

Micronutrients and Fetal Growth^{1,2}

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ABSTRACT Fetal undernutrition affects large numbers of infants in developing countries, with adverse consequences for their immediate survival and lifelong health. It manifests as intrauterine growth retardation (IUGR), defined as birth weight <10th percentile, which probably underestimates the number failing to achieve full growth potential. Birth weight is a crude measure of the dynamic process of fetal growth and does not capture effects of fetal undernutrition on body composition and the development of specific tissues. The link between maternal nutrition and fetal nutrition is indirect. The fetus is nourished by a complex supply line that includes the mother's diet and absorption, endocrine status and metabolism, cardiovascular adaptations to pregnancy and placental function. Micronutrients are essential for growth, and maternal micronutrient deficiency, frequently multiple in developing countries, may be an important cause of IUGR. Supplementation of undernourished mothers with micronutrients has several benefits but there is little hard evidence of improved fetal growth. However, this has been inadequately tested. Most trials have only used single micronutrients and many were inconclusive because of methodological problems. Several food-based studies (some uncontrolled) suggest benefits from improving maternal dietary quality with micronutrient-dense foods. One trial of a multivitamin supplement (HIV-positive mothers, Tanzania) showed increased birth weight and fewer fetal deaths. Well-conducted randomized controlled trials of adequate sample size and including measures of effectiveness are needed in populations at high risk of micronutrient deficiency and IUGR and should include food-based interventions and better measurements of fetal growth, maternal metabolism, and long-term outcomes in the offspring. *J. Nutr.* 133: 1747S–1756S, 2003.

KEY WORDS: • *maternal nutrition* • *micronutrients* • *fetal growth* • *fetal death* • *review*

Low birth weight affects large numbers of infants in developing countries (1). Premature delivery makes a major contribution (2) but unlike the situation in developed countries, intrauterine growth retardation (IUGR)⁴ is the predominant cause. Prematurity is frequently complicated by IUGR (2). The incidence of IUGR, defined as a birth weight below the 10th percentile for gestational age based

on a standard population (1), is as high as 40% in some developing countries, compared with less than 10% in most developed countries. The highest rates are in south Asia and parts of sub-Saharan Africa (**Fig. 1**). Good population-based data are not available for many developing countries because of frequent home births where newborns are not routinely weighed.

IUGR carries both short- and long-term disadvantages for the infant. The disadvantages show continuous trends into the normal range of birth weight, suggesting that the division of newborns into two groups, small or appropriate based on a fixed cutoff point, may underestimate the occurrence of fetal growth retardation. Fetal growth is suboptimal at, for example, a birth weight of 3 kg if that infant had the potential to achieve 3.5 kg.

Short-term consequences of IUGR include an increased risk of fetal, neonatal and infant death and impaired postnatal growth, immune function and intellectual development (3). The exponential rise in relative risk for neonatal mortality at birth weights below 2.5–3.0 kg is similar in all populations although absolute death rates are considerably higher in developing countries (4).

Long-term consequences include an increased risk of adult chronic disease (cardiovascular disease and type 2 diabetes) (5,6). This increased risk has been attributed to permanent

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⁴ Abbreviations used: BMI, body mass index; CI, confidence interval; GLV, green leafy vegetable; IUGR, intrauterine growth retardation; OR, odds ratio; RR, relative risk; WIC, Women, Infants and Children.

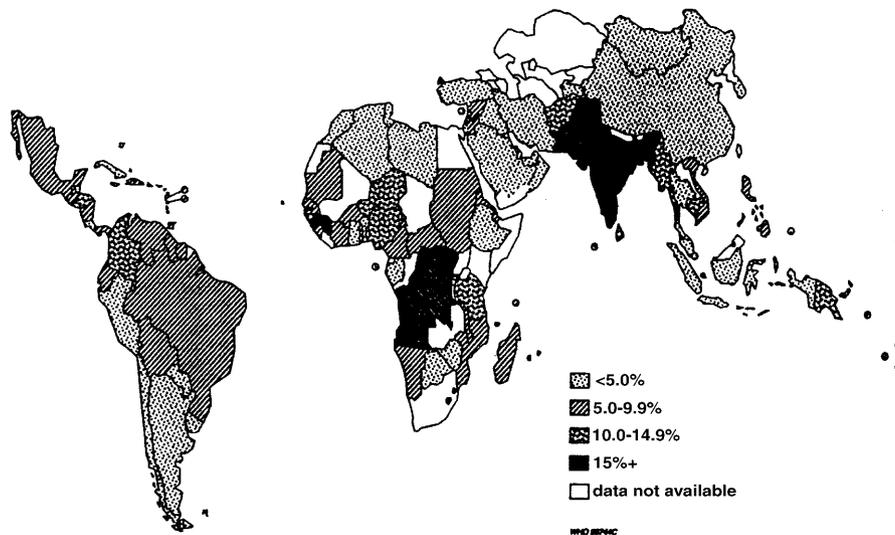


FIGURE 1 Prevalence of intrauterine growth retardation in developing countries (birth weight <10th percentile for gestational age, World Health Organization criteria). Used with permission from reference 1.

changes in structure and metabolism resulting from undernutrition during critical periods of early development (the fetal origins of adult disease hypothesis) (5). An inadequate supply of nutrients forces the fetus to adapt, down-regulate growth and prioritize the development of essential tissues. Adaptations include preferential blood flow to the brain and reduced flow to the abdominal viscera, altered body composition (reduced muscle mass) and reduced secretion of and sensitivity to the fetal growth hormones (insulin-like growth hormone and insulin). These adaptations enhance immediate survival but may carry a long-term price. An association between low birth weight and later insulin resistance, a strong risk factor for both cardiovascular disease and type 2 diabetes, is a consistent finding in a number of populations (7). We have shown the same association in both adults and children in India (8,9) (Fig. 2) and there are similar findings from China (10) and Jamaica (11). Low birth weight has also been linked to higher blood pressure in children (9,12) and coronary heart disease in adults (13) in developing countries. The combination of low birth weight followed by obesity in later life appears to carry the greatest risk of insulin resistance (Fig. 2). The persisting high incidence of IUGR, along with a worldwide increase in obesity, may therefore contribute to the epidemic rise of cardiovascular disease and type 2 diabetes in developing countries (14,15).

IUGR also has adverse consequences for future generations. It forms part of an intergenerational vicious cycle of deprivation (16). For example the poor postnatal growth of low-birth-weight girls increases their own risk of producing low-birth-weight infants.

Limitations of birth weight alone as a measure of fetal growth

Birth weight is sometimes the only feasible measurement of fetal growth but it has limitations. Growth is a dynamic process and similar birth weights may be achieved by different fetal growth trajectories that result in different body composition and organ development (17) (Fig. 3). Rapid early growth, occurring because the embryo senses adequate nutrition or because of genetic drive, may increase nutritional requirements beyond the capacity for supply later in pregnancy (18). In

experimental animals, nutritional impairment disrupts the growth of fast-growing more than slow-growing fetuses (19).

We have shown that infants born in India, lighter and thinner than United Kingdom infants, also have a different pattern of body composition (20,21). Neonatal muscle mass and abdominal circumferences are reduced but head circumference and truncal fat are substantially spared. This may reflect inadequate nutrition at critical times in gestation for the growth of muscle and abdominal viscera. Fat-sparing may occur because it confers a survival advantage in the neonatal period or because of metabolic incompetence and inability to lay down other tissues because of inadequate substrate. The long-term consequences of this neonatal phenotype are unknown, but excess fat may persist postnatally and lead to increased insulin resistance whereas a low muscle mass and impaired development of abdominal viscera (liver, pancreas, kidneys) could have a number of adverse metabolic effects (20).

Accurate measurement of gestational age is essential for assessment of fetal growth. Reliable dates for last menstrual periods are difficult to obtain and dating based on ultrasound scans may also be misleading. Fetuses that have grown poorly from early gestation may not be identified as such but may be re-dated to an earlier gestation, underestimating the degree of growth retardation (22).

Although the main focus of this paper is fetal undernutrition and low birth weight, recent data show that problems occur at both extremes of the birth weight distribution (23). Maternal diabetes, which causes fetal overnutrition (macrosomia) and well-described perinatal complications, is associated with an increased risk of adult obesity and type 2 diabetes (24–26). As obesity becomes more common in all populations, gestational diabetes is likely to increase (27). A recent study showed a high incidence (6%) in mothers in urban south India (21). Because of the low overall birth weight in this population (2.7 kg), the macrosomic infants of diabetic mothers—who had markedly increased body fat and skeletal measurements compared with infants of nondiabetic women—had an apparently healthy mean birth weight of 3.3 kg.

Maternal nutrition and fetal growth

Fetal growth depends on the uptake of nutrients, which occurs at the end of a complex maternal supply line (17) that

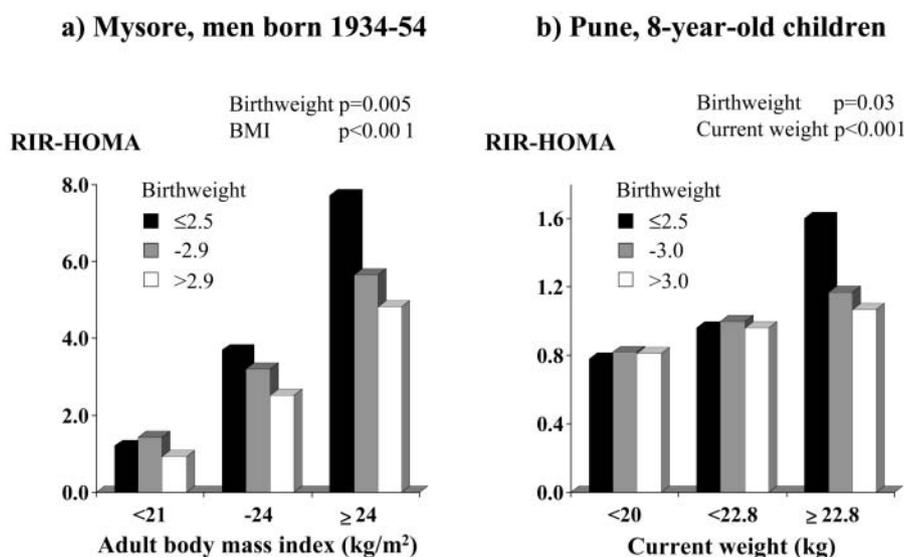


FIGURE 2 Relative insulin resistance–homeostasis model assessment (RIR-HOMA) in a) men born in Holdsworth Memorial Hospital, Mysore, South India 1934–54 ($n = 266$) and b) 8-y-old children, Pune, India ($n = 477$). BMI, body mass index. Data in panel a are from reference 8. Data in panel b are from reference 9.

begins with the mother's intake (appetite, diet, absorption). Nutrients arriving at the placenta depend on the mother's intermediary metabolism and endocrine status; her partitioning of nutrients among storage, use and circulation; the capacity of circulating transport proteins; and cardiovascular adaptations to pregnancy, such as plasma volume expansion, which determine uterine blood flow. These are influenced by her nutritional status and infection load in ways that are poorly understood. Nutritional factors are also likely to influence placental function, including vascular structure; the efficiency of placental transport systems; and the partitioning of nutrients among mother, placenta and fetus. Thus the link between maternal nutrition and fetal nutrition is indirect; they are not the same.

IUGR can be produced in experimental animals by reducing maternal intakes of energy and protein (17). Maternal energy and protein deficiency are also clearly associated with IUGR in humans (28,29). Like other forms of undernutrition, IUGR should be preventable. However, attempts to increase birth weight by giving mothers high-density protein supplements have consistently reduced fetal growth (30). Balanced energy and protein supplements have led to increased birth weight but effects have been disappointingly small (28) with the notable

exception of a trial in undernourished women in The Gambia (31). Recent interest has turned to micronutrients as possible limiting factors for fetal growth. Some micronutrients are structural components of body tissues. Others are essential for the processes of growth, including energy and protein metabolism, gene transcription, endocrine function and nutrient transport (32).

The Pune Maternal Nutrition Study, India

During 1994–1997 we carried out a prospective observational study of maternal diet and neonatal outcome among women living in rural villages near Pune, Maharashtra, India: the Pune Maternal Nutrition Study (20,33–36). Data from India's National Nutrition Monitoring Bureau and National Institute of Nutrition show that poor rural and urban women have low intakes of a range of vitamins and minerals and of micronutrient-dense foods such as green leafy vegetables (GLVs), fruits and dairy products (37–39). The study was therefore designed to measure consumption of these foods as well as macronutrient intakes.

Prepregnancy anthropometry and dates for serial last menstrual periods were recorded for all 2466 women of reproductive age living in 6 villages. For the 797 who became pregnant during the 3-y study, dietary intakes and physical activity were recorded at 18 and 28 wk gestation using methods developed specifically for this community (33,36). Consumption of micronutrient-dense foods (GLVs, fruit, meat, fish and dairy products) were recorded by food frequency questionnaire and energy, protein, fat, and carbohydrate intakes were recorded by using semiweighed 24-h recall. Erythrocyte folate and plasma vitamin C status were also measured.

The mothers were short and thin (mean height and body mass index [BMI; expressed as kg/m^2): 152 cm and 18.1). Their main staple was millet *roti* (bread) eaten with *dal* (pulses) and vegetables, and there was a marked lack of day-to-day variety in the diet. Energy and protein intakes were low and did not increase during pregnancy (7.4 MJ and 45 g/d at 18 wk gestation). Intakes of nonvegetarian foods were low; 32% reported never eating meat, fish or eggs and 40% of those reporting some intake ate them less than once per week. There were large variations among mothers in intakes of GLVs, fruit

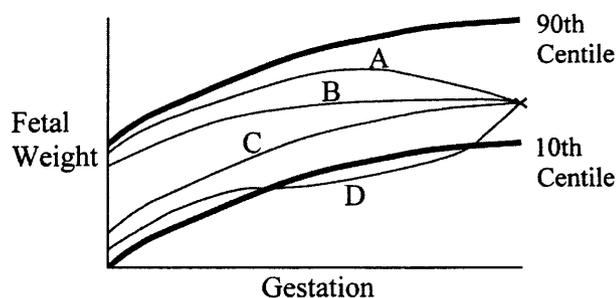


FIGURE 3 Fetal growth trajectories. A fetus can reach a given birth weight via a variety of possible different growth trajectories. These fetuses may have a different body composition, and later disease risk. A: Late growth restriction and fetal wasting; B: early growth restriction; C: normal growth; D: late growth restriction followed by catch-up growth. Used with permission from reference 17.

and milk but many had low intakes (Table 1). The most commonly eaten GLVs were fenugreek leaves, spinach, coriander and colocasia, usually as cooked preparations.

The mean full-term birth weight was 2.7 kg. Birth weight and other birth measurements were not associated with maternal energy, protein or carbohydrate intakes. Higher fat intakes at 18 wk were associated with longer neonatal length and larger skinfold thickness. The most striking finding was that mothers with higher intakes of milk at 18 wk and of GLVs and fruits at 28 wk had larger infants (Table 1). The trends were particularly strong for GLVs; women eating GLVs at least every alternate day had infants almost 200 g heavier than those who never ate them, and their odds ratio (OR) for low birth weight was 0.43 (95% confidence interval [CI]: -0.12 to 0.99). Higher GLV intakes at 28 wk were also associated with higher maternal fasting plasma insulin and triglyceride concentrations and lower albumin concentrations, all of which were related to increased neonatal size. The trends for all birth measurements (with GLVs) and head circumference and placental weight (with fruit and milk) remained significant after adjustment for confounding factors that were carefully measured in this study, including maternal prepregnancy weight, weight gain during pregnancy, socioeconomic status and energy intakes. The strength of the associations with GLV and fruit intakes was increased (approximately doubled) in women of low BMI (33). Neonatal measurements were also related to maternal folate and vitamin C status (Table 2) independent of food intakes. For example, although folate status was correlated with GLV intakes, the folate and GLV effects on neonatal measurements remained after adjustment of each for the other. According to the National Nutritional Anemia Control Program, women were given 100 tablets of iron (60 mg) and folic acid (500 µg) at 18 wk gestation. It was not possible to measure compliance, and

thus to control in the analysis for iron and folate intakes from supplements. However, we formed a strong impression that compliance was low. Detailed measurements were made of these infants at birth, and we have already described their body composition (above). As shown in Table 1, all measurements were larger, including skeletal measurements, muscle, abdominal girth and fat, in infants of mothers with higher GLV intakes. Maternal blood folate and vitamin C concentrations showed no significant effect on neonatal body fat (Table 2).

The study also showed effects of maternal workload on neonatal size (36). Higher total physical activity scores were associated with lower maternal weight gain, birth weight and neonatal head and mid-upper-arm circumferences. Again these trends were independent of major confounding factors and stronger in low-BMI women.

As with all observational studies, causal effects cannot be inferred, but one interpretation of the Pune Maternal Nutrition Study findings is that in this population low intakes of the micronutrients supplied by GLVs, fruit and milk limit fetal growth, especially in energy-deficient mothers.

Review of published evidence: micronutrients and fetal growth and survival

To avoid overlap with Villar's birthweight meta-analysis in this supplement (32), the following review focuses on data from studies in developing countries, includes measurements other than birth weight and effects within subgroups of mothers (e.g., those with a low BMI). We also describe effects on fetal loss (miscarriages, spontaneous abortions and stillbirths) where data were available. The literature linking maternal micronutrient intakes or status with fetal growth is dominated by studies of single micronutrients, and the evidence relating to each

TABLE 1

Pune Maternal Nutrition Study: Relation between frequency of maternal intake of green leafy vegetables and fruits at 28 wk gestation and milk at 18 wk gestation, and neonatal anthropometry

Food group	Frequency	No.	Neonatal measurements								
			Birthwt (g)	Length (cm)	Head circ (cm)	MUAC (cm)	Abdo circ (cm)	Triceps skinfold (mm)	Subscap skinfold (mm)	Placental weight (g)	
Green leafy vegetables at 28 wk	Never	60	2571	47.0	32.6	9.6	28.2	3.9	3.9	347	
	<1/wk	175	2601	47.5	32.9	9.6	28.2	4.0	4.1	354	
	1+/wk	225	2675	48.0	33.2	9.7	28.6	4.1	4.0	358	
	≥Alternate days	149	2742	47.9	33.3	9.9	29.1	4.4	4.3	371	
			p*	<0.001	<0.01	<0.001	<0.05	<0.001	<0.001	<0.05	<0.05
Fruits at 28 wk	<1/wk	44	2598	47.5	32.7	9.7	28.6	4.1	4.2	352	
	1+/wk	363	2633	47.5	32.9	9.6	28.5	4.1	4.1	353	
	≥Once/d	202	2721	48.1	33.4	9.8	28.8	4.1	4.2	370	
			p*	<0.01	<0.01	<0.001	0.09	0.2	0.4	0.7	<0.05
			p#	0.1	0.2	<0.01	0.8	0.5	0.4	0.99	0.07
Milk products at 18 wk	Never	95	2643	47.5	32.9	9.6	28.5	4.2	4.1	354	
	<1/wk	134	2618	47.6	33.0	9.7	28.6	4.1	4.1	348	
	1+/wk	116	2639	47.6	33.0	9.5	28.5	4.1	4.1	352	
	≥Alternate days	281	2704	48.0	33.2	9.8	28.8	4.1	4.1	371	
			p*	<0.05	<0.05	<0.01	<0.05	0.2	0.9	0.4	<0.01
		p#	0.1	0.1	<0.01	0.1	0.5	0.5	0.5	<0.01	

p* adjusted for sex, parity and gestational age at delivery; p# adjusted additionally for prepregnancy weight, weight gain, energy intake, physical activity, socioeconomic status and relevant macro- and micronutrients (for green leafy vegetables, erythrocyte folate concentration; for fruits, serum vitamin C concentration; for milk products, fat intake).

Birthwt, birth weight; Head circ, head circumference; MUAC, mid-upper-arm circumference; Abdo circ, abdominal circumference; Subscap skinfold, subscapular skinfold.

Data are from reference 33.

TABLE 2

Pune Maternal Nutrition Study: relation between maternal erythrocyte folate and serum vitamin C concentrations at 28 wk, and neonatal anthropometry

Blood nutrient	Concentration	No.	Neonatal measurements							
			Birthwt (g)	Length (cm)	Head circ (cm)	MUAC (cm)	Abdo circ (cm)	Triceps skinfold (mm)	Subscap skinfold (mm)	Placental weight (g)
Erythrocyte folate (nmol/L)	<816	171	2616	47.5	33.0	9.5	28.4	4.1	4.2	347
	–1147	171	2637	47.7	32.9	9.7	28.6	4.1	4.1	356
	≥1147	172	2727	48.1	33.3	9.8	28.9	4.2	4.1	374
		p*	<0.001	<0.05	<0.05	<0.005	<0.005	0.2	0.6	<0.001
		p#	<0.005	0.08	0.1	<0.01	<0.01	0.3	0.8	<0.001
Serum vitamin C (μmol/L)	<4	254	2647	47.6	33.0	9.7	28.5	4.1	4.1	356
	–19	169	2679	47.8	33.1	9.6	28.7	4.2	4.1	360
	≥19	146	2688	47.9	33.1	9.8	29.0	4.2	4.2	367
		p*	0.2	<0.05	0.7	<0.01	<0.01	0.07	0.6	0.9
		p#	0.3	0.07	0.7	<0.05	<0.05	0.2	0.8	0.8

p* adjusted for sex, parity and gestational age at delivery; p# adjusted additionally for prepregnancy weight, weight gain, energy intake, physical activity and socioeconomic status.

Birthwt, birth weight; Head circ, head circumference; MUAC, mid upper arm circumference; Abdo circ, abdominal circumference; Subscap skinfold, subscapular skinfold.

Data are from reference 33.

vitamin or mineral is reviewed. However, the circumstances that lead to undernutrition are frequently associated with multiple micronutrient deficiencies (40). Thus, for developing countries, the reductionist approach seems illogical. There is no such thing as a key micronutrient, and a single micronutrient supplement would be expected to produce an effect only if it were the sole nutrient limiting fetal growth. We therefore conclude with a review of current evidence for multiple micronutrient supplementation with either pharmaceutical preparations or micronutrient-dense foods.

Single-micronutrient studies

Observational studies, recently reviewed by Ramakrishnan et al. (41), show for almost every vitamin and mineral, associations between poor maternal micronutrient status and IUGR or other adverse pregnancy outcomes. Confounding factors (often not measured) limit the usefulness of these studies for determining causal effects. Available trial evidence is often unsatisfactory. Ramakrishnan et al. (41) calculated the sample size needed to detect a difference in birth weight between control and intervention groups. Realistic effects on birth weight (≈ 150 g) are unlikely to be detected with sample sizes under about 200 per group. Most trials have been too small.

Other frequent problems are inadequate randomization and allocation concealment, lack of data on the equivalence of subjects in intervention and control groups, uncertain compliance or use of non-compliers as controls, no placebo in the control group (leading to biases in compliance and response to treatment), inadequate gestational age assessment, lack of data on changes in maternal micronutrient status, large losses to follow up, nonblinded measurement of outcomes and failure to carry out statistical analysis by intention to treat. Trials are expensive and require high levels of training. These resources are scarce, and poor quality trials are most common, in developing countries.

An important public health question is whether benefits result from intervening pre- or periconceptionally. Most trials started after the confirmation of pregnancy, usually from midgestation onward.

Fat-soluble vitamins. *Vitamin A.* Two large randomized trials of vitamin A in Nepal (42) and Indonesia (43) have not reported birth weight as an outcome. The Nepal study (17,373 pregnancies) was a rare example of preconceptional intervention. It reported a significant fall in maternal mortality but no effect on fetal loss, defined as the sum of miscarriages, stillbirths and losses due to maternal death (92.0, 97.5 and 95.0 per 1000 pregnancies in placebo, vitamin A and β -carotene supplement groups, respectively). Trials among HIV-positive mothers in Tanzania (44) and South Africa (45) showed no effect of vitamin A alone on fetal growth or fetal loss.

Vitamin D. Data are insufficient for evaluating the effects of vitamin D on birth weight or fetal loss. Of several published trials, all were small, and only two, giving conflicting results, were of adequate quality to be included in the Cochrane review of vitamin D in pregnancy (46). One, in white mothers in France, showed no difference in birth weight between infants born to mothers randomly assigned to receive 1000 IU vitamin D daily (3370 g; $n = 21$), 200,000 IU vitamin D monthly (3210 g; $n = 27$) or placebo (3460 g; $n = 29$). The second, in Asian mothers in the United Kingdom, more likely to be vitamin D deficient, showed a higher mean birth weight in the vitamin D group (1000 IU daily; $n = 59$, 3157 g, IUGR 15%) than in the placebo group ($n = 67$, 3034 g, IUGR 29%). These differences were not statistically significant and length, head circumference or skinfold thickness did not increase. The intervention group had fewer cases of neonatal hypocalcemia.

Vitamin E. The one trial of vitamin E supplementation in pregnancy focused on hypertensive disorders (47). UK mothers ($n = 283$) with abnormal uterine artery Doppler waveforms or a previous history of preeclampsia were randomly assigned to receive vitamins C and E or a placebo. The supplement was associated with a significant decrease in the incidence of preeclampsia (8% versus 17%, $p = 0.02$). The intervention group had fewer small-for-gestational-age infants (23% versus 32%; $p = 0.12$) but showed no effect on mean birth weight (supplement 3100 g versus placebo 3160 g). Both groups had similar but small numbers of intrauterine deaths.

Water-soluble vitamins. *Thiamin (vitamin B-1), riboflavin (vitamin B-2) and niacin (vitamin B-3).* These are essential cofactors for energy metabolism. Deficiency in pregnancy might

be expected to result in marked metabolic effects in the mother and impaired fetal growth. Specific active transport systems across the placenta for thiamin and riboflavin maintain concentration gradients in favor of the fetus (48,49). We identified no trials that studied these vitamins in pregnancy. Observational studies show widely differing findings and there are few data from developing countries despite evidence that deficiency in pregnancy is common (50,51). Better riboflavin status was associated with higher birth weight in a study in Kenya (52).

Vitamin B-6 (pyridoxine). Most observational studies have found no correlation with birth weight (41). Taiwanese mothers supplemented with 2 mg/d had larger birth weight infants (53).

Vitamin B-12. Because vitamin B-12 is derived mainly from animal sources, deficiency is more common in vegetarians (54). A recent study reported metabolic evidence of vitamin B-12 deficiency in 75% of young urban Indian men and women, both vegetarians and nonvegetarians (55). We identified no trials of vitamin B-12 in pregnancy.

Folic acid. There is a large body of literature, mainly from developed countries, reporting observational studies and randomized controlled trials of folic acid in pregnancy. These have been reviewed by Ramakrishnan et al. (41), de Onis et al. (56) and Mahomed (57). Some observational studies have shown positive associations between maternal folate status and birth weight but the evidence is inconsistent (33,41). Twenty-one trials of folate supplementation were included in the Cochrane review (57), which concluded that despite a significant reduction in maternal anemia, there was only a small and nonsignificant effect on the incidence of low birth weight (relative risk [RR] 0.73; 95% CI: 0.47–1.13). de Onis et al. (56) included five folate trials in their review of nutritional interventions to prevent IUGR; they found a significant reduction in low birth weight but commented on the poor quality of much of the data. Although better-quality trials, all in developed countries, have shown no effect on birth weight, two from developing countries [India (58) and South Africa (59)] showed large increases.

Folic acid was used in trials of periconceptional supplementation to prevent neural tube defects, mostly in developed countries (60). Supplements were stopped at the end of the first trimester. The Cochrane review (60) concludes that there was no effect on rates of miscarriage (RR 1.12; 95% CI: 0.98–1.29) or stillbirth (RR 0.78; 95% CI: 0.34–1.78). There was some evidence that folate supplements increase the risk of multiple births (RR 1.40; 95% CI: 0.93–2.11), which although not statistically significant was a consistent finding in three studies. These trials do not report birth weight as an outcome. A trial of preconceptional and first-trimester folic acid and multivitamin supplementation carried out by the Indian Council of Medical Research showed no effect on abortions or stillbirths and a nonsignificant effect on low birth weight (folic acid 12.5% versus placebo 15.6%) (61).

Vitamin C. Several observational studies showed positive correlations between maternal vitamin C status and birth weight (33,62,63). Only one trial was identified, in which U.K. women were supplemented with vitamins E and C (see under vitamin E above) (47).

Minerals. Iron. Several large studies in developed countries have shown a U-shaped relationship between maternal hemoglobin concentration and birth weight, with higher birth weights at both ends of the distribution; the highest birth weights were at levels of hemoglobin below the cutoff for anemia (64,65). This may reflect plasma volume expansion as a favorable maternal adaptation to increase uteroplacental

perfusion. However, evidence from developing countries, where iron deficiency anemia is common, shows that maternal iron deficiency is associated with low birth weight and poor obstetric outcome (64,65).

Trials of iron (66) or iron plus folate (67) supplementation have been summarized in Cochrane reviews. Although iron reduces maternal anemia, no evidence exists that either iron alone or with folate affects birth weight or fetal survival in developed countries. Data from developing countries are not sufficient for drawing conclusions; the few randomized controlled trials are inconclusive because of small sample size, problems with compliance and large losses to follow-up. Small trials in The Gambia, Nigeria and India showed no significant effects on birth weight (68–71).

Zinc. Zinc fingers (loops within the DNA binding domain of receptors for hormones, other vitamins and protein transcription factors) are active in embryogenesis, cell differentiation and proliferation. Zinc deficiency states are characterized by impaired growth. Data from over 40 observational studies, many from developing countries, were reviewed by King (72) and Shah and Sachdev (73). Approximately one-half show associations between low zinc status and low birth weight. Thirteen trials of zinc supplementation in pregnancy were reviewed (72–74). Although there is some evidence of benefit, the data are inconclusive. Many trials had an inadequate sample size and other methodological problems; the Cochrane review (75) excluded all studies from developing countries on this basis. A large well-conducted study of 580 U.S. women with low serum zinc concentrations showed a significant increase in birth weight (+126 g), head circumference (+0.4 cm) and limb length in the supplemented group (76). Both supplement and control groups also received a multivitamin preparation. The effect size was greater in thinner women. There were no effects on fetal deaths. Recent large trials of zinc in low-income women in Bangladesh (77) and Peru (78) showed no effects on birth weight or other birth measurements.

Iodine. Levels of iodine in soil (and therefore in plant foods) are highly variable, with pockets of deficiency in mountainous and flooded areas. All the trial evidence of iodine supplementation comes from such areas (New Guinea, Zaire, Malawi, Algeria, Bhutan, Peru) but most do not report data on birth weight or fetal loss. A nonrandomized trial in Algeria showed reduced rates of abortion and stillbirth and significantly higher birth weight in iodine-supplemented mothers (79).

Calcium. We identified 10 trials of calcium supplementation in pregnancy, all focusing on hypertension and preeclampsia (80–83). Most found no effect on birth weight, other birth measurements or incidence of abortion and stillbirth. However, a small trial from India (84) showed an increase in mean birth weight (calcium 2731 g [$n = 103$] versus placebo 2626 g [$n = 98$]; $p = 0.01$) as did a trial with Iranian mothers (85) (calcium 3316 g [$n = 15$] versus placebo 2764 g [$n = 15$]; $p < 0.05$). The Cochrane meta-analysis (80) showed a significant effect on low birth weight (RR 0.83; 95% CI: 0.71–0.98) probably because of prolongation of gestation rather than enhanced fetal growth. In the only micronutrient trial with long-term follow up, Belizan et al. (86) found that children whose mothers were supplemented with calcium in pregnancy had lower blood pressures.

Magnesium. The Cochrane review (87) included 6 trials of magnesium, mainly focusing on hypertension as an outcome. The meta-analysis showed a beneficial effect on low birth weight and smallness for gestational age. The only trial from a developing country (Angola) had birth weight data inadequate for drawing conclusions.

Other trace elements: copper, selenium, chromium, manganese, molybdenum. The role of these trace elements in human pregnancy is unknown and we identified no trials. Evidence from observational studies of copper and selenium are reviewed by Ramakrishnan et al. (41) and Keen et al. (88).

Multiple micronutrient supplements and studies of improved food quality

In 1936 Boyd Orr et al. (89) compared pregnant rats fed a diet approximating "the average diet eaten by a working class-community in Scotland" with rats given the same diet supplemented with green vegetables and milk. He observed increased appetite in the supplemented rats, larger litter weight and fewer stillbirths. The pups were heavier at weaning and had a marked reduction in postnatal infection-related deaths. Pups in the supplemented group did better even if mothers were fed the nonsupplemented diet postnatally.

This intervention has never been tested in humans, but in the 1940s and 1950s there were a number of studies of improved diet quality in human mothers. For example, Ebbs (90) supplied Toronto mothers on a poor diet with either a placebo (corn oil capsules) or food (milk, cheese, eggs, oranges, tomatoes and wheat germ). Supplemented mothers had a lower risk of miscarriage, stillbirth and low birth weight (2% versus 8%, no test of statistical significance). Mean birth weight was similar in both groups. Postnatally the infants had a lower risk of respiratory infection, rickets, anemia and death. Typically of similar studies at that time, the sample size was small, randomization procedures were not described, the groups differed in important baseline characteristics and results were not analyzed statistically. From a modern standpoint they can best be described as inconclusive. Nonetheless pregnant women were increasingly advised to eat a mixed diet including micronutrient-rich foods. In the United States multiple micronutrient tablets are routinely prescribed in pregnancy, and trials of these are now considered unethical, at least in women with poor-quality diets (91).

Recent data provide some support for nutrient-dense diets in pregnancy, although hard evidence of benefit is still lacking. The U.S. federal Women, Infants, and Children's (WIC) Program provides low-income mothers with vouchers for milk, eggs, cheese, fruit juice, cereals, pulses, and peanut butter. This intervention has not been assessed in a randomized trial, and only evaluated retrospectively. In a critical review, Rush (92) concluded that participation in WIC was associated with reduced pre-term delivery and fetal death, and small but significant increases in mean birth weight (+22.7 g) and head circumference. Retrospective analyses of mothers treated by the Montreal Diet Dispensary, which provided milk, eggs,

and oranges to mothers with poor diets, showed higher mean birth weight compared with retrospectively matched controls (+40 g) (92) and siblings (+107 g) (93). There were stronger effects in thinner mothers.

The large trials of periconceptional multiple micronutrient supplements for prevention of neural tube defects stopped supplements in midpregnancy (60). Most did not examine birth weight as an outcome, but there was no increase in birth weight in the large Hungarian trial (94).

Data from developing countries are equally fragmentary. A small ($n = 43$) trial in a poor rural population of Thailand studied the effect on birth weight of improving the mothers' dietary quality (95). Mothers were randomly distributed into 3 groups to receive either extra food (preprepared cooked food containing 1) legumes, sesame, groundnuts and sugar or 2) rice, oil, groundnuts, shrimp and sugar) or 3) no extra food. Babies were heavier in the 2 intervention groups than in the control group (birth weight 3089 g, 3104 g and 2853 g, respectively). In the Narangwal Study (Punjab, India) villages were allocated to receive nutrition care, health care, both or neither (96). In the villages that received nutrition care, pregnant mothers received bulgar wheat porridge, sugar, milk powder and oil. Although birth weights were not recorded, stillbirths were reduced by 40% compared with control villages. In a trial in Chile, mothers received either powdered milk or milk fortified with vitamins and minerals. Mean birth weight was higher in the fortification group (+73 g; $p < 0.05$) and the percentage of IUGR infants was lower (32% versus 44%; $p < 0.05$) (97). In Bogota, Columbia, families living in an urban slum were randomly assigned to receive extra food (milk, fortified bread and vegetable oil) (98). There were large losses to follow-up, but birth weight was increased among male newborns in supplemented families (+95 g; $p < 0.05$). In South Africa, infants born to women who received a supplement of maize porridge; skimmed milk; and added vitamin A, thiamin, riboflavin and calcium had higher birth weights (+300 g) (99). A major flaw of the study was that mothers in this group also had a higher mean baseline body weight.

Two studies that stand out are a large trial of a high-energy biscuit in The Gambia (31) and a multivitamin supplementation trial in HIV-positive mothers in Tanzania (44). In The Gambia, the locally made biscuit was designed to provide balanced energy and protein but contained groundnuts and therefore micronutrients including calcium and iron. It resulted in improved maternal weight gain, an increase in birth weight of 136 g (201 g in mothers supplemented in the hungry rainy season) and a reduction in stillbirths (OR 0.47; 95% CI: 0.23–0.99) and deaths up to 28 postnatal days (OR 0.57; 95% CI: 0.38–0.88). In Tanzania, mean birth weight was higher (+100 g; $p < 0.01$) in mothers who received a multivitamin capsule,

TABLE 3

Randomized controlled trial of a multivitamin supplement among HIV-positive mothers in Tanzania (n = 1021)

	Multivitamins n (%)	No multivitamins n (%)	RR (95% CI)	p
Miscarriage	12 (2.3)	18 (3.5)	0.66 (0.32–1.36)	0.3
Stillbirth	18 (3.5)	31 (6.1)	0.58 (0.33–1.02)	0.05
Fetal death	30 (5.9)	49 (9.6)	0.61 (0.39–0.94)	0.02
Preterm <34/40 wks	28 (6.2)	44 (10.2)	0.61 (0.38–0.96)	0.03
SGA	39 (10.0)	66 (17.6)	0.57 (0.39–0.82)	0.002
Birthweight <2500 g	36 (8.8)	62 (15.8)	0.56 (0.38–0.82)	0.003

Mean birth weight and placental weight: 3,048g and 526g (multivitamins), 2,948g and 507g (no multivitamins)

SGA, small for gestational age; RR, relative risk; CI, confidence interval.

Used with permission from reference 44.

which provided between three and 10 times the recommended daily intake (RDI) for thiamin; riboflavin; niacin; vitamins B-6, B-12, C and E; and folate. The incidence of IUGR was also reduced (10% versus 18%; $p = 0.002$) and there were fewer fetal deaths (5.9% versus 9.6%; $p = 0.02$) (Table 3). These effects were limited to HIV-negative infants.

Some energy-plus-protein supplementation trials have included micronutrients incidentally. The well-known Guatemala and Bacon Chow (Taiwan) trials provided multiple mineral and vitamin supplements to participants in both intervention and control groups. In Guatemala there was no difference in birth weight between the two groups but the most compliant mothers (total ingestion >20,000 kcal of either supplement) had heavier infants (+56 g) (100). In Taiwan, there was a significant increase in birth weight, similar in both treatment groups (male infants only) (101). These results were attributed to increased energy intakes but could equally have been an effect of micronutrients present in both supplements.

In summary, there is no good evidence that single micronutrient supplements lead to improvements in fetal growth and survival in undernourished populations. The more logical approach of multiple micronutrient supplements or improved overall micronutrient quality of mothers' diets has been inadequately tested. Well-designed trials are needed to address this specifically.

Food versus pharmacology

The provision of micronutrients using pharmaceutical products is simpler and arguably more convenient and certain than using food. Pharmaceutical supplements are expensive (102,40) but so is good food, and indeed the incremental cost of adding micronutrients to current iron supplements may be relatively small. It has been argued that food is unable to provide sufficient micronutrients, especially in vegetarian diets or in areas with depleted soil, although this has been contested (103).

Strong arguments have been made in favor of trials of food-based as well as pharmaceutical interventions. Concerns exist about interactions, in terms of side-effects and absorption, among nutrients packaged together pharmaceutically (102). A pharmaceutical supplement may lack crucial nutrients, for example, fatty acids, phytonutrients or others so far unidentified (104). Toxicity is a risk, both to the mother and fetus and potentially (in accidental overdose) to other children in the family. More compelling considerations are acceptability and sustainability. In the Pune Maternal Nutrition Study, it was clear that many women did not comply with the iron and folate supplements provided free to all pregnant women in India. Given the choice, women would prefer a good diet (for themselves and their families) to tablets. It would be an attractive prospect to find solutions to undernutrition that encourage local agricultural production and methods of preserving food quality (103). Pharmaceutical supplements, in the short term only likely to reach low-income women as handouts, are disincentives to this process.

Future research

- Good quality trials are needed in populations at high risk of micronutrient deficiency and low birth weight. Food-based and multiple micronutrient interventions would be more appropriate than single micronutrient interventions. Pre-conceptional supplementation should be evaluated.
- Trials should incorporate assessment of baseline and post-intervention nutritional status, markers of maternal in-

fection, accurate gestational age assessment, serial measurements of fetal growth and measurements of neonatal body composition. Measurement of micronutrient effects on maternal glucose tolerance and insulin resistance is essential in populations at high risk of gestational diabetes.

- The infrastructure required for trials of good quality should be used to support more detailed studies in subgroups of mothers and to examine mechanisms including micronutrient effects on the maternofetal supply line, maternal metabolism and body composition, adaptations to pregnancy and infection.
- Studies are needed into synergistic and antagonistic reactions between micronutrients, bioavailability and differences between food and pharmaceutical supplements.
- Medium- and long-term outcomes, including morbidity and mortality, and risk factors for cardiovascular disease and type 2 diabetes should be studied in childhood and adult life.

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