# M I R A  J a n a k p u r

## 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.**

# S t u d y  p r o t o c o l

Painting by Rebti Mandal

# MIRA Janakpur

## 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.

## 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 [1]

## Iron and folic acid tablets

The supplements contain iron 60 mg and folic acid 400 mcg, recommended for pregnant women by His Majesty's Government, Nepal [2 3].

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.

## 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.

## Ethics and agreement

The study follows the CONSORT guidelines for randomised controlled trials [4], 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

Antenatal care

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.

Gestational dating by ultrasound biometry

Explanation and consent

## Follow-up

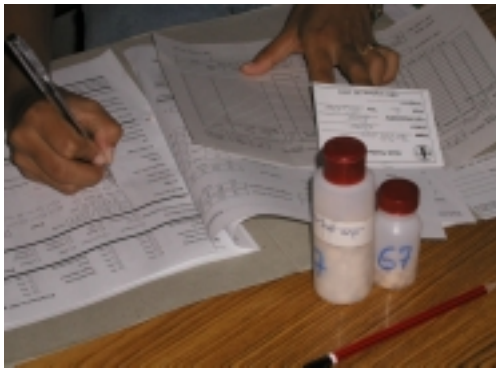
Participants attend for antenatal care at the clinic every month.

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.

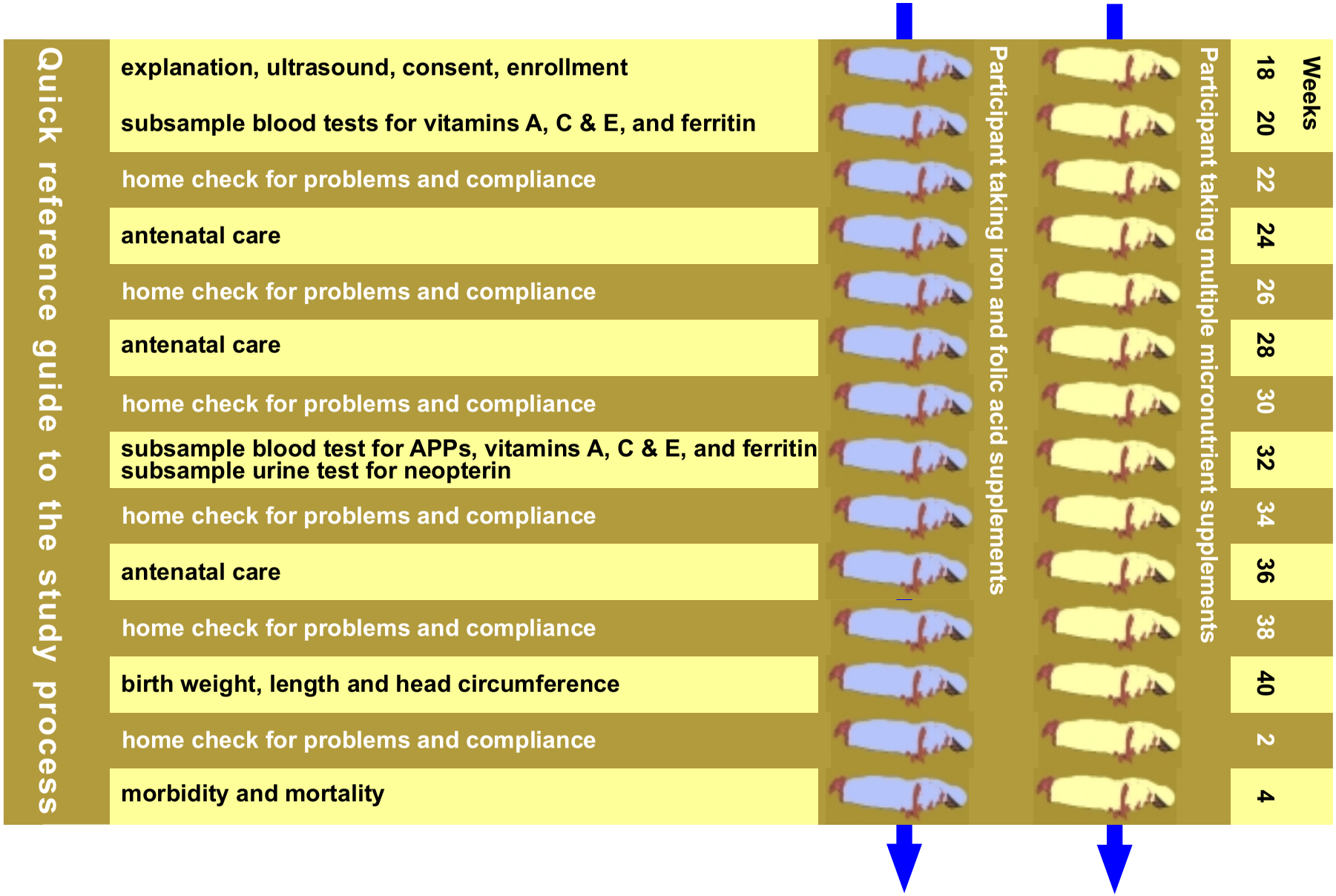
Because of the flat terrain and relatively good road system in this part of Nepal, follow-up of women and their children in the community is feasible.

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.


Individualised supplements



Follow-up


Quick reference guide to the study process
explanation, ultrasound, consent, enrollment
subsample blood tests for vitamins A, C & E, and ferritin
home check for problems and compliance
antenatal care
home check for problems and compliance
antenatal care
home check for problems and compliance
subsample blood test for APPs, vitamins A, C & E, and ferritin
subsample urine test for neopterin
home check for problems and compliance
antenatal care
home check for problems and compliance
birth weight, length and head circumference
home check for problems and compliance
morbidity and mortality
Participant taking iron and folic acid supplements
Participant taking multiple micronutrient supplements
Weeks
18
20
22
24
26
28
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32
34
36
38
40
2
4

# Information about the background to the study

## 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 [5]. 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.

## Recommendations for multiple micronutrient supplementation

Recent reviews [6 7] 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 [1]. 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.

Low birth weight newborn baby in the paediatric unit

## 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 [8]). 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

Indonesia. The MIRA Janakpur study is the fifth in this group. Investigators from all five studies constitute a working group which aims to produce comparable and combinable results for the sake of meta-analysis. The MIRA Janakpur study will augment the findings by (a) studying effects on gestational age on the basis of accurate gestational dating, and (b) studying biological markers of perinatal infection and immunocompetence.

## 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 [9], as has the high prevalence of stunting [10]. 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 [11] which goes some way to explain the disappointingly limited impact of macronutrient supplementation and dietary advice for pregnant women [12-16].

Previous investigators have identified deficiencies of selenium and zinc in the diets of Nepalese women [17], deficiencies compounded by the reduced bioavailability associated with high fibre and phytate intakes [18]. 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 [19]. Insert information from Nepal Micronutrient Status Survey [20].

Waiting to give birth in the labour ward

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 [21]. Malnutrition rates among mothers and children are high, but goitre prevalence is low, so that sub-clinical 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%.

## 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)

Pyridoxine appears to play an important role in the development of the central nervous system. Gestational pyridoxine deficiency in the rat has been associated with lower body weights in offspring, impaired physical and neuromotor development and neurological symptoms [46]. Maternal serum pyridoxine levels have not been

shown to correlate with birth weight, although cord levels may be lower in LBW infants [27]. A possible association of pyridoxine deficiency with lower Apgar scores has been reported several times [47-49]. A Cochrane review of trials of intrapartum supplementation for effects on neonatal outcome includes no relevant studies [45].

### 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 [50] and low birth weight [27], 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 [51]. Severe gestational deficiency may also be associated with intrauterine death [52]. We know of no trials of supplementation with respect to fetal outcome.

### Folate (reviewed in 53-55)

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 [27 56] and preterm birth [56]. 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 [31]. Two Brazilian studies, however, suggest a lack of relation between serum or erythrocyte folate and intrauterine growth retardation [35 57]. An early supplementation trial suggested that folate supplementation might have an effect on birth weight in malnourished women [58]. Although supported by studies of varying quality [59 60], this has not been confirmed [61]. There is some evidence that supplementation prolongs gestation [62]. 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 [53]. We know of no trials which look at neonatal mortality, apart from an as yet unpublished one from southern Nepal.

### 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 [64 65 66], and serum ascorbate concentrations have been weakly positively associated with gestational duration [40]. 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 [68]). A nonrandomised trial of third trimester supplementation in India was associated with increases in birth weight and length [69]. This was supported by a trial in UK Asians which suggested a reduced prevalence of low birth weight [70], but not by another study [71]. Routine supplementation has not been an issue: the focus has generally

been on populations at risk of neonatal hypocalcaemia. A Cochrane review includes two trials, and supports the administration of cholecalciferol to vulnerable groups in later pregnancy [67].

### 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 hypozincaemia) 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 [19 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

[127], reduces the incidence of preterm delivery [125 128 129], increases gestational duration [125 126 128 130-132], and improves Apgar scores [125]. Again, however, other studies demonstrate no effect on birth weight [128-136], small for gestational age [125], preterm [130 131 134-137], gestational duration [136], or Apgar scores [133 134]. It is possible that beneficial effects would only be seen in poorer populations [138]: a Cochrane review of five trials [81] finds that reductions in the prevalence of low birth weight and small for gestational age were only significant in groups selected for low zinc status [126 127].

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].

### Iron (reviewed in 7 55 139-141)

That gestational iron supplementation improves haematological indices is not in question [140 142]. The U-shaped relation of gestational anaemia with fetal outcome makes assumptions about benefits questionable, however [143], and the ethics of placebo-controlled trials where supplementation is routinely recommended cloud the subject further [139]. 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 [145]. 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 delvery [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].

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### Magnesium (reviewed in 7 55 151)

Dietary assessment suggests that magnesium intake is positively associated with birth weight [40]. While some studies show no association of maternal plasma magnesium [102] or cord plasma magnesium [25] with low birth weight, one study suggests that higher serum magnesium levels are seen in mothers of growth retarded infants [152]. Lower serum magnesium levels have also been questionably associated with preterm labour [153]. Some magnesium supplementation studies have shown benefits to rates of preterm birth [154-156]; others have not [157-159]. Some studies have shown benefits for low birth weight [156] and small for gestational age [154 156]; others have not [157-159]. Supplementation seems to have no effect on Apgar scores [157] or admission for special care [157 159]. A Cochrane review of six controlled trials (heavily weighted by two studies [154 155]) concludes that supplementation starting before the third trimester results in a lower incidence of preterm birth and a lower prevalence of low birth weight and small for gestational age. No significant effect was found on fetal or neonatal mortality [151].

### Selenium (reviewed in 160)

Selenium participates in antioxidant cellular protection and energy metabolism. Frank deficiency is associated with a juvenile cardiomyopathy and a chondrodystophy. 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 [113], and plasma selenium has been found to be higher in preterm than term infants [148]. There is a putative association of deficiency with neonatal respiratory morbidity [161]. There is presently little evidence for direct effects of deficiency on the fetus, other than in conjunction with iodine deficiency [162], and we know of no supplementation trials.

### Multiple micronutrients (reviewed in 6 7 55 163)

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 [164]. 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) [8]. 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 [165]. The status of infection in the aetiology of preterm birth, however, remains unclear [166 167]. There is evidence that infection can affect pregnancy outcome and infant development through changes in cytokine balance [168 169], 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 [170] and TNFa [171]; chorioamnionitis and preterm delivery are associated with increased IL-6 production [172 173]; and spontaneous abortion and fetal growth faltering with increased IFNc [174].

Painting by Anuragi Jha

If micronutrient supplementation during pregnancy leads to a reduction in the incidence of preterm delivery [175] or of pre-eclampsia [176], 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 [177 178].

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 [186 187]. Subclinical mastitis appears a marker of both poor lactation practice associated with slow infantgrowth [186] and of maternal infection or micronutrient deficiency [187]. Supplementation of Tanzanian women with vitamin E and essential fatty acid-rich sunflower oil decreased milk Na/K ratio [187]. Antioxidant micronutrients are used in the dairy industry to reduce mastitis in herds [188]. 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 [179], 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 [173 180]. Neopterin - released by macrophages stimulated primarily by IFNc - is excreted in urine [181] and is an established indicator of cell-mediated immune activation during many diseases including infection, malignancy [182] and sterile respiratory inflammation [183]. It has recently been quantified in pregnancy and the postpartum period, and has been suggested as a marker of high risk for complications [184 185].

## References


1. UNICEF/WHO/UNU. Composition of a multi-micronutrient supplement to be used in pilot programmes among pregnant women in developing countries. New York: United Nations Children's Fund, 1999.
2. Ministry of Health. National maternity care guidelines. Kathmandu: Ministry of Health, His Majesty's Government of Nepal, 1996.
3. Family Health Division. Reproductive health clinical protocols. Kathmandu: Health Services Department, Ministry of Health, His Majesty's Government, Nepal, 1999.
4. Moher D, Schulz K, Altman D. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials. *JAMA* 2001;285:1987-91.
5. Costello A, Manandhar D. Current status of newborn care in developing countries. In: Manandhar D, editor. *Improving newborn infant health in developing countries*. London: Imperial College Press, 1999.
6. Huffman S, Baker J, Shumann J, Zehner E. The case for promoting multiple vitamin/mineral supplements for women of reproductive age in developing countries. Washington DC: Linkages, Academy for Educational Development, 1998.
7. Ramakrishnan U, Manjrekar R, Rivera J, Gonzales-Cossio T, Martorell R. Micronutrients and pregnancy outcome: a review of the literature. *Nutr Res* 1999;19:103-59.
8. Fawzi W, Msamanga G, Spiegelman D, Urassa E, McGrath N, Mwakagile D, et al. Randomised trial of effects of vitamin supplements on pregnancy outcomes and T cell counts in HIV-1-infected women in Tanzania. *Lancet* 1998;351:1477-82.
9. Banji M, Lakshmi A. Less recognized micronutrient deficiencies in India. *Bull Nutr Foundation India* 1998;19:5-8.
10. Osmani S. Poverty and nutrition in South Asia. In: Nutrition US-Co, editor. *Nutrition and Poverty. Papers from the ACC/SCN 24th Session Symposium, Kathmandu, March 1997*. 16 ed. Geneva: ACC/SCN, 1997:23-51.
11. Jonsson U. Malnutrition in South Asia. In: Nutrition US-Co, editor. *Nutrition and Poverty. Papers from the ACC/SCN 24th Session Symposium, Kathmandu, March 1997*. 16 ed. Geneva: ACC/SCN, 1997:53-67.
12. Kramer M. Nutritional advice in pregnancy (Cochrane Review). *Cochrane Library. Oxford: Update Software* 1999(1).
13. Kramer MS. Effects of energy and protein intakes on pregnancy outcome: an overview of the research evidence from controlled clinical trials. *Am J Clin Nutr* 1993;58:627-35.
14. Ceesay SM, Prentice AM, Cole TJ, Foord F, L.T. W, Poskitt EME, et al. Effects on birth weight and perinatal mortality of maternal dietary supplements in rural Gambia: 5 year randomised controlled trial. *Br Med J* 1997;315:786-90.
15. Gulmezoglu M, de Onis M, Villar J. Effectiveness of interventions to prevent or treat impaired fetal growth. *Obstet Gynecol Surv* 1997;52:139-49.
16. Kramer M. Balanced protein/energy supplementation in pregnancy (Cochrane review). *Cochrane Library. Oxford: Update Software* 1999(1).
17. Moser P, Reynolds R, Acharya S, Howard M, Andon M, Lewis S. Copper, iron, zinc, and selenium dietary intake and status of Nepalese lactating women and their breast-fed infants. *Am J Clin Nutr* 1988;47:729-34.
18. King J, Stein T, Doyle M. Effect of vegetarianism on the zinc status of pregnant women. *Am J Clin Nutr* 1981;34:1049-55.
19. Caulfield L, Zavaleta N, Shankar A, Merialdi M. Potential contribution of maternal zinc supplementation during pregnancy to maternal and child survival. *Am J Clin Nutr* 1998;68 (suppl):S499-508.
20. Ministry of Health. Nepal Micronutrient Status Survey 1998. Kathmandu: Ministry of Health, Child Health Division, HMG/N, New ERA, Micronutrient Initiative, UNICEF Nepal, WHO, 2001.
21. Nepal South Asia Centre. Nepal human development report 1998. Kathmandu: Nepal South Asia Centre, 1998.



22. Underwood B. Maternal vitamin A status and its importance in infancy and early childhood. *Am J Clin Nutr* 1994;59 (suppl):517S-24.
23. Underwood B, Arthur P. The contribution of vitamin A to public health. *FASEB J* 1996;10:1040-8.
24. Dibley M, Jeacocke D. Vitamin A in pregnancy: impact on maternal and neonatal health. *Food Nutr Bull* 2001;22:267-84.
25. Ghebremeskel K, Burns L, Burden T, Harbige L, Costeloe K, Powell J, et al. Vitamin A and related essential nutrients in cord blood: relationships with anthropometric meaurements at birth. *Early Hum Dev* 1994;39:177-88.
26. Crosby W, Metcoff J, Costiloe J, Mameesh M, Sandstead H, Jacob R, et al. Fetal malnutrition: an appraisal of correlated factors. *Am J Obstet Gynecol* 1977;128:22-31.
27. Baker H, Thind I, Frank O, DeAngelis B, Caterini H, Louria D. Vitamin levels in low-birth-weight newborn infants and their mothers. *Am J Obstet Gynecol* 1977;129:521-4.
28. Metcoff J, Costiloe J, Crosby W, Bentle L, Seshachalam D, Sandstead H, et al. Maternal nutrition and fetal outcome. *Am J Clin Nutr* 1981;34:708-21.
29. Howells D, Haste F, Rosenberg D, Brown I, Brooke O. Investigation of vitamin A nutrition in pregnant British Asians and their infants. *Hum Nutr Clin Nutr* 1986;40C:43-50.
30. Neel N, Alvarez J. Chronic fetal malnutrition and vitamin A in cord serum. *Eur J Clin Nutr* 1990;44:207-12.
31. Tamura T, Goldenberg R, Johnston K, Cliver S, Hoffman H. Serum concentrations of zinc, folate, vitamins A and E, and proteins, and their relationships to pregnancy outcome. *Acta Obstet Gynaecol Scand* 1997;165 suppl:63-70.
32. Shenai J, Chytil F, Jhaveri A, Stahlman M. Plasma vitamin A and retinol-binding protein in premature and term neonates. *J Pediatr* 1981;99:302-5.
33. Shah R, Rajalakshmi R. Vitamin A status of the newborn in relation to gestation age, body weight and maternal nutritional status. *Am J Clin Nutr* 1984;40:794-800.
34. Shah R, Rajalakshmi R, Bhatt R, Hazra M, Patel B, Swamy N, et al. Liver stores of vitamin A in human fetuses in relation to gestational age, fetal size and maternal nutritional status. *Br J Nutr* 1987;58:181-9.
35. Rondo P, Abbott R, Rodrigues L, Tomkins A. Vitamin A, folate, and iron concentrations in cord and maternal blood of intra-uterine growth retarded and appropriate birth weight babies. *Eur J Clin Nutr* 1995;49:391-9.
36. Suharno D, West C, Muhilal, Karyadi D, Hautvast J. Supplementation with vitamin A and iron for nutritional anaemia in pregnant women in West Java, Indonesia. *Lancet* 1993;1 342:1325-8.
37. Coutsoudis A, Pillay K, Spooner E, Kuhn L, Coovadia H. Randomized trial testing the effect of vitamin A supplementation on pregnancy outcomes and early mother-to-child HIV-1 transmission in Durban, South Africa. South African Vitamin A Study Group. *AIDS* 1999;13:1517-24.
38. Katz J, West K, Khatry S, Pradhan E, LeClerq S, Christian P, et al. Maternal low-dose vitamin A or beta-carotene supplementation has no effect on fetal loss and early infant mortality: a randomized cluster trial in Nepal. *Am J Clin Nutr* 2000;71:1570-6.
39. Christian P, West K, Khatry S, LeClerq S, Kimbrough-Pradhan E, Katz J, et al. Maternal night blindness increases risk of mortality in the first 6 months of life among infants in Nepal. *J Nutr* 2001;131:1510-12.
40. Doyle W, Crawford M, Wynn A, Wynn S. Maternal magnesium intake and pregnancy outcome. *Magnesium Res* 1989;2:205-10.
41. Heller S, Salkeld R, Korner W. Vitamin B1 status in pregnancy. *Am J Clin Nutr* 1974;27:1221-4.
42. Heinze T, Weber W. Determination of thiamine in maternal blood during normal pregnancies and pregnancies with IUGR. *Zeitschrift fur Ernahrungswissenschaft* 1990;29:39-46.
43. Badart-Smook A, van Houwelingen A, Al M, Kester A, Hornstra G. Fetal growth is associated positively with maternal intake of riboflavin and negatively with maternal intake of linoleic acid. *J Am Diet Assoc* 1997;97:867-70.
44. Heller S, Salkeld R, Korner W. Riboflavin status in pregnancy. *Am J Clin Nutr* 1974;27:1225-30.
45. Mahomed K, Gulmezoglu A. Pyridoxine (B6) in pregnancy (Cochrane Review). *Cochrane Library. Oxford: Update Software* 1999(1).
46. Alton-Mackey M, Walker B. Graded levels of pyridoxine in the rat diet during gestation and the physical and neuromotor development of offspring. *Am J Clin Nutr* 1973;26:420-8.
47. Roepke J, Kirksey A. Vitamin B6 nutriture during pregnancy and lactation. I. Vitamin B6 intake, levels of the vitamin in biological fluids, and condition of the infant at birth. *Am J Clin Nutr* 1979;32:2249-56.
48. Schuster K, Bailey L, Mahan C. Vitamin B6 status of low-income adolescent and adult pregnant women and the condition of their infants at birth. *Am J Clin Nutr* 1981;34:1731-5.
49. Schuster K, Bailey L, Mahan C. Effect of maternal pyridoxine-HCL supplementation on the vitamin B-6 status of mother and infant and on pregnancy outcome. *J Nutr* 1984;114:977-88.
50. Temperly I, Meehan M, Gatenby P. Serum folic acid levels in pregnancy and their relationship to megaloblastic marrow change. *Br J Haematol* 1968;14:13.
51. Frery N, Huel G, Leroy M, Moreau T, Savard R, Blot P, et al. Vitamin B12 among parturients and their newborns and its relationship with birth weight. *Eur J Obstet Gynaecol Reprod Biol* 1992;45:155-63.

52. Shojania A. Folic acid and vitamin B12 deficiency in pregnancy and in the neonatal period. *Clin Perinatol* 1984;11:433-59.
53. Mahomed K. Routine folate supplementation in pregnancy (Cochrane Review). *Cochrane Library. Oxford: Update Software* 1999(1).
54. Scholl T, Johnson W. Folic acid: influence on the outcome of pregnancy. *Am J Clin Nutr* 2000;71(suppl):1295S-303S.
55. Black R. Micronutrients in pregnancy. *Br J Nutr* 2001;85 (Suppl 2):S193-7.
56. Scholl T, Hediger M, Schall J, Khoo C, Fischer R. Dietary and serum folate: their influence on the outcome of pregnancy. *Am J Clin Nutr* 1996;63:520-5.
57. Rondo P, Tomkins A. Folate and intrauterine growth retardation. *Ann Trop Paediatr* 2000;20:253-8.
58. Baumslag N, Edelstein T, Metz J. Reduction of incidence of prematurity by folic acid supplementation in pregnancy. *Br Med J* 1970;1:16-17.
59. Iyengar L, Rajalakshmi K. Effect of folic acid supplement on birth weights of infants. *Am J Obst Gynecol* 1975;122:332-6.
60. Rolschau J, Date J, Kristoffersen K. Folic acid supplement and intrauterine growth. *Acta Obstet Gynecol Scand* 1979;58:343-6.
61. Giles P, Harcourt A, Whiteside M. The effect of prescribing folic acid during pregnancy on birth-weight and duration of pregnancy. A double-blind trial. *Med J Aust* 1971;2:17-21.
62. Blot I, Papiernik E, Kalwasser J, Werner E, Tchernia G. Influence of routine administration of folic acid and iron during pregnancy. *Gyn Obs Invest* 1981;12:294-304.
63. Matthews F. Antioxidant nutrients in pregnancy: a systematic review of the literature. *Nutr Res Rev* 1996;9:175-95.
64. Wideman G, Baird G, Bolding O. Ascorbic acid deficiency and premature rupture of fetal membranes. *Am J Obstet Gynecol* 1964;88:592-5.
65. Casanueva E, Magana L, Pfeffer F, Baez A. Incidence of premature rupture of membranes in pregnant women with low leucocyte levels of vitamin C. *Eur J Clin Nutr* 1991;45:401-5.
66. Barrett B, Sowell A, Gunter E, Wang M. Potential role of ascorbic acid and beta carotene in the prevention of preterm rupture of fetal membranes. *Int J Vit Nutr Res* 1994;64:192-7.
67. Mahomed K, Gulmezoglu A. Vitamin D supplementation in pregnancy (Cochrane Review). *The Cochrane Library. Oxford: Update Software* 1999(1).
68. Brunvand L, Quigstad E, Urdal P, Haug E. Vitamin D deficiency and fetal growth. *Early Hum Dev* 1996;45:27-33.
69. Marya R, Rathee S, Lata V, Mudgil S. Effects of vitamin D supplementation in pregnancy. *Gynecol Obstet Invest* 1981;12:155-61.
70. Brooke O, Brown I, Bone C, Carter A, CLeeve H, Maxwell P, et al. Vitamin D supplements in pregnant Asian women: effects on calcium status and fetal growth. *Br Med J* 1990;1:751-4.
71. Mallet E, Gugi B, Brunelle P, Henocq A, Basuyau J, Lemeur H. Vitamin D supplementation in pregnancy: a controlled trial of two methods. *Obstet Gynecol* 1986;68:300-4.
72. Basu T, Dickerson J. *Vitamins in health and disease*. Wallingford, Oxon: CAB International, 1996.
73. Tateno M, Oshima A. The relation between serum vitamin E levels in the perinatal period and the birth weight of the neonate. *Acta Obstet Gynaecol Jap* 1973;20:177-81.
74. von Mandach U, Huch R, Huch A. Maternal and cord serum vitamin E levels in normal and abnormal pregnancy. *Int J Vit Nutr Res* 1993;63:26-32.
75. Jagadeesan V, Prema K. Plasma tocopherol and lipid levels in mother and umbilical cord; influence on birth weight. *Br J Obstet Gynaecol* 1980;87:908-10.
76. Ibeziako P, Ette S. Vitamin E levels in pregnant Nigerian women and newborn. *J Trop Med Hyg* 1982;85:265-8.
77. Villa Elizaga I, Da Cunha Ferreira R. Zinc, pregnancy and parturition. *Acta Pediatr Scand Suppl* 1985;319:150-7.
78. Simmer K, Thompson R. Maternal zinc and intrauterine growth retardation. *Clin Sci* 1985;68:395-9.
79. Swanson C, King J. Zinc and pregnancy outcome. *Am J Clin Nutr* 1987;46:763-71.
80. Tamura T, Goldenberg R. Zinc nutriture and pregnancy outcome. *Nutr Res* 1996;16:139-81.
81. Mahomed K. Zinc supplementation in pregnancy (Cochrane Review). *Cochrane Library. Oxford: Update Software* 1999(1).
82. King J. Determinants of maternal zinc status during pregnancy. *Am J Clin Nutr* 2000;71(suppl):1334S-43S.
83. Brown K, Wuehler S. Zinc and human health. Results of recent trials and implications for program interventions and research. Ottawa: Micronutrient Initiative, International Development Research Centre, 2000.
84. Shah D, Sachdev H. Effect of gestational zinc deficiency on pregnancy outcomes: summary of observation studies and zinc supplementation trials. *Br J Nutr* 2001;85 (2 Suppl):S101-8.
85. Scholl T, Hediger M, Schall J, Fischer R, Khoo C-S. Low zinc intake during pregnancy: its association with preterm and very preterm delivery. *Am J Epidemiol* 1993;137:1115-24.
86. Meadows N, Smith M, Keeling P, Ruse W, Day J, Scopes J, et al. Zinc and small babies. *Lancet* 1981;ii:1135-7.
95. Neggers Y, Cutter G, Acton R, Alvarez J, Bonner J, Goldenberg R, et al. A positive association between maternal serum zinc concentration and birth weight. *Am J Clin Nutr* 1990;51:678-84.
96. Bahl L, Chaudhuri L, Pathak R. Study of serum zinc in neonates and their mothers in Shimla hills (Himachal Pradesh). *Ind J Pediatr* 1994;61:571-5.
97. Kirksey A, Wachs T, Yunis F, Srinath U, Rahmanifar A, McCabe G, et al. Relation of maternal zinc nutriture to pregnancy outcome and infant development in an Egyptian village. *Am J Clin Nutr* 1994;60:782-92.
98. Mukherjee M, Sandstead H, Ratnaparkhi M, Johnson L, Milne D, Stelling H. Maternal zinc, iron, folic acid, and protein nutriture and outcome of human pregnancy. *Am J Clin Nutr* 1984;40:496-507.
99. Fehily D, Fitzsimmons B, Jenkins D, Cremin F, Flynn A, Soltan M. Association of fetal growth with elevated maternal plasma zinc concentration in human pregnancy. *Hum Nutr Clin Nutr* 1986;40C:221-7.
100. Kapoor R, Misra P, Dixit S, Wakhlu I, Sharma B, Seth T. Zinc and intrauterine growth. *Ind Pediatr* 1988;25:972-6.

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87. Jones R, Keeling P, Hilton P, Thompson R. The relationship between leucocyte and muscle zinc in health and disease. *Clin Sci* 1981;60:237-9.
88. Meadows N, Ruse W, Keeling P, Scopes J, Thompson R. Peripheral blood leucocyte zinc depletion in babies with intrauterine growth retardation. *Arch Dis Child* 1983;58:807-9.
89. Hambidge K, Droegemueller W. Changes in plasma and hair concentrations of zinc, copper, chromium and manganese during pregnancy. *Obstet Gynecol* 1974;44:666-72.
90. McMichael A, Dreosti I, Gibson G, Hartshorne J, Buckley R, Colley D. A prospective study of serial maternal serum zinc levels and pregnancy outcome. *Early Hum Dev* 1982;7:59-69.
91. Prema K. Predictive value of serum copper and zinc in normal and abnormal pregnancy. *Ind J Med Res* 1980;71:554-60.
92. Swanson C. Reduced serum zinc concentration during pregnancy. *Obstet Gynecol* 1983;62:313-8.
93. Tuttle S, Aggett P, Campbell D, MacGillivray I. Zinc and copper nutrition in human pregnancy: a longitudinal study in normal primigravidae and in primigravidae at risk of delivering a growth retarded baby. *Am J Clin Nutr* 1985;41:1032-41.
94. Tamura T, Goldenberg R, Johnston K, DuBard M. Maternal plasma zinc concentrations and pregnancy outcome. *Am J Clin Nutr* 2000;71:109-13.
101. Jeswani R, Vani S. A study of serum zinc levels in cord blood of neonates and their mothers. *Ind J Pediatr* 1991;58:683-7.
102. Bogden J, Thind I, Kemp F, Caterini H. Plasma concentrations of calcium, chromium, copper, iron, magnesium, and zinc in maternal and cord blood and their relationship to low birth weight. *J Lab Clin Med* 1978;92:455-62.
103. Campbell-Brown M, Ward R, Haines A, North W, Abraham R, McFadyen I. Zinc and copper in Asian pregnancies - is there evidence for a nutritional deficiency? *Br J Obstet Gynaecol* 1985;92:875-85.
104. Marsal K, Furgyik S. Zinc concentrations in maternal blood during pregnancy and post partum, in cord blood and amniotic fluid. *Acta Obstet Gynecol Scand* 1987;66:653-6.
105. Higashi A, Tajiri A, Matsukura M, Matsuda I. A prospective survey of serial maternal serum zinc levels and pregnancy outcome. *J Pediatr Gastroenterol Nutr* 1988;7:430-3.
106. Lazebnik N, Kuhnert B, Kuhnert P, Thompson K. Zinc status, pregnancy complications, and labor abnormalities. *Am J Obstet Gynecol* 1988;158:161-6.
107. Bro S, Berendtsen H, Norgaard J, Host A, Jorgensen P. Serum zinc and copper concentrations in maternal and umbilical cord blood. Relation to course and outcome of pregnancy. *Scand J Clin Lab Invest* 1988;48:805-11.

108. Okonofua F, Amole F, Emofurieta W, Ugwu N. Zinc and copper concentration in plasma of pregnant women in Nigeria. *Int J Gynaecol Obstet* 1989;29:19-23.
109. Okonofua F, Isinkaye A, Onwudiegwu U, Amole F, Emofurieta W, Ugwu N. Plasma zinc and copper in pregnant Nigerian women at term and their newborn babies. *Int J Gynaecol Obstet* 1990;32:243-5.
110. Lao T, Loong E, Chin R, Lam C, Lam Y. Zinc and birth weight in uncomplicated pregnancies. *Acta Obstet Gynecol Scand* 1990;69:609-11.
111. Sikorski R, Juszkiewicz T, Paszkowski T. Zinc status in women with premature rupture of membranes at term. *Obstet Gynecol* 1990;76(675-7).
112. Yasodhara P, Ramaraju L, Raman L. Trace minerals in pregnancy. 1. Copper and zinc. *Nutr Res* 1991;11:15-21.
113. Arnaud J, Preziosi P, Mashako L, Galan P, Nsibu C, Favier A, et al. Serum trace elements in Zairian mothers and their newborns. *Eur J Clin Nutr* 1994;48:341-8.
114. Jameson S. Effects of zinc deficiency in human reproduction. *Acta Med Scand Suppl* 1976;593:3-89.
115. Cavdar A, Arcasoy A, Baycu T, Himmetoglu O. Zinc deficiency and anencephaly in Turkey. *Teratology* 1980;22:141.
116. Bergmann K, Makosch G, Tews K. Abnormalities of hair zinc concentration in mothers of newborn infants with spina bifida. *Am J Clin Nutr* 1980;33:542-4.
117. Cherry F, Bennett E, Bazzano G, Johnson L, Fosmire G, Batson H. Plasma zinc in hypertension/toxemia and other reproductive variables in adolescent pregnancy. *Am J Clin Nutr* 1981;34:2367-75.
118. Soltan M, Jenkins D. Maternal and fetal plasma zinc concentration and fetal abnormality. *Br J Obstet Gynaecol* 1982;89:56-8.
119. Buamah P, Russell M, Bates G, Milford Ward A, Skillen A. Maternal zinc status: a determination of central nervous system malformation. *Br J Obstet Gynaecol* 1984;91:788-90.
120. Velie E, Block G, Shaw G, Samuels G, Scheffer D, Kulldoff M. Maternal supplemental and dietary zinc intake and the occurrence of neural tube defects in California. *Am J Epidemiol* 1999;150:605-16.
121. Stoll C, Dolt B, Alembik Y, Koehl C. Maternal trace elements, vitamin B12, vitamin A, folic acid and fetal malformations. *Reprod Toxicol* 1999;13:53-7.
122. Islam M, Hemalatha P, Bhaskaram P, Kumar P. Leukocyte and plasma zinc in maternal and cord blood: their relationship to period of gestation and birth weight. *Nutr Res* 1994;14:353-60.
123. Wells J, James D, Luxton R, Pennock C. Maternal leucocyte zinc deficiency at start of third trimester as a predictor of foetal growth retardation. *Br Med J* 1987;294:1054-6.
124. Jepsen L, Clemmensen K. Zinc in Danish women during late normal pregnancy and pregnancies with intra-uterine growth retardation. *Acta Obstet Gynecol Scand* 1987;66:401-5.
125. Garg H, Singhal K, Arshad Z. A study of the effect of oral zinc supplementation during pregnancy on pregnancy outcome. *Ind J Physiol Pharmacol* 1993;37:276-84.
126. Goldenberg R, Tamura T, Neggers Y, Copper R, Johnston K, DuBard M, et al. The effect of zinc supplementation on pregnancy outcome. *JAMA* 1995;274:463-8.
127. Simmer K, Lort-Phillips L, James C, Thompson R. A double-blind trial of zinc supplementation in pregnancy. *Eur J Clin Nutr* 1991;45:139-44.
128. Cherry F, Sandstead H, Rojas P, Johnson L, Batson H, Wang X. Adolescent pregnancy: associations among body weight, zinc nutriture, and pregnancy outcome. *Am J Clin Nutr* 1989;50:945-54.
129. Castillo-Duran C, Marin V, Alcazar L, Iturralde H, Ruz M. Controlled trial of zinc supplementation in Chilean pregnant adolescents. *Nutr Res* 2001;21:715-24.
130. Ross S, Nel E, Naeye R. Differing effects of low and high bulk maternal dietary supplements during pregnancy. *Early Hum Dev* 1985;10:295-302.
131. Kynast G, Saling E. Effect of oral zinc application during pregnancy. *Gynecol Obstet Invest* 1986;21:117-23.
132. Osendarp S, van Raaij J, Arifeen S, Wahed M, Baqui A, Fuchs G. A randomized, placebo-controlled trial of the effect of zinc supplementation during pregnancy on pregnancy outcomes in Bangladeshi urban poor. *Am J Clin Nutr* 2000;71:114-9.
133. Hunt I, Murphy N, AE C, Faraji B, Swendseid M, Coulson A, et al. Zinc supplementation during pregnancy: effects on selected blood constituents and on progress and outcome of pregnancy in low-income women of Mexican descent. *Am J Clin Nutr* 1984;40:508-21.
134. Mahomed K, James D, Golding J, McCabe R. Zinc supplementation during pregnancy: a double blind randomised controlled trial. *Br Med J* 1989;299:826-30.
135. Jonsson B, Hauge B, Larsen M, Hald F. Zinc supplementation during pregnancy: a double blind randomised controlled trial. *Acta Obstet Gynecol Scand* 1996;75:725-9.
136. Caulfield L, Zavaleta N, Figueroa A, Leon Z. Maternal zinc supplementation does not affect size at birth or pregnancy duration in Peru. *J Nutr* 1999;129:1563-8.
137. Hunt I, Murphy N, Cleaver A, Faraji B, Swendseid M, Browdy B, et al. Zinc supplementation during pregnancy in low-income teenagers of Mexican descent: effects on selected blood constituents and on progress and outcome of pregnancy. *Am J Clin Nutr* 1985;42:815-28.

138. de Onis M, Villar J, Gulmezoglu M. Nutritional interventions to prevent intrauterine growth retardation: evidence from randomized controlled trials. *Eur J Clin Nutr* 1998;52(Supplement 1):83-93.
139. Scholl T, Hediger M. Anemia and iron-deficiency anemia: compilation of data on pregnancy outcome. *Am J Clin Nutr* 1994;59(suppl):492S-501S.
140. Mahomed K. Routine iron supplementation during pregnancy (Cochrane Review). *The Cochrane Library: Oxford, Update Software* 1998(3).
141. Mahomed K. Routine iron and folate supplementation in pregnancy (Cochrane Review). *The Cochrane Library. Oxford: Update Software* 1999(1).
142. Hemminki E, Starfield B. Routine administration of iron and vitamins during pregnancy: review of controlled clinical trials. *Br J Obstet Gynaecol* 1978;85:404-10.
143. Garn S, Ridella S, Petzold S, Falkner F. Maternal haemoglobin levels and pregnancy outcomes. *Sem Perinatol* 1981;5:155-62.
144. Preziosi P, Prual A, Galan P, Daouda H, Boureima H, Hercberg S. Effect of iron supplementation on the iron status of pregnant women: consequences for newborns. *Am J Clin Nutr* 1997;66:1178-82.
145. Keen C, Uriu-Hare J, Hawk S, Jankowski M, Daston G, Kwik-Uribe C, et al. Effect of copper deficiency on prenatal development and pregnancy outcome. *Am J Clin Nutr* 1998;67 (Suppl):1003S-11S.
146. Breskin M, Worthington-Roberts B, Knopp R, Brown Z, Plovie B, Mottet N, et al. First trimester serum zinc concentrations in human pregnancy. *Am J Clin Nutr* 1983;38:943-53.
147. Kiiholma P, Gronroos M, Rkkola R, Pakarinen P, Nanto V. The role of calcium, copper, iron, and zinc in preterm delivery and premature rupture of foetal membranes. *Gynecol Obstet Invest* 1984;17:194-201.
148. Wasowicz W, Wolkanin P, Bednarski M, Gromadzinska J, Sklodowska M, Grzybowska K. Plasma trace element (Se, Zn, Cu) concentrations in maternal and umbilical cord blood in Poland: relation with birth weight, gestation age, parity. *Biol Trace Element Res* 1993;38:205-15.
149. Thilly C, Delange F, Lagasse R, Bourdoux P, Ramidul L, Berguist H, et al. Fetal hypothyroidism and maternal thyroid status in severe endemic goitre. *J Clin Endocrinol Metab* 1978;47:354-60.
150. Chaouki M, Benmilloud M. Prevention of iodine deficiency disorders by oral administration of lipiodol during pregnancy. *Eur J Endocrinol* 1994;130:547-51.
151. Makrides M, Crowther C. Magnesium supplementation during pregnancy (Cochrane Review). *The Cochrane Library. Oxford: Update Software* 1999(1).
152. Nieto-Diaz A, Villar J, Matorras-Weinig R, Valenzuela-Ruiz P. Intrauterine growth retardation at term: association between anthropometric and endocrine parameters. *Acta Obstet Gynecol Scand* 1996;75:127-31.
153. Kurzel R. Is low serum magnesium associated with premature labor? *Ann NY Acad Sci* 1993;678:350-2.
154. Kovacs L, Molnar E, Huhn E, Bodis L. Magnesium substitution in der Schwanggerschaft. *Geburtsch Frauenheilk* 1988;48:595-600.
155. Spatling L, Spatling G. Magnesium supplementation in pregnancy. A double-blind study. *Br J Obstet Gynaecol* 1988;95:120-5.
156. D'Ameida A, Carter J, Antol A, Prost C. Effects of a combination of evening primrose oil (gamma linolenic acid) and fish oil (eicosapentaenoic + docosahexaenoic acid) versus magnesium and placebo in preventing pre-eclampsia. *Women and Health* 1992;19:117-31.
157. Sibai B, Villar L M, Bray E. Magnesium supplementation during pregnancy: a double-blind randomized controlled clinical trial. *Am J Obstet Gynecol* 1989(115-9).
158. Martin R, Perry K, Hess L, Martin J, Morrison J. Oral magnesium and the prevention of preterm labor in a high-risk group of patients. *Am J Obstet Gynecol* 1992;166:144-7.
159. Arikan G, Panzitt T, Gucer F, Boritsch J, Trojovski A, Haeusler M. Oral magnesium supplementation and the prevention of preterm labour. *Am J Obstet Gynaecol* 1997;176:S45.

160. Combs G. Selenium in global food systems. *Br J Nutr* 2001;85:517-47.
161. Darlow B, Inder T, Graham P, Sluis K, Malpas T, Taylor B, et al. The relationship of selenium status to respiratory outcome in the very low birth weight infant. *Pediatrics* 1995;96:314-9.
162. Arthur J, Beckett G, Mitchell J. Interactions between iodine and selenium deficiencies in man and animals. *Nutr Res Rev* 1999;12:57-75.
163. Keen C, Zidenberg-Cherr S. Should vitamin-mineral supplements be recommended for all women with childbearing potential? *Am J Clin Nutr* 1994;59(suppl):532S-9S.
164. Czeizel A. Controlled studies of multivitamin supplementation on pregnancy outcomes. *Ann NY Acad Sci* 1993;687:266-75.
165. Schmidt K. Antioxidant vitamins and beta-carotene: effects on immunocompetence. *Am J Clin Nutr* 1991;53(Supplement 1):S383-5.
166. Taylor D, Kenyon S, Tarnow-Modi W. Infection and preterm labour. *Br J Obstet Gynaecol* 1997;104:1338-40.
167. Brocklehurst P. Infection and preterm delivery. *Br Med J* 1999;318:548-9.
168. Dammann O, Leviton A. Maternal intrauterine infection, cytokines, and brain damage in the preterm newborn. *Pediatr Res* 1997;42:1-8.
169. Fried M, Muga R, Misore A, Duffy P. Malaria elicits type 1 cytokines in the human placenta: IFNg and TNFa associated with pregnancy outcomes. *J Immunology* 1998; 160:2523-30.
170. Saito S, Umekage H, Sakamoto Y, Sakai M, Tanebe K, Sasaki Y, et al. Increased T-helper-1-type immunity and decreased T-helper-2-type immunity in patients with preeclampsia. *Am J Reprod Immunol* 1999;41: 297-306.
171. Kupferminc M, Peaceman A, Wigton T, Rehnberg K, Socol M. Tumor necrosis factor-a is elevated in plasma and amniotic fluid of patients with severe preeclampsia. *Am J Obstet Gynecol* 1994;170: 1752-9.
172. Ghidini A, Jenkins C, Spong C, Pezzullo J, Salafia C, Eglinton G. Elevated amniotic fluid interleukin-6 levels during early second trimester are associated with greater risk of subsequent preterm delivery. *Am Jour Reprod Immunol* 1997;37: 227-31.
173. Maeda K, Matsuzaki N, Fuke S, Mitsuda N, Shimoya K, Nakayama M, et al. Value of the maternal interleukin 6 level for determination of histologic chorioamnionitis in preterm delivery. *Gynecol Obstet Invest* 1997;43:225-31.
174. Marzi M, Vigano A, Trabattoni D, Villa M, Salvaggio A, Clerici E, et al. Characterization of type 1 and type 2 cytokine production profile in physiologic and pathologic human pregnancy. *Clin Exp Immunol* 1996;106:127-33.
175. Scholl T, Hediger M, Bendich A, Schall J, Smith W, Krueger P. Use of multivitamin/mineral prenatal supplements: influence on the outcome of pregnancy. *Am J Epidemiol* 1997;146:134-41.
176. Chappell L, Seed P, Briley A, Kelly F, Lee R, Hunt B, et al. Effect of antioxidants on the occurrence of pre-eclampsia in women at increased risk: a randomised trial. *Lancet* 1999;354:810-6.
177. Grimble R. Nutritional modulation of cytokine biology. *Nutrition* 1998;14:634-40.
178. Long K, Santos J. Vitamins and the regulation of the immune response. *Pediatr Inf Dis J* 1999;18:283-90.
179. Orvieto R, Ben-Rafael Z. The role of cytokines in early detection of preeclampsia. *Med Hypoth* 1994;43:315-8.
180. Thompson D, Milford-Ward A, Whicher J. The value of acute phase protein measurements in clinical practice. *Ann Clin Biochem* 1992;29:123-31.
181. Fuchs D, Weiss G, Reibnegger G, Wachter H. The role of neopterin as a monitor of cellular immune activation in transplantation, inflammatory, infectious, and malignant diseases. *Crit Rev Clin Lab Sci* 1992;29:307-41.
182. Fuchs D, Stahl-Henning C, Gruber A, Murr C, Hunsmann G, Wachter H. Neopterin - its clinical use in urinalysis. *Kidney Int* 1994;47 Supplement:S8-11.
183. Filteau S, Raynes J, Simmank K, Wagstaff L. Vitamin A status does not influence neopterin production during illness or health in South African children. *Br J Nutr* 1998;80:75-9.
184. Burns D, Nourjah P, Wright D, Minkoff H, Landesman S, Rubinstein A, et al. Changes in immune activation markers during pregnancy and postpartum. *J Reprod Immunol* 1999;42:147-65.
185. Radunovic N, Kuczynski E, Rebarber A, Nastic D, Lockwood C. Neopterin concentrations in fetal and maternal blood: a marker of cell-mediated immune activation. *Am J Obstet Gynecol* 1999;181: 170-3.
186. Filteau S, Rice A, Ball J, Chakraborty J, Stoltzfus R, de Francisco A, et al. Breast milk immune factors in Bangladeshi women supplemented postpartum with retinol or b-carotene. *Am J Clin Nutr* 1999;69:953-8.
187. Filteau S, Lietz G, Mulokozi G, Bilotta S, Henry C, Tomkins A. Milk cytokines and subclinical breast inflammation in Tanzanian women: effects of dietary red palm oil or sunflower oil supplementation. *Immunol* 1999;97:595-600.
188. Hogan J, Weiss W, Smith K. Role of vitamin E and selenium in host defence against mastitis. *J Dairy Sci* 1993;76: 2795-2803.

## Ethical clearance

The MIRA Janakpur study has been reviewed and supported by the ethical committees of the Nepal Health Research Council and the Institute of Child Health, London.

The MIRA Janakpur study follows the CONSORT guidelines for randomised controlled trials [4], and is monitored by a Trial Monitoring Committee.

## Agreements

The MIRA Janakpur study operates under a joint agreement with His Majesty's Government, Nepal, Ministry of Health.

Painting by Rebti Mandal

## Funding

The MIRA Janakpur study is funded by a research grant from The Wellcome Trust, UK.

Approximately ten percent of the multiple micronutrient supplements used in the study were donated by UNICEF, Nepal.

## Acknowledgements

The MIRA Janakpur team would like to thank the following for their generous help in setting up the study:

Dr LL Sah, Medical Superintendent

Dr VK Singh, Previous Superintendent

Dr M Sharma Adhikari, Obstetrician

Dr KP Yadav, Medical Officer

Dr RN Pandit, Obstetrician

Dr RK Mahto, Pathologist

## International team

Ramesh Adhikari
*Dean and Professor of Paediatrics, Institute of Medicine, Kathmandu*

Dharma S Manandhar
*President, MIRA. Professor and Head, Department of Paediatrics, Kathmandu Medical College*

Anthony Costello
*Professor of International Child Health, International Perinatal Care Unit, Institute of Child Health, London*

Andrew Tomkins
*Professor and Head of Department, Centre for International Child Health, Institute of Child Health, London*

Suzanne Filteau
*Senior Lecturer in Nutrition, Centre for International Child Health, Institute of Child Health, London*

David Osrin
*Clinical Research Fellow, International Perinatal Care Unit, Institute of Child Health, London*

## Janakpur team

Ram Bahadur Baniya
*Programme Manager*

Anjana Vaidya
*Clinical Coordinator*

Yagya Shrestha
*Senior Technical Officer*

Pusker Manandhar
*Administrative Officer*

Bechan Chaudhary
*Field Supervisor*

Sunita Yadav
*Counsellor*

Binaya Karki
*Field Officer*

## Contact details


Kathmandu miraorg@wlink.com.np
Janakpur mira@jncs.cjb.net
London ipu@ich.ucl.ac.uk