MIRA Janakpur

Multiple Micronutrient Supplementation Study

The effects of antenatal multiple micronutrient supplementation on birthweight, gestation and infection: a double blind, randomised controlled trial conducted in Nepal.

Study protocol

Painting by Rebi Mandal
A guide to the study and its conduct

The study question
Does antenatal multiple micronutrient supplementation for pregnant women have beneficial effects on birthweight, gestation and perinatal infection?

The study hypotheses
Second and third trimester supplementation with a multiple micronutrient regime will increase birthweight.
Second and third trimester supplementation with a multiple micronutrient regime will prolong gestation.
Second and third trimester supplementation with a multiple micronutrient regime will make mothers less susceptible to infection.

Dimensions of the study
The study will enroll 1200 pregnant women, 600 in each of two groups. Each participant will randomly receive either routine iron and folic acid supplement tablets or multiple micronutrient supplement tablets for the duration of her pregnancy. The two groups will be compared at 32 weeks’ gestation, at the time of birth and one month later.

Primary outcomes
1200 participants
Birthweight, length and head circumference are measured within 24 hours of birth. Gestation at birth is calculated on the basis of ultrasound at enrollment.

Micronutritional outcomes
200 participants
Venous blood is collected at enrollment and at 32 weeks’ gestation for measurement of plasma vitamins A, C and E and ferritin.

Immunological outcomes
600 participants
Clinical indicators of infection are assessed at every clinical contact. Venous blood is collected at 32 weeks’ gestation for measurement of plasma acute phase proteins. Urine is collected at 32 weeks gestation for measurement of neopterin. Placentae are examined at birth for macroscopic and microscopic evidence of infection. Breastmilk is collected at one month postpartum for measurement of sodium/potassium ratio.

Pregnant women who are invited to participate
Pregnancy at gestations up to and including 20 weeks 0 days. No pre-existing maternal illness of a nature likely to affect pregnancy. Single live pregnancy detected by obstetric ultrasound at enrollment. Residence potentially accessible for home follow-up.

Reasons for exclusion of potential participants
Pregnancy at gestations greater than 20 weeks 0 days. Pre-existing maternal illness of a nature likely to affect pregnancy. Multiple pregnancy detected by obstetric ultrasound at enrollment. Major fetal anomalies detected by obstetric ultrasound at enrollment. Residence potentially inaccessible for home follow-up.

The study site
The study is being conducted in collaboration with Janakpur Zonal Hospital, Dhanusha District, Nepal.
Measurements used in the study

Ultrasound is carried out at enrollment to estimate gestational age, using standard tables of crown-rump length, biparietal diameter, head circumference and femur length. Birth weight is measured within 24 hours on electronic scales accurate to 10 g. Birth length is measured within 24 hours using a standard length board (Kiddimeter). Head circumference is measured within 24 hours using a plastic tape. Vitamins A, C and E and ferritin are measured in plasma samples at enrollment and 32 weeks’ gestation as an index of adherence. Acute phase proteins are measured in a plasma sample at 32 weeks’ gestation. Urine neopterin is measured at 32 weeks’ gestation. Breastmilk sodium and potassium are measured at one month postpartum.

Iron and folic acid tablets

The two types of tablet are identical in size, shape, colour, consistency, smell and taste. Participant identification numbers have been randomised off-site to one of the two types of supplement, in permuted blocks of 50. Each participant is allocated an individual, numbered bottle of supplements that will last her for the duration of her pregnancy. She receives a number of tablets from this bottle at each fortnightly visit (either at home or at the antenatal clinic). The participant is therefore unaware of which type she is taking, as are all the local members of the study team. Participants are encouraged to take one tablet daily, after the evening meal, and also advised not to take other forms of supplement unless recommended by an obstetrician affiliated with the study.

Multiple micronutrient tablets

The supplements contain vitamin A 800 mcg, vitamin B1 1.4 mg, vitamin B2 1.4 mg, vitamin B6 1.9 mg, vitamin B12 2.6 mcg, vitamin C 70 mg, vitamin D 5 mcg, vitamin E 10 mg, niacin 18 mg, folic acid 400 mcg, iron 30 mg, zinc 15 mg, copper 2 mg, selenium 65 mcg and iodine 150 mcg. These levels have been recommended for pregnant women by WHO and UNICEF.

Vitamins A, C and E and ferritin are measured in plasma samples at enrollment and 32 weeks’ gestation as an index of adherence. Acute phase proteins are measured in a plasma sample at 32 weeks’ gestation. Urine neopterin is measured at 32 weeks’ gestation. Breastmilk sodium and potassium are measured at one month postpartum.

Ethics and agreement

The study follows the CONSORT guidelines for randomised controlled trials, and is monitored by a Trial Monitoring Committee. The study has ethical clearance from the Nepal Health Research Council and the Ethics Committee of the Institute of Child Health, London. It operates under a joint agreement with His Majesty’s Government, Nepal, Ministry of Health. The study is funded by The Wellcome Trust, UK.

Enrollment

All pregnant women attending for antenatal care at gestations up to and including 20 weeks are invited to participate. They are offered obstetric ultrasound examination to confirm fetal viability, exclude major congenital anomalies and assess gestation.
Potential participants who meet the inclusion criteria are briefed in detail on the nature of the study and invited to enroll. The study is explained in Nepali or Maithili depending on the participant’s wish, written materials being available in both languages. Enrollment takes place after participants have given their signed consent, preferably in the presence of a family member and always in the presence of two members of the study team.

At enrollment, each participant answers a series of questions dealing with demographics, socioeconomic status, medical history and obstetric history.

She is offered a unique identification number, an identity card, a client-based maternity record, recommended antenatal blood tests (haemoglobin, group and rhesus status, and rapid plasma reagin test), and a personal bottle of supplement tablets.

**Follow-up**

Participants attend for antenatal care at the clinic every month. They are seen every two weeks to monitor their tablet consumption and any problems, and to top up their supply, either at the hospital or through home visits by team members.

Because of the flat terrain and relatively good road system in this part of Nepal, follow-up of women and their children in the community is feasible.

Participants receive routine antenatal care, delivery and postnatal care. If they have any risk factors or develop complications, they are referred for management by the obstetric team. The costs of all clinical care required for the duration of pregnancy, including emergency services, are met by the study team.
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<td>Quick reference guide to the study process</td>
<td>participant taking multiple micronutrient supplements</td>
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<td>subsample blood test for APPs, vitamins A, C &amp; E, and ferritin</td>
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Newborn infants in Nepal

Nepal has high rates of perinatal and neonatal mortality. In low-income countries, the perinatal period remains a source of unacceptable morbidity and mortality for women and their infants. South Asia bears the largest burden of perinatal mortality and morbidity. High rates of intrauterine growth retardation, stillbirth and preterm birth are compounded by a lack of effective perinatal care for mothers and infants. The neonatal period now accounts for 65% of deaths in infancy in South Asia. Low birth weight, as a result of intrauterine growth retardation or preterm birth, is the major underlying cause of neonatal mortality. Intrauterine growth is currently a priority area for intervention by international agencies.

Information about the background to the study

MIRA Janakpur

Recommendations for multiple micronutrient supplementation

Recent reviews have recommended the introduction of multiple micronutrient supplements in developing countries because for most pregnant women several micronutrients are limiting factors and they might act synergistically. The content of such supplements has been recommended by after expert consultations among United Nations agencies and the World Health Organization. However, the potential benefits of supplementation have not been explored in settings where they are most relevant. The magnitude of the health benefits need clarification before supplementation programmes are introduced in poor communities. The rationale for the MIRA Janakpur study is that length of gestation, birth weight, immunocompetence and neonatal mortality might all be affected by micronutrient status.

Other current investigations

Three studies have already been completed: in Mexico (U Ramakrishnan and colleagues, Emory University), in Nepal (NNIPS 3, K West and colleagues, Johns Hopkins University) and in Tanzania (W Fawzi and colleagues, Harvard School of Public Health). The Mexican study took place in a population with lower indices of mortality and a lower prevalence of LBW. The Nepal study is currently being analysed. The Tanzanian study suggested important effects of supplementation on indices of mortality, but took place in a population of pregnant women infected with the HIV virus.

Four other studies are currently underway: in Guinea Bissau, in Bangladesh, in Pakistan, and in
Janakpur Zonal Hospital is situated in Dhanusha District in the Central Development Region. Dhanusha has a Human Development Index of 0.329 (below the national average of 0.378) and a Gender Sensitive Development Index of 0.272. Malnutrition rates among mothers and children are high, but goitre prevalence is low, so that subclinical iodine deficiency is not likely to be a confounding factor.

The hospital has 100 inpatient beds and provides a range of services including paediatrics and obstetrics. There is a dedicated Mother and Child Health (MCH) department staffed by obstetricians, medical officers, nurses and auxiliary nurse midwives. There are over 200 deliveries per month within the unit. The Caesarian section rate is about 8% and the prevalence of LBW is at least 25%.

Previous investigators have identified deficiencies of selenium and zinc in the diets of Nepalese women, deficiencies compounded by the reduced bioavailability associated with high fibre and phytate intakes. A recent review estimates that 82% of women worldwide have inadequate zinc intakes, and suggests that supplementation trials be carried out in populations with assumptive risks. Insert information from Nepal Micronutrient Status Survey.

Women in Nepal are likely to be deficient in several micronutrients

Local diets centre on rice, pulses and some vegetables. Meat is reserved for special occasions and use of oils is limited. Micronutrient and macronutrient deficiencies in this and other dietetically similar lacto-ovo-vegetarian populations have been well described, as has the high prevalence of stunting. Dietary precedence follows a hierarchy from male family members, via senior women and children, to younger women, such that women of reproductive age are least likely to achieve satisfactory nutritional status. Existing household structures tend to ensure that food entering the home reaches family members with a keener demand which goes some way to explain the disappointingly limited impact of macronutrient supplementation and dietary advice for pregnant women.

Waiting to give birth in the labour ward

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What is the evidence for beneficial effects from micronutrient supplementation?

The present body of work on multiple micronutrient interventions is not sufficient for us to draw conclusions on their effects on neonatal wellbeing. Because studies have generally concentrated on single micronutrients and a range of outcomes, we summarise the findings for each nutrient. We limit our discussion to outcomes for the infant, particularly birth weight, preterm delivery and neonatal mortality.

Vitamins

**Vitamin A** (reviewed in 7 22-24)

In animal models, vitamin A deficiency has been associated with reduced fetal survival and reversible squamous metaplasia of the respiratory tract epithelium. Some observational studies have described an association between serum vitamin A or carotenoids and birth weight 25 26, while others have failed to do so 27-31. Associations with head circumference, length and gestational duration 25 have likewise not been confirmed 30: serum vitamin A, serum retinol binding protein and fetal liver retinol levels are lower in preterm infants, but are probably effects rather than causes 32-35. Serum vitamin A levels probably do not correlate with intrauterine growth retardation, maternal infection, or neonatal Apgar scores 31. The trials of antenatal vitamin A supplementation have been carried out either in developing countries, or with selected poor or malnourished women in industrialised ones.

Supplementation has no significant effect on cord plasma retinol 29, birth weight 8 29 36, length 29, head circumference 29, or skinfold thickness 29. A possible effect on preterm birth 37 has not been replicated 38. The large cluster randomised trial in Nepal showed no effect of vitamin A supplementation on neonatal mortality or mortality in the first 6 months 38, although subanalysis suggests that there may have been a trend towards an effect 39.

**Vitamin B1 (thiamine)**

The potential role of thiamine as an antiteratogenic nutrient has been inconclusively explored in animal models. Although thiamine intake has been linked with birth weight on the basis of dietary assessment in the first trimester 40, there has been no observational association of thiamine levels with stillbirth, birth weight or length 27 41. One study has drawn an association between lower maternal third trimester erythrocyte thiamine levels and intrauterine growth retardation 42. We know of no observational studies from developing countries, and no trials of supplementation.

**Vitamin B2 (riboflavin)**

Riboflavin intake has been weakly correlated with gestational duration on the basis of dietary assessment in the first trimester 40, and with birth weight on the basis of dietary assessment in the second 43. The latter association is not supported by other studies 27 44. We know of no observational studies from developing countries, and no trials of supplementation.

**Vitamin B6 (pyridoxine)** (reviewed in 7 45)

In animal models, vitamin A deficiency has been associated with reduced fetal survival and reversible squamous metaplasia of the respiratory tract epithelium. Some observational studies have described an association between serum vitamin A or carotenoids and birth weight 25 26, while others have failed to do so 27-31. Associations with head circumference, length and gestational duration 25 have likewise not been confirmed 30: serum vitamin A, serum retinol binding protein and fetal liver retinol levels are lower in preterm infants, but are probably effects rather than causes 32-35. Serum vitamin A levels probably do not correlate with intrauterine growth retardation, maternal infection, or neonatal Apgar scores 31. The trials of antenatal vitamin A supplementation have been carried out either in developing countries, or with selected poor or malnourished women in industrialised ones.

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The key role of folate in DNA synthesis means that deficiency is associated with dysfunction in rapidly dividing cells. The relationship between periconceptional folate deficiency and neural tube defects is now well established, as is the benefit of supplementation. Observational studies have suggested that lower maternal serum folate levels are associated with low birth weight and preterm birth. A large US study suggests an association between higher maternal serum folate at 30 weeks gestation and lower risk of intrauterine growth retardation, higher birth weight and higher Apgar scores. Two Brazilian studies, however, suggest a lack of relation between serum or erythrocyte folate and intrauterine growth retardation. An early supplementation trial suggested that folate supplementation might have an effect on birth weight in malnourished women. Although supported by studies of varying quality, this has not been confirmed. There is some evidence that supplementation prolongs gestation. A Cochrane review of trials in non-anaemic women finds no association between folate supplementation and stillbirth or preterm delivery. A nonsignificant reduction in the prevalence of low birth weight is deemed plausible. We know of no trials which look at neonatal mortality, apart from an as yet unpublished one from southern Nepal.

Vitamin B12 (cobalamin) (reviewed in 7)

The megaloblastic anaemia of cobalamin deficiency highlights its association with defects in DNA synthesis, cell multiplication and metabolism. Low serum cobalamin levels have been associated with preterm birth and low birth weight, and it has been suggested that the interrelation between cobalamin and lipid metabolism may play a part in causing the low birth weight associated with smoking in pregnancy. Severe gestational deficiency may also be associated with intrauterine death. We know of no trials of supplementation with respect to fetal outcome.

Vitamin C (ascorbate) (reviewed in 7 63)

The involvement of ascorbate in collagen stabilisation and protection from reactive oxygen species support a role for it in maintaining membranes: lower plasma and leucocyte ascorbate have been associated with premature rupture of membranes, and serum ascorbate concentrations have been weakly positively associated with gestational duration. We know of no trials that have looked at either fetal growth or mortality.

Vitamin D (cholecalciferol) (reviewed in 7 67)

Because of its relationships with parathyroid hormones and calcium homeostasis, maternal cholecalciferol status might affect fetal growth (although putative effects on fetal length may be mediated through calcium availability). A nonrandomised trial of third trimester supplementation in India was associated with increases in birth weight and length. This was supported by a trial in UK Asians which suggested a reduced prevalence of low birth weight, but not by another study. Routine supplementation has not been an issue: the focus has generally...
Vitamin E (tocopherol) (reviewed in 7 63)

The antioxidant properties of tocopherol might protect membranes from damage by reactive oxygen species. Tocopherol deficiency has also been associated with malformation and fetal death 72. Two studies found an association between maternal plasma or serum tocopherol and birth weight 73 74. Other studies, however, have found no association with birth weight 31 75 76, intrauterine growth retardation 31, length 25, head circumference 25, gestational duration 25, or Apgar scores 31. Although several studies have examined the effect of supplementation on pre-eclampsia, we know of no trials that have looked at neonatal mortality.

Minerals

Zinc (reviewed in 19 55 77-84)

Since zinc interacts with over 300 enzymes and proteins, the effects of deficiency are wide ranging. Zinc deficiency in animal models has been associated with fetal wastage, stillbirth, delivery complications and neonatal death. This relates at least to some degree to the deleterious effects of deficiency on DNA synthesis, skeletal abnormalities and growth, and CNS malformations. However, much of this work was done in the presence of severe zinc deficiency. In a situation of marginal deficiency, no effects were seen on pregnancy outcome or fetal malformation, although there may be an association with preterm rupture of membranes. The knowledge that maternal acrodermatitis enteropathica (an inborn defect of zinc absorption associated with severe hypozincemia) leads to a reversible propensity for fetal malformation led to wider concern about the possibility of zinc deficiency in the general population. Indeed, lower zinc intakes have been associated with lower birth weight and preterm delivery 85. The observational studies on zinc in pregnancy are, however, confusing. A central issue is the interpretation of plasma or serum zinc levels during pregnancy. It was suggested early on that gestational plasma or serum zinc concentrations are not always useful indicators of zinc status 86 88. Maternal plasma zinc levels decline with gestation to a plateau in the third trimester 89-94, and may be difficult to interpret unless sampling time, laboratory methods and the underlying zinc status of the population are accounted for 99 82.

The extensive literature on zinc and fetal growth is inconclusive to the degree that some systematic reviews suggest that it is beneficial, some that the benefits are unproven, and all that more work is required. Some studies have described positive associations between maternal plasma zinc and birth weight 25 26 95-97; some have described negative associations 86 90 91 98-101; and others have found no association 28 31 78 86 94 99 101-113. Some studies have found lower maternal plasma zinc to be a risk factor for congenital malformations 114-120; others have not 121. Some studies have found lower maternal plasma zinc to be a risk factor for preterm delivery 111; others have not 94 122. Maternal leucocyte zinc has been put forward as a better indicator of status, and some studies have found a positive association with intrauterine growth 86 78 88 123; others studies have not 122 124.

Some supplementation trials have shown that supplementation increases birth weight 125 126, reduces the prevalence of small for gestational age leads to a reversible propensity for fetal malformation led to wider concern about the possibility of zinc deficiency in the general population. Indeed, lower zinc intakes have been associated with lower birth weight and preterm delivery 85. The observational studies on zinc in pregnancy are, however, confusing. A central issue is the interpretation of plasma or serum zinc levels during pregnancy. It was suggested early on that gestational plasma or serum zinc concentrations are not always useful indicators of zinc status 86 88. Maternal plasma zinc levels decline with gestation to a plateau in the third trimester 89-94, and may be difficult to interpret unless sampling time, laboratory methods and the underlying zinc status of the population are accounted for 99 82.

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Some supplementation trials have shown that supplementation increases birth weight 125 126, reduces the prevalence of small for gestational age
Iron (reviewed in 7 55 139-141)

Although the results of three trials have since been published 132 135 136, disaggregation on the basis of developing or industrialised, poor or affluent populations has not clarified the issue. It is conceivable that gestational zinc supplementation could have longer term effects on mortality because of benefits to immunocompetence 132. In the short term, however, two supplementation studies in poor US populations found no effect on perinatal deaths 133, stillbirths, neonatal deaths or admission for special care 128. A recent trial of supplementation showed reductions in both fetal loss and neonatal mortality 144, but a Cochrane review of routine iron supplementation in pregnancy cannot draw conclusions about either beneficial or harmful effects to mother or baby 140.

Copper

Copper deficiency affects many cuproenzymes, leading to defects in ATP production, lipid peroxidation, hormone activation, angiogenesis and abnormalities of vasculature, skeleton and lung 148. Although maternal serum copper level rises over pregnancy 103 108 112 146, its validity as an index of copper status is questionable 113. Several studies have failed to describe an association of maternal serum copper with birth weight 102 103 108, although one study found an association of low maternal plasma copper with preterm rupture of membranes 147. Cord serum copper has been negatively associated with birth weight and head circumference 25 109 112, small for gestational age 107 and preterm delivery 148. We know of no supplementation trials.

Iodine (reviewed in 7 55)

Iodine-dependent thyroid hormones increase cell proliferation, synapse formation and microtubular assembly. The beneficial effect of iodine supplementation on endemic cretinism and goitre has been well established, and deficiency disorders are now understood to manifest across a spectrum that includes the subclinical. A trial in Zaire suggested improvements in birthweight and infant mortality as well 149; this is supported by a study of lower quality from Algeria 150.
Selenium participates in antioxidant cellular protection and energy metabolism. Frank deficiency is associated with a juvenile cardiomyopathy and a chondrodystrophy. Maternal serum selenium does not correlate with birth weight, length or head circumference. Cord serum selenium has been found to be lower in low birth weight infants and plasma selenium has been found to be higher in preterm than term infants. There is a putative association of deficiency with neonatal respiratory morbidity. There is presently little evidence for direct effects of deficiency on the fetus, other than in conjunction with iodine deficiency, and we know of no supplementation trials.

Multiple micronutrients

It is tempting to aggregate the possible beneficial effects of individual micronutrients described above, and to add the findings of dietary intake studies which suggest a relationship between the intake of a range of micronutrients and rates of low birth weight. However, to generalise from observational studies is unwise. The results of two multiple micronutrient supplementation trials have been published. A double blind randomised controlled trial in Hungary involving 4753 women and three supplemental combinations of vitamins and minerals suggested improvements in stillbirth rates and birth weight. A double blind, factorial randomised controlled trial in Tanzania suggested improvements in fetal death rates, birth weight, low birth weight prevalence (by 44%) and small for gestational age (without concomitant changes in gestational duration). The study involved only women with HIV infection.

Do micronutrients have significant immunological effects?

There are multidirectional interactions between micronutrients, cytokine production and infection during pregnancy and lactation. The status of infection in the aetiology of preterm birth, however, remains unclear. There is evidence that infection can affect pregnancy outcome and infant development through changes in cytokine balance, these changes falling into two broad categories: excessive production of inflammatory cytokines such as interleukins 1 and 6 and tumour necrosis factor alpha (IL-
1, IL-6 and TNFa), and an increase in type 1 cytokines such as interferon-gamma (IFNc) relative to type 2 cytokines such as IL-10. Pregnancy-induced hypertension, for example, is associated with increased plasma IFNc and TNFa; chorioamnionitis and preterm delivery are associated with increased IL-6 production; and spontaneous abortion and fetal growth faltering with increased IFNc.

If micronutrient supplementation during pregnancy leads to a reduction in the incidence of preterm delivery or of pre-eclampsia, the mechanisms are unknown. They may involve changes in cytokine production which either directly affect the maintenance of pregnancy or increase immunocompetence with a consequent decrease in infection. Vitamins C and E and pyridoxine have been implicated in cell-mediated immunity, vitamin D in both cell-mediated and humoral immunity. The antioxidant activities of the former affect both inflammatory cytokine levels and tissue damage. An additional marker is available for assessing maternal morbidity postpartum, a period of high mortality. Subclinical mastitis, which we define as raised milk sodium/potassium ratio and IL-8 concentration in the absence of symptoms of mastitis, has been shown to be common in several populations. Subclinical mastitis appears a marker of both poor lactation practice associated with slow infant growth and of maternal infection or micronutrient deficiency. Supplementation of Tanzanian women with vitamin E and essential fatty acid-rich sunflower oil decreased milk Na/K ratio. Antioxidant micronutrients are used in the dairy industry to reduce mastitis in herds. Therefore, milk Na/K ratio will be used as a non-specific marker of mother-infant pairs at risk and its response to micronutrient supplementation will be investigated.

Although cytokine levels might be used to monitor women at risk for obstetric complications, they are difficult to measure under field conditions. Acute phase proteins (APPs) have longer plasma half-lives and are easier to assay than the inflammatory cytokines which induce them, and their usefulness in monitoring clinical and subclinical inflammation is well established. Neopterin - released by macrophages stimulated primarily by IFNc - is excreted in urine and is an established indicator of cell-mediated immune activation during many diseases including infection, malignancy and sterile respiratory inflammation. It has recently been quantified in pregnancy and the postpartum period, and has been suggested as a marker of high risk for complications.
References


130. Ross S, Nel E, Naeye R. Differing effects of low and high bulk foods on zinc status in Mexican adolescents.


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