

ORIGINAL ARTICLE

Haematological effects of multimicronutrient supplementation in non-pregnant Gambian women

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Background/Objectives: The use of multimicronutrient (MMN) supplementation to reduce the burden of anaemia in non-pregnant women of reproductive age has been little studied, particularly in Africa. The objective of the study was to evaluate haematological outcomes in non-pregnant, rural Gambian women of reproductive age, receiving daily MMN supplements for 1 year.

Subjects/Methods: The study in 293 women aged from 17 to 45 years old was nested within a double-blind, randomized placebo-controlled trial of periconceptional MMN supplementation [ISRCTN 13687662], using the United Nations International Multiple Micronutrient Preparation (UNIMMAP), received daily for 1 year or until conception. Red cell parameters and free erythrocyte protoporphyrin concentration were measured at baseline and after 12 months in those women who did not conceive.

Results: Anaemic women (haemoglobin concentration <12 g per 100 ml) were more likely to be older and in economic deficit at baseline. Mean change in haemoglobin concentration was $+0.6 \pm 1.4$ g per 100 ml in the intervention arm and -0.2 ± 1.2 g per 100 ml in the placebo arm ($P < 0.001$). After supplementation with MMN, the relative risk of anaemia (<12 g per 100 ml) was 0.59 (0.46, 0.76) compared with placebo. Anaemic subjects at baseline showed an increase in mean haemoglobin from 10.6 g per 100 ml to 11.8 g/l ($P < 0.001$) after MMN supplementation.

Conclusions: MMN supplementation should be considered as a strategy for improving the micronutrient and haematological status of non-pregnant women of reproductive age.

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Introduction

Many women in low-income countries suffer from sub-optimal micronutrition as a consequence of eating diets that are often low in animal source foods and that do not meet dynamic micronutrient requirements throughout the reproductive life cycle (Bartley *et al.*, 2005). Although iron deficiency is the most common cause of nutritional morbidity worldwide (Stoltzfus, 2003), in women of reproductive age concurrent deficiencies of vitamins A, C, B and

folate also frequently occur and all contribute to an increased anaemia risk (Huffman *et al.*, 1998). The prevention of multimicronutrient (MMN) deficiencies as a strategy to reduce the risk of prenatal anaemia has been little studied, and attention has tended to focus instead on iron supplementation during pregnancy, with conflicting results (Revez *et al.*, 2007). There are few large intervention trials of MMN supplementation in non-pregnant women of reproductive age and none have been conducted in Africa.

In The Gambia, as in other parts of sub-Saharan Africa, seasonal MMN deficiencies are common (Bates *et al.*, 1984; Powers and Bates, 1987), and maternal anaemia and its sequelae remain frequent problems, particularly in rural areas (Topley, 1968; Powers and Bates, 1987). To assess the effectiveness of MMN supplementation in anaemia prevention in rural Gambian women, we analysed a sub-sample of

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participants enrolled as part of a large MMN, placebo-controlled supplementation trial.

Materials/subjects and methods

Study design and intervention

This study was nested within the Periconceptional Multimicronutrient Supplementation Trial (PMMST) [ISRCTN 13687662], based in 31 rural Gambian villages of Kiang West under demographic surveillance by the UK Medical Research Council field station in Keneba and which ran between February 2005 and June 2008. Between March and July 2006, resident females aged 17–45 years were identified from the demographic database and pre-randomized to receive daily supplementation with United Nations International Multiple Micronutrient Preparation (UNIMMAP) or placebo. For ease of administration in the field, randomization was to one of the four colour-coded groups (two for intervention; two for placebo), stratified by age. Researchers were blinded to subject allocation scheme, and subjects and field staff knew only the colour-coded allocation groups. UNIMMAP is a balanced preparation of 15 vitamins and minerals formulated by an Expert Committee of the United Nations Children's Fund, World Health Organization and the United Nations University, primarily for use by pregnant and lactating women. The composition of UNIMMAP is shown in Table 1 (UNICEF/WHO/UNU, 1999). Eligible females were invited to attend a recruitment clinic, where informed consent was sought in writing from individuals or guardians, in the cases of girls under 18 years of age. Consenting non-pregnant women who were menstruating, not breastfeeding an infant, not using contraception or not severely anaemic (haemoglobin concentration <7 g per

100 ml) were included in the trial. Exclusions were referred for clinical management as required. Women received 30 tablets according to their allocated intervention group and were instructed to take one tablet daily. They were also asked to attend weekly supplementation clinics held in their own villages when they were re-supplied with supplements. Compliance with supplementation was assessed by pill counts (compliance factor = ratio of the number of tablets apparently consumed/number of days enrolled in study). Information on morbidity and menstrual patterns was also recorded at these visits. The subjects were asked to notify fieldworkers of suspected pregnancy and after confirmation of pregnancy by study midwives, women were instructed to stop supplementation and no further supplements were issued. These women received a standard antenatal preparation of 60 mg elemental iron and 250 µg of folic acid daily. They were followed in the antenatal study clinic at regular intervals until delivery. Women who did not conceive while enrolled in the PMMST had received at least 1 year of MMN supplementation or placebo and were still resident in the selected villages in Kiang West, were eligible for inclusion in this study. Data collection for this study took place between July and August 2007.

Permission for this study was obtained from the London School of Hygiene and Tropical Medicine Ethics Committee and the Gambian Government/MRC Joint Ethics Committee. The parent trial was subjected to the scrutiny of an independent Data Safety and Monitoring Board and operated under the auspices of Good Clinical Practice guidelines (MRC, 1998). MRC Keneba offers free primary health care to anyone who consults, in collaboration with the Gambian Government Lower River Divisional Health Team. Apart from the structured clinical contacts outlined (during which subjects were given transport and a meal), no additional benefits were provided to trial participants.

Table 1 Composition of the UNIMMAP

Micronutrient	Dose ^a
Vitamin A	800 RE
Vitamin D	200 IU
Vitamin E	10 mg
Vitamin C	70 mg
Vitamin B1	1.4 mg
Vitamin B2	1.4 mg
Niacin	18 mg
Vitamin B6	1.9 mg
Vitamin B12	2.6 mg
Folic acid	400 µg
Iron	30 mg
Zinc	15 mg
Copper	2 mg
Selenium	65 µg
Iodine	150 µg

Abbreviation: UNIMMAP, United Nations International Multiple Micronutrient Preparation.

^aDoses are based on US/Canadian-recommended daily allowances in pregnancy (UNICEF/WHO/UNU, 1999).

Study location and population

Kiang West is a semi-arid area of Savannah scrubland with a rural population of 14 000 people, predominantly of the Mandinka tribe. HIV (human immunodeficiency virus) status was not assessed during this study, but national sentinel data from antenatal clinics indicates a HIV prevalence of around 1% (Schim van der Loeff *et al.*, 2003). The sub-sample was drawn from 12 of the original 31 villages, which was chosen on the grounds of their larger size and easier accessibility. They included the core research villages of the Keneba, Kanton Kunda and Manduar, and nine of the villages to the east and west of Keneba. This provided a broad, yet-manageable sample frame from within the original trial cohort, given the logistical constraints under which this study was carried out. Consenting subjects who were present in the village on an allotted day were recruited. Anthropometry and blood samples were collected from participants during village visits

Sample size

Mean recruitment haemoglobin for the PMMST study was 11.7 g per 100 ml (s.d. 1.5) for a population of 1156. A sample of 150 for each trial arm was required to detect a change in haemoglobin of 0.5 g per 100 ml between study arms with 5% significance and 80% power.

Data and sample collection

Individual informed consent was obtained in the local language by Gambian MRC fieldworkers. A thumbprint was requested from women who were unable to write. An information sheet in English was provided for women to take away. A questionnaire assessing marital status and socioeconomic status was carried out. Economic situation was assessed by the ownership of radio and/or bicycle and information on perceived economic status. Participants were asked whether they considered the household's economic situation in the preceding year to have been always in deficit, occasionally deficit, balanced or surplus. A household was categorized as in 'economic deficit' if the subject answered either always deficit or occasionally deficit (Hyder *et al.*, 2004). A 1-week dietary history, menstrual and medical history were completed for each subject. A stool sample was requested and sterile universal containers provided at the study visit following the trial. At initial recruitment within the main study, baseline stool samples had not been collected. Venous blood was placed in EDTA, sedivette and serum tubes (Sarstedt Monovette, Leicester, UK). Height was measured to the nearest centimetre using a stadiometer (Seca Leicester, UK), and weight to the nearest 100 g using an electronic scale (Tanita, Middlesex, UK). Mid-upper arm circumference was measured at the mid-point of the left arm using standard tapes (TALC, Herts, UK). Data were double entered into the MRC Keneba database (MS Access, Microsoft, Redmond, WA, USA).

Laboratory analysis

Haematological analysis included haemoglobin concentration, white blood cell count, mean cell volume, mean cell haemoglobin, reticulocyte count (Cel-Dyn 3700 Analyser, Abbott Diagnostics, Abbott Park, IL, USA and Medonic CA530, Clinical Diagnostic Solutions Inc., Plantation, FL, USA) and zinc protoporphyrin concentration (AVIV ZP Hematofluorometer Model 206, Analis Scientific Instruments, Namur, Belgium). The erythrocyte sedimentation rate (ESR) (Sarstedt) and C-reactive protein (CRP) (Vitros system 250, Johnson & Johnson, Rochester, NY, USA) were measured. Malaria blood films were prepared and stained with Giemsa's stain, and 100 microscopic fields were examined to determine parasite count. Wet stool samples were prepared for examination by light microscopy for the presence of helminths.

Both Cell-dyn and Medonic CA530 analysers were used for processing of haematological samples because of a technical fault with the Cell-dyn machine. The correlation coefficient between the results for the two machines was 0.96 ($P < 0.001$).

Statistical analysis

Data were blinded until completion of the analysis, which was performed on an intention-to-treat basis. Data were processed and analysed using SPSS for Windows v15. Log transformation was used for non-normal distributions. Anaemia was defined as haemoglobin concentration < 12 g per 100 ml (WHO/UNU/UNICEF, 2001). Mean haemoglobin concentration change (baseline to follow-up) was compared between treatment groups with analysis of covariance. Variables that did not require adjustment for baseline values were compared using an independent *t*-test for continuous variables, Wilcoxon's rank sum for non-normally distributed variables (CRP) and a χ^2 test of proportions for categorical variables. The odds ratio (OR) and relative risk (RR) for anaemia in relation to MMN supplement, age and ESR were computed using logistic regression. Differences were considered significant at $P < 0.05$.

Results

Of 1156 women recruited for the original trial, 421 were considered to be eligible for this study according to the above-mentioned criteria. Of these, 293 subjects were identified and recruited (Figure 1). The subjects who could

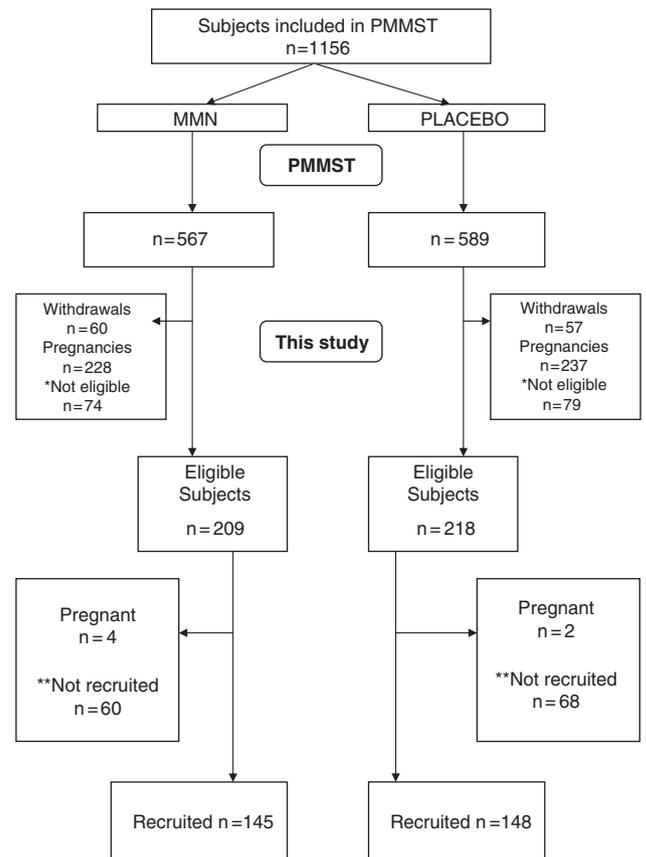


Figure 1 Trial profile: subjects entering PMMST and further enrolment into the haematological sub-study.

not be traced by the field teams or who did not attend after direct invitation were not recruited ($n=128$). Baseline characteristics of recruited ($n=293$) and non-recruited ($n=128$) women are summarized in Table 2. Untraced women were more likely to be nulliparous, to have lower mean anthropometric indices and were more likely to have received formal schooling. There were no differences in haematological parameters. Baseline characteristics of the intervention and placebo groups were not different except for total white cell concentration at baseline (Table 3). The estimate of compliance did not differ between treatment groups (MMN: 0.96 ± 0.18 ; placebo: 0.92 ± 0.19 , $P=0.15$). The mean time of follow-up was similar in both groups (MMN: 14.1 ± 1.2 months, placebo 14.0 ± 1.2 months). Baseline predictors of anaemia, after adjustment (Table 4), included older age (OR = 2.01 (1.16, 3.48)) and economic deficit (OR = 1.98 (1.21, 3.25)).

After the intervention period, mean haemoglobin concentration was higher in women receiving micronutrients (MMN: 12.3 ± 1.1 g per 100 ml, placebo: 11.5 ± 1.4 g per 100 ml), with a mean change in haemoglobin concentration of $+0.6 \pm 1.4$ g per 100 ml (MMN) and -0.2 ± 1.2 g per 100 ml (placebo) ($P < 0.001$). The increase in haemoglobin concentration in the intervention group relative to placebo was more marked in women who were anaemic at the start of the study (Figure 2). Although age and economic deficit were predictors of anaemia at baseline, in a subanalysis these groups did not respond differently to supplementation. Table 3 includes the haematological findings at follow-up. Supplementation was associated with increased haemoglobin concentration and mean cell volume and reduced zinc protoporphyrin concentration (all $P < 0.001$). The reduction in reticulocyte count was marginally

significant ($P=0.04$). ESR was also noted to be lower in the MMN group than that in the placebo group (22 ± 13.5 mm/h versus 26 ± 15.5 mm/h, $P=0.02$). Raised ESR values (>20 mm/h) were less frequent in supplemented women (OR = 0.71 (0.55, 0.91)).

There were no differences in anthropometric indices (BMI, weight and mid-upper arm circumference) between the two groups (Table 3). No subjects had malaria parasitaemia at follow-up. Median CRP was 0 mg/l for both groups, and there were no differences in proportion of women with an increased CRP (>10 g/l) in either group. Only one stool sample showed hookworm infection.

At baseline, anaemia prevalence was 50.7% (MMN group) and 54.1% (placebo group). After supplementation, these were 36 and 62%, respectively, generating RR = 0.59 (0.46, 0.76) for anaemia in the MMN group. Anaemia was less frequent in the MMN group ($P < 0.001$) and more frequent in older women ($P=0.01$), and there was no significant interaction between age and intervention group.

There were no significant differences in combined or system-specific morbidity profiles between study groups (data not shown).

Discussion

To our knowledge, this is the first African trial to evaluate the effect of MMN supplementation on red cell parameters and iron status among non-pregnant women of reproductive age. Twelve months of daily MMN supplementation significantly improved haemoglobin concentration in this group of women. The associated decrease in zinc protoporphyrin concentration and increased mean cell volume in

Table 2 Characteristics of recruited and non-recruited eligible subjects before intervention period

	Untraced women (n = 128)	Traced women (n = 293)	P-value
<i>Characteristics</i>			
Age (years)	25.2 ± 7.9	32.4 ± 8.7	<0.001
BMI (kg/m ²)	21.1 ± 3.2	22.3 ± 4.4	0.03
Weight (kg)	54.6 ± 9.5	57.1 ± 11.5	0.007
MUAC (mm)	259.5 ± 28.6	268.9 ± 37.9	0.012
Proportion nulliparous ^a	0.66 (0.58, 0.74)	0.30 (0.25, 0.35)	<0.001
Formal schooling (proportion) ^a	0.33 (0.24, 0.41)	0.10 (0.07, 0.13)	<0.001
Meat or fish eaten (days/week)	3.9 ± 2.0	4.5 ± 1.8	0.04
Haemoglobin ^b (g per 100 ml)	11.9 ± 1.5	11.7 ± 1.5	0.18
MCV (fl) ^b	80.6 ± 8.02	81.7 ± 7.91	0.22
MCH (pg) ^b	27.6 ± 3.3	27.9 ± 3.28	0.30
ZnPP (µmol per mol haeme) ^{b,c}	97.9 (87.5, 109.3)	87.9 (79.3, 97.3)	0.09
Malaria film positive (no) ^d	0	3	0.89
Total white cells (× 10 ⁹ /l)	5.19 ± 1.21	4.87 ± 1.33	0.02
Reticulocyte (%) ^b	1.64 ± 0.68	1.58 ± 0.52	0.30

Abbreviations: BMI, body mass index; CI, confidence interval; MCH, mean cell haemoglobin; MCV, mean cell volume; MUAC, mid-upper arm circumference; ZnPP, zinc protoporphyrin concentration.

^aProportions and 95% CIs given, χ^2 test performed.

^bOnly 127 in the untraced group and 292 in the traced group baseline haematological samples not being available.

^cGeometric means.

^dOnly 127 in the untraced group and 283 in the traced group baseline samples not being available.

Table 3 Characteristics of subjects at baseline and after intervention

Characteristics	MMN		Placebo		P-value ^b
	Baseline (n = 145)	After intervention (n = 142) ^a	Baseline (n = 148)	After intervention (n = 148)	
Age (years)	33 ± 8.6	—	34 ± 8.8	—	—
Proportion nulliparous ^c	0.35 (0.27, 0.43)	—	0.25 (0.18, 0.32)	—	—
Formal schooling (proportion) ^c	0.12 (0.06, 0.17)	—	0.09 (0.04, 0.13)	—	—
Meat or fish eaten (days/week)	4.6 ± 1.8	—	4.4 ± 1.8	—	—
No of months in study	14.1 ± 1.2	—	14.0 ± 1.2	—	—
Malaria film positive (no) ^d	0	0	3	0	—
BMI (kg/m ²)	22.4 ± 5.0	23 ± 5.2	22.1 ± 3.8	22.6 ± 4.2	0.49
Weight (kg)	57.4 ± 12.1	59.0 ± 12.9	56.7 ± 10.9	58.0 ± 11.8	0.49
MUAC (mm)	269.7 ± 41.2	280.1 ± 44.0	267.8 ± 34.6	275.1 ± 37	0.30
Haemoglobin (g per 100 ml)	11.7 ± 1.5	12.3 ± 1.1	11.6 ± 1.5	11.5 ± 1.4	<0.001
MCV (fl)	81.2 ± 8.5	81.9 ± 6.2	82.1 ± 7.3	80.6 ± 7.2	<0.001
MCH (pg)	27.8 ± 3.5	32.0 ± 3.9	28.1 ± 3.1	32.0 ± 4.4	0.76
ZnPP (µmol per mol haeme) ^{e,f}	89.5 (86.0, 93.0)	54.0 (52.2, 56.8)	86.4 (82.8, 90.0)	72.2 (68.5, 75.9)	<0.001
Total white cells (× 10 ⁹ /l)	5.02 ± 1.30	5.03 ± 2.1	4.71 ± 1.35	4.50 ± 2.0	0.08
Reticulocyte (%)	1.64 ± 0.62	1.60 ± 0.56	1.51 ± 0.40	1.56 ± 0.41	0.04
ESR (mm/h) ^g	—	22 ± 13.5	—	26 ± 15.5	0.02 ^h
CRP ⁱ	—	0	—	0	0.73

Abbreviations: BMI, body mass index; CI, confidence interval; CRP, C-reactive protein, ESR, erythrocyte sedimentation rate; MCH, mean cell haemoglobin; MCV, mean cell volume; MUAC, mid-upper arm circumference; ZnPP, zinc protoporphyrin concentration.

^aA total of 142 samples as baseline sample not available for one subject, and two subjects did not have samples taken at follow-up.

^bAnalysis of covariance unless otherwise stated. P-values refer to change in mean values between baseline and after intervention and not to the absolute values that are given in the table.

^cProportions and 95% CIs given, χ^2 performed.

^dFisher's exact test.

^eOnly 144 subjects in the MMN group at baseline, as one sample was not available.

^fGeometric means given.

^gOnly 147 samples in the placebo group due to a clotted sample.

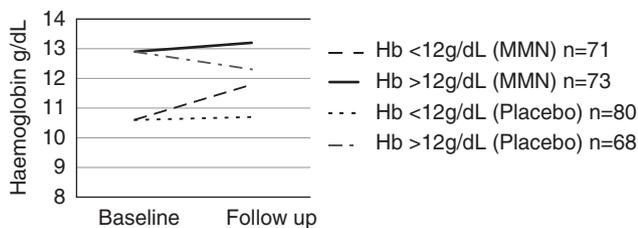
^hStudent's *t*-test.

ⁱNon-parametric tests, Wilcoxon's rank sum, median values quoted.

Table 4 Predictors of haemoglobin < 12 g per 100 ml at baseline

	Odds ratio (CI)	P-value	Adjusted odds ratio (CI)	P-value
Age > 35 years	2.37 (1.54, 3.80)	<0.001	2.01 (1.16, 3.48)	0.012
Nulliparous	0.48 (0.2, 0.80)	0.005	0.74 (0.40, 1.32)	0.29
Economic deficit	2.05 (1.27, 3.33)	0.04	1.98 (1.21, 3.25)	0.007
MUAC (< 21 cm)	1.22 (0.27, 5.54)	0.799	—	—
Meat (> once a week)	0.53 (0.19, 1.50)	0.23	—	—
No formal schooling	1.75 (0.81, 3.77)	0.16	—	—

Abbreviation: MUAC, mid-upper arm circumference.

**Figure 2** Comparison of haemoglobin change in MMN and placebo groups in relation to baseline haemoglobin concentration.

supplemented women suggests that the iron content of the supplements underpinned the improved haematological profile, whereas the differences in reticulocyte count suggest

a reduction in red cell turnover among MMN supplemented women. Anaemia prevalence was almost halved in supplemented women, and anaemic women showed the greatest increase in haemoglobin concentration after supplementation (+ 1.2 g per 100 ml). This would be expected, as subjects who are iron-depleted use iron more effectively than those who are iron-replete (Moriarty-Craige *et al.*, 2004).

ESR was measured in this study. ESR is a nonspecific, indirect measurement of plasma acute-phase protein and systemic inflammatory status that is known to correlate with haemoglobin and plasma fibrinogen concentrations (Kanfer and Nicol, 1997). It is influenced by the size, shape and number of erythrocytes, as well as by other plasma

constituents such as immunoglobulins (Brigden, 1999; Gabay and Kushner, 1999). Interpretation of the effect of supplementation on ESR is complex (Breckenridge and Okpanachi, 1976), but the reduction in ESR after supplementation could indicate an ameliorating effect of MMN on systemic inflammation. However, there was no direct evidence that CRP concentration was affected by micronutrient supplementation. More detailed studies of inflammatory markers and MMN supplementation are required to understand their roles in the aetiology of anaemia in this population of women, particularly in areas of high malarial endemicity.

The study was restricted to a sub-sample of geographically selected villages but this does not appear to have biased the results of the study, as the placebo and intervention groups were comparable at baseline. The high compliance, regular follow-up and long duration of our study further increase confidence in the findings. The placebo control group allowed for an absolute evaluation of the effects of micronutrients on haematological parameters. The primary analysis was powered to assess changes in haemoglobin concentration, which limited the scope of this study to the functional effect of MMN supplementation on nutritional anaemia. This outcome represented a simple, clinically relevant measure of composite micronutrient status, which was achievable within the time and resource constraints under which the nested study was carried out. However, an analysis of the effects of supplementation on broader micronutrient status in this population would have been of interest and is a specified outcome in the main trial, which will be reported elsewhere. There was a low prevalence of malaria parasitaemia in this study, which is likely to have been a consequence of cross-sectional sampling of partially immune adults early in the wet season, in an area of highly seasonal endemicity. Although there was no difference in the rate of clinical infection diagnosis between the two arms of the parent trial, longitudinal malaria exposure may have been an effect modifier or confounding variable in this study and ought to be assessed formally in future work.

Iron deficiency is an important risk factor for increased maternal mortality, low birth weight and preterm delivery (Brabin *et al.*, 2001), and the availability of calcium, iodine, folate and vitamins A and D may modulate fetal development (Bartley *et al.*, 2005). To achieve Millennium Development Goals, new strategies to address nutritional problems in women of reproductive age are required. Several recent studies in poorer countries have already shown improved parameters of fetal growth and gestational length with various prenatal MMN supplementation regimens, although effects have been inconsistent and the mechanisms of action are unclear (Christian *et al.*, 2003; Kaestel *et al.*, 2005; Fawzi *et al.*, 2007). There are concerns about possible detrimental interactions between micronutrients combined within a single supplement, but experimental data are sparse and the potential synergistic and positive effects of particular combinations should not be ignored (Sandstrom, 2001). The

timing of supplementation may likewise be a crucial factor (Owens and Fall, 2008). For example, the fact that prenatal folate supplementation substantially reduces the risk of neural tube defect only if it is taken preconceptually is well known, but improving vitamin A status before pregnancy has also been shown to have a dramatic and as-yet unexplained effect on maternal morbidity and mortality (West *et al.*, 1999). Optimizing the pre- and inter-pregnancy micronutritional status of women, particularly in low-resource settings could be extremely beneficial (Huffman *et al.*, 1998; Allen, 2000; Bartley *et al.*, 2005; Owens and Fall, 2008) and may lead to improved subsequent maternal and pregnancy outcomes.

Earlier studies in Mexico and Bangladesh have shown no additional benefit on haematological parameters when women of reproductive age and adolescents were given MMN compared with iron-folate supplements (Moriarty-Craige *et al.*, 2004; Ahmed *et al.*, 2005). The duration of supplementation in these studies was short, different MMN formulations were used and sample sizes were smaller than in this study. Neither trial used a placebo-controlled design, limiting the interpretation of the study results. This study achieved a greater improvement in haemoglobin concentration and a greater decline in the prevalence of anaemia compared with those in earlier reports. The findings from the Bangladesh study did, however, provide additional support for the use of combinations of micronutrients in preference to iron alone, as the prevalence of other nutrient deficiencies, including folic acid, riboflavin and vitamin B12, A and C, were also decreased in addition to improved values for haematological parameters (Ahmed *et al.*, 2005).

In Indonesia, the haemoglobin concentration of adolescent girls improved on supplementation with combinations of iron, folic acid, retinol and vitamin C, over placebo, but did not respond better to daily rather than weekly supplementation, although serum ferritin increased more in the daily-supplemented group (Angeles-Agdeppa *et al.*, 1997). The intervention period was only 3 months compared with 12 months in this study, and there was a high dropout rate.

A study of Bangladeshi adolescents receiving a micronutrient fortified beverage 6 days/week reported haematological effects that were of a similar magnitude to those in this study (Hyder *et al.*, 2007). An increase in serum retinol and improved anthropometric status (weight, height and BMI) in supplemented girls were also recorded (Hyder *et al.*, 2007), indicating the potential benefits of MMN supplementation for a risk group attempting to satisfy increased nutritional requirements for pubertal growth while commonly manifesting a substantial degree of undernutrition. It has frequently been suggested that adolescents should be the focus of targeted intervention strategies, which aim to improve reproductive health particularly before first pregnancies (Leenstra *et al.*, 2007). Although the issue of supplementation in younger women is critical, they may be difficult to target over a long period of time and may not begin their reproductive lives proximal to the supplementation period.

The number of adolescents in this study was small ($n = 23$), and conclusions cannot readily be drawn regarding the haematological responses of this sub-group. By contrast, subjects were generally older, better nourished, multiparous and poorly educated. Two-thirds of untraced women were nulliparous compared with one-third of the traced women, characterizing a group of younger, more mobile but less reproductively active women, akin to that in the Bangladeshi study (Hyder *et al.*, 2007).

We have rather shown that older, anaemic multiparae who are experiencing recurrent demands of pregnancy and lactation under seasonally adverse conditions (Billewicz and McGregor, 1981; Anya, 2004) clearly benefit from MMN supplementation. The findings of this study provide evidence of the efficacy of MMN supplementation and support its promotion in non-pregnant women as a means of optimizing haematological and micronutrient status in between pregnancies, especially in relation to iron status. Community antenatal and paediatric clinics, which often already serve these populations, can readily be used to identify those at particular risk on finger-prick testing and target them for supplementation.

There is now a small but growing body of data supporting the efficacy of prenatal MMNs in improving selected perinatal outcomes of women and babies in resource-poor settings. Studies specifically utilizing UNIMMAP benefit from and contribute to data sets based on a uniform and targeted intervention, facilitating the interpretation and reproducibility of their results. Future studies should compare placebo, iron folate and UNIMMAP supplementation to assess the efficacy and effectiveness of pre- or inter-pregnancy supplementation on later maternal anaemia and pregnancy outcome, as well as the impact on broader micronutrient status and other functional outcomes in this vulnerable group.

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