showed you. In many human studies, we do longitudinal studies, so when we are looking at the whole-body metabolism, we are representing the whole body. In humans, we cannot easily isolate tissues or organ systems. We have done that sometimes, but we can’t do that in pregnant populations or newborn babies. So, the data I presented in relation to pregnancy and babies are for whole-body metabolism, and we extrapolate to tissue and to organ systems based on what is known in the literature, or from our own experiments in non-pregnant populations, where we can use a little more invasive techniques. The question I suppose you are alluding to is, where is the methionine getting oxidized? The transsulfuration pathway is very interesting in that it is not expressed in every organ and tissue in the body, and the major organs are the liver, gut, pancreas and kidney. Our impression is that when we see changes in transsulfuration, it’s probably changing most in the liver itself rather than any other tissue. In contrast, transmethylation of methionine is ubiquitous, it is expressed in every cell, every tissue and everywhere else. Now going to phenylalanine, I didn’t show you any oxidation data; there, we look at conversion to tyrosine; phenylalanine is not metabolized in the skeletal muscle, and so it is probably again metabolized in the liver itself. In animal studies, we did parallel experiments measuring the expression of the enzymes and the enzyme activity in the liver and other tissues, and these are probably the major sites of oxidation.

*Dr. Van Goudoever:* I have two questions, one relates to the transmethylation rates you demonstrated in the 3 different trimesters of pregnancy, and you said you were surprised that transmethylation rates went up in the last trimester. If I recall correctly, these were absolute rates, so isn’t that to be expected that if you have an increase in your cell mass and an increase in your methylation rates, the transmethylation rates would go up as well?

*Dr. Kalhan:* We looked at those things, we did that in relation to phenylalanine, and it was impressively out of proportion, that is a 3- to 4-fold increase. When I look at other situations, I don’t see that kind of change.

*Dr. Van Goudoever:* But is it not just reflecting the amount of methyl groups you basically need?

*Dr. Kalhan:* The need for methyl groups is primarily for two things, to make creatine and phosphatidylcholine. We have done some studies in late pregnancy looking at changes in creatine synthesis, and we didn’t see much change, so we don’t think that the change in transmethylation as we see it is a consequence of the big methylation demand. It’s probably my bias that this need must be due to the fetus, liver and placenta.
Dr. Van Goudoever: My second question relates to your protein restriction experiments. When you did the gene arrays, you actually saw many genes upregulated, and many of them downregulated; in the upregulated genes, there was a very small upregulation in glutathione genes, but then if you look at the enzyme activity, there was a huge increase, so this does not correspond basically to what you would expect on the basis of your upregulation of your genes. Do you have an explanation?

Dr. Kalhan: No, that is a well-known phenomenon. That is my argument with the molecular biologists that the correlation in expression, translation, protein and activity doesn’t hold. If you look at the molecular biology literature, they will often say these are expressed and therefore the pathway is moving on; no, that’s not true, and when you look at the other studies that measured PEPCK protein and used the enzyme activity to correlate this, it does not occur. Actually, it turns out that we have got enzyme activity which is plenty to move the pathways along, except in very few rate-controlling enzymes, and those are very few left.

Dr. Banerjee: You said the transport of serine is lower in human beings than in sheep. Is there any specific difference, which might be due to the serine transport that is found in the sheep and the human being? Is there any specific problem in serine transport due to this difference?

Dr. Kalhan: The short answer to that is no.

Dr. Banerjee: But if any work is done in this paradigm do you feel that anything might come out, any positive things which would help our knowledge?

Dr. Kalhan: What I have described here is physiology, and we are not at the stage yet where the consequences of these perturbations are known. What I am describing to you is that methyl groups may be an important component of it, and they may be impaired as a consequence of these things and provide the mechanisms. It is possible that these may ultimately turn out to be biomarkers or we may do some interesting functional tests out of these observations so we can identify populations with nutrient insufficiency and therefore we can do a targeted intervention. For example, I showed you the protein malnutrition data where homocysteine is a nice biomarker looking at the population here, so we can say this is a protein-malnourished person and therefore that’s what we need to do.

Dr. Ramakrishnan: You showed increased methionine turnover in preterm infants and explained that this partly based on feeding, on the fact that the infants may have been given total parenteral nutrition (TPN). In all of those studies, did you have information on feeding patterns and when they were measured? In other words, whether preterm infants were switched to breast milk
later on versus continued parenteral feeding. Would you see a different pattern depending on when you measured methionine turnover? The second question is a broader question. You showed that we eat food and not nutrients, and a lot of the rationale for the multiple micronutrient work came from poor diet quality. With regard to the relationship where you talked about diets being isocaloric but reducing the protein, can you comment on protein quality? In other words, even within the changing world, the older literature showed that people don’t get enough food to eat, so you have balanced deficiency with low protein and low energy intakes; but what’s happening is we may be moving towards meeting energy needs but not getting quality protein. I just would like to hear your comments on the protein quality if you had only 20 g of protein in your experiments.

**Dr. Kalhan:** To answer the first question is very easy for those of us who do these studies on babies and newborn, and I do them myself. The babies who are on parenteral nutrition receive this in the first week after birth, and they don’t receive anything else other than parenteral nutrition. They all have larger content in methionine; the goal of administration of TPN was that the amino acid concentration in the blood should be similar to that of a breastfed baby, and therefore they didn’t look at fluxes or anything like that. All that was wanted was that blood pH stayed stable and the amino acid levels were optimal. Now the time has come to move further, and these are the kind of experiments which will help to do that. Full-term babies are very difficult to study, and these studies were all done within the first 48 h. In general, across the world, we do not study breastfed babies because there are emotional discussions about separating the babies from the mothers and all those kind of things, so they are all formula-fed babies. Now coming to isocaloric protein-restricted animals, those were all casein-based diets, so they had a little less cysteine, but were supplemented with methionine and cysteine so that the amino acid component in the control and protein-restricted animal is almost the same. The sulfur amino acid intake was the same. You asked me about the large picture, the quality of protein. That one is a little difficult to answer because the quality of protein changes from one part of the world to another, and the study I showed you was from Chad in Africa [3]; my guess is that their protein was animal protein, but of a low quality. In the vegetarians, the animal protein intake was very low. Beyond that, I cannot say much about this.
References


Influence of Maternal Vitamin B\textsubscript{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\textsubscript{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\textsubscript{12} exaggerated the risk of insulin resistance. Low maternal vitamin B\textsubscript{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 B₁₂**

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<sub>12</sub> 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<sub>12</sub> [5].

We then investigated the relationship between maternal circulating concentrations of tHcy, vitamin B<sub>12</sub> 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<sub>12</sub> 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<sub>12</sub> 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<sub>12</sub> concentration during each of the three trimesters of pregnancy had significantly higher risk of IUGR [7].
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].

![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.](image-url)
In a cohort of 785 women attending an antenatal clinic in Mysore, India, low plasma vitamin B₁₂ concentrations (<150 pM) were observed in 43% of women and low plasma folate concentrations (<7 nM) in 4%. Vitamin B₁₂-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 B₁₂-sufficient women. Among vitamin B₁₂-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 B₁₂ 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 B₁₂ 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 B₁₂, 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 B₁₂ 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 B₁₂ 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 B₁₂ 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).
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)

**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 B12 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’ (B12, folate 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, B12 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 B12 Deficiency in India, Nutrition Transition and Dual Teratogenesis**

In Indians, hyperhomocysteinemia and vitamin B12 deficiency are common. Hyperhomocysteinemia in Indians is predominantly contributed by vitamin B12 deficiency rather than folate deficiency [17]. Vitamin B12 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 B12 in nature is microbes, and this perhaps explains why vitamin B12 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
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.

**References**

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 B₁₂ 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 B₁₂, 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 B₁₂ 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 B₁₂ 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 B₁₂ 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-
Influence of Maternal Vitamin B\textsubscript{12} and Folate on the Offspring

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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{12} deficiency is much more common than folate. The original tablet for antenatal supplementation, which was devised in the 1960s, included vitamin B\textsubscript{12} along with iron and folic acid, but because vitamin B\textsubscript{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 \( \text{B}_12 \) and low birthweight, and did not find an association. I have to accept that; we did not have any association of vitamin \( \text{B}_12 \) 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.

References


Abstract
Optimal fetal growth resulting in a ‘normally grown’ term infant is of paramount importance for assuring a healthy start for postnatal growth and development. Fetal, infant and childhood growth restriction is an important clinical problem for obstetricians, neonatologists, pediatricians and globally, for public health. Worldwide, an estimated 20 million infants are born with low birthweight and a substantial proportion are small for gestational age. Many advances have been made in defining growth restriction by prenatal techniques, thus allowing the recognition of intrauterine growth restriction. Distinguishing infants who are small but have appropriate growth potential from those with growth restriction is important in order to apply obstetric surveillance, anticipate neonatal problems and plan for post-neonatal guidance. It is clear that the fetus in growth-restricted pregnancies has limited supply of nutrients and oxygen. The resultant changes, if involving the placenta as well, can lead to circulatory and metabolic changes affecting both short- and long-term survival and development. In this paper, the causes and immediate consequence of being born with low birthweight, intrauterine growth restriction or small for gestational age will be discussed.

Under optimal circumstances, fetal growth occurs in sequential patterns of growth (tissue and organ) and maturation. Uteroplacental function, maternal environment and genetic factors play a role in modulating this growth [1–4]. Alteration in any of these factors may become rate limiting resulting in altered growth resulting in intrauterine growth restriction (IUGR). A clear distinction needs to be made between IUGR which may affect survival in utero, alter ad-
aptations to labor and delivery stressors as well as neonatal transition and small-for-gestational-age (SGA) and low-birthweight (LBW) infants. IUGR indicates a reduction in the expected growth trajectory in utero and is traditionally based on fetal weight; fetal growth at term may be predicted based on ultrasonographic findings in the second trimester, and growth curves are available based on maternal weight and height, parity, ethnicity and fetal gender. SGA denotes an infant whose weight is lower than –2 or –3 standard deviations from population norms. It is important to remember that an accurate estimate of gestation age and definition of SGA is essential for postnatal care since it is associated with significant antenatal and postnatal pathology. SGA also includes constitutional smallness which does not carry the same risks as true pathological SGA. SGA often represents placental dysfunction and may be associated with preeclampsia, preterm labor, placental abruption, intrapartum complications and fetal mortality [5]. Birthweight, in both developing and developed countries, is considered the single most important factor affecting neonatal and postneonatal mortality [6]. LBW is defined by the WHO as a birthweight <2,500 g; however, plots of cumulative frequency distribution of birthweight show two different normal distributions, and 2,000 g as a lower cutoff point has been suggested [7]. Nonetheless, LBW is caused by either premature birth or IUGR or both. The various growth curves used to define appropriateness of growth for gestational age, fetal metabolism and maternal metabolic adjustments during pregnancy are beyond the scope of this paper and are discussed elsewhere in this monograph.

It is however important to briefly mention the long-term consequences of reduced birthweight. LBW in the fetus has been associated with adult-onset hypertension, type II diabetes, obesity and cardiovascular disease. These consequences occur in infants born small and exhibit increased growth including catch-up growth. The fetal origin hypothesis states that poor nutritional status in the mother produces reduced birthweight and results in ‘reprogramming’ that results in adult-onset diseases. The risk for the adult diseases is not universal and may be two to three times that of a normal population. It is also recognized that twins (who may not have placental dysfunction causing reduced body size) do not have a higher incidence of adult diseases compared with other adults. Increased weight gain in infancy and childhood either as part of catch-up growth or excessive growth is becoming an established risk for the development of metabolic syndrome although this entity is not well defined in children. The increased growth is primarily central fat and not lean body mass; this altered growth is associated with insulin resistance. Alternative hypotheses have been put forth where genetic factors or poor intrauterine nutrition could result in insulin resistance and make the individual susceptible to diabetes and heart disease.
On the other hand, nutritional status of women in both developing and developed countries could lead to insulin resistance or obesity [9]. Risk factors for LBW are summarized in Table 1. It should be emphasized that many of the listed factors are common for both IUGR and prematurity.

### Table 1. Factors associated with growth restriction

<table>
<thead>
<tr>
<th>Classification</th>
<th>Factors</th>
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<tbody>
<tr>
<td>Demography</td>
<td>Black, Lower socioeconomic status</td>
</tr>
<tr>
<td>Prenatal factors</td>
<td>Short stature, Poor nutritional intake, Chronic disease, Transgenerational (low birthweight of mother)</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Multiple gestation, Placental dysfunction (chronic diseases affecting delivery of nutrients), Altitude, Infections, Fetal factors (genetic, renal agenesis etc.)</td>
</tr>
<tr>
<td>Other</td>
<td>Habitual smoking, Alcohol and illicit drug use, Short interpregnancy interval</td>
</tr>
</tbody>
</table>

Modified from Committee to Study the Prevention of Low Birthweight, Institute of Medicine [25].

[8].

Scope of the Problem

Worldwide, more than 20 million infants are born with a birthweight <2,500 g, and of these, it is estimated that 30–40% are SGA at term gestation. SGA status at lower gestational ages is not known on a population level, although it is known that a great majority of extreme premature infants who survive are born SGA. Population means and SD may be misleading since there may be less variability within a family with respect to birthweight rather than a population. Accurate assessment requires proper definition of growth and hence, weight, and that depends on the following: accurate dating of a pregnancy; use of appropriate growth standards, and growth and weight standards need to be free of confounding factors that affect them, for example, smoking, diabetes, etc. Fetal growth restriction originates early in gestation in approximately 20% of the infants, and these infants are usually growth restricted in a symmetric fashion: weight, length and head circumference are all affected. Late-onset growth restriction occurs due to uteroplacental insuf-
ficiency and, therefore, limited transfer of nutrients and oxygen, during the last trimester of pregnancy. The resultant growth restriction generally spares the head to the greatest extent and the length to a more limited extent (table 2).

### Anticipated Problems and Management Prior to Delivery

There are numerous studies demonstrating reduced umbilical blood flow in growth-restricted fetuses and that the reduction can occur quite early in gestation. In addition, umbilical blood flow closely approximates the growth of the fetus and decreases slightly if expressed per kg as gestation progresses in normal pregnancies [10]. The ductal shunt (ductus venosus) is however increased in growth-restricted fetuses. Thus, an increase in ductal shunt combined with a decrease in umbilical flow in growth-restricted fetuses can result in hepatic injury unrelated to infection in growth-restricted neonates [11, 12]. Maternal undernutrition and an increase in ductal shunting have also been reported [13].

Management principles for the care of a pregnancy with a suspected growth-restricted fetus are as follows:
- Appropriate maternal and fetal care and close monitoring of the fetus
- Limit maternal activity
- Improve fetal oxygenation by providing oxygen to the mother if needed
- Assessment of fetal well-being
- Coordinate delivery with the neonatal team

### After Birth

A neonate who is SGA may develop several neonatal problems. If marginal placental perfusion is suspected, uterine contractions and prolonged labor may compromise fetal gas exchange. Myocardium of the fetus may have diminished

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<td>Intrinsic fetal problems</td>
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glycogen stores and less so if premature also, and these fetuses may not withstand asphyxia. Meconium passage in utero may also occur due to fetal hypoxia and stress. The infant may exhibit physical features of the disease process that resulted in IUGR/SGA/LBW. Among SGA infants with perinatal stress, up to 10% continue to have hypoglycemia beyond the first week of life [14]. This hyperinsulinism is responsive to diazoxide, and exact pathogenic mechanisms are not clear.

The perinatal mortality of IUGR infants is 10–20 times higher than that of a normal-weight infant. As alluded to previously, these infants are at high risk for perinatal asphyxia and its sequelae including multiorgan dysfunction and failure. Concomitant with these, there may be metabolic problems including hypoglycemia. Physiologically normal appropriate-for-gestation-age infants are susceptible to the development of hypoglycemia if feedings are delayed in the first few hours of life. The susceptibility to transient hypoglycemia is associated with developmental lags for both hepatic ketogenesis and gluconeogenesis and is aggravated in infants with limited glycogen stores [15]. A delay in PEP-CK, carnitine palmitoyl-transferase 1 and β-hydroxy-β-methylglutaryl-coenzyme A synthase has been described in the guinea pig and the rat, suggesting these changes as a cause of the hypoglycemia [16]. SGA infants have also demonstrated similar mechanisms for the development of hypoglycemia, low glycogen stores, delayed oxidation of FFA and induction of PEP-CK [17, 18]. Hypocalcemia may occur due to excess phosphate release from damaged cells or acidosis, but is not a consistent feature of SGA per se unless accompanied by prematurity.

Fasting hypoglycemia is very common, and the incidence is greatest during the first 3 days of life, largely due to reduced hepatic glycogen content [19]. Glycogenolysis is limited due to lack of glycogen stores, and glucose production occurs from incorporation of lactate and specific amino acid precursors into glucose. Hypoglycemic SGA infants have elevated alanine and lactate levels, and have been demonstrated to have an inability to raise blood glucose levels after alanine administration [20]. Immediately after birth, hypoglycemic SGA infants may also exhibit lower plasma FFAs and fasting glucose levels correlate with FFA and ketone body levels. In addition, as parenteral nutrition is established, intolerance to intravenous lipids is evidenced by raised triglyceride concentrations in plasma. However, ketone body formation is attenuated, suggesting that oxidation of FFA and triglycerides are limited in SGA infants. The latter, deficient oxidation of fatty acids may be responsible, in part, for the fasting hypoglycemia observed in these infants [21]. Hypoglycemia may also be due to hyperinsulinemia or excessive sensitivity to insulin in infants who are SGA. In addition, glucagon fails to enhance blood glucose levels suggesting
an abnormality in counter-regulatory mechanisms. Provision of glucose infusions at 4–8 mg/kg per min generally alleviates hypoglycemia.

**Temperature Regulation**

Temperature regulation becomes an important clinical issue in infants who are born after uteroplacental insufficiency since these infants may manifest hyperthermia because of failure of heat-eliminating mechanisms. On exposure to the usually cold environment in the delivery room, SGA infants increase heat production; however, with continued exposure to a cold environment, core temperature decreases since heat production is less than heat loss. Initially, brown adipose tissue which is not necessarily lost in IUGR fetuses accounts for the increase in heat production. Infants who are SGA have a narrower neutral thermal environment than term or preterm infants and should be managed accordingly. Both hypoglycemia and hypoxia interfere with heat production and contribute to thermal instability. Thus, maintenance of appropriate body temperature is an important clinical issue since postnatal weight gain would be affected by continued hypothermia.

**The Metabolic Changes**

The metabolic changes with respect to respiratory quotient that occurs in SGA infants are similar to those observed in AGA infants. These infants demonstrate a shift towards free fatty oxidation and a lower respiratory quotient. Basal oxygen consumption may be decreased similar to that observed in IUGR infants in utero suggesting a decrease in oxidizable substrate. Once substrates are provided in the form of exogenous nutrition, an increase in oxygen consumption is observed. In addition, SGA infants do not demonstrate the usual 10–15% weight loss as observed in premature infants. In two studies with a small number of infants (n = 7 and 5), SGA infants <35 weeks’ gestation were noted to have a maximal weight loss of 2–5% [22, 23]. Moreover, infants in the second study were reported to have regained their birthweight dissimilar to AGA infants. The resumption of adequate energy intakes in these SGA infants is accompanied by an increase in oxygen consumption and may represent energy cost of growth. One of the nutritional factors generally not considered clinically is that SGA infants demonstrate increased fecal fat excretion, less energy storage and protein loss. There are SGA infants, however, who do not demonstrate these increases and demonstrate lower rates of weight gain. It is possible that this lack of weight loss and subsequent weight gain may have implications for the observed adult-onset diseases seen in SGA infants.
Blood Volumes

Blood volumes vary with methodology (plasma vs. red cell labels), time of cord clamping and may vary with delivery complications. There are no significant differences between term and preterm neonates with regard to blood volumes. IUGR does not appear to affect blood volume or red blood cell mass. However, in SGA infants with polycythemia, blood volume is increased (108 ml/kg vs. 86 ml/kg) compared to AGA infants [24]. Implications of the polycythemia-hyperviscosity syndrome as a result of fetal hypoxia (increased erythropoietin synthesis) or a placental-fetal transfusion include hypoglycemia and hypoxia, hyperbilirubinemia and associated risks with increased red cell mass.

To summarize, the neonatal effects include hypoglycemia, hypocalcemia, hypothermia, perinatal depression/asphyxia, meconium passage in utero (therefore, higher risk for meconium aspiration syndrome), and polycythemia.

Prognosis is obviously dependent on the etiology, duration of the effects causing SGA/IUGR, and includes increased mortality compared to appropriate-for-gestational-age infants, increased risk for perinatal complications, increased risk for neurological dysfunction, increased risk for adult-onset hypertension, glucose intolerance, obesity and long-term growth problems depending on cause.

Disclosure Statement

JB has no financial or other conflict of interest in relation to the content of the chapter. AG is a consultant to Mead Johnson Nutritionals, Evansville, IN, USA.

References

Intrauterine growth restriction (IUGR) is defined as a rate of fetal growth that is less than normal for the growth potential of a specific infant. An enormous number and variety of established and possible causes have been identified. Potentially, any aberration of biological activity in the fetus can lead to growth failure. The most common identifiable cause is fetal undernutrition due to placental insufficiency. IUGR infants have a significant risk of increased morbidity and mortality. Neonates born at term weighing between 1,500 and 2,500 g (<10th percentile) have a 5 to 30 fold increase in perinatal morbidity and mortality compared with infants with weight between the 10th and 90th percentile [1]. Those less than the 3rd percentile (<1,500 g) at term have a 70 to 100 fold increase in poor perinatal outcomes [2]. Survivors also experience an excess of neurodevelopmental problems during later childhood. It is important to keep in mind that infants with IUGR may or may not be small for gestation age (SGA), and infants who are SGA may not have been affected by growth restricting processes that cause IUGR. The decreased fetal growth rate in IUGR is an adaptation to an unfavorable intrauterine environment and may result in permanent alterations in metabolism, growth, and development [3]. Onset of growth restriction early in gestation results in symmetrical growth restriction with weight, length, and head circumference equivalently affected. Causes include genetic disorders, dwarf syndromes, congenital viral infections, some inborn errors of metabolism, and intrauterine drug exposure. Growth restriction of later onset is usually related to impaired uteroplacental function (preeclampsia, chronic hypertension, class D and F diabetes) or nutrient deficiency. In these cases, fetal weight is affected, and there is relative sparing of the head and length.
The acute neonatal consequences of IUGR are perinatal asphyxia and poor neonatal adaptation. The list of problems is long and can involve any system. Common problems in term and late preterm IUGR babies include asphyxia, hypothermia, hypoglycemia, respiratory distress and polycythemia. Perinatal asphyxia is the initial concern in the IUGR fetus. Careful obstetric surveillance and timely delivery can prevent it. Major respiratory complication seen in these infants is meconium aspiration syndrome with persistent pulmonary hypertension. These babies are at increased risk for problems with thermoregulation [4]. Thus, a neutral thermal environment should be maintained. Fasting hypoglycemia is an issue of major concern. It is primarily due to decreased glycogen stores with a possible contribution from diminished glucose production in the liver from alanine and lactate via gluconeogenesis [5]. Furthermore, hyperinsulinism or excessive sensitivity to insulin can exacerbate hypoglycemia and in some cases can lead to the need for high glucose infusion rates and prolonged hypoglycemia [6]. Chronic intrauterine hypoxia induces synthesis of erythropoietin causing an increase in red cell mass [7]. In addition, or alternatively, placental-fetal transfusions can occur in labor or during asphyxia, resulting in a shift of placental blood to the fetus. Both of these mechanisms can account for polycythemia in the IUGR fetus.

Preterm babies with very low birthweight who are SGA have higher mortality rates than their AGA counterparts and are at significant risk for reduced postnatal growth and development as well as acute and chronic morbidities such as respiratory distress syndrome, bronchopulmonary dysplasia, retinopathy of prematurity and necrotizing enterocolitis (NEC) [8]. The incidence of NEC is increased in infants with fetal absent or reversed end diastolic flow in the umbilical artery [9]. Superior mesenteric artery Doppler studies on the first day of life show reduced blood flow in SGA infants.

Not only short term complications affect term and preterm IUGR babies, they are also at increased risk of poor neurodevelopment outcome as compared to their AGA counterparts [10].

In conclusion, both preterm and term growth restricted babies are at higher risk of both neonatal and long term morbidities. Specific cause of growth restriction is an important factor in assessing risk for poor outcomes. Treatments to reduce risk of adverse outcome include more advanced antenatal assessment to determine time of delivery, optimized pre and postnatal growth and nutrition and other early intervention strategies.

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Naveen P. Gupta
References


Abstract
Functional outcome of preterm infants is highly related to the quality and quantity of nutrients provided during the first few weeks of life. New guidelines, as published by the ESPGHAN in 2010, have provided means to prevent undernutrition in the NICU. Especially proteins and amino acids seem to play a pivotal role, and the optimal regimen has not yet been determined. New data on the intrauterine nutrient supply suggest a high amino acid intake during the fetal period. How these results might translate into improvement of especially neurocognitive outcome needs to be investigated.

Introduction
Undernourishment during the early phase of life leads to reduced neurocognitive functioning in term infants [1]. Also in low birthweight and preterm infants, observational studies have suggested that inadequate nutrient intakes in the first few days to weeks following birth have adverse effects on long-term neurocognitive function [2–4]. Unfortunately, only a few prospective studies have been conducted in preterm infants. These studies, most originating in the 1980s, have shown that both quantity and quality of the provided nutrients to preterm children and subsequent growth have pronounced effects on neurocognitive function [5], cardiovascular health [6, 7], and metabolic and endocrine status [8, 9].
These effects seem to persist over a long period, well beyond childhood [9–11], and justify all efforts to provide especially preterm infants with adequate nutrients in early life.

**Different Approaches in Determining Requirements for Preterm Infants**

Two approaches can be used in determining adequate requirements for infants following premature birth. Since preterm infants have not yet matured well enough, their organs are still developing and growth is extremely rapid, it follows that the supply to the fetus of similar gestational age can be regarded as suitable. However, some drawbacks to that theory can be made. The fetus thrives in an entirely different environment, despite care for these newborns in warmed and humidified incubators. Furthermore, the fetus hardly uses the lungs or intestines, and the maturation pattern is different due to less stressors from outside, including a potentially hostile microbial environment. Also, most preterm infants are ill, certainly at birth, requiring mechanical ventilation, antibiotic therapy and sometimes vasopressors. It is likely that these conditions influence nutrient requirements. Altogether, it is unlikely that the nutrient supply via the umbilical cord adequately reflects the nutrient requirement of prematurely born infants being treated at the neonatal intensive care unit.

The other approach is based on the composition of human milk and the factorial approach. The factorial approach is a method in which the growth rate and the composition of newly formed tissues of a fetus of a certain gestational age are estimated. The composition of the fetal tissues is derived from carcass analyses of deceased fetuses [12]. These data are rather old, originating from 1890s onwards, with little knowledge on the health status of the fetomaternal dyade. The other factor in this method is the composition of human milk. Own mother’s milk is the optimal source of nutrients for term-born children, but both quantity and content vary considerably. Hindmilk has a different fat content than foremilk, and milk produced in the first week of life has a higher protein content than mature human milk. Mothers with a high fish diet, e.g. from Japan or Greece, have different long-chain polyunsaturated fatty acid concentrations in their milk than mothers from a different region. Consequently, the mean concentration of nutrients and a mean volume intake are just rough estimates of what the actual intake is. Therefore, also the factorial approach and human milk composition are not likely to provide the exact requirements for all nutrients of the prematurely born infant.

So, both methods have their drawbacks in estimating the exact nutrient requirements of prematurely born children who are taken care of in an incubator with all kinds of accompanying illnesses. Knowledge on both approaches is how-
ever pivotal in developing the appropriate nutritional management, which should be based upon estimates using both methods and subsequent well-conducted clinical trials. Since hardly any data were available on the first approach, the fetal nutrient supply, we undertook a few studies to determine these.

**Fetal Nutrient Intake**

In a series of studies, we were able to determine the fetal amino acid uptake of fetuses around term gestational age [13–15]. Prior to caesarean section, pregnant women received intravenously administered amino acids that were labeled with stable isotopes. The measurement of flow rate at the umbilicus and the measurement of both arterial and venous umbilical blood directly after birth enabled us to quantitate the actual uptake of the studied amino acids by the fetus just prior to birth.

We were able to demonstrate that the placenta provides the fetus with a great surplus of amino acids. Only 20% of the available phenylalanine is utilized by the fetus, of which a part is hydrolyzed to tyrosine (fig. 1) [13]. That is important information, since tyrosine was considered a conditionally essential amino acid, especially following birth. These data show that even a fetus is capable of hydroxylizing phenylalanine, so that tyrosine can be considered a non-essential amino acid.
acid. Based upon valine and leucine tracers, we estimate that up to half of these amino acids taken up from the umbilical cord are oxidized instead of being used for anabolism [14]. This demonstrates that just as in animal models like sheep [16], amino acids should be regarded as a significant energy source during fetal life. This directly also shows a disadvantage of the factorial approach as only accreted nutrients are counted in, and not those oxidized for energy generation.

**Current Practice**

Undernutrition, with subsequent growth failure, occurs predominantly during the first week of life of premature infants. Recent recommendations for enteral nutrition prevent undernutrition during the enteral phase [17, 18]. However, many neonatologists are reluctant to start with high nutrient intakes directly following birth. They fear that the renal and hepatic systems are not developed well enough to handle high amounts of (par)enterally administered nutrients and their metabolites. That seems logical since in utero, the placenta and the mother have large capacities to handle off possible detrimental metabolites. However, several trials on early amino acid administration showed improvements of nitrogen balances, and thus anabolism, without clinical significant adverse events [19–21]. According to current guidelines, 2–3 g amino acids/kg per day should be started as soon as possible after birth, with increments to a maximum dose of 4 g/kg per day in the next few days [22–24]. Present amino acid mixtures and lipid emulsions are improved when compared to mixtures that were marketed years ago. Marked differences in enteral feeding practices and consequently also in parenteral policies were found in recent surveys, evaluating clinical practices amongst over 100 NICUs around the world [25–29]. Nevertheless, despite newer and possibly beneficial alternatives [30], the lipid emulsion still used most often in NICUs is a 100% soybean emulsion, of which the composition has not changed during almost half a century except from increasing its concentration from 10 to 20%.

**Quality of Amino Acid Mixtures**

The quality of parenteral amino acid mixtures is difficult to judge since we do not know the exact requirements for individual amino acids in parenterally fed preterm infants. For term neonates, some requirements (threonine, methionine, sulfur amino acids) are known [31–33]. Consequently, the composition of current pediatric amino acid solutions differs widely among the different brands. Some brands used plasma amino acid concentrations of healthy, term, breastfed infants as a guiding
reference, while others derived the composition from fetal and neonatal cord blood amino acid concentrations. Overall, amino acid mixtures are low in tyrosine and cysteine due to poor solubility or stability in parenteral nutrition. Future studies should indicate the optimal composition of amino acid mixtures for preterm infants.

**Amino Acid Tolerance**

Specific parameters for amino acid intolerance are lacking. Biochemical parameters, such as acidosis, elevated plasma urea concentrations, increased ammonia concentrations and concentrations of potentially (neuro)toxic amino acids above reference ranges for term infants are often used as a proxy for intolerance. However, these parameters are also influenced by the general clinical status of the neonate. Like in fetuses who use amino acids for both protein synthesis and energy generation (oxidation), elevated urea concentrations in preterm neonates are the result of functional amino acid oxidation and not purely a sign of amino acid intolerance. In addition, correlations between urea concentrations and amino acid intake are inconsistently found. Therefore, elevations in plasma urea concentration should not automatically lead to withholding of amino acids in preterm infants.

**Early Amino Acid Administration**

In a randomized controlled trial, we aimed to find a difference in nitrogen balance, a proxy for anabolism, on day 2 of life by supplying 2.4 g of amino acids per kilogram per day from birth onwards, whereas the control group received dextrose only [20]. The latter group started after 36 h with 1.2 g of amino acids, with an increase to 2.4 g on day 3 of life. Despite very low energy intakes (30–50 kcal/kg per day), the preterm infants (average birthweight of 1 kg) were able to use the provided amino acids and increase whole-body protein synthesis [34] and albumin synthesis [35], and turn nitrogen balance from negative (catabolic state) to positive (anabolic state) [20]. In addition, the synthesis rate of the main intracellular antioxidant, glutathione, was higher as well [36]. We could not detect any negative side effects. So, this study showed that supplementing preterm infants with 2.4 g amino acids per kg per day resulted in a clear beneficial effect in the short term. Studies where amino acid administration was started not directly following birth but within the first few days yielded similar results [37]. Other trials questioned the efficiency and warned of possible detrimental effects [38, 39]. Very recently, long-term follow-up from one of these studies (not powered for long-term follow-up though) became available, showing diminished growth dur-
ing the first 2 years of life and cumulative and single plasma AA concentrations negatively correlating with MDI and postnatal growth [40]. However, these data are derived from only 16 infants in each group who were available at age 18 months (63% of surviving infants). Nevertheless, their data indicate we should be careful infusing the most immature infants (<25 weeks’ gestation) with high-dose amino acids (4 g/kg per day) without further investigations.

Two-year follow-up of our own trial (follow-up n = 111, 97% of those surviving) revealed no detrimental effects. Even the opposite, preterm boys had significantly more often normal outcome (survival without major handicaps) when they were supplemented with high amino acid intakes from birth onwards. No significant effect was noticed in girls [manuscript submitted].

**Fetal Albumin Synthesis Rate**

In the same series on fetal metabolism as described above, we infused several $^{13}$C-labeled amino acids in a staggered manner to pregnant women prior to (preterm or term) caesarean section, allowing for the determination of fetal protein synthesis, such as fetal albumin [41]. Albumin, the major transport protein with antioxidative capacities, is frequently found at low concentrations in premature infants. A low concentration results in low colloid osmotic pressure with subsequent edema. Albumin serves as an indicator of nutritional status, although concentrations are not as sensitive as synthesis rates. The use of stable isotopes enabled us to determine fetal albumin synthesis rates and compare those with rates found following admission to the NICU after different amino acid intakes (fig. 2). The administration of amino acids from birth onwards at a rate of 2.4 g/kg per day improved synthesis rates significantly when compared to the unsupplemented infants, but the rates remained below those obtained in utero. This might be related to neonatal stress or illness following birth, but might also illustrate that nutrient supply in these infants failed to meet the requirement [15]. Nevertheless, and most importantly, it was shown that the liver of a premature fetus is capable of synthesizing protein at very high rates and regarding this aspect, the liver should not be regarded as immature.

**Meeting the Needs Directly following Birth**

Very recently, we finished our second trial on early nutritional support of small infants. One hundred and forty-four infants were included, with different lipid and amino acid intakes from birth onwards. The highest intake group (3.6 g/kg
per day amino acids and 2 g/kg per day lipids) was most anabolic as was shown by classic nitrogen balances. These results need further evaluation but confirm the results obtained by Ibrahim et al. [19].

**Conclusion**

Optimal nutritional strategies for preterm infants are continuously being developed. Suboptimal quality and quantity of nutrients, even during only the first few days of life, are likely to have long-term consequences. Providing high amounts of nutrients form birth onwards should however be carefully evaluated, and randomized trials with ample power should be conducted. Not only short-term proxy outcome variables should be measured, but also long-term follow-up should be included as end points of such trials to evaluate the choices made in feeding this vulnerable population.

**Disclosure Statement**

The authors have no conflict of interest to declare that has a relationship with this paper. The hospital of J.B.v.G. received a fee for the presentation of the results described in this paper at a Nestlé Nutrition Institute workshop held in March 2012, Goa, India.
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Delivering adequate amounts of nutrients to premature infants at all times is challenging because the infant’s immature gastrointestinal tract is initially unable to accept feedings, necessitating the use of parenteral nutrition. Although the rate of survival of these infants has improved, growth failure is nearly universal, and multiple recent studies have documented the poor growth and nutritional deficits in this population of infants. At the time of birth, about 18% of extremely low-birthweight infants are less than the 10th percentile for weight and length, but at 36 weeks’ corrected gestational age or before discharge, most of these same babies are below their birth centiles for weight and length. In addition, many remain very small throughout infancy [1]. Protein deficits, especially those occurring in the first week of life contribute substantially to poor growth [2]. Delaying amino acid (AA) supplementation for even few days in the postnatal period can result in negative nitrogen balance, and approximately 1.1–2.5 g/kg per day of AA intake with caloric intake of 30–60 kcal/kg per day can change protein balance from significantly negative to neutral or positive [3, 4]. The consistencies of these findings are despite differences in the composition of the AA solutions used in the studies. Therefore, early administration of AA at a rate equal to or slightly exceeding the rate of protein losses (1.5–2 g/kg per day) is an important clinical strategy in preterm infants to preserve body protein stores, even if caloric intakes remain low.

Safety is often cited as a reason to delay initiation of AA, particularly in the sickest, most immature infants. Specific concerns include changes in acid-base status and elevations in blood urea nitrogen (BUN) and ammonia levels. It is, therefore, reassuring that multiple studies have demonstrated no differences in

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**Commentary on Amino Acid Homeostasis in the Preterm Infant**


metabolic acidosis, BUN, or ammonia concentrations in infants who received early AA compared with those who received none. It is interesting to note that in early postnatal life, BUN primarily reflects fluid status and does not reflect AA intake.

Traditionally, AA were started as 1 g/kg per day on day 1 of life and then increased sequentially to 3–3.5 g/kg per day in increments of 0.5–1 g/kg per day [5]. However, the preference for a stepwise procedure is solely empirical, based on fluid limitations, worries about intolerance, and fear of hyperglycemia in case of mixed glucose/AA solutions. Since even few days of delay can result in negative nitrogen balance and significant protein deficits that can have long-term implications in terms of growth, many investigators have tried supplementing AA at high dose starting from day 1 of life. Trials show that doing this leads to conversion to neutral or positive nitrogen balance suggestive of anabolism, increased albumin synthesis, and increased glutathione synthesis [6–8]. Negative side effects observed with early high-dose AA infusion such as increased mean peak serum indirect bilirubin, lower base excess, lower concentrations of bicarbonate, and increased plasma urea nitrogen were without clinical implications. In these studies, AA were given on day 1 of life to a maximum of 2.5 g/kg per day.

Although the short-term metabolic safety of early AA administration is well established, less information is available assessing long-term outcomes. Beneficial long-term effects on neurodevelopment have been difficult to prove.

Up until now, studies investigating the effect of high-dose parenteral AA administration to preterm infants do not exceed 2 years’ follow-up and show mixed results. Blanco et al. [9] showed that extremely preterm infants who received high-dose infusion of AA (up to 4.0 g/kg per day on day 3 compared to standard supplementation; n = 32) had lower long-term anthropometric measurements and lower cognitive development at 18 months’ corrected gestational age, although the difference disappeared at 2 years of age. In contrast, Stephens et al. [10] showed that increased first-week protein and energy intakes were associated with higher Mental Development Index scores and lower likelihood of length growth restrictions at 18 months of age in 148 extremely low-birthweight infants. Follow-up data of nutritional intervention of protein supplementation in infants (NIPI 1) study presented by Dr. van Goudoever showed better Mental Development Index scores in boys at 18 months’ corrected age.

Since transplacental transfer of protein in the fetus in the last trimester is somewhere between 3.5–4 g/kg per day, some of the investigators have questioned whether starting protein supplementation at 2.5 g/kg per day on the first day of life is sufficient or these babies need much higher intake. Nutritional intervention of protein supplementation in infants (NIPI 2) evaluated two different AA administration rates (3.5 g/kg per day with lipids against 2.6 g/kg per day
without lipids on day 1). Initial results presented by Dr. van Goudoever showed better phenylalanine and tyrosine kinetics and more positive nitrogen balance in the group receiving high AA and lipids. Hence, supplementing AA at 3.6 g/kg per day and lipids at 2 g/kg per day on day 1 can be considered safe.

In conclusion, AA supplementation should be started on day 1 of life. Starting at 2.5 g/kg per day has been shown to be safe by studies looking at both short-term and long-term outcomes. Starting AA at 3.6 g/kg per day along with lipids at 2 g/kg per day can be considered safe and beneficial, as shown in some studies looking at short-term outcomes; still, long-term outcomes need to be seen before making it a standard of care.

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Naveen P. Gupta

References

Clinical Outcome of Low Birthweight, Long-Term Consequences


Interventional Strategies to Promote Appropriate Growth

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Abstract

Appropriate growth of premature infants can be defined as growth that is not associated with adverse consequences in the short and the long term. Growth failure is associated with neurocognitive impairment. The goal of nutritional management therefore is the achievement of appropriate growth by ensuring that nutrient intakes are maintained at all times at adequate levels. Many impediments stand in the way of this goal. Parenteral administration of nutrients must begin immediately at birth and needs to be continued until enteral nutrition is fully established. While nutritional support is provided by parenteral nutrition, gut priming, also beginning at birth, stimulates the immature gastrointestinal tract to undergo maturation. Human milk is the preferred agent for gut priming because it is more effective and safer than alternative agents. As a source of nutrients, however, human milk is incomplete for the premature infant and requires supplementation (fortification) with nutrients. At the authors’ institution, commercial human milk fortifiers and additional sources of protein are being used in efforts to achieve appropriate growth. Data from the authors’ institution indicate that nutrient intakes, especially intakes of protein, have improved in recent years and are approaching adequate levels. Accordingly, growth of infants has improved to the point where on average only a mild degree of postnatal growth failure is observed.

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Why Is Appropriate Growth Necessary?

Postnatal growth failure among premature infants began to receive serious attention only in the late 1990s [1, 2]. It was soon realized that the occurrence of growth failure implied inadequate nutrient intakes. This led to efforts to improve nutrient intakes, which succeeded in diminishing the extent of growth
failure. Yet, defying all efforts, growth failure has remained a substantial problem to this day [3–8]. The importance of postnatal growth failure lies of course in the fact that it is associated with poor neurocognitive development. One of the largest and most important studies documenting the dose-dependent association of growth failure with impairment of neurocognitive development was reported in 2006 by Ehrenkranz et al. [9]. The association between growth failure and poor developmental outcome has been confirmed independently in a number of localities [10–13], leaving no doubt that growth failure has deleterious effects on the later neurodevelopment of premature infants. Growth failure also exerts a negative effect on retinopathy of prematurity [14].

Since there is good evidence that growth failure is mainly, if not in its entirety, the consequence of inadequate nutrient intakes, efforts aimed at preventing growth failure have focused on achieving adequate nutrient intakes through parenteral as well as enteral venues. Many impediments, mostly imaginary, to the provision of appropriate nutrient intakes have been removed. But many more remain and ensure that the achievement of adequate nutrient intakes remains an elusive goal.

**When Is Growth Appropriate?**

Although growth failure is easily recognized as such when it is severe, lesser degrees of growth failure are not always easy to define. The question of when growth is normal (appropriate) and when it is no longer normal (growth failure) is therefore important. Ideally, appropriate growth should be defined as growth that is not associated with any short-term or long-term adverse consequences. Unfortunately, we lack the data to describe appropriate growth according to this definition. Less ambitiously, we can define appropriate growth as growth that follows the fetus closely. After all, the growing fetus provides the model we use for estimating nutrient requirements. Fetal growth is well described by published growth curves [15, 16]. How close to the fetus should weight of the premature infant be in order to be considered ‘appropriate’? The transition from fetal to extrauterine life involves a contraction of extracellular fluid space that is presumed to be permanent [17, 18]. If we assume that extracellular fluid, which accounts for about 50% of bodyweight, shrinks by 10%, a decrease in bodyweight by 5% may be considered physiologic in that it can be explained solely by loss of water without involvement of non-aqueous tissues. According to this view, appropriate growth could be defined as weight that parallels fetal weight on a trajectory about 5% below the weight trajectory of the fetus. Appropriate weight would be defined as equal to or greater than 95% of expected fetal weight.
Although it is likely that a mild degree of growth failure (weight deficit slightly greater than 5%) is innocuous, the available data unfortunately do not permit us to delineate any degree of growth failure that is free of adverse consequences. Therefore, we must strive to avoid any degree of growth failure.

**What Is Causing Growth Failure?**

Identification of the cause(s) of growth failure has to rely on observational data as evidence from controlled trials is lacking. Inadequate nutrient intakes are implicated as the main, if not the sole, cause of growth failure because intakes have been found to be less than adequate (see below) wherever they have been determined [1, 17–20]. Since the effects of low nutrient intakes in slowing growth are well established, there can be little doubt that the main cause of growth failure is inadequacy of nutrient intakes. Although the possibility that non-nutritional factors play a role in the causation of growth failure cannot be ruled out completely, such factors could at most play a minor role.

An important study linking nutrient intakes during the first week of life directly to neurocognitive outcomes at 2 years of age was reported by Stephens et al. [21]. Poor neurocognitive outcomes were found to be associated with low first week nutrient intakes in a dose-dependent fashion. This finding provides a strong rationale for initiating adequate nutrient intakes very soon after birth.

**What Are Adequate Nutrient Intakes?**

The nutrients limiting for growth are protein and energy. By consensus [22], the recommended intake of protein, which is assumed to permit fetal growth, is 4.0–4.5 g/kg per day for infants weighing <1,000 g and 3.5–4.0 g/kg per day for infants weighing 1,000–1,800 g. For parenterally fed infants, somewhat lesser (by about 10%) intakes are probably adequate. Recommended energy intakes are 110–135 kcal/kg per day, again with somewhat lesser intakes being adequate for parenterally fed infants. Intakes of all other nutrients must also be met at all times.

**Why Are Nutrient Intakes Often Inadequate?**

Historically, the predominant reasons explaining inadequate nutrient intakes have been concerns regarding the safety of the administration of nutrients both parenterally and enterally. Parenteral nutrition was for a long time considered
too risky in the early days of life and was therefore withheld and introduced late and cautiously. Especially lipid emulsions were introduced very hesitantly. This did not change until studies began to suggest that parenteral nutrition in premature infants was safe. Key studies documenting the complete safety as well as efficacy of parenteral nutrition initiated within hours of birth did not appear until 2004 [23] and 2005 [24]. Today, near-full or full parenteral nutrition is started within hours of birth and no serious adverse effects are encountered.

Enteral feedings similarly were thought to carry risks, in this case mainly the risk of necrotizing enterocolitis, and for this reason were withheld for periods ranging from days to weeks. Trophic feedings (gut priming) began to be introduced earlier in life in the mid-1990s, but progress has been slow, and today withholding of feedings is still widely practiced, if for shorter periods than in the past. Also, there continue to be lingering concerns about the safety of ‘high’ intakes of protein, with ‘high’ not being defined in quantitative terms and in the absence of any evidence supporting this concern. As safety concerns have faded, other reasons why nutrient intakes remain inadequate have come into clearer focus. There have been and continue to be misperceptions regarding the amount of protein that is required for appropriate growth. Finally, there is a lack of tools necessary for achieving adequate intakes. The prime example is human milk fortifiers that, with one exception, provide far too little protein and thereby make it nearly impossible to achieve adequate intakes.

**Strategy**

Overall, the strategy to prevent growth failure aims at providing adequate nutrient intakes at all times. Growth approximating that of the fetus in rate and composition can only be expected if nutrient intakes are adequate at all times, i.e. match intakes estimated to permit duplication of fetal growth. Feeding practices employed to provide nutrients to the premature infant have evolved over the years.

**Parenteral Nutrition**

For the first days of life, all premature infants depend on parenteral nutrition because immaturity of their intestinal tract precludes any substantial enteral nutrient administration. Safety and efficacy of immediate parenteral nutrition have been established [23, 24]. Parenteral nutrition should be started within 2 h of birth with a dose of amino acids no less than 3.0 g/kg per day. Intravenous lipids
need to be started within 24 h of birth at no less than 1.0 g/kg per day and advanced to 2.0 g/kg per day or more. The glucose infusion rate should be increased periodically as long as euglycemia is maintained.

During the next 1–3 weeks, parenteral nutrition typically remains the dominant, or at least a major, source of nutrients. It is gradually replaced by enteral nutrition. Parenteral nutrition should be discontinued only when enteral nutrition is almost complete (>90% of full).

**Gut Priming**

The objective of gut priming is solely to move the intestinal tract from its immature state at birth to a functionally mature state. Maturation is brought about by small amounts of food, preferably colostrum and/or human milk. The immature gut is devoid of normal motility [25], which is manifested clinically as persistent gastric residuals [20]. The presence of residuals does not preclude the administration of small amounts of feedings. Gut priming should start on the day of birth or the following day as any delay in initiation may lead to gut atrophy. Instability of the infant is not a contraindication to gut priming. There is insufficient information to decide whether gut priming should use constant low feeding volumes for a set number of days or whether volumes should be increased as residual size and frequency are declining. At the authors’ institution, the latter approach is followed on the presumption that the decline of gastric residuals is a marker of gut maturation.

The choice of priming agent is important. Human milk (initially colostrum), by virtue of its trophic and immune-protective properties, is the agent of choice. Human milk matures the gut more rapidly and in a safer way than formula. When maternal milk is not available, or not available in sufficient quantity, donor human milk should be used. Formula is a distant third choice, but is still preferable to no gut priming. At the authors’ institution, donor milk is frequently used as priming agent in the first few days until maternal milk comes in.

**Enteral Feeding**

Human milk, the preferred feeding for the preterm infant, does not provide the necessary amounts of protein and of most other nutrients when fed in volumes that can be handled by the infant, e.g. ≤200 ml/kg per day. Therefore, human milk must be supplemented (fortified) with nutrients. Fortification is typically initiated at a feeding volume of 100 ml/kg per day. Commercial fortifiers provide protein from
bovine milk, energy from carbohydrate and/or lipid, minerals, especially Ca and P, and vitamins. Fortifiers raise the caloric density to 80 kcal/100 ml (24 kcal/oz) and increase the levels of most nutrients to where intakes meet needs for growth. Fortifiers do not increase osmolality to any significant extent. The amount of protein provided by fortifiers is, with some notable exceptions, less than needed for appropriate growth. Additional protein is therefore often provided. Several methods to accomplish this have been proposed, two of which (adjustable fortification [26] and targeted fortification [27]) have been shown to be effective and safe.

After the first few weeks of life and when full feedings have been tolerated for some time, it is safe to use formulas as an alternative to human milk. Standard premature formulas provide protein in a concentration of 3.0 g/100 kcal, which is satisfactory for infants weighing more than 1,500 g. Formulas with protein concentrations between 3.3 and 3.6 g/100 kcal (‘high protein’) are available and should be used for infants weighing less than 1,500 g. In general, formulas are more likely to provide adequate protein intakes than fortified human milk.

**Current Iowa Nutritional Practices**

The authors have periodically provided descriptions of their nutritional practices [1, 28–30]. The present report presents an update on practices, together with nutrient intake data in 2001 and 2010 and growth outcomes for 2010.

**Parenteral Nutrition**

Parenteral nutrition is started within 2 h of birth using an incomplete (‘starter’) nutrient solution that, in a volume of 60 ml/kg per day, administers amino acids in a dose of 3.0 g/kg per day and glucose at 4 mg/kg per min. Within 24–36 h, the starter solution is replaced by a complete neonatal parenteral nutrition solution. Amino acid administration is maintained at 3.0–3.5 g/kg per day, whereas the glucose infusion rate is increased daily in stepwise fashion by 1–2 mg/kg per min as long as euglycemia is preserved. Intravenous lipids are started within 24 h of birth at a dose of 1 g/kg per day and increased in stepwise manner to a rate of 2 g/kg per day. This regimen is continued until enteral feeds are advanced beyond gut priming amounts, at which point the phase-out of parenteral nutrition begins. As parenteral nutrition volume is weaned, the amino acid concentration of the solution is advanced in order to maintain total amino acid intakes of ≥3.0 g/kg per day. Once parenteral volume falls below 60 ml/kg per day, amino acid concentration is maintained at 5 g/dl, and amino acid intake gradually
declines as volume declines. Intravenous lipids are maintained at goal dose until ∼1 day prior to discontinuation of parenteral amino acids. At that time lipids are reduced to 1 g/kg per day or discontinued. Typically, the amino acid/dextrose portion of parenteral nutrition is not discontinued until enteral feedings have reached approximately 90% of ‘full’ feedings.

**Gut Priming**

Gut priming with small amounts (1–2 ml) of human milk is initiated on the day of birth or the following day. Priming is performed initially every 8 h. If there is not enough colostrum, or when the mother is not expressing her milk, donor milk is used, which is replaced by the mother’s own milk as soon as it is available. Gut priming is not interrupted regardless of the size of gastric residuals, but may be held if residuals are bilious and/or GI obstruction is suspected. As residuals diminish in size, priming is increased in frequency and, somewhat later, in volume in stepwise fashion.

**Human Milk Fortification**

We initiate fortification when total feed volume reaches 25 ml/day. Once feed volume reaches 120–130 ml/kg per day, or sooner, we increase fortification to 6 packets per 100 ml milk instead of the standard 4 packets. We do this in order to increase the level of protein fortification. By increasing the amount of fortifier we also increase caloric density to about 90 kcal/100 ml (27 kcal/oz) and increase the level of Ca and P and of all other nutrients provided by the fortifier to levels that exceed the levels intended by standard fortification. In all infants weighing <1,000 g and in infants weighing <1,500 g who receive donor milk and demonstrate weight gain below goal, we add, in addition to 6 packets of HMF, some protein (Beneprotein) and term formula concentrate. This results in fortified donor milk with a caloric density of 100 kcal/100 ml (30 kcal/oz) and a protein concentration of 3.5 g/100 kcal.

**Growth Goals**

The goal for weight gain is generally 15–20 g/kg per day (e.g. a 1,000-gram infant should gain 15–20 g/day, a 2,000-gram infant 30–40 g/day). As infants approach term, growth goals decline to ∼10 g/kg per day. Weight gain of infants is calcu-
lated weekly, and weight is plotted weekly on fetal growth charts so that infant growth may be visually compared to fetal (expected) growth.

**Current Iowa Results: Nutrient Intakes**

One of the authors has since 1994 recorded intakes of nutrients (protein and energy) as well as weight of infants cared for in our NICU. The methods are essentially as reported before [1]. In brief, actual intakes of energy and protein are recorded every 7th day beginning on day 7 (day of birth = day 0). Intakes are for that day (24 h) and not for any period bordered by that day. Weight is obtained from hospital records. Human milk is uniformly assumed to provide 67 kcal/dl and 1.0 g/dl of protein, regardless of whether it is maternal milk or donor milk. All other nutrient values, for example of human milk fortifiers, are taken from manufacturer’s information.

We report data for 95 infants born in calendar 2010 with birthweight <1,250 g. This included 26 SGA infants born between 26 and 34 weeks gestation, 36 AGA infants born before 27 weeks gestation and 33 AGA infants born between 27 and 31 weeks gestation. For comparison, we also report nutrient intakes and growth for all 86 infants born in calendar 2000.

Figure 1 summarizes protein intakes in 2010 for days of life 7, 14, 21 and 28, broken down into parenteral and enteral intakes. Average total protein intake was 3.2 g/kg per day on day 7 and increased to 3.4 g/kg per day by day 28. Protein intake from enteral sources was miniscule on day 7, but by 28 days account-
ed for most of the protein, with only a minority of infants still receiving parenteral nutrition. In 2000 (data not shown) protein intakes were around 3.0 g/kg per day regardless of age and were thus somewhat lower than in 2010.

Energy intakes in 2010 are summarized in figure 2. The picture is similar to that for protein except that total intakes were initially low but increased substantially with age. At 28 days, a minority of infants were still receiving energy parenterally. In 2000 (data not shown) energy intakes were quite similar to intakes in 2010.

Figure 3 presents total protein intakes for the first 8 weeks of life for 2000 and 2010. As the figure shows, protein intakes were substantially higher in 2010 than in 2000. Also, in 2010 protein intakes increased substantially with age, whereas in 2000 no such increase was evident. Breakdown of protein intakes by infant group shows only trivial differences between groups (data not shown).

**Current Iowa Results: Growth**

Figure 4 shows mean weight of the three groups of infants born in 2010 plotted against fetal growth (indicated by the 10th, 50th and 90 percentiles of Fenton [15]). Although average weight is generally approaching fetal weight, it is evident that there still is some fall-off from fetal growth. However, conversion of weight to z scores (not shown) indicates that, following the weight loss of the first week of life, there is actually very little change in weight z score for the next 7 weeks. This seems to indicate that average weight growth parallels fetal growth.
The data of Senterre and Rigo [5] similarly show an initial drop in weight z scores, perhaps somewhat deeper than in our infants, but one that is followed by no further fall-off but rather a progressive return toward zero relative weight loss.

Table 1 summarizes weight and weight status of infants at 36 weeks’ postmenstrual age. Included is the percentage of infants who met our definition of

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**Fig. 3.** Intake of protein from all sources in 2000 and 2010 during the first 56 days of life.

**Fig. 4.** Mean weight of infants born in 2010 from birth to 36 weeks’ postmenstrual age plotted against 10th, 50th and 90th centiles of fetal growth.
**Table 1.** Weight and weight status at 36 weeks’ postmenstrual age (mean ± SD)

<table>
<thead>
<tr>
<th>Subject groups</th>
<th>Calendar 2000</th>
<th>Calendar 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>weight, g</td>
</tr>
<tr>
<td>AGA &lt;27 weeks</td>
<td>27</td>
<td>2,251±434</td>
</tr>
<tr>
<td>AGA 27–31 weeks</td>
<td>39</td>
<td>2,225±335</td>
</tr>
<tr>
<td>SGA</td>
<td>20</td>
<td>1,583±233</td>
</tr>
<tr>
<td>All</td>
<td>86</td>
<td>2,074±462</td>
</tr>
</tbody>
</table>

Appropriate weight is defined as 95% of expected fetal weight.

Appropriate growth, i.e. who were 5% or less below the expected fetal weight. Somewhat surprisingly, AGA infants born at <27 weeks do a little better than 27- to 31-week AGA infants. All parameters show improvement between 2000 and 2010. However, the percentage of infants born AGA who dropped below the 10th centile remains substantial. In 2000, 100% of SGA infants remained SGA at 36 weeks, whereas in 2010 that percentage had decreased to 80%. The percentage of infants with appropriate weight at 36 weeks increased between 2000 and 2010, especially for SGA infants.

**Disclosure Statement**

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

**References**

Comments by Discussant

Postnatal growth restriction in preterm infants has been recognized to be a major issue globally despite applying standard nutritional practices. There has been a concern particularly with respect to its effects on neurocognitive outcomes. The problem of growth restriction is likely to be more exaggerated in developing countries, where nutritional products like optimal infant formulas and milk fortifiers are still unavailable. The issue brought up by Dr. Ziegler raises the question whether poor growth affects neurocognition or whether inadequate nutrition affects both growth as well as neurocognition simultaneously. Nonetheless, attempts to improve postnatal growth or at least decrease neonatal/postneonatal growth restriction are necessary.

Optimal growth postnatally is debatable in view of varying degrees of postnatal weight loss, degree of in utero growth restriction and varying early nutritional practices. Setting the right benchmark is essential for deciding the nutritional strategies. It is established that it is difficult to match in utero growth due to metabolic differences in accretion of nutrients after birth. Dr. Ziegler has suggested that growth trajectory of 5% below fetal growth curve is both reasonable and achievable in the postnatal period. The presentation included several strategies currently used to improve growth in preterm infants.

Early Parenteral Nutrition with Higher Nutrient Intakes

Several recent studies have demonstrated the safety and efficacy of early introduction of parenteral nutrition (within hours of birth) with amino acids up to 3 g/kg per day and lipids 3 g/kg per day in small ELBW infants. Metabolic bal-
ance studies indicate good tolerance of higher nutrient intakes, thus establishing this as a routine practice in ELBW care.

Early Enteral Feeds and Grading Up

Fear of necrotizing enterocolitis delays introduction and slows grading up of feeds in preterm/LBW infants, though evidence in this regard is insufficient. Benefits of early introduction of enteral feeds (MEN) are known, and grading up by at least 20 ml/kg per day is advisable while monitoring for feed intolerance in infants at risk.

Optimizing Nutrient Intakes by Fortification

Recent ESPGHAN guidelines on preterm nutrition emphasize the need for higher protein intakes in preterm infants. Achieving 3.5–4 g/kg per day protein intake has been shown to be the prime determinant for driving postnatal growth in these infants. Providing such protein intakes is a challenge considering low protein content of breast milk, infant formulas and fortifiers. Dr. Ziegler demonstrated the possibility of early addition of fortifier to enteral feeds at feed volumes of 25 ml/kg for maintaining higher protein intake, especially while weaning off PN. Addition of extra amounts of fortifier than standard recommendation (6 sachets instead of 4) was shown to be tolerated. Protein fortification with specific products (such as Beneprotein) has also shown good tolerance and achievement of adequate protein intakes. Significant improvement in postnatal growth has been demonstrated in Iowa infants over the last decade as a result of change in nutritional practices (of particular significance is the marked change in SGA growth). This opens up the opportunity for improving growth of SGA babies in developing countries. However, issues of long-term implications of accelerated growth in early life in SGA infants will continue to haunt researchers until clear data emerge.

Umesh Vaidya

Interactive Discussion

*Dr. Bloomfield:* Our nutritional practices include earlier achievement of full feeds in ELBWs (11 days), and we provide higher feed volumes (180 ml/kg per day). We also use a higher protein intake TPN solution in first 2–3 days (4 g/kg
in 60 ml/kg fluid). Growth showed zero change in weight and 0.6 SD loss in length at 36 weeks.

**Dr. Ziegler:** I appreciate the superb growth outcome in your babies. I suggest that maybe 3.5 g/kg protein should be adequate in first few days.

**Dr. Van Goudoever:** Appropriate feeding in the first week of life will prevent these babies from falling off their growth centiles. This will avert the need for too much of catch up, thus reducing the need for very high protein intakes.

**Dr. Kramer:** Strong association between postnatal growth and later cognitive development raises issues of confounding factors and reversed causality effect. Several factors such as neonatal sickness and morbidity could lead to poor nutrition and mental deficits. How would you address these?

**Dr. Ziegler:** I agree with the confounding effects of other factors on cognitive outcomes. However, sickness itself precludes adequate feeding, thus accentuating nutritional effects on cognition.

**Dr. Renner:** With higher protein intakes that you described, were babies affected by osmolar load, higher urea and acidosis?

**Dr. Ziegler:** As we predominantly used breast milk and isotonic formulas, we did not encounter metabolic acidosis or osmolar load.

**Dr. Solomons:** With respect to long-term growth in the small preterm infants, do we know about proportionality of height loss such as sitting height or leg growth?

**Dr. Ziegler:** Most of these babies are short at 10 years, but proportionality has not been studied.

**Dr. Gumbo:** What is the role of thermoneutral environment on growth? Do you feel that this affects neurocognitive outcomes?

**Dr. Ziegler:** All our babies have been managed in a thermoneutral environment, so it is not likely that this is a factor influencing neurocognition. However, current standard of care would suggest maintaining normal temperature and avoiding hypothermia in sick infants.
Abstract
Low birthweight (LBW), defined as birthweight <2,500 g, is a major global public health problem and is associated with lifelong cognitive and behavioral problems. Very LBW (VLBW) infants (<1,500 g) are at high risk of multiple macro- and micronutrient deficiencies, but most LBW infants are larger (1,500–2,500 g), and the most common nutritional problem of those infants is iron deficiency (ID). Globally, about 25% of pre-school children have ID anemia (IDA), the most severe form of ID, and there is good evidence that ID is associated with impaired brain development. However, adverse effects of excessive iron supplementation have been observed. Delayed umbilical cord clamping, which increases infant iron stores, should be recommended for all newborns. There is good evidence that intakes of 2 mg of dietary iron per kg daily prevents IDA in LBW infants without causing adverse effects. A recent study shows that this dose of iron supplementation also reduces the risk of behavioral problems at 3 years in infants with birthweights 2,000–2,500 g. VLBW infants need 2–3 mg/kg per day. To achieve these intakes, breastfed LBW infants should receive iron supplements, and formula-fed LBW infants should receive an iron-fortified infant formula.
imize later morbidity in LBW infants. However, there is still a severe lack of knowledge on this subject – not least with regard to micronutrients – and, consequently, a large variation in nutritional practices.

LBW infants include both term, small-for-gestational-age infants and preterm infants. Most LBW infants have only marginally or moderately LBW (1,500–2,500 g). Recent advances in neonatal care have significantly improved the survival of very LBW (VLBW) infants (<1,500 g), who are at high risk of multiple macro- and micronutrient deficiencies. However, this review will mainly focus on the larger LBW infants, and the most common nutritional problem of those infants is iron deficiency (ID).

**Iron Deficiency**

ID is the most common single-nutrient deficiency worldwide [4]. Young children, and especially LBW infants are at high risk of ID since their rapid growth leads to high iron requirements. Globally, about 25% of pre-school children are estimated to have ID anemia (IDA), the most severe form of ID, and the highest prevalence is found in South Asia and Africa, where up to 50% of young children have IDA in socioeconomically disadvantaged areas [4]. In Europe, the prevalence of IDA in toddlers is 3–4% in the general population [5] but much higher in subpopulations at risk, including those with LBW.

Growth and development of the central nervous system is rapid during the first years of life, and iron is critical for this process. The human brain almost triples its weight from birth to 3 years of age, and has at that age reached 85% of its adult size. Animal studies have shown that iron is required for several aspects of brain development: myelination, monoamine neurotransmitter function and neuronal and glial energy metabolism [6].

Several well-performed case-control studies in children have shown a consistent association between IDA in infancy and long-lasting poor cognitive and behavioral performance [7]. In summary, even though there still is limited data from human intervention studies [8], the available evidence suggests that it is important to prevent ID in infants to ensure optimal neurodevelopment.

However, it is important to note that iron, in contrast to most other nutrients, cannot be actively excreted by humans, and the risk of iron overload must therefore be considered. We and others have shown that iron supplementation of iron replete infants may have adverse effects, e.g. increased risk of infection and impaired growth [9]. Increased risk of severe infections seems to be restricted to malarious regions (see below), while the risk of impaired growth has been observed also in European infants [10, 11]. A single study has even suggested that
Iron and Other Micronutrient Deficiencies in LBW Infants  199

a high iron intake may have adverse effects on long-term cognitive outcomes in iron-replete infants [12]. Furthermore, iron is a potent pro-oxidant, and non-protein-bound iron has been suggested to cause formation of reactive oxygen species, especially in newborns before 2 weeks of age, and possibly increase the risk for e.g. retinopathy of prematurity [13, 14].

It is therefore important to identify iron requirements in infants to avoid both ID and iron overload.

**Estimated Iron Requirements**

At birth, most of the body iron is found in blood hemoglobin (Hb), but a term, healthy, normal-birthweight infant also has some iron stores, corresponding to about 25% of total body iron (fig. 1). When the newborn emerges from the relatively hypoxic environment of the uterus out into the oxygen-rich atmosphere, Hb synthesis is halted, and the Hb falls from an average of 170 g/l to about 120 g/l during the first 6 weeks of life [9]. Due to recirculation of iron from senescent erythrocytes, iron is transferred from Hb to iron stores, which thereby increase in size. During the following months, as the baby continues to grow and expand its blood volume, iron is transferred back from stores to the blood compartment, making the normal infant virtually self-sufficient with regard to iron during the first 6 months of life (fig. 1). This is compatible with the very low concentration of iron in breast milk (0.3 mg/l).

**Fig. 1.** Body iron compartments and total body iron in a term infant with a birthweight of 3,500 g.
LBW infants clearly have lower iron stores due to their lower bodyweight. The body iron content at birth has been calculated to be 75 mg/kg at weights ranging between 0.2 and 4 kg, based on fetal body composition studies from the 1950s [15]. As shown in figures 2 and 3, LBW infants have higher iron requirements during the first months of life due to more rapid postnatal growth. Based on expected growth and assuming negligible iron losses, the increase in total body iron corresponds to dietary iron requirements of 1–2 mg/kg per day between 6 weeks and 6 months of age in an LBW infant with a birthweight of 2,000
g (fig. 2), and 2–3 mg/kg per day between 2 weeks and 6 months of age in a VLBW infant with a birthweight of 1,000 g (fig. 3). These conclusions are compatible with previous calculations using a similar factorial approach when iron requirements for VLBW infants have been estimated to be about 2 mg/kg per day over the course of the first 12 months of life [16].

The timing of umbilical cord clamping is of great importance for the amount of blood transfused from the placenta to the newborn. We and others have shown that delayed cord clamping increases iron stores and prevents ID at 3–6 months of age in normal-birthweight infants [17, 18]. This may be even more important in preterm infants. A Cochrane review concluded that delayed cord clamping of preterm infants was associated with less need for blood transfusions [19].

In VLBW infants, blood losses and blood transfusions related to neonatal intensive care will greatly influence iron status and iron requirements. Iron losses due to phlebotomy have been estimated to be 6 mg/kg per week [20]. On the other hand, each red blood cell transfusion typically adds 8 mg/kg of iron. Hepatic iron stores as well as serum ferritin concentrations in preterm infants are highly correlated with the number of blood transfusions received [21]. Erythropoietin treatment results in greatly increased iron requirements, and high doses of oral or parenteral iron is recommended as an adjunct to this therapy. Thus, local practice regarding blood sampling, blood transfusions and erythropoietin treatment will greatly influence iron requirements of VLBW infants. It is useful to follow serum ferritin concentrations in infants who have received multiple blood transfusions to assure that they do not develop iron overload.

In normal birthweight infants, virtually no external iron is required during the first 6 months of life, but dietary iron requirements are high at 6–24 months of age, corresponding to about 0.1 mg/kg per day of absorbed iron or 1 mg/kg per day of dietary iron, assuming a fractional absorption of 10% [22]. Assuming an optimal diet and normal growth, LBW infants should have similar iron requirements as normal-birthweight infants after 6 months corrected age.

Effects of Interventions

There are relatively few published randomized intervention trials comparing different doses of iron supplements or fortification of human milk or formula given to LBW infants.

A meta-analysis has shown that prophylactic iron at a dose of 2 mg/kg per day given to LBW infants, most of which with birthweights 1,500–2,500 g, leads to significantly reduced incidence of anemia at 6 months [23].
In a study by Friel et al. [24], 58 infants with an average birthweight of 1,500 g were randomized to different infant formulas resulting in iron intakes of 3–6 versus 2–3 mg/kg per day up to 9 months of age. There was no difference in anemia or neurodevelopment at 12 months. However, the high-iron group had higher glutathione peroxidase concentrations (a marker of oxidative stress), lower plasma zinc and copper levels and a higher number of respiratory tract infections, suggesting possible adverse effects with the higher iron intakes.

It is not clear at what postnatal age iron supplements should be initiated in order to prevent ID in LBW infants. Two randomized trials in VLBW infants with an average birthweight of 0.9–1.2 kg have shown that early initiation of iron supplementation or fortification (2 weeks postpartum, compared to 6–8 weeks) may reduce the need of blood transfusions [25, 26].

Iron Requirements of Marginally LBW Infants

We have recently performed a randomized, controlled, blinded trial (n = 285) of iron supplements at the following doses: 0 (placebo), 1 or 2 mg/kg per day, given from 6 weeks of age to 6 months of age in a population of otherwise healthy Swedish marginally LBW infants with birthweights 2,000–2,500 g.

We could show that iron supplements at a dose of 2 mg/kg per day, compared to placebo, significantly reduced the risk of IDA at 6 months [27]. In the placebo group, 36% developed ID and 10% developed IDA, as compared to 4 and 0% in the 2 mg group. About half of these infants were mostly breastfed during the intervention and the other half received iron-fortified formula. No adverse effects of iron supplements were observed with regard to infant growth, infections or other morbidity. There were significant differences in iron status between those who had received 1 or 2 mg/kg per day of iron but there were no significant differences in the proportion of infants with ID or IDA in those two groups. When considering all dietary iron sources and iron supplements, including compliance, we could show that an actual iron intake of 0.25 mg/kg per day was sufficient to prevent IDA, and an intake of 1 mg/kg per day prevented ID [27].

Interestingly, when we followed up the LBW children from the above RCT at 3.5 years of age, we found a significantly higher proportion of abnormal behavioral scores in the placebo group [28]. Using a validated questionnaire (Achenbach Child Behavior Checklist), the prevalence of children with behavioral scores above the US subclinical cutoff was 12.7, 2.9 and 2.7% in the placebo, 1 mg, 2 mg, and control group respectively, as compared to 3.2% in a reference group of children with normal birthweight. Adjusting for socioeconomic con-
founders, the risk of behavioral problems was 4.5 times higher (95% CI: 1.3–15.8) in placebo-treated compared to iron-supplemented children. However, no significant differences were observed in cognitive scores.

**International and National Recommendations for Iron Intake in LBW Infants**

The WHO previously recommended 2 mg/kg per day of iron from 2–23 months of age for all LBW infants [29], and more recently recommended 2–3 mg/kg per day from 6–8 weeks to 12 months of age [30]. The American Academy of Pediatrics recommends 2 mg/kg per day from 1–12 months to breastfed LBW infants [31]. The European Society for Paediatric Gastroenterology, Hepatology and Nutrition recommends 2–3 mg/kg per day from 2–6 weeks to 6–12 months.

**Iron Supplements in Malaria-Endemic Areas**

Supplementation with iron, which is an essential nutrient also for pathogens, may increase the risk of diarrhea and malaria [32, 33].

In 2003, a large RCT of iron supplementation in Pemba, Tanzania, which has a high prevalence of malaria, had to be terminated due to serious adverse effects [10]. In this trial, 24,000 children aged 1–35 months were randomized to daily oral supplementation with iron (12.5 mg) and folic acid or placebo. The dose of iron was halved in infants <12 months. In the groups receiving iron and folic acid, there was a 15% increased risk of death and an 11% increased risk of hospital admission. A substudy suggested that the risk for serious adverse events was higher in infants who were initially iron replete, i.e. those with higher Hb and lower zinc protoporphyrin. A similar study performed in Nepal showed that such adverse effects are not observed in a non-malarious region [34].

This led to a joint statement by the World Health Organization and the United Nations Children’s Fund in 2007 advising that, in regions with high prevalence of malaria and other infections, iron/folic acid supplementation should be limited to those identified as iron deficient [35]. A more recent Cochrane review in 2011 concluded that iron supplementation alone or with antimalaria treatment does not increase the risk of clinical malaria or death when regular malaria surveillance and treatment services are provided [36]. However, such services are not generally available in all low-income countries.

Since LBW infants are at high risk of ID, and can be assumed not to be iron replete, the risk of adverse effects is likely to be lower, and iron supplements can be recommended for this patient group, preferably of course in combination with
malaria surveillance and treatment. If breast milk is not available, iron-fortified infant formula is a good option, since it has not been associated with increased risk of malaria.

**Other Micronutrient Deficiencies in LBW Infants**

Similarly to normal-birthweight infants, LBW infants are at risk of deficiencies of vitamin K and vitamin D, and should therefore receive prophylactic vitamin K soon after birth and vitamin D supplementation during infancy if they do not receive a vitamin D-fortified infant formula.

VLBW infants have extraordinarily high macro- and micronutrient requirements and a high risk of general malnutrition, severe growth failure, visual impairment and cognitive/behavioral problems. Recent studies have suggested that low macronutrient intakes, especially protein and energy, during the early postnatal period are associated with poor neurodevelopment and retinopathy of prematurity, a major cause of visual impairment and blindness in VLBW children [37, 38]. Micronutrient deficiencies are also common in these infants, and clinical deficiencies of sodium, calcium, phosphorus, iron, zinc, copper, vitamin A, vitamin D, vitamin E, vitamin K, riboflavin and folic acid have all been described in this patient group [39]. Even in modern neonatal units, where micronutrient supplemented parenteral and enteral nutrition is routinely used, it is difficult to achieve recommended intakes of macro- and micronutrients [40]. Preliminary data from a population-based Swedish cohort of extremely preterm infants born 2004–2007 showed that average intakes during the first 4 weeks of life were lower than recommended for calcium, phosphorous, zinc, copper, iodine and magnesium [unpubl. data]. Such suboptimal micronutrient intakes may have negative short- and long-term effects on growth, bone health and immune function even though this has not been sufficiently studied.

**Conclusions**

LBW infants are at risk of ID, which is associated with impaired brain development and later behavioral and cognitive problems. Delayed umbilical cord clamping, which increases infant iron stores, should be recommended for all newborns, including LBW infants. There is good evidence that intakes of 2 mg of dietary iron per kg daily starting at 2–6 weeks of age prevent IDA in LBW infants without causing adverse effects. VLBW infants need 2–3 mg/kg per day starting at 2 weeks of age. To achieve these intakes, breastfed LBW infants should receive iron supplements,
and formula-fed LBW infants should receive an infant formula fortified with iron to a concentration of approximately 12 mg/l. Iron supplements should be continued up to 6–12 months of age, depending on the diet and growth of the infant and the local prevalence of IDA. After discontinuation of supplements, LBW infants should follow dietary recommendations for normal-birthweight infants.

**Disclosure Statement**

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

**References**

Comments by Discussant

The presentation highlighted that micronutrient deficiencies, particularly iron deficiency, are very common in low birthweight infants and extend into later life. Of particular concern is the high incidence (25–50%) of iron deficiency reported in pre-school children and its deleterious effects on cognition and neurobehavior. It thus becomes imperative to prevent iron deficiency in early infancy and childhood to have optimal neurodevelopment.

While term infants have sufficient iron stores (which shift between storage pool and hemoglobin), most infants will be self-sufficient in iron until 6 months, needing supplementation thereafter. The preterms would need higher doses and earlier supplementation in view of limited stores and rapid growth. Risks of iron excess such as increased infection and impaired growth have to be kept in mind during supplementation. Cord clamping, blood transfusions, phlebotomy would affect iron status of these infants and need to be considered during iron supplementation.

The current published studies quoted in the presentation suggest 2 weeks as the optimal timing for iron supplementation in LBW infants. The Swedish study presented by Dr. Domellöf is particularly significant as it includes the marginally LBW infants (2,000–2,500 g), a large population less well studied in this respect. The Swedish infants showed less risk of IDA at 6 months with 2 mg/kg per day supplementation from 6 weeks to 6 months as compared to placebo (no iron
supplementation). No differences were seen between the groups receiving 1 versus 2 mg/kg iron. Interestingly, at 3.5 years the placebo group showed higher proportion of abnormal behavioral scores, demonstrating the long-term relevance of iron supplementation.

International recommendations (WHO, AAP, ESPGHAN) are 2–3 mg/kg per day iron starting at 6–8 weeks in breastfed LBW infants and continued for at least 12 months. The presentation also touched on iron supplements in malaria-endemic areas. It seems prudent to provide iron/folic acid supplements only to iron-deficient populations due to demonstrated increased risk of mortality and hospitalization in iron-replete individuals. It was also emphasized that other micronutrient deficiencies such as vitamin K, vitamin D, calcium, phosphorus, zinc and copper need special attention in LBW infants to avert negative short- and long-term effects on growth, bone health and immune function.

Umesh Vaidya

Interactive Discussion

Dr. Koletzko: Would provision of iron as supplements or as food make a difference on iron status?

Dr. Domellöf: This is a very interesting issue, and currently we are in the process of evaluating iron supplements versus iron in fortified food with respect to absorption, metabolism and incorporation in erythrocytes using stable isotopes.

Dr. Osendarp: I have a comment and a question. A cohort of normal-birthweight iron-deficient infants in the US (study by Betsy Lozoff) demonstrated differential responses to socioemotional problems at 9–10 months. These were mediated by the impact of iron on dopamine production. My question pertains to the significance of poor iron absorption in the first few weeks and whether this leads to adverse effects.

Dr. Domellöf: We found similar results as Betsy Lozoff, but case-control studies are prone to confounders. With respect to iron absorption, preterm infants have higher iron absorption as they have higher iron requirement.

Dr. Solomons: We must decide whether adding iron to infant formulas should be called as supplementation or fortification.

Dr. Domellöf: We should call it fortification rather than supplementation. It is intriguing to look at differences of adverse effects between fortification and supplementation. Increased risk of infections and poor neurodevelopment are more likely with supplementation than fortified foods, though contrary data do exist.
Dr. Solomons: With increasing number of elective caesarean sections, are we depriving babies of the benefit of placental transfusion?

Dr. Domellöf: It is possible though not well studied.

Dr. Gibson: In your study, was there an increase in behavioral problems in those receiving iron?

Dr. Domellöf: No. In fact, the placebo group had 12% behavioral problems as compared with the two iron-supplemented groups.

Dr. Hernell: Is iron metabolism fully developed in preterm infants? On the basis of previous hepcidin studies, can one conclude that iron metabolism or absorption is well regulated?

Dr. Domellöf: There are huge changes in iron status and metabolism during the first year of life. It seems that most infants would have adequate iron absorption irrespective of their iron status.

Dr. Gupta: In LBW infants, ferritin levels on umbilical cord are normal, suggesting sufficiency. Why start supplementing earlier? Are the criteria for initiating supplementation different for multiply transfused infants?

Dr. Domellöf: We do not want to start supplementation after deficiency has developed. So, I would suggest starting iron even if ferritin is normal on umbilical cord sample. However, in transfused infants I would withhold supplementation if serum ferritin is above 300 mg/dl.

Dr. Lapillonne: In your study, there were two groups of infants, term SGA and preterm SGA. Was there any difference between the two groups?

Dr. Domellöf: There were no differences in iron deficiency or iron deficiency anemia in the two groups. We suggest iron supplementation is needed in both groups.

Dr. Lapillonne: In view of wide variations in ferritin levels in VLBW babies, is it correct to fix a uniform recommendation for all infants, or should it be individualized on the basis of ferritin levels?

Dr. Domellöf: Though it is better to individualize on the basis of ferritin levels, it would be difficult in resource-limited situations.

Dr. Renner: Is cord clamping at 3 min advisable for extremely small preterm infants? Would it delay resuscitation and affect other neonatal outcomes?

Dr. Domellöf: We follow the practice; we define delayed cord clamping as after 2–3 min. For very small and sick babies, 3 strokes of milking have been shown to improve iron status and also neonatal outcomes, but further studies are needed.

Dr. Renner: Is there any risk of hypervolemia or polycythemia with milking of the cord?

Dr. Domellöf: Japanese studies have found it to be safe and effective, but more studies are needed.
Dr. Alves Filho: Do low levels of erythropoietin in these babies matter?

Dr. Domellöf: Early anemia of prematurity is influenced by erythropoietin, though such therapy is now not routine standard care. The late anemia is influenced greatly by nutritional factors, particularly iron deficiency.

Dr. Karim: How should one deal with iron supplementation in malaria-endemic areas? Should supplementation stop during infections?

Dr. Domellöf: The trial in Tanzania has shown increased mortality due to severe infections in babies receiving iron/folate supplementation particularly in the group which was more iron replete. Most infants, especially low birthweight, are not iron replete between 0 and 6 months, and hence supplementation would be safe. A Cochrane review has confirmed that supplementation is safe in babies and young children in malaria-endemic areas. Regarding iron supplementation during acute infections, increased risk of severe infection and diarrhea has been shown, though there is no evidence that supplements need to be stopped during airway or other infections.
Clinical Outcome of Low Birthweight, Long-Term Consequences


Abstract

Infants born with low birthweight (LBW) have poorer neurodevelopmental outcomes compared with their term counterparts with appropriate weight for gestational age. The perinatal period is a time of high energy and high nutrient needs, and any process, such as preterm birth, poor nutrition or placental insufficiency, that interrupts the concentrated flow of nutrients to the fetus may result in babies with LBW. Therefore, it makes logical sense that at least part of the cognitive deficits may be explained by nutritional deprivation. The nutrients commonly deficient in LBW infants include protein and energy and micronutrients such as iron, zinc and long chain polyunsaturated fatty acids. In this review, we aimed to determine the effect of nutrient supplementation on neurodevelopment in LBW infants. While few trials have supported the hypothesis that nutritional supplementation improves neurodevelopment, many studies are limited by sample size and methodological shortcomings. Further large-scale rigorously designed intervention trials, with long-term neurodevelopment follow-up, are required to determine the optimal nutritional supplements and the timing of their administration to LBW infants.

Improving the Neurodevelopmental Outcomes of Low-Birthweight Infants

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Abstract

Infants born with low birthweight (LBW) have poorer neurodevelopmental outcomes compared with their term counterparts with appropriate weight for gestational age. The perinatal period is a time of high energy and high nutrient needs, and any process, such as preterm birth, poor nutrition or placental insufficiency, that interrupts the concentrated flow of nutrients to the fetus may result in babies with LBW. Therefore, it makes logical sense that at least part of the cognitive deficits may be explained by nutritional deprivation. The nutrients commonly deficient in LBW infants include protein and energy and micronutrients such as iron, zinc and long chain polyunsaturated fatty acids. In this review, we aimed to determine the effect of nutrient supplementation on neurodevelopment in LBW infants. While few trials have supported the hypothesis that nutritional supplementation improves neurodevelopment, many studies are limited by sample size and methodological shortcomings. Further large-scale rigorously designed intervention trials, with long-term neurodevelopment follow-up, are required to determine the optimal nutritional supplements and the timing of their administration to LBW infants.

The trajectory of growth is greatest during the perinatal period, which is the time that includes late pregnancy and early postnatal life. During the last trimester of pregnancy the normal fetus will grow from approximately 900 to
3,400 g, more than tripling in weight. The newborn term baby will double birthweight in the first 4 months of life and will again nearly double in size over the next 24 months, but will not achieve adult size until late adolescence. The brain, on the other hand, will achieve adult size (approximately 1,200 g) by about 2 years of age. In the last trimester of pregnancy, brain weight will increase from approximately 150 to 400 g, and between term birth and 6 months of age the brain will again double in size, reaching approximately two thirds of adult brain weight [1]. The composition and structure of the brain also changes dramatically during this time [2], and these changes continue well into childhood.

The perinatal period is characterized by high energy and high nutrient requirements necessary to sustain the high growth rates. It is therefore not surprising that any process, such as preterm birth, poor nutrition or placental insufficiency, that interrupts the concentrated flow of nutrients to the fetus will result in babies with low birthweight (LBW). The brain appears particularly sensitive to the nutrient deprivation associated with LBW, as infants who are born with LBW are more likely to have lower scores on neurodevelopmental tasks into childhood compared with term, normal-birthweight infants [3]. Preterm infants are at particular risk for long-term cognitive and educational problems directly proportional to their degree of prematurity, with those most preterm demonstrating a mean intelligence quotient (IQ) of 0.8–1.5 standard deviations (SD) lower than children who were born at term [4]. Recent evidence also suggests there are even higher rates of developmental delay in preterm infants born growth restricted compared with appropriately grown preterm infants [5, 6]. Children born at term and with LBW are twice as likely to have an IQ that is 2 SD lower than term-born appropriately grown infants [7].

Strategies to improve the neurodevelopmental outcome of children born with LBW are important, and many interventions have focused on nutritional approaches applied during the period immediately after birth, especially for preterm infants who are born before the last trimester of pregnancy is complete and the concentrated nutrient supply delivered across the placenta is prematurely ceased. The nutrients that are commonly deficient in LBW infants include protein and energy as well as micronutrients such as iron, zinc and long-chain polyunsaturated fatty acids (LC-PUFA) [2]. The focus of this review, therefore, is to determine the effect of nutrient supplementation on neurodevelopment in LBW infants. The review is limited to randomized controlled trials (RCTs) designed to assess the effects of enteral nutrition interventions during the postnatal period. Other study designs have not been considered because complex neurodevelopmental outcomes are influ-
enced by many factors including sex, perinatal morbidity as well as social and environmental influences [4], and RCTs remain the gold standard methodology to minimize the biases of these other factors and show cause and effect relationships.

A comprehensive literature search was undertaken to identify systematic reviews of RCTs or RCTs including postnatal protein-energy, micronutrient or LC-PUFA supplementation in LBW infants and reported neurodevelopmental outcomes.

**Protein-Energy Supplementation of Preterm Infants**

The effect of protein-energy enrichment in the postnatal period on neurodevelopment of preterm infants has been summarized in five Cochrane Systematic Reviews [8–12] involving 9 RCTs conducted between mid-1960 to mid-2000 [13–21]. The early trials compared differing concentrations of protein in formula or breast milk while in hospital [13–18], and the more recent trials investigated the effect of providing protein-energy-enriched formula or breast milk after discharge [19–21].

**In-Hospital Supplementation of Formula Compared with Donor Human Milk**

In a systematic review of formula versus donor human milk (either as the sole diet or as a supplement to mother’s own milk) only one trial was included [9]. This seminal study conducted by Lucas et al. [15, 22, 23] in the 1980s compared preterm formula (2 g protein and 80 kcal/dl; 2.5 g protein/100 kcal) with donor breast milk (1.07 g protein and 46 kcal/dl; 2.3 g protein/100 kcal). In this parallel RCT, women who chose not to breastfeed were randomized to preterm formula or donor breast milk as the sole diet (trial 1), and women who chose to breastfeed were randomized to receive preterm formula or donor breast milk as a supplement to breast milk (trial 2) [15]. At 9 months of age, on combining trials 1 and 2, a significant benefit was found in developmental quotients (DQ) with the preterm formula (preterm formula 100.4, SD 10.7, versus banked donor human milk 97.9, SD 9.6; 95% CI: 0.4–4.6) [23]. At 18 months’ corrected age, no benefit to mental development was found in either trial 1 or trial 2 or on meta-analysis of both trials (n = 387, weighted mean difference, WMD, 1.24, 95% CI: –2.62 to 5.09) [9].
In-Hospital Supplementation with Protein and Energy Using Human Milk Fortifiers

While human milk is the preferred base feed for preterm infants because of its immunological properties, it is well recognized that protein, energy and micronutrient supplementation is needed to achieve appropriate growth rates. However, in a systematic review of multicomponent fortification of human milk versus no fortification, there was only one trial that assessed neurodevelopment [10]. In this trial, 275 infants born weighing <1,850 g whose mothers chose to breastfeed were randomized to receive 0.7 g protein and 14 kcal added to 100 ml of breast milk or no additional protein-energy supplement [18]. Neurodevelopment was assessed at 18 months’ corrected age with no significant difference found (MD 2.2, 95% CI: -3.35 to 7.75) [10].

In-Hospital Supplementation with Protein and Energy Using Infant Formulas

Lucas et al. [16] in another well-known study compared term formula vs. preterm formula in preterm infants. This study included two parallel trials in which infants born weighing <1,850 g were randomly allocated to receive a protein-energy-enriched formula containing 2.0 g protein and 80 kcal/dl (2.5 g protein/100 kcal) or standard formula of 1.45 g protein and 68 kcal/dl (2.1 g protein/100 kcal) [16]. The first trial (trial A) included infants who were not receiving any breast milk, so the test formulas were the sole diets, and the second trial (trial B) included infants who were receiving some breast milk so that the test diets were a supplement to mother’s milk. In trial A, psychomotor development, but not mental development, at 18 months’ corrected age was higher with protein energy enrichment (mean difference, MD, 14.7, 95% CI: 8.7–20.7) [16]. There were no neurodevelopmental differences found in trial B. When trials A and B were combined at 18 months, there were no differences found in mental development, but the improvement in psychomotor development remained (MD 6.2, 95% CI: 2.4–10.0) [16], and there was a significant sex by diet interaction such that boys fed the protein-energy-enriched formula had an 8-point gain in mental development (95% CI: 2–13) [16].

When the same cohort was assessed at 7.5–8 years of age (84% follow-up rate) no differences in any IQ measures were found in either trial A or trial B or when trials A and B were combined [24]. However, the verbal IQ of children who were fed the protein-enriched formula in trial A was higher compared with control but did not reach statistical significance (MD 4.8, 95% CI: -0.6 to 10.02) [24]. Al-
though no sex by diet interaction was reported, post hoc analyses indicated that boys in trial A who had protein-energy-enriched formula as the sole diet had a 12.2 increase in verbal IQ (95% CI: 3.7–20.6) compared to boys who had standard formula [24]. We have excluded from this review results for a subset (n = 95) of the children assessed as neurologically normal at 7.5–8 years of age and who were assessed again at 16 years of age. This highly selected population now constitutes an exploratory analysis with a high risk of bias [25]. Although the neurodevelopmental data are not strong with trial A including the smallest numbers and extensive exploratory analyses, nutrient-enriched preterm formulas are now common practice for all preterm infants who require formula complements.

Nevertheless, there is significant interest in further enriching protein concentration based on growth and other metabolic studies. A systematic review of the effects of higher versus lower protein intakes in exclusively formula-fed infants included three trials reporting developmental outcome [8]. These trials, beginning early in the postnatal period in LBW infants, have yielded mixed results on neurodevelopmental outcomes [8]. The earliest and largest trial included in the review was conducted in the 1960s and included infants <2,000 g (n = 304) [13]. This study reported no difference in overall IQ at 3 and 5–7 years of age between infants fed very high protein content formula (5 g protein/100 kcal providing 6.0–7.2 g/kg per day) compared with lower protein content formula (2.5 g protein/100 kcal, providing 3.0–3.6 g/kg per day). However, in infants with birthweights <1,300 g, the infants fed the higher protein formula had a significantly increased incidence of IQ <90 at 3 (RR 0.30, 95% CI: 0.14–0.64) and 5–7 years of age (RR 0.31, 95% CI: 0.15–0.66) when compared to infants fed lower protein formula [8]. The formula was isocaloric; however, the very high protein formula had 17% more minerals. Results of this early study have led to a cautious approach to increasing protein concentration and the recommendation from the Cochrane Systematic Review that protein intakes greater than 4 g/kg per day should be considered experimental [8].

The two remaining trials included in the review were limited primarily by small sample sizes and by either inadequate description of trial details/procedures or large loss to follow-up. One trial (n = 48), fed very LBW preterm infants isocaloric formula of 3.0 g protein/100 kcal to provide 3.2 g/kg per day protein compared with 2.3 g/100 kcal providing 2.6 g/kg per day from 3 weeks of age [14]. No difference in neurodevelopmental assessments (tests not specified) were reported at 6 months, 1 or 2 years of age [8]. The remaining trial (n = 26) fed isocaloric formula yielding 2.6 g/kg per day (2.2 g protein/100 kcal) of protein compared with 3.1 and 3.8 g/kg per day (2.7 and 3.2 g/100 kcal) of protein to very LBW infants [17]. Infants receiving the higher protein performed significantly better on orientation (p = 0.0003), habituation (p = 0.003) and auto-
nomic stability (p = 0.01) clusters of the Neonatal Behavior Assessment Scale when assessed at approximately 36–37 weeks’ postmenstrual age [8].

Postdischarge Supplementation with Protein and Energy for Breast Milk and Formula

As almost all of the in-hospital studies did not continue supplementation beyond discharge, it is possible that the intervention period was too short to ‘catch up’ the nutrient deprivation associated with prematurity and hence see consistent neurodevelopmental advantages. With this rationale, postdischarge protein and energy supplementation has been investigated for preterm infants. The key trials are summarized in two systematic reviews, one comparing term formula versus preterm formula [11] and one comparing fortified with unfortified human milk [12]. Only two trials of preterm versus term formula have assessed neurodevelopment at 18 months’ corrected age [11]. The trials included preterm infants with birthweight <1,750 g. In one trial, only infants who were growing normally with a rate of weight gain ≥25 g/day at time of discharge were eligible to participate [19]; in the other trial, infants had to weigh <3,000 g at time of discharge [20]. Infants were randomized to receive nutrient-enriched formula (72–80 kcal and 1.85–2.2 g protein/dl) or standard term formula from discharge to 6 [19] or 9 [20] months after term. A meta-analysis of data from both trials (n = 299) showed no significant difference in mental (WMD 0.23, 95% CI: −2.99 to 3.45) or psychomotor development (WMD 0.55, 95% CI: −1.95 to 3.05) [11]. Only one small trial (with a high loss to follow-up) of postdischarge fortification of human milk was found for inclusion in a systematic review [12]. Thirty-nine preterm infants born at <33 weeks’ gestation, with birthweight 750–1,800 g were included in the trial [26]. Infants were randomized to have half of the daily breast milk intake fortified to achieve an approximate protein and energy content of 2.2 g and 81 kcal/100 ml or no fortification (1.3 g protein and 68 kcal/100 ml) for 12 weeks. No significant difference in developmental outcome was found at 18 months’ corrected age [intervention mental development index (MDI) 100 (1st to 3rd centile; 72–102.5) versus control 91 (1st to 3rd centile; 77–107)] [12].

Protein-Energy Supplementation of Term Growth-Restricted Infants

Limited data exist for term-born infants who are born small for gestational age (SGA). The benefits of a nutrient-enriched formula compared with standard formula for SGA term infants (>37 weeks and birthweight <10th percentile for
sex/age) also require more clarification. At 9 months of age, infants fed the nutrient-enriched formula from week 1 after birth until 9 months of age, had a significant disadvantage (MD –2.5, 95% CI: –4.6 to 0.4) in overall DQ as assessed with the Knobloch Developmental Screening Inventory. There was a significant sex by treatment interaction with girls fed the nutrient-enriched formula performing significantly poorer (MD –5.1, 95% CI: –7.8 to –2.4) compared to boys (MD 0.9, 95% CI: –2.4, 4.2). At 18 months, however, the same cohort of infants assessed using the Bayley Scales of Infant Development (BSID) showed that the enriched formula had no significant effect on mental or psychomotor development compared with standard formula [27].

Although protein-energy supplementation for LBW infants has been widely studied, there are surprisingly few well-conducted trials with neurodevelopmental outcomes that have adequate sample sizes to draw robust conclusions regarding the direct effect of protein-energy supplementation on cognitive and psychomotor outcomes.

**Micronutrient Supplementation**

Individual micronutrient building blocks, which do not contribute energy, are also considered important for brain development, and a number of studies have specifically investigated individual nutrient supplementation for LBW infants, although LC-PUFA have been the most widely studied in relation to neurodevelopment.

**Zinc and Iron**

Zinc supplementation and neurodevelopment has been investigated in two trials of LBW term infants in low-income families in Brazil and India [28, 29]. In one trial in an impoverished community in India, 1,250 infants were randomized to receive a daily supplement of: (1) 5 mg of zinc sulfate, in a micronutrient mix of riboflavin, calcium, phosphorus, folate and iron supplement, or (2) the same micronutrient mix without zinc, or (3) 5 mg of zinc sulfate with riboflavin or (4) riboflavin only [29]. Treatment was given from 30 days of age to 9 months. 200 infants were randomly selected from groups 1 and 2 for neurodevelopmental assessment at 6 and 10 months of age. No significant difference in mental development was found at either time point (MDI intervention 83, SD 9 vs. control 82, SD 9; intervention 86, SD 5 vs. control 86, SD 5, at 6 and 10 months, respectively) [29].
In the only other trial of zinc supplementation where neurodevelopment was measured, the intent was to randomize 250 infants to receive a 5-mg oral solution of zinc compared with no supplementation form birth to 8 weeks of age [28]. There was an error in the manufacture of the solution such that it only contained 1 mg of zinc rather than 5 mg. At this stage, 134 infants had been randomized. The study continued with a further 71 infants enrolled to receive 5 mg of zinc, i.e. not randomized, to give a total sample size of 134. No significant difference in either mental or psychomotor development was found at 6 or 12 months of age between placebo, 1 mg of zinc and 5 mg of zinc supplement (12 months MDI: 1 mg zinc 107, SD 11; 5 mg zinc 107, SD 12; placebo 109, SD 12; p = 0.6) [28].

Iron supplementation has been investigated in a small trial of LBW infants born weighing <2,500 g. Fifty-eight infants were randomized to receive a high-iron-containing formula (21 mg/l) compared with formula containing 13.4 mg/l [30]. The infants’ neurodevelopment was assessed using the Griffiths Developmental Assessment at 3, 6, 9 and 12 months of age, with no significant difference found at any time point (12 months DQ higher iron 118, SD 11 vs. control 118, SD 10, n = 42).

**Long-Chain Polyunsaturated Fatty Acids**

Supplementation of infant formula with LC-PUFA for preterm infants has been the focus of two recent systematic reviews [31, 32]. Both reviews found that supplemented formula had no significant effect on DQ compared with no supplementation. At 12 months’ corrected age in a meta-analysis of 4 trials including 364 preterm infants, the WMD in BSID MDI was 0.96 (95% CI: −1.42 to 3.34) [31]. At 18 months’ corrected age, a 2.4-point improvement in DQ was found, but again this was not significant (95% CI: −0.33 to 5.12) [31].

Different versions of the BSID were used in the included trials, and because of this, Smithers et al. [32] conducted a subgroup analysis according to BSID version. The second version of the BSID included more language and problem solving items for 12- to 18-month-old children. This, along with differences in scoring and administration, may have introduced systematic differences in assessing neurodevelopment [32]. Accordingly, when trials using the same version of the BSID were considered as a separate subgroup, the cognitive DQ of LC-PUFA-supplemented infants assessed using version II of the Bayley Scales was significantly higher than control [32]. The meta-analysis included 5 trials of 879 infants and demonstrated a mean difference in MDI of 3.4 points (95% CI: 0.56–6.31).

Beyond 18 months, only one study has followed children into early childhood to determine cognitive effects of LC-PUFA supplementation in infancy [33]. This
trial of 238 infants randomized to formula supplemented with 0.5% DHA as total fatty acids versus unsupplemented, given from enrolment to 9 months of age assessed children when 10 years of age. They found no difference in IQ but did find suggestions of sex-specific and diet-specific effects, i.e. girls who received supplemented formula performed significantly better at single word reading accuracy and spelling than girls who received unsupplemented formula. In infants who did not receive any breast milk, those who were fed supplemented formula performed significantly better on a number of cognitive outcomes including IQ than infants who received unsupplemented formula [33]. However, given the very large losses to follow-up (55%), interpretation and generalization is difficult.

Most preterm formula has been supplemented with LC-PUFA since early 2000. Of more current clinical relevance are two recent trials in which DHA doses reflective of the estimated in utero accretion rate were used [34, 35]. These trials also included infants fed human milk. Both trials reported improvements in neurodevelopment. Henriksen et al. [34] studied 141 very LBW infants (<1,500 g) and demonstrated an improvement in problem solving at 6 months’ corrected age (intervention 53.4, SD 7.0 vs. control 49.5, SD 9.5; p = 0.02; n = 105). In a further follow-up at 20 months of age, they showed no difference in MDI (intervention 103, SD 10 vs. control 101, SD 13, p = 0.4; n = 92) but reported a significant improvement in sustained attention in free play activities [36]. The small sample size and large losses to follow-up make interpretation difficult.

The best evidence comes from the largest trial [35]. Although there were no significant differences in overall cognitive DQ at 18 months’ corrected age (MD 1.9; 95% CI: –1.0 to 4.7), girls had a significant 4.5-point (≈0.3 SD) improvement in cognitive DQ (95% CI: 0.5–8.5) [35]. In post hoc analyses, significant mental delay (MDI <70) was reduced from 10.5% in the control group to 5% in the higher DHA group (RR 0.50; 95% CI: 0.26–0.93). These children are currently being followed at 7 years’ corrected age to determine the effect of early LC-PUFA supplementation on cognitive outcome in early childhood. While suggestion of benefit is evident for LC-PUFA supplementation at 18 months of age, the long-term benefits of LC-PUFA supplementation in preterm children remain unclear.

**Conclusion**

LBW infants have well-documented cognitive deficits compared with their term, normal-birthweight counterparts. While it makes logical sense that at least part of these cognitive deficits may be explained by nutritional deprivation and that nutritional enrichment may improve the longer term neurodevelopmental outcomes of LBW children, few studies have been able to support this hypothesis. However,
the lack of support for the hypothesis linking nutritional supplementation and neurodevelopmental outcome is largely because the available studies were too small or had methodological shortcomings, limiting their ability to draw robust conclusions. Further large-scale rigorously designed intervention trials, with long-term neurodevelopment follow-up, are required to determine the optimal nutritional supplements and the timing of their administration to LBW infants.

Disclosure Statement

Maria Makrides serves on advisory boards for the Nestlé Nutrition Institute, Fonterra and Danone, and associated honoraria are paid to her institution to support the continuing education of early and mid-career researchers. Robert Gibson serves on advisory boards for Fonterra, and associated honoraria are paid to his institution to support the continuing education of early and mid-career researchers. For Carmel Collins and Amanda Anderson, there are no financial or other conflicts to report.

References

Brain of the fetus undergoes remarkable physical and functional development. Brain weight increases from 150 to 400 g in last trimester and it again doubles in weight in the first 6 months of life. At 6 months, it reaches approximately two thirds of adult brain weight [1]. Low birthweight includes babies born with birthweight <2,500 g. These babies may be preterm appropriate for gestational age (AGA), term small for gestational age (SGA) or preterm SGA. Intrauterine growth-restricted (IUGR) babies have a substantially higher rate of minor handicaps that include broad spectrum of cognitive and learning disorders. The latter includes deficits in attention, memory processing and verbal abilities. A 6- to 8-point intelligence quotient decrement has been documented in term IUGR babies. Preterm babies who are IUGR as compared to their AGA counterparts are at higher risk of poor neurodevelopmental outcomes [2]. Nutritional intervention in postnatal period is documented as one of the strategies to improve neurodevelopmental outcome in these babies.

Nutritional supplementation involves protein energy and micronutrients like docosahexaenoic acid (DHA), iron and zinc. Protein energy interventions based on human milk or infant formula do not show any significant improvement in neurodevelopmental outcome at 2 years of age [3, 4]. However, standard term formula and unsupplemented human milk are now regarded as nutritionally insufficient for preterm infants, and it is unethical to conduct these studies in today’s era. In some developing countries like India, the existing preterm formulas and human milk fortifiers do not supplement protein as recommended by nutritional committees [5].

Studies involving iron and zinc supplementation also showed no difference in outcome.
Long-chain polyunsaturated fatty acids (LC-PUFAs) are important for normal eye and brain development, and feeding infants with formulas deficient in the specific LC-PUFA DHA is associated with poorer visual acuity and perhaps alterations in the cognitive function in preterm infants.

Two meta-analyses evaluated outcomes at 12–18 months’ corrected age in LC-PUFA-supplemented babies [6, 7]. No significant difference in mental development index (MDI) or psychomotor development index was seen. However, when trials were assessed separately using two different versions of Bayley scale, trials involving BSID version 2 showed a 3-point significant increase in MDI, whereas trials involving BSID version 1 showed no significant difference in MDI [7].

The DINO trial evaluated supplementation of high (1% of total fat) versus normal (0.3% of total fat) DHA in babies <1,250 g. Study enrolled 657 babies, 94% of which were followed at 18 months of age. There was no significant difference in developmental quotients, but minor and major cognitive delay was less in high DHA-supplemented group.

In conclusion, looking at fetal accretion of proteins in the third trimester, it seems that the protein needs of preterm babies are much higher than those of term babies. So, it seems essential to supplement preterm babies with proteins and micronutrients like DHA. It should be kept in mind that nutritional intervention is only one of and not the only intervention to improve outcomes of these babies.

Neelam Kler
Naveen P. Gupta

References
On behalf of my co-chairs Zulfiqar Bhutta and Jatinder Bhatia, my thanks to the Nestlé Nutrition Institute and specifically to Ferdinand Haschke, Natalia Wagemans and Sanjeev Ganguly for organizing this workshop in the beautiful surroundings of Goa, for asking us to co-chair and for inviting you all to participate. Like all previous NNI workshops, this also has been a scientifically stimulating meeting with outstanding discussions by the participants. We heard state-of-the-art talks on maternal and infant nutrition particularly in the first 1,000 days of the baby’s development that can have immediate and long-term consequences. The invited speakers, who are renowned experts in their respective fields, gave excellent talks emphasizing the critical importance of nutrition and environment during the first 1,000 days between conception and the child’s second birthday in influencing the health and future development of the baby. During this critical window, recognition of nutrient insufficiencies, impaired fetal and infant’s growth and appropriate interventions can have profound impact on child’s growth and development, have long-term consequences like chronic noncommunicable diseases in adulthood, and can impact society’s health and prosperity. The following are some of the salient conclusions of the workshop.

In session 1, chaired by Zulfiqar Bhutta, we discussed the epidemiology and prevention of low birthweight (LBW). The speakers discussed the need for a careful distinction between LBW due to short gestation (prematurity) and that due to intrauterine growth retardation, recognition of the global burden of LBW and its impact on infants’ and societal health and economy, the possible methods of prevention including the specific critical periods of intervention, scaling of intervention at the societal levels and the effect of multiple micronutrient supplementation during pregnancy on LBW.

Michael Kramer started the morning with a discussion of the epidemiology of LBW. It is important to recognize that all cases of LBW are not the same and
that LBW as a result of shorter duration of gestation (premature birth) should be separated from babies born ‘too small’ for their gestational age (small for gestational age, SGA). Preterm and SGA infants have very different prognosis for survival, morbidity, and development. In addition, SGA infants are at increased risk for stillbirth and higher frequency of chronic disease of adulthood. He further discussed the global burden of LBW and showed that the incidence of SGA and prematurity is country specific and biased by the definition of LBW and confounded by temporal trends and the growth charts used. While the etiology of spontaneous preterm birth does not appear to differ amongst rich, middle- or low-income countries, the etiology of SGA differs considerably amongst countries and regions and is related to nutrition, pregnancy-related complications, smoking and other specific societal factors. He suggested that neonatal mortality is affected far more by preterm birth than by SGA birth and that prevention of LBW has proved difficult and is not necessary to reduce neonatal mortality or stillbirth.

Zulfiqar Bhutta discussed the importance of intervening in the preconception period in order to impact pregnancy outcome. He underscored that the preconception care that begins in adolescence and is provided before and between pregnancies has the potential to impact millions of women and their babies, and can ensure that the newborns receive the healthiest start possible. He defined preconception care as ‘any intervention provided to women and couples of childbearing age, regardless of pregnancy status or desire, before pregnancy, to improve health outcomes for women, newborn and children’. In order for preconception care to have an impact, it must begin in adolescence. This is important since adolescent girls are particularly vulnerable and are not equipped to face challenges to their health because of lack of appropriate education, skills and other necessary social and emotional support. Many of these girls do not receive any health care after childhood until their first pregnancy. Zulfiqar Bhutta presented the evidence that preconception intervention leads to improved pregnancy outcomes, improves health of the newborn and children and ultimately impacts the society with higher economic productivity. He then cited specific examples where simple interventions have resulted in better outcomes. These include school-based educational programs aimed at prevention of dating violence, empowerment of women, promotion of human rights, personal development programs that include skill building, provision of contraceptives, and others related to teaching parenting skills, etc. He discussed the significant impact of community programs aimed at correction of micronutrient deficiencies, identification of chronic medical conditions such as diabetes and hypertension, mental health problems and infectious diseases. In addition, particular attention needs to be given to risky behavior and use of illicit substances. The evidence
clearly points to the need to make preconception care as the important part of continuum so that the women are healthy and not suffering from nutrient deficiency before they become pregnant. Finally, he emphasized that preconception care is particularly effective when men are involved and care is provided in the community setting.

Ricardo Uauy then gave a comprehensive review of the possible intervention strategies for preventing LBW babies in developing countries, while emphasizing that multiple interactive factors need to be considered. He gave us a brief overview of the various nutritional, genetic and environmental factors that contribute to LBW. He underscored the need to separate prematurely born babies from those who had suffered growth retardation in utero, because of the differences in short-term and long-term outcomes in these infants. In addition, the proposed interventions should be cause specific and context specific. In many resource-poor countries, the establishment of timing of conception may be difficult, and even if established the predictive models are not established for these populations. He then reviewed the various contributors to the impaired fetal growth. These include amongst others, the genetic factors, and the role of placenta, maternal nutrition, chronic illnesses, intrauterine infections, gene-nutrient interactions and other environmental influences.

He also briefly reviewed the long-term consequence of impaired fetal growth and its relation to the developmental origin of health and disease hypothesis. He stressed the need to develop appropriate growth parameters that are to be used as ‘gold standard’ because they are not only important for clinical monitoring of the individual but also critical for assessment of societal and generational effects. Inadequate standards may have unintended and possibly detrimental consequence on the health of future generations. Using the wrong fetal growth charts for example results in increased maternal antenatal hospitalization, unjustified neonatal intensive care, unnecessary interventions such as cesarean section, etc. which could impose risk on mother as well as the baby. The recently developed and soon to be reported INTERGROWTH prescriptive standards (charts) will serve to objectively describe normal fetal growth including whole body, brain, liver, and long bones. Developed in a contemporary multi-ethnic sample, these fetal growth standards will be an important contribution for the evaluation of the effect of early life events on later growth, health and well-being.

Usha Ramakrishnan presented the results of a meta-analysis of randomized controlled trials on the effects of prenatal multiple micronutrient supplements (≥5 micronutrients) on infants’ birthweight. The analysis showed that prenatal multiple micronutrient supplementations significantly reduced the incidence of LBW and SGA when compared with iron-folate supplement alone. The effect on birthweight was variable, cumulative data showed a mean increase of 55 g in the
multiple micronutrient-supplemented groups. Although the effect size on birth-weight was small and may not be significant for the individual baby, it may be significant for the population and for making policy recommendations. They also observed a higher incidence of neonatal death among those who began intervention after the first trimester, thus raising caution for supplementation in settings where prenatal care of the pregnant women is not started early.

The last speaker, Caroline Fall, discussed the long-term consequence of fetal malnutrition. She pointed out that the human data are mostly observational epidemiological data of associations and do not provide mechanistic insights. However, these studies have shown that lower birthweight is associated with a wide range of adverse outcomes in later life such as poor ‘human capital’ (short stature, lower cognition), increased risk for type 2 diabetes, hypertension, etc. Higher birthweight such as associated with maternal diabetes is also associated with increased risk of obesity, diabetes and cancer. Animal models have been helpful in identifying the possible mechanism of fetal metabolic programming at the molecular level. The intervention studies in humans have only shown a weak evidence of any beneficial effects in prevention of the metabolic programming and prevention of long-term consequences.

The excellent presentations by the outstanding speakers underscored the importance of rigid definition of LBW in relation to gestation, that LBW is the consequence of a number of contributors, that these contributors are different in different societies and therefore the interventions should be context specific, and for the interventions to be successful they will have to start early, even prior to pregnancy and may need to consider the nutrition of the fathers as much as that of the mother at the time of conception.

Session 2, co-chaired by Berthold Koletzko and Satish Kalhan, was devoted to epigenetic factors before and during pregnancy and the long-term consequences for the baby. To place it in context, we discussed the endocrine and nutritional regulation of fetal growth, and how stress and perturbations in one-carbon metabolism may result in altered fetal growth, metabolic reprogramming and long-term consequences.

Abigail Fowden started the morning with an excellent discussion of the endocrine interactions in the control of fetal growth. She underscored the role of hormones as environmental and maturational signals in the regulation of tissue accretion and differentiation during late gestation. By their interaction, the growth-stimulatory and growth inhibitory hormones regulate the fetal-placental transcriptome to ensure that the fetal growth is matched to the nutrient supply. She pointed out that by producing an epigenome specific to the intrauterine environment, hormones can modify fetal growth, and lead to phenotypical consequences long after birth.
Frank Bloomfield in his review of nutritional regulation of fetal growth pointed out that during most of the first trimester, fetal nutrition is histiotrophic from the uterine glands; homeotrophic nutrition is established only after placentation, and the delivery of nutrients to the fetus is controlled by uterine blood flow, placental function, umbilical blood flow and the fetal hormonal milieu. He suggested that this may explain why nutritional supplementation after the first trimester has not been too successful. He reviewed the various factors during the time of conception including nutrition that have been shown to impact fetal growth, length of gestation, and fetal development trajectory and long-term consequences for the offspring. Some of these effects appear to be mediated via epigenetic changes (both DNA methylation and histone modifications) in specific organs and tissues in the developing fetus and persist into postnatal and adult life and are associated with increased fat mass and may predispose to lifelong risk of metabolic syndrome and obesity.

Pathik Wadhwa presented a detailed analysis of how stress-related biological factors by influencing hypothalamic-pituitary-adrenal axis and inflammation may influence nutrition at several levels including energy intake, selection of food types, and metabolic fate of the energy. All these may exert direct effects on fetal targets of programming of body composition and metabolic function. He proposed the concept that maternal-placental-fetal endocrine and immune/inflammatory stress biology represents a candidate mechanism that may underlie the long-term effects of adverse intrauterine exposure on subsequent risk of child and adult obesity and metabolic dysfunction.

Satish Kalhan summarized the available data on one-carbon and methionine metabolism during pregnancy, fetus and neonate. Studies in human pregnancy show unique adaptive changes in methionine metabolism; increased transsulfuration early in gestation and higher transmethylation late in pregnancy. These are accompanied by a lower concentration of plasma homocysteine and changes in circulating levels of folate, B\textsubscript{12} and other methyl donors in maternal plasma. He highlighted the unique characteristics of the fetal one-carbon metabolism. A number of physiological and environmental factors can influence one-carbon metabolism and can have profound effects on cellular metabolism, cell proliferation and growth by influencing the cellular methylation status and thereby causing epigenetic modifications. The impact of nutrient insufficiencies specifically chronic protein and micronutrient insufficiency or that of metabolic and endocrine dysregulation due to chronic disease, etc. on one-carbon metabolism in the mother and its consequence for human newborn have not been carefully examined. Future studies using contemporary (omics) technologies along with physiological and epidemiological cohort studies will address these critical questions and help develop intervention strategies in vulnerable periods during development.
**Chittaranjan Yajnik** discussed the relation between alterations in maternal one-carbon metabolism and birthweight and long-term consequences. Data from the Pune Maternal Nutrition study showed that higher plasma homocysteine concentration during pregnancy was associated with smaller size of the neonate attributed to both growth restriction and short gestation. In addition, there was a strong association between maternal B12 insufficiency, higher methylmalonic acid concentration and insulin resistance at age 6 years. Recent observational data also showed that maternal vitamin B12 insufficiency was also associated with increased risk of gestational diabetes and neural tube defects. All these data underscore the role of perturbations in one-carbon metabolism of the mother in the development of immediate and long-term consequences for both the mother and the offspring.

This extremely exciting and scientifically stimulating session carried the audience through the potential nutritional and environmental contributors to the alteration in the fetal growth and a discussion of the possible mechanism involved. The speakers identified the critical periods during development in the first 1,000 days, from conception to infancy, when nutritional, endocrine and environmental influences could impact the growing baby and emphasized that the mechanisms are likely to be different at different stages of development. Recent evidence suggests that the nutritional and environmental influences act through modification of the transcriptome, cause epigenetic changes that persist through adult life and predispose the offspring to chronic noncommunicable diseases such as hypertension, obesity and metabolic syndrome. Additionally, it is not only the mother’s nutrition and health that can impact the embryo; recent data from studies in animals suggest that the father’s diet at the time of conception can also have long-lasting effects on the developing embryo [1].

Session 3 was entitled Clinical Outcome of LBW, Long-Term Consequences. It was chaired by **Jatinder Bhatia**. The speakers gave excellent talks on the immediate (neonatal) metabolic consequences of intrauterine growth restriction and LBW, intervention strategies to promote appropriate growth during the neonatal period, iron and micronutrient deficiencies at lower birthweight and strategies to improve long-term neurocognitive outcomes of LBW infants.

**Jatinder Bhatia** presented a broad overview of the neonatal consequences of intrauterine growth restriction and LBW. He again underscored the importance of separating LBW due to prematurity from that due to intrauterine growth restriction because of differences in neonatal and long-term morbidity. He discussed the need for identification of growth restriction during pregnancy and possible interventions. Following birth, the LBW babies have special needs for monitoring their metabolism, glucose, calcium, oxygenation, temperature regulation and nutritional care. He emphasized the need for both careful antepartum and neonatal care of these babies for appropriate growth and prevention of morbidities.
Johannes van Goudoever presented a number of elegant studies from his group on protein and amino acid homeostasis in the preterm infant. As he stated, observational data show that inadequate nutrition, in particular the quality and quantity of protein, in the first few days after birth has adverse effects on neurocognitive function, cardiovascular health, and on the metabolic status, and that undernutrition, with subsequent growth failure, occurs predominantly in the first few weeks after birth. Towards this end, his group has examined the effects of early administration of higher amounts of amino acids (protein) on protein metabolism, nitrogen balance, and growth of the LBW infants. The studies were done using a combination of clinical evaluation with state-of-the-art sophisticated tracer methodologies. Their data showed that higher amino acid intake was associated with improved nitrogen balance, albumin synthesis, whole-body protein synthesis, and did not have any adverse effects. A second trial of early nutrient supplement of small babies showed most anabolic effects with higher protein and energy intake. These are exciting data which may need to be supplemented with studies from other centers, and require detailed and careful follow-up through infancy and adolescence.

Ekhard Ziegler presented an intervention strategy to promote appropriate growth and prevent postnatal growth failure. He introduced us to a novel definition of appropriate growth as that ‘not associated with adverse consequences in the short and long term’. He reinforced the need for appropriate protein and energy administration soon after birth by parenteral nutrition, need for priming the gut in order to stimulate the intestinal tract towards a functionally mature state, and introduction of enteral feed with human milk as soon as possible, and the requirement for the fortification of human milk with nutrients in order to meet the protein requirement of the LBW infant. He then shared with us the current Iowa nutrition practices. The key elements include administration of proteins at 3.0 g/kg per day, glucose at 4 mg/kg per min within 2 h of birth and progressing to a complete neonatal parenteral nutrition solution within 24–36 h. Gut priming with human milk is initiated on the first day or the following day. The goal is a weight gain of 15–20 g/kg per day. He then showed the impressive data on growth following the implementation of the protocol. The results showed that infants under this protocol appear to parallel the fetal weight gain, although there was some fall-off from fetal growth. Other institutions will have to examine whether such impressive results can be accomplished elsewhere and identify the possible impediments to achieving the goal.

Magnus Domellöf discussed iron and other micronutrient deficiencies in LBW infants. Very LBW infants are at high risk for multiple micro- and macronutrient deficiencies. Iron deficiency represents the most common nutrient problem in the LBW infants and has been associated with impaired brain devel-
development, and later behavioral and cognitive problems. He underscored the need to practice delayed clamping of umbilical cord in order to increase the infant’s iron stores. He then discussed the various dose regimens for iron that can prevent anemia without causing adverse effects while cautioning against excessive iron supplement.

Maria Makrides discussed the nutritional strategies to improve the neurodevelopmental outcomes of LBW babies. She postulated that cognitive deficit seen in LBW infants may in part be explained by nutritional deficits at critical periods of development and that nutritional enrichment may improve the long-term neurodevelopmental outcome of these babies. However, few studies have been able to support this hypothesis, largely due to methodological shortcomings and small sample size. Larger rigorously designed studies are required to address this question.

The session ended with an interesting discussion of the clinical implementation of the knowledge gained in both the neonatal units and the society at large and in the development of policy. In addition, all the speakers emphasized the need for the assessment of neurodevelopment and cognition as an important outcome measure.

It was a wonderful, stimulating and participatory workshop in beautiful surroundings where a small group of clinical, developmental, epidemiological and other scientists were brought together to discuss a critical period of development, the first 1,000 days, that has important implications for not only the individual but for the society at large.

My sincere thanks to all the participants, speakers, my co-chairs, the organizers and the Nestlé Nutrition Institute.

Satish C. Kalhan

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