Effect of provision of daily zinc and iron with several micronutrients on growth and morbidity among young children in Pakistan: a cluster-randomised trial

Sajid Soofi, Simon Cousens, Saleem P Iqbal, Tauseef Akhund, Javed Khan, Imran Ahmed, Anita K M Zaidi, Zulfiqar A Bhutta

Summary

Background Powders containing iron and other micronutrients are recommended as a strategy to prevent nutritional anaemia and other micronutrient deficiencies in children. We assessed the effects of provision of two micronutrient powder formulations, with or without zinc, to children in Pakistan.

Methods We did a cluster randomised trial in urban and rural sites in Sindh, Pakistan. A baseline survey identified 256 clusters, which were randomly assigned (within urban and rural strata, by computer-generated random numbers) to one of three groups: non-supplemented control (group A), micronutrient powder without zinc (group B), or micronutrient powder with 10 mg zinc (group C). Children in the clusters aged 6 months were eligible for inclusion in the study. Powders were to be given daily between 6 and 18 months of age; follow-up was to age 2 years. Micronutrient powder sachets for groups B and C were identical except for colour; investigators and field and supervisory staff were masked to composition of the micronutrient powders until trial completion. Parents knew whether their child was receiving supplementation, but did not know whether the powder contained zinc. Primary outcomes were growth, episodes of diarrhoea, acute lower respiratory tract infection, fever, and incidence of admission to hospital. This trial is registered with ClinicalTrials.gov, number NCT00705445.

Results The trial was done between Nov 1, 2008, and Dec 31, 2011. 947 children were enrolled in group A clusters, 910 in group B clusters, and 889 in group C clusters. Micronutrient powder administration was associated with lower risk of iron-deficiency anaemia at 18 months compared with the control group (odds ratio [OR] for micronutrient powder without zinc=0·20, 95% CI 0·11–0·36; OR for micronutrient powder with zinc=0·25, 95% CI 0·14–0·44). Compared with the control group, children in the group receiving micronutrient powder without zinc gained an extra 0·31 cm (95% CI 0·03–0·59) between 6 and 18 months of age and children receiving micronutrient powder with zinc an extra 0·56 cm (0·29–0·84). We recorded strong evidence of an increased proportion of days with diarrhoea (p=0·001) and increased incidence of bloody diarrhoea (p=0·003) between 6 and 18 months in the two micronutrient powder groups, and reported chest indrawing (p=0·03). Incidence of febrile episodes or admission to hospital for diarrhoea, respiratory problems, or febrile episodes did not differ between the three groups.

Interpretation Use of micronutrient powders reduces iron-deficiency anaemia in young children. However, the excess burden of diarrhoea and respiratory morbidities associated with micronutrient powder use and the very small effect on growth recorded suggest that a careful assessment of risks and benefits must be done in populations with malnourished children and high diarrhoea burdens.

Funding Bill & Melinda Gates Foundation.

Published **Online**
April 18, 2013
http://dx.doi.org/10.1016/S0140-6736(13)60437-7

Department of Paediatrics and Child Health, Division of Women and Child Health, the Aga Khan University, Karachi, Pakistan (S Soofi FCPS, S P Iqbal MSc, T Akhund MSc, J Khan MSc, I Ahmed MSc, Prof A K M Zaidi FAAP, Prof Z A Bhutta PhD)**; and London School of Hygiene and Tropical Medicine, London, UK** (Prof S Cousens DipMathStat, Prof Z A Bhutta)

Correspondence to:
Prof Zulfiqar A Bhutta, Division of Women and Child Health, Aga Khan University, Karachi 74800, Pakistan
zulfiqar.bhutta@aku.edu

Introduction

Worldwide, about 165 million children younger than 5 years were stunted in 2011.[1] Nearly half of all children aged 0–59 months are estimated to be anaemic[2] and 17% of the world's population is estimated to be at risk of zinc deficiency because of low dietary intake of zinc.[3] Zinc supplementation has been identified as an effective intervention to reduce morbidity[4] and improve growth.[5] However, there is no consensus on suitable delivery strategies or vehicles for daily zinc supplementation, and hence no preventive zinc supplementation programmes. The development of micronutrient powders containing microencapsulated iron administered on a daily or regular basis offers a way to provide a regular dose of key micronutrients, particularly iron, to children at risk of deficiency and has been recommended by WHO as a delivery strategy for prevention of iron-deficiency anaemia in at-risk populations.[6] However, most of the evidence relating to micronutrient powders derives from small studies[7] and there is little evidence of the benefit of inclusion of zinc within micronutrient powders. In a cluster-randomised trial[8] of daily administration of micronutrient powders containing 10 mg zinc gluconate among children aged 6–12 months in Cambodia, despite slightly higher serum zinc concentrations at 12 months of age in children who received the powder compared with those who did not, the investigators noted no evidence of improved growth. Although micronutrient powders containing iron are generally regarded as safe[9] and without the concerns associated with the use of iron

supplements in malaria-endemic areas,[10] micronutrient powders have not been assessed in large scale trials with prospective morbidity assessment.[11]

We assessed the effects of provision of two micronutrient powder formulations, with or without zinc, to children aged between 6 and 18 months in urban and rural Pakistan on their growth, micronutrient status, and morbidity.

Methods

Study design and participants

We did a cluster randomised trial with three groups in a cohort of children from urban and rural populations in Sindh. Bilal colony is an urban squatter settlement in Karachi with a population of 75 000. Matiari district, the location of the rural study site, is about 200 km from Karachi with an estimated population of 500 000. Both sites have functional health centres and research infrastructure, and are broadly representative of urban and rural Pakistan.

See Online for appendix

A baseline census was done in the entire urban site and six union councils of the rural district. We identified clusters for inclusion in the trial on the basis of the census and geographic maps, to ensure that clusters were either administratively separated (urban clusters) or geographically distinct (rural clusters). A total of 256 clusters were identified (111 clusters in Bilal Colony and 145 clusters in Matiari) and were allocated to one of three groups: group A was the control group, receiving basic infant and young child feeding messages based on UNICEF/WHO recommendations,[9] namely promotion of exclusive breastfeeding up to 6 months and continued breastfeeding with appropriate complementary feeding with locally available foods thereafter; group B—as group A plus receiving a fortnightly supply of micronutrient powders without zinc for daily use between 6–18 months of age; and group C—as group A and receiving a fortnightly supply of micronutrient powders with zinc (10 mg per day) for daily use between 6–18 months of age.

All children younger than 5 months in the three groups were eligible for inclusion in the study. A medical officer provided information about the study to the families and obtained written informed consent from a parent or guardian. The protocol was approved by the Ethics Review Committee of Aga Khan University (752-Peds/ERC-07). A randomly selected subset of children in each group, roughly every third child, were followed-up weekly for in-depth assessment of intestinal permeability, faecal microbial isolates, and microflora (data to be presented elsewhere).

Randomisation and masking

Clusters were randomly allocated within urban and rural strata, by computer-generated random numbers, to one of the three groups. Sachets of micronutrient powder were identical apart from their colour (group B=brown, group C=green). The colour coding used was known only to the Manager of Genera Pharmaceuticals (Islamabad), who produced the powders under licence, and the Chair of the trial's Data Monitoring Committee. Investigators and field and supervisory staff were masked to composition of the micronutrient powder preparations until after the results of the trial had been presented to the independent Data Monitoring Committee. Since we did not use a placebo powder in the control group, parents knew whether their child was receiving supplementation, but did not know whether the powder contained zinc.

Procedures

The two micronutrient powder formulations were prepared as individual daily dose sachets. They were of identical composition—containing iron, vitamin C, vitamin A, vitamin D, and folic acid—(appendix) with the exception of the addition of 10 mg elemental zinc as zinc gluconate in the formulation administered to group C.

To simulate potential programme settings, 100 community health workers with at least 12 years of schooling were hired at both sites. They received 1 week's training in the delivery of educational messages to the families, using standardised flip charts to promote exclusive breastfeeding (until 6 months) and appropriate complementary feeding with locally available foods thereafter. Community health workers were also trained to advise families on the use of oral rehydration solution and zinc for the treatment of acute diarrhoea and on continued breastfeeding and appropriate complementary feeding during episodes. In the intervention clusters only (groups B and C) the community health workers were trained to distribute a 14 day supply of micronutrient powder sachets to the families of enrolled children every 2 weeks between 6 and 18 months of age with instructions to use only one sachet daily mixed with the child's usual weaning foods. Families were advised to continue micronutrient powders during common illnesses in the same dose. Community health workers counted empty and remaining sachets at each fortnightly visit and replenished supplies.

12 independent teams of data collectors and supervisors received 7 days training in data collection, using standardised data collection instruments, validated for assessment of diarrhoea and respiratory illnesses.[12,13] These teams collected information from mothers of participating children from recruitment to 24 months of age through regular household visits. At each visit information was obtained from families on presence or absence of diarrhoea, including the presence of visible blood in the child's stools; respiratory signs; and fever in the preceding week or 2 weeks. Trained medical officers did monthly anthropometric assessments using standard methods.[14] Length or height, depending on the child's ability to stand, was measured with stadiometers (Shorr Boards, Shorr Productions, Olney, MD, USA). Weight was measured with digital weighing scales (sensitivity 10 g, LAICA, Barbarano, Italy). All data collectors were provided with refresher training at

6-monthly intervals and rotated between clusters to avoid differential interviewer bias across clusters.

A separate clinical team of two medical officers assessed children who were reported to them by community health workers with present diarrhoea, respiratory signs, and other illnesses. No home-based therapy was provided other than promotion of oral rehydration salts for the treatment of diarrhoea and a 10 day course of treatment with zinc (20 mg daily).[15] In the case of serious illness or lack of improvement, families were advised to seek treatment in the nearest available public sector facility.

A data quality assurance team consisting of a physician and a community health worker revisited a random sample of (5%) households about 3 days after the original data collection visit. Compliance and morbidity data were collected and anthropometric assessments done. The overall technical error estimated from these repeat measurements by physicians and community health workers[16] were 1·15% for height and 2·03% for weight measurements.

Diarrhoea was recorded by data collectors on the basis of maternal or care-provider reports of three or more loose stools per day. A diarrhoeal episode was defined as a minimum of 2 days with diarrhoea followed by at least 2 diarrhoea-free days.

Signs (fast breathing, chest indrawing) of acute respiratory illness were recorded as reported by the

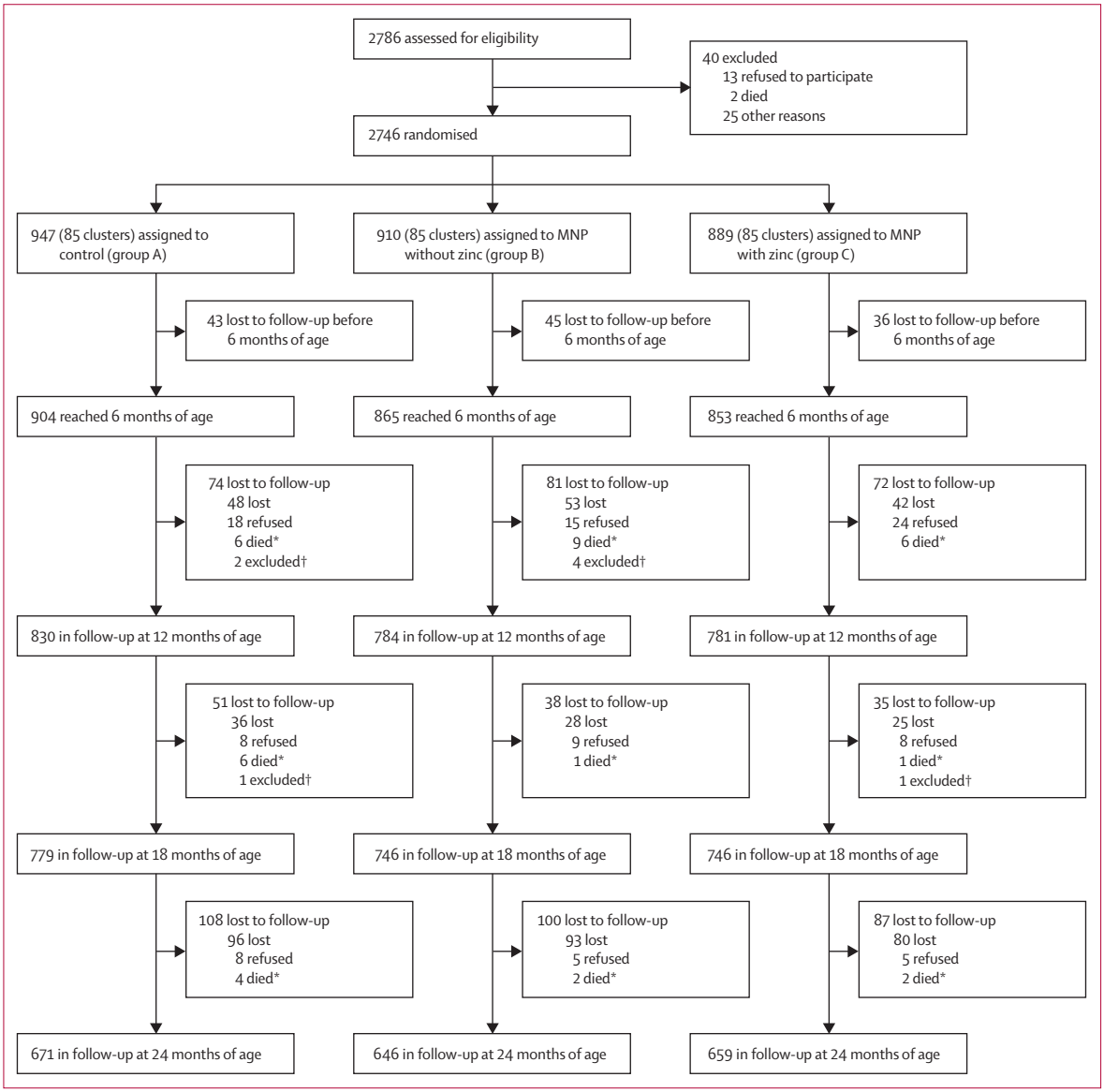

Figure 1: **Trial profile**

MNP=micronutrient powder. *When known, causes of death were diarrhoea, pneumonia, meningitis, sepsis, accident, congenital heart disease, and hepatitis. †Reasons for exlusion were congential abnormalities or birth defects (such as heart disease, microcephaly, hydrocephalus, meningomyocele, Down's syndrome) and chronic illlnesses.

	Bilal colony (urban)			Matiari (rural)			Overall (urban plus rural)		
	Group A: control	Group B: MNP without zinc	Group C: MNP with zinc	Group A: control	Group B: MNP without zinc	Group C: MNP with zinc	Group A: Control	Group B: MNP without zinc	Group C: MNP with zinc
Number of households	3674	3598	3591	8418	8135	7620	12 092	11 733	11 211
Total population	25 908	24 831	25 164	58 435	55 577	53 588	84 343	80 408	78 752
Infants 0–3 months	0·8% (216/25 908)	0·8% (206/24 831)	0·8% (206/25 164)	0·7% (386/58 435)	0·8% (429/55 577)	0·7% (377/53 588)	0·7% (602/84 343)	0·8% (635/80 408)	0·7% (583/78 752)
Children 0–59 months	14·4% (3739/25 908)	14·5% (3590/24 831)	14·4% (3635/25 164)	14·7% (8582/58 435)	14·7% (8179/55 577)	14·7% (7858/53 588)	14·6% (12 321/84 343)	14·6% (11 769/80 408)	14·6% (11 493/78 752)
Piped drinking water	42·5% (1562/3674)	38·9% (1401/3598)	42·8% (1536/3591)	49·3% (4154/8418)	58·3% (4744/8135)	48·4% (3686/7620)	47·3% (5716/12 092)	52·4% (6145/11 733)	46·6% (5222/11 211)
Underground sewerage	96·0% (3528/3674)	94·6% (3404/3598)	93·8% (3370/3591)	3·5% (292/8418)	4·2% (344/8135)	4·2% (320/7620)	31·6% (3820/12 092)	31·9% (3748/11 733)	32·9% (3690/11 211)
Household monthly income (hundreds of rupees)	95·5 (76·2)	99·8 (71·1)	103·5 (76·9)	87·8 (165·8)	90·7 (177·4)	86·9 (170·5)	89·8 (148·0)	93 (157·5)	91·5 (150·4)
Children with diarrhoea in past 2 two weeks	8·1% (304/3739)	8·7% (312/3590)	9·1% (329/3635)	8·8% (758/8582)	9·3% (760/8179)	10·2% (800/7858)	8·6% (1062/12 321)	9·1% (1072/11 769)	9·8% (1129/11 493)
Admitted to hospital for diarrhoea	12·2% (37/304)	13·1% (41/312)	9·4% (31/329)	3·6% (27/758)	3·4% (26/760)	3·0% |(24/800)	6·0% (64/1062)	6·3% (67/1072)	4·9% (55/1129)
Children with respiratory illness in past 2 weeks	13·0% (487/3739)	11·1% (397/3590)	10·8% (392/3635)	10·7% (917/8582)	10·8% (886/8179)	12·5% (979/7858)	11·4% (1404/12 321)	10·9% (1283/11 769)	11·9% (1371/11 493)
Admitted to hospital for respiratory illness	10·5% (51/487)	7·3% (29/397)	8·2% (32/392)	1·2% (11/917)	2·1% (19/886)	1·8% (18/979)	4·4% (62/1404)	3·7% (48/1283)	3·6% (50/1371)

Data are N, % (n/N), or mean (SD). MNP=micronutrient powder.

Table 1: **Baseline characteristics according to study sites and randomised groups**

mother. An acute respiratory illness episode was defined as a minimum of 2 days with signs followed by a sign-free interval of at least 7 days.

For the subset of children visited weekly, routine blood and stool specimens were collected and analysed in the Aga Khan University Research laboratories (Karachi). Blood samples were collected at recruitment and at 6 months and 18 months of age by venepuncture in zinc free tubes and tested with an automated haematology analyser (Beckman Coulter, Galway, Ireland) to measure haemoglobin concentrations and haematocrit.[17] Serum was separated from blood specimens. Serum C-reactive protein (CRP) and ferritin were measured with an immunoturbidimetric method on a Roche/Hitachi 902 (Tokyo, Japan) automated clinical chemistry analyser.[18,19] Serum retinol was measured (by reverse phase high performance liquid chromatography with ultra violet detection at 325 nm [Series 200 HPLC, Agilent Technologies, Boebligen, Germany][20]) and serum zinc by Flame Atomic Absorption Spectrophotometry (Model MK II-6 Thermo Electron Corporation, Cambridge, UK, with three slots burner and Zeeman's Background correction).[21] Ferritin, retinol, and zinc measurements were adjusted in the presence of raised CRP (>95·2 nmol/L): ferritin was multiplied by a factor of 0·8, retinol by a factor of 1·11, and zinc by a factor of 1·2.[22]

Routine stool samples were collected at recruitment, and at 6, 12, 18 and 24 months of age for microbiological assessment. Additionally, diarrhoeal stool samples were collected when mothers reported acute diarrhoea to visiting data collectors and transported within 6 h in transport medium to the Department of Paediatrics Infectious Diseases Research Laboratory at Aga Khan University for assessment of possible bacterial and viral cause for a range of pathogens (appendix), with standard methods.[23]

Statistical analysis

Our original assumption was that the rural clusters each would contribute about 50 child years of observation and that the average diarrhoea incidence in the absence of zinc would be 3·4 episodes per child year. We estimated that variation in incidence of diarrhoea across rural clusters would range from one to six episodes per child year (coefficient of variation=0·37). Similarly, we estimated that each urban cluster would contribute about 25 child years of observation with the average diarrhoea incidence rate of 4·0 episodes per child year (coefficient of variation 0·31). Separate sample size estimations were made to work out the minimum required number of clusters in urban and rural sites.[24] We estimated that 50 clusters per group in the rural site and 36 clusters per group in the urban site would provide 80% power to detect in each site a 20% reduction in diarrhoea incidence with the administration of micronutrient powders and zinc. In practice, the number of children recruited per cluster was much lower than expected, yielding on average about 8 child years per cluster. We therefore did not do separate analyses of rural and urban clusters.

The primary outcomes were growth, episodes of diarrhoea, acute lower respiratory tract infection, fever,

and incidence of admission to hospital. Micronutrient status was a secondary outcome. We analysed data on an intention-to-treat basis, using Stata (version 12). We used a random effects models to account for repeated measures or episodes within children and multiple children in each cluster, fitting models including both child-level and cluster-level random effects when appropriate. For analyses of disease incidence we used random effects Poisson regression. For the analysis of binary outcomes (eg, longitudinal prevalence, stunting) we used random effects logistic regression. In all these analyses we included fixed effects for sex, present age (grouped in 3-month age bands), urban or rural location, and treatment group and controlled for baseline measures of the outcome (before or at 6 months of age) at the cluster level (for morbidity outcomes) or individual level (micronutrient status, anthropometric status). When modelling growth (height, weight) we used restricted cubic splines with knots at 6, 18, and 24 months of age and allowed growth patterns to vary between boys and girls and between urban and rural populations with child-level and cluster-level random intercepts and coefficients.

This trial is registered with ClinicalTrials.gov, number NCT00705445.

Role of the funding source

The study was funded by the Bill & Melinda Gates Foundation who provided funding for the study on the basis of the original project design. The funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report. A Data Monitoring Committee was established at the outset of the study and met four times during the course of the study to assess results, provide clearance to proceed, and to unmask data locking and analysis at the conclusion of the study. ZAB and SS had full access to all data. ZAB had the final responsibility to submit the manuscript for publication.

Results

The study was done between Nov 1, 2008, and Dec 31, 2011. 2786 children in 256 clusters were eligible for the study and after informed consent 2746 entered the study at 6 months of age (figure 1). The appendix shows characteristics of the three groups of children at 6 months of age and initiation of micronutrient powders. Of the 2746 children, 2395 (87%) remained under follow-up at age 12 months, 2271 (83%) at age 18 months, and 1976 (72%) at age 24 months. No clusters were lost.

The three intervention groups were much the same at baseline within the urban and rural populations for a range of indicators including socioeconomic status, and environmental indicators such as access to safe water and sanitation (table 1). The proportion of children with diarrhoea in the previous 2 weeks was similar across urban and rural settings and much the same across treatment groups. A higher proportion of children in the

	Group A: control	Group B: MNP without zinc	Group C: MNP with zinc	p value*
Haemoglobin (g/dL)				
6 months				
N	184	191	194	..
Mean (95% CI)	10·5 (10·3–10·7)	10·5 (10·3–10·7)	10·5 (10·3–10·7)	..
18 months				
N	211	217	210	..
Mean (95% CI)	9·2 (8·9–9·4)	10·2 (10·0–10·4)	10·1 (9·9–10·3)	<0·0001†
Percentage (95% CI) anaemic (Hb <11 g/dL) at 18 months	92% (89–96)	76% (69–83)	72% (66–78)	..
Geometric mean ferritin (ng/mL)				
6 months				
N	161	172	178	..
Mean (95% CI)	22·4 (19·6–25·7)	16·4 (13·8–19·5)	18·5 (15·8–21·6)	..
18 months				
N	131	159	158	..
Mean (95% CI)	9·6 (8·1–11·3)	19·1 (16·5–22·0)	16·0 (13·7–18·9)	0·0001‡
Percentage (95% CI) with iron deficiency (adjusted ferritin <12 ng/mL) at 18 months	64% (55–74)	26% (19–34)	35% (26–43)	..
Prevalence of iron-deficiency anaemia				
6 months				
N	164	173	185	..
% (95% CI)	17·1% (11·9–22·3)	26·6% (18·8–34·4)	24·9% (18·1–31·6)	..
18 months				
N	135	166	162	..
% (95% CI)	57·0% (47·6–66·5)	22·9% (15·7–30·1)	26·5% (19·4–33·7)	0·0001§
Zinc (µg/dL)				
6 months				
N	169	185	185	..
Mean (95% CI)	75·2 (71·0–79·5)	84·0 (78·4–89·6)	84·8 (78·5–91·4)	..
18 months				
N	199	198	203	
Mean (95% CI)	69·4 (65·7–73·0)	68·9 (64·3–73·4)	67·1 (63·1–71·1)	0·39
Percentage (95% CI) zinc deficient (<60 µg/dL) at 18 months	38% (29–46)	44% (36–52)	43% (36–51)	..
Retinol (mmol/L)				
6 months				
N	172	181	182	..
Mean (95% CI)	1·05 (0·96–1·14)	0·92 (0·84–1·00)	0·94 (0·84–1·03)	..
18 months				
N	196	176	190	..
Mean (95% CI)	1·11 (1·03–1·20)	0·95 (0·85–1·04)	0·95 (0·86–1·03)	0·08
Percentage (95% CI) retinol deficient (<0·7 mmol/L) at 18 months	22% (16–29)	40% (33–47)	35% (27–42)	..
Proportion with CRP >1 mg/dL				
6 months				
N	177	190	188	..
% (95% CI)	19% (12–26)	10% (6–14)	9% (5–13)	..
18 months				
N	205	204	205	..
% (95% CI)	9% (5–13)	9% (6–13)	13% (8–18)	0·71

(Continues on next page)

	Group A: control	Group B: MNP without zinc	Group C: MNP with zinc	p value*
(Continued from previous page)				
Mean CRP (mg/dL)				
6 months				
N	177	190	188	..
Mean (95% CI)	0·69 (0·45–0·92)	0·47 (0·36–0·59)	0·59 (0·33–0·85)	..
18 months				
N	205	204	205	..
Mean (95% CI)	0·42 (0·25–0·59)	0·62 (0·25–0·99)	0·54 (0·31–0·79)	0·64

Data are adjusted for acute phase response measured by C-reactive protein estimation. MNP=micronutrient powder. CRP=C-reactive protein. *Testing null hypothesis of no differences between the three groups, adjusted for baseline values, sex, urban or rural location, and cluster randomisation. †MNP without zinc versus control p<0·0001, MNP with zinc versus control p<0·0001, MNP without zinc versus MNP with zinc p=0·56. ‡MNP without zinc versus control p<0·0001, MNP with zinc versus control p=0·001, MNP without zinc versus MNP with zinc p=0·45. §MNP without zinc versus control p<0·0001, MNP with zinc versus control p=0·001, MNP without zinc versus MNP with zinc p=0·43.

Table 2: **Haemoglobin and serum micronutrient status by treatment group**

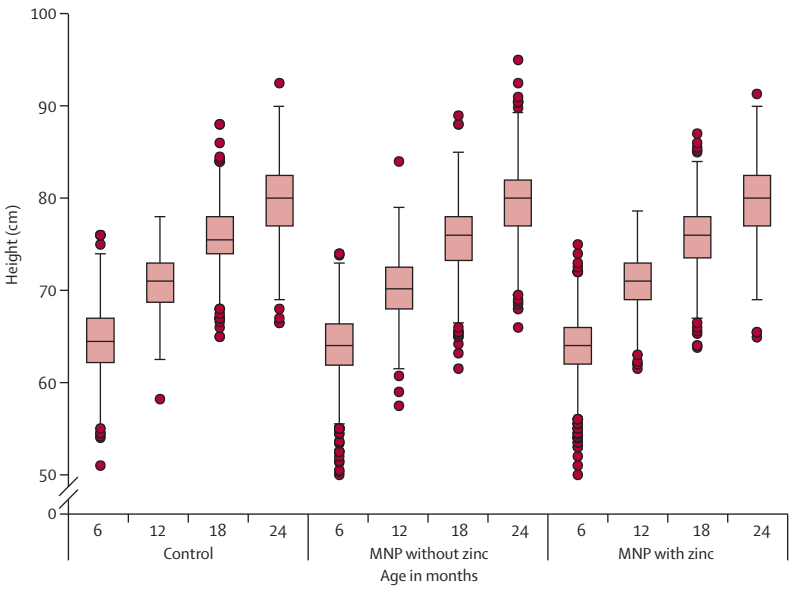

Figure 2: **Measured lengths or heights at 6, 12, 18, and 24 months of age, by treatment group**
MNP=micronutrient powder.

urban setting had been hospitalised with diarrhoea (table 1), presumably indicative of better access compared with the rural setting. Consistent with findings from other similar districts in Pakistan,[25] use of oral rehydration solution was around 40% in all groups but fewer than 2% of children were reported to have received zinc for the treatment of diarrhoea.

The mean number of micronutrient powder sachets consumed each month between ages 6 and 18 months by children in the group without zinc was 16·8 (SD 11·7) and in the group with zinc was 15·2 (11·9). We noted no evidence of reduction in consumption over time nor reports of excess vomiting in either group (appendix).

Table 2 shows mean haemoglobin, and adjusted serum ferritin, retinol, and zinc concentrations at 6 months and 18 months of age. Haemoglobin and ferritin concentrations were much the same across all three treatment groups at 6 months of age, before the start of the intervention, but substantially lower in the control group than in the two micronutrient powder groups at 18 months of age (p<0·0001). In the control group the proportion of children with iron deficiency anaemia (defined as haemoglobin <11 and CRP-adjusted serum ferritin <12) among control children trebled from 17·1% at age 6 months to 57·0% by 18 months of age (table 2). By contrast, the proportions of children with iron-deficiency anaemia at 18 months in the two micronutrient powder groups changed little (table 2; p=0·0001). After taking account of iron deficiency status at 6 months the odds ratios (ORs) of iron deficiency at 18 months were much lower in both micronutrient powder groups than in the control group (OR for micronutrient powder without zinc=0·20, 95% CI 0·11–0·36; OR for micronutrient powder with zinc=0·25, 0·14–0·44). We noted no suggestion that children in the group receiving micronutrient powder with zinc had improved serum zinc status compared with children in either the control group or in the group receiving micronutrient powder without zinc (table 2). In all three groups mean serum retinol changed little between 6 months of age and 18 months of age, providing no evidence that children in either of the micronutrient powder groups had better serum retinol status than children in the control group (table 2).

Figure 2 shows the pattern of linear growth over the course of the study. We identified evidence of small differences in the shapes of the growth curves across the three groups (p=0·0017). Compared with the control group, children in the group receiving micronutrient powder without zinc gained an extra 0·31 cm (95% CI 0·03–0·59) between 6 and 18 months and children in the group receiving micronutrient powder with zinc gained an extra 0·56 cm (0·29–0·84). At 24 months of age the differences in length gain were much the same: 0·44 cm (95% CI 0·04 to 0·84) for micronutrient powder without zinc versus control; 0·53 cm (0·13 to 0·93) for micronutrient powder with zinc versus control.

We identified similar patterns for weight gain (appendix). Children in the micronutrient powder groups gained slightly more weight than did controls during the supplementation period: 0·15 kg (95% CI 0·05–0·24) for micronutrient powder without zinc; 0·13 kg (0·03–0·23) for micronutrient powder with zinc. At 24 months of age the corresponding values were 0·10 kg (–0·02 to 0·22) and 0·06 kg (–0·06 to 0·18), respectively.

Table 3 summarises the anthropometric indicators (stunting, wasting, underweight) for the three treatment groups over the course of the study. After controlling for height-for-age *Z* score at 6 months, we noted some evidence of a beneficial effect of micronutrient powders on odds of stunting at 18 months (p=0·03). At 6 months the prevalence of stunting was lower in the control group than in the micronutrient powder groups, whereas at

18 months stunting prevalence was much the same across all three groups (p=0·60).

Longitudinal prevalence of any diarrhoea in the two micronutrient powder groups was somewhat higher than that in the control group before initiation of micronutrient powder supplementation (table 1). After taking account of cluster-level diarrhoea prevalence before the intervention, we identified strong evidence that the longitudinal prevalence of diarrhoea was higher in the two intervention groups than in the control group between 6 and 18 months of age (table 4; p=0·001). We noted no evidence of differences between the three groups after cessation of micronutrient powder at 18 months of age (table 4; p=0·29). Table 5 shows incidence and rate ratios for reported diarrhoeal episodes (any, bloody, severe [≥6 stools per day], and persistent [>14 days] diarrhoea) for the three treatment groups. We recorded strong evidence of an increased incidence of bloody diarrhoea reported between 6 and 18 months in the two micronutrient powder groups (p=0·003) and weaker evidence (p=0·07) of an increase in severe diarrhoea (≥6 stools per day) among children receiving micronutrient powders (table 5). After 18 months of age, when supplementation ceased, we noted no evidence that either bloody diarrhoea (p=0·41) or severe diarrhoea (p=0·51) were more frequent in either of the micronutrient powder groups than in the control group (table 5).

Between ages 6 and 24 months the use of oral rehydration solution to treat diarrhoea episodes was much the same in the three groups: 30% (1225 of 4110) of episodes in the control group; 32% (1341 of 4157) of episodes in the micronutrient powder without zinc group); 33% (1421 of 4352) of episodes in the micronutrient powder with zinc group. Treatment with zinc was more frequent in the group receiving micronutrient powder without zinc (39% [1627 of 4157] of episodes) than in the control group (24% [986 of 4110] of episodes) or in the group receiving micronutrient powder with zinc (27% [1161 of 4352] of episodes; p=0·003).

From more than 3000 episodes of diarrhoea per group between 6–18 months of age, we were able to obtain stool specimens for microbiology for about 600 episodes per group. A higher proportion of children receiving micronutrient powder had *Aeromonas* spp isolated from their stools compared with controls (5·9% [35 of 593], control group; 7·3% [44 of 606], micronutrient powder without zinc; 11·3% [65 of 577], micronutrient powder with zinc; p=0·002; appendix). Enteropathogenic *Escherichia coli*

	Group A: control group		Group B: MNP without zinc		Group C: MNP with zinc		p value†
	Proportion stunted	Prevalence OR	Proportion stunted	Prevalence OR	Proportion stunted	Prevalence OR	
Age 6 months							
Stunting	23·3% (181/777)	1·0	30·1% (221/734)	1·35 (0·98–1·85)	26·9% (191/709)	1·19 (0·86–1·63)	..
Wasting	21·0% (163/777)	1·0	23·6% (173/733)	1·14 (0·86–1·50)	22·0% (156/708)	1·05 (0·79–1·39)	..
Underweight	30·0% (241/803)	1·0	36·3% (276760)	1·29 (0·98–1·68)	34·7% (255/734)	1·26 (0·96–1·65)	..
Age 18 months							
Stunting	51·4% (321/625)	1·0	51·9% (320/617)	0·75 (0·54–1·04)	49·8% (310/622)	0·65 (0·47–0·89)	0·03‡
Wasting	16·8% (105/624)	1·0	16·9% (104/614)	0·86 (0·58–1·27)	21·1% (131/622)	1·25 (0·86–1·83)	0·15
Underweight	36·7% (260/709)	1·0	39·5% (271/687)	0·90 (0·64–1·28)	38·6% (267/691)	0·96 (0·68–1·36)	0·85
Age 24 months							
Stunting	55·8% (344/616)	1·0	56·9% (341/599)	0·93 (0·63–1·35)	55·4% (330/596)	0·94 (0·64–1·38)	0·92
Wasting	16·2% (100/616)	1·0	15·4% (92/599)	0·87 (0·59–1·28)	17·5% (104/596)	0·96 (0·65–1·41)	0·76
Underweight	43·0% (281/653)	1·0	42·4% (271/639)	0·76 (0·55–1·05)	46·9% (299/638)	1·01 (0·73–1·38)	0·15

Data are % (n) or OR (95% CI) unless otherwise specified. For all categories, *Z* score <–2. MNP=micronutrient powder. OR=odds ratio. *ORs at 6 and 18 months are adjusted for the children's *Z* scores at 6 months of age, and urban or rural location and sex. †p value for null hypothesis that ORs in MNP groups are both 1 versus the alternative that they are not both 1. ‡MNP without zinc versus control p=0·08, MNP with zinc versus control p=0·009, MNP without zinc versus MNP with zinc p=0·37.

Table 3: **Anthropometric status at 6, 18, and 24 months of age by trial group***

	Group A: control group		Group B: MNP without zinc		Group C: MNP with zinc		p value†
	Proportion of days with diarrhoea	OR	Proportion of days with diarrhoea	OR	Proportion of days with diarrhoea	OR	
Age 6·0–17·9 months	5·7% (13 913)	1·0	6·5% (15 836)	1·15 (1·00–1·33)	6·7% (16 166)	1·31 (1·13–1·51)	0·001‡
Age 18·0–23·9 months	4·3% (4718)	1·0	3·8% (3964)	0·85 (0·66–1·08)	4·3% (4501)	1·01 (0·77–1·28)	0·29

Data are % (number of days with diarrhoea) or OR (95% CI) unless otherwise specified. MNP=micronutrient powder. OR=odds ratio. *ORs, CIs, and p values are adjusted for age, sex, urban or rural location, and for cluster-level prevalence at baseline (for children older than 6 months). †Testing the null hypothesis that longitudinal prevalence is equal in all three groups versus the alternative that it is not equal in all three groups. ‡MNP without zinc versus control p=0·06, MNP with zinc versus control p=0·0002, MNP without zinc versus MNP with zinc p=0·08.

Table 4: **Reported diarrhoea longitudinal prevalence by trial group and age group***

was less frequently detected in children in the group receiving micronutrient powder without zinc (9·2%, 50 of 542) than the other two groups (13·2%, [71 of 537], control group; 14·1% [71 of 503], micronutrient powder with zinc; p=0·04).

Table 6 summarises data for respiratory signs and febrile episodes for the three groups after initiation of supplementation. The reported incidence of respiratory episodes involving either rapid breathing or chest indrawing was higher in the group receiving micronutrient powder with zinc than either of the other two groups. This difference was seen between both 6 and 18 months of age (p<0·0001) and, less strongly, between 18 and 24 months of age (p=0·004). The reported incidence of episodes with chest indrawing was higher in the two intervention groups than in the control group between

	Group A: control group		Group B: MNP without zinc		Group C: MNP with zinc		p value†
	Incidence per child year	IRR	Incidence per child year	IRR	Incidence per child year	IRR	
Age 6·0–17·9 months							
Any diarrhoea	3·73 (3030)	1·0	4·16 (3229)	1·05 (0·94–1·17)	4·32 (3323)	1·12 (1·01–1·26)	0·12
Bloody diarrhoea	0·08 (69)	1·0	0·16 (124)	1·63 (1·12–2·39)	0·17 (132)	1·88 (1·29–2·74)	0·003‡
Severe diarrhoea (≥6 stools per day)	1·31 (1063)	1·0	1·94 (1503)	1·28 (1·03–1·57)	1·69 (1304)	1·17 (0·95–1·45)	0·07
Persistent diarrhoea (>14 days)	0·06 (51)	1·0	0·10 (75)	1·41 (0·87–2·28)	0·09 (68)	1·33 (0·82–2·16)	0·34
Admission to hospital with diarrhoea	0·04 (29)	1·0	0·05 (37)	1·30 (0·71–2·38)	0·04 (31)	1·01 (0·54–1·90)	0·63
Age 18·0–23·9 months (after intervention)							
Any diarrhoea	3·13 (1080)	1·0	2·80 (928)	0·90 (0·77–1·05)	3·08 (1029)	0·99 (0·85–1·16)	0·31
Bloody diarrhoea	0·15 (52)	1·0	0·14 (48)	1·01 (0·61–1·69)	0·11 (38)	0·73 (0·42–1·25)	0·41
Severe diarrhoea (≥6 stools per day)	1·35 (468)	1·0	1·36 (453)	1·02 (0·80–1·30)	1·23 (411)	0·89 (0·70–1·14)	0·51
Persistent diarrhoea (>14 days)	0·05 (16)	1·0	0·05 (17)	1·07 (0·47–2·43)	0·07 (25)	1·65 (0·77–3·54)	0·36
Admission to hospital with diarrhoea§	0·01 (4)	1·0	0·03 (9)	2·48 (0·69–8·87)	0·01 (3)	0·83 (0·17–4·00)	0·19

Data are incidence (number of episodes) or IRR (95% CI) unless otherwise specified. MNP=micronutrient powder. IRR=incidence rate ratio. *IRRs, CIs, and p values are adjusted for age, sex, urban or rural location, and for cluster-level baseline diarrhoea rates. †Testing the null hypothesis that incidence is equal in all three groups versus the alternative that incidence is not equal in all three groups. ‡MNP without zinc versus control p=0·01, MNP with zinc versus control p=0·001, MNP without zinc versus MNP with zinc p=0·42. §Unadjusted analysis because small number of events.

Table 5: **Reported diarrhoea incidence by trial group and period after intervention***

	Group A: control group		Group B: MNP without zinc		Group C: MNP with zinc		p value†
	Incidence per child year	IRR	Incidence per child year	IRR	Incidence per child year	IRR	
Age 6·0–17·9 months							
Rapid breathing or chest indrawing	2·03 (1636)	1·0	2·17 (1674)	0·85 (0·69–1·04)	3·11 (2376)	1·61 (1·32–1·96)	<0·0001‡
Chest indrawing	0·23 (185)	1·0	0·30 (232)	1·36 (1·01–1·84)	0·32 (245)	1·49 (1·10–2·01)	0·03§
Admission to hospital with suspected pneumonia or severe pneumonia	0·02 (13)		0·02 (14)	1·15 (0·44–3·00)	0·02 (14)	1·08 (0·41–2·84)	0·96
Fever alone	0·46 (378)	1·0	0·55 (431)	1·20 (0·93–1·54)	0·46 (355)	0·95 (0·73–1·22)	0·14
Admission to hospital with fever alone	0·004 (3)	..	0·004 (3)	..	0 (0)	..	
Age 18·0–23·9 months (after intervention)							
Rapid breathing or chest indrawing	1·14 (387)	1·0	1·37 (445)	0·93 (0·63–1·38)	1·47 (480)	1·66 (1·14–2·41)	0·004¶
Chest indrawing‖	0·03 (10)	1·0	0·04 (13)	1·79 (0·62–5·18)	0·05 (16)	2·13 (0·76–6·00)	0·57
Admission to hospital with pneumonia or severe pneumonia	0·008 (3)	..	0·006 (2)	..	0·008 (3)	..	
Fever alone	0·45 (154)	1·0	0·52 (173)	1·10 (0·80–1·51)	0·55 (184)	1·05 (0·76–1·44)	0·85
Admission to hospital with fever alone	.. (0)	..	.. (0)	..	.. (0)	..	..

Data are incidence (number of episodes) or IRR (95% CI) unless otherwise specified. MNP=micronutrient powder. IRR=incidence rate ratio. *IRRs, CIs, and p values are adjusted for age, sex, urban rural or location, and baseline cluster-level disease rates unless otherwise specified.†Testing the null hypothesis that incidence is equal in all three groups versus the alternative that incidence is not equal in all three groups. ‡MNP without zinc versus control p=0·11, MNP with zinc versus control p<0·0001, MNP without zinc versus MNP with zinc p<0·0001. §MNP without zinc versus control p=0·05, MNP with zinc versus control p=0·01, MNP without zinc versus MNP with zinc p=0·55. ¶MNP without zinc versus control p=0·73, MNP with zinc versus control p=0·008, MNP without zinc versus MNP with zinc p=0·003. ||Unadjusted analysis because small number of events.

Table 6: **Reported incidence of respiratory signs and fever by trial group and period after intervention***

6 months and 18 months of age (p=0·03). Between 18 and 24 months of age there were too few episodes involving chest indrawing to establish whether this pattern was maintained. We noted no clear evidence that the incidence of episodes of fever alone differed between the three groups (p=0·14, 6–18 months; p=0·85, 18–24 months).

Discussion

Provision of micronutrient powder from age 6 months was associated with lower risk of iron-deficiency anaemia at 18 months compared with no supplementation. Compared with the control group, children in the group receiving micronutrient powder without zinc gained about an extra 3 mm in height and children receiving micronutrient powder with zinc an extra 6 mm between 6 and 18 months of age. However, we recorded strong evidence of an increased proportion of days with diarrhoea, increased incidence of bloody diarrhoea, and increased reporting of chest indrawing in children in the micronutirent powder groups compared with those in the control group. Incidence of admission to hospital and other severe illnesses did not differ between the three groups.

As far as we are aware, our trial is the largest safety study and cluster randomised trial of micronutrient powders up to now. The reported effect of micronutrient powders on anaemia and iron-deficiency anaemia is broadly consistent with previous findings[26–30] and supports the contention that a longer period of supplementation might result in a greater effect on iron status and anaemia.[31] The reported adherence with periodic supply of micronutrient powders is much the same as that reported in other studies[30] and in view of the duration of the study, might be the best that can be achieved in programme settings. However, even a 12 month period of supplementation did not eliminate iron-deficiency anaemia and the overall effect on mean haemoglobin was modest. The absence of a beneficial effect of micronutrient powders on serum retinol could relate to the reportedly high rates of biannual vitamin A supplementation (>90%) in this region.

An important objective of the study was to assess the effectiveness of this approach to delivery of a daily 10 mg dose of zinc to children at high risk of zinc deficiency in improving zinc status and hence growth, as few studies of micronutrient powders have assessed effects on linear growth or weight gain.[7] No effect on serum zinc concentrations was detected and although the reported benefits on growth were much the same as those estimated from a meta-analysis[5] of zinc supplementation trials using 10 mg daily for 6 months, the biological benefit of the 3 mm extra length gain in the micronutrient powder plus zinc group over 12 months is unclear. Our finding of little effect of micronutrient powder supplementation (with or without additional zinc) on growth is consistent with the results of a recent study in Cambodia,[8] which did not detect an effect of micronutrient powder on growth although a small improvement in serum zinc concentrations (3 µg/dL) was reported. Notably, however, background rates of undernutrition were lower in the Cambodian study than in the present study, and most children in Cambodia consumed a rice-based diet, which probably has a lower phytate content than the largely wheat-based and maize-based diets in Pakistan.[8]

The findings of increased morbidity (especially bloody diarrhoea and acute respiratory signs) in both micronutrient powder supplementation groups are new and potentially worrying. As far as we are aware, this is the first large scale study of micronutrient powders to have systematically collected information on morbidity over a 12 month period of supplementation. Most previous studies of micronutrient powders have involved a shorter duration of supplementation, typically around 2–3 months, and micronutrient powder programmes do not collect such information through regular household surveillance. That iron supplementation might be associated with an increase in diarrhoea has been noted previously. In a meta-analysis of 28 trials of iron supplementation Gera and Sachdev[32] documented an 11% increase in diarrhoea (incidence rate ratio 1·11; 95% CI 1·01–1·23; p=0·04) but did not judge this excess of diarrhoea to be programmatically relevant because the difference in incidence was 0·05 episodes per child year. In our study the difference between micronutrient powder groups and the control group in incidence for bloody diarrhoea was around 0·08 per child year which corresponds to about one additional episode of bloody diarrhoea per year for every 12–13 children treated. Consistent with our findings, a 49% excess in dysenteric episodes among infants has been reported from a study of daily oral iron (15 mg elemental iron) supplementation in Bangladesh.[33]

Iron supplementation has been shown to alter the microbial flora of African children leading to a preponderance of Gram negative bacteria[34] and has been shown to increase the pathogenic potential of Gram negative bacteria at the intestinal epithelial interface.[35] In a study[36] of multiple micronutrient supplementation with or without zinc among Tanzanian children (aged 6–60 months) the micronutrient group had an excess of diarrhoea (hazard ratio 1·19; 95% CI 0·94–1·50), particularly in children with asymptomatic giardiasis at baseline (2·03; 1·24–3·32). Although we do have microbiological information on a proportion (about 20%) of diarrhoeal episodes in all groups, we did not identify evidence of an excess of any specific pathogen other than *Aeromonas* spp in the micronutrient powder supplemented groups. Aeromonas is widely regarded as a true pathogen[37] and has been noted to be a very common cause of diarrhoea and dysentery in children in Pakistan and Bangladesh in a large global enteric multicentre study (Zaidi AKM, unpublished).

We do not have an explanation for the reported excess of respiratory morbidity, which was consistently high during the period of supplementation and thereafter.

This relation has not been examined in previous studies of supplementation with micronutrient powder. Potential iron toxicity has been reported from a malaria endemic area,[38] and it should be noted that this included an excess of admissions to hospitals and deaths attributed to non-malarial febrile illness, including pneumonia in children who were iron replete or sufficient at baseline, which has never been satisfactorily explained. The use of micronutrient powders has been generally deemed safe in malaria-endemic areas[39] because of lower absorption of the microencapsulated form of iron compared with other pharmacological iron preparations.[40] The WHO guideline on use of micronutrient powders in at-risk children[6] and the systematic review accompanying it,[7] do not discuss the possibility of adverse effects.

Several limitations should be recognised in considering our data, especially the coverage achieved. We sought to replicate programmatic delivery strategies that could be used by public sector community health workers such as in the Lady Health Workers programme[41] and hence we used fortnightly replenishment of supplies. Although attrition rates were higher between 18–24 months of age when no intervention was being delivered compared with the invention groups, they were 13–14% at 18 months of age. Although receipt of health care and uptake of diarrhoea treatment was much the same in all groups, a higher proportion of children in the micronutrient powder without zinc group received zinc as part of diarrhoea therapy compared with the other two groups. This finding could have reduced the difference in overall zinc intake between the micronutrient powder without zinc and micronutrient powder with zinc groups. We noted no excess of reported refusals or vomiting in the micronutrient powder with zinc group, but the slightly lower intake in this group overall suggests that taste masking might have been less than perfect, leading to an average 10% lesser micronutrient powder consumption in the micronutrient powder with zinc group.

Our findings with regard to diarrhoea and respiratory morbidity are based on maternal recall, with no placebo used in the control group. Mothers were thus aware of their child's allocation to micronutrient powders or not. The absence of masking could have resulted in differential recall between the control group and the micronutrient powder groups. To account for increased reported morbidity in the micronutrient powder groups this outcome would have required control mothers to under-report or intervention group mothers to over report morbidity, or both. Although we cannot entirely exclude this possibility, in reality, if mothers in the control group felt dissatisfied that their child was not receiving an intervention, we believe any differential misclassification is more likely to have occurred in the opposite direction. The agreement between data collectors' observation and reported maternal fast breathing on the day of the visit by the community health worker was 95% (data not shown). We did not do an objective verification of blood in the stools or bedside stool guaiac testing; however, the data collection instruments and questions specifically queried mothers on fresh blood as opposed to dark or black stools. Since neither urban nor rural study areas were high malaria burden areas, we did not do nor collect information on malaria tests or antimalarial therapy during the course of this study.

Missing data are also a limitation. Among children with follow-up beyond 6 months of age (ie, into the intervention period), the average number of days of follow-up between 6 and 18 months of age was about 280 (of 365). Rates of follow-up were very similar across the three groups but if so-called missingness mechanisms

Panel: Research in context

Systematic review

The most up-to-date Cochrane review[7] assessed the effects and safety of fortification with micronutrient powders consumed by children younger than 2 years towards improving health outcomes and was followed by a set of WHO recommendations and guidelines for use.[6] The review included eight trials (3748 participants; six micronutrient powders versus nothing or placebo and two trials comparing micronutrient powders with iron drops) of micronutrient powders containing between five and 15 micronutrients and were done in low-income countries in Asia, Africa, and the Caribbean. Home fortification with micronutrient powder reduced anaemia among infants and young children by 31% (six trials, 1447 participants, risk ratio [RR] 0·69; 95% CI 0·60–0·78) and iron deficiency by 51% (four trials, 586 participants, RR 0·49; 95% CI 0·35–0·67) but no effect on growth could be shown. We systematically reviewed scientific literature to include new studies published up to November, 2012. We searched PubMed, Cochrane Libraries, Embase, and WHO Regional Databases to identify all published and unpublished trials. We used the combinations of the search terms "micronutrient*" OR "multiple micronutrient" OR "multi-vitamin" OR "multi-mineral" OR "micronutrient powder" OR "MNP" OR "sprinkle" AND "Fortifi*" OR "food fortifi*" OR "point of use" OR "home fortification". Additional studies were identified by hand searching references from included studies.

We identified an additional seven studies,[8,11,42–46] excluding the present study. Pooled analysis from these studies suggested that micronutrient powders significantly reduced the prevalence of anaemia by 34% (11 trials, 5734 participants; RR 0·66, 95% CI 0·57–0·77), iron-deficiency anaemia by 59% (six trials, 1905 participants; 0·41, 0·29–0·58), and retinol deficiency by 21% (three trials, 1468 participants; 0·79, 0·64–0·98). Effects were non-significant for serum zinc (three trials, 1549 participants, standard mean difference [SMD] –0·22, 95% CI –0·52 to 0·09), zinc deficiency (two trials, 1058 participants; RR 1·02, 95% CI 0·87–1·19), and linear growth (three trials, 524 participants; height-for-age *Z* score [HAZ] SMD 0·04, 95% CI –0·13 to 0·22).

Interpretation Inclusion of the findings from the present study with the above results confirm the significant benefit of micronutrient powder on reduction of prevalence of anaemia and iron deficiency anaemia by 34% and 57%, respectively, with no benefits on linear growth or weight gain. However, consistent with the findings from previous meta-analysis of liquid iron supplementation studies in children,[34] administration of micronutrient powder is associated with significant increase in diarrhoea (four trials, 3466 participants, RR 1·04, 95% CI 1·01–1·06) with non-significant effects on fever and acute respiratory infections. Although supportive of the continued use of micronutrient powders in programmes as potential vehicles for delivery of multiple micronutrients and iron for prevention of iron deficiency anaemia in susceptible populations, these findings suggest that careful risk benefit estimation be done to assess the significance of the findings of excess diarrhoea and respiratory morbidity versus benefits on micronutrient status.

differed between treatment groups this discrepancy could have biased our results. However, our analysis did control for cluster-level, pre-intervention morbidity rates, age, sex, and urban or rural status and we do not believe that missing data invalidate our findings (appendix).

Notwithstanding the above limitations, our study has several strengths, key among these strengths being the large sample size and the prospective nature of data collection with regular home visits to collect information on morbidity and measure growth, unlike other recent cluster randomised trials that have relied on cross-sectional data[8] and a non-randomised comparison of recipients and non-recipients.[11]

The context of our study must be borne in mind. Prevalence of both stunting and wasting were high in children in our trial, being about 25% at 6 months of age with stunting increasing to around 50% at 18 months of age. Although such prevalences are not unusual in south Asian populations, they are indicative of a very poorly nourished population. Our trial confirms that micronutrient powder reduces iron-deficiency anaemia among at-risk children in urban and rural Pakistan; however, the reported excess morbidity and very small effect on growth suggest that a careful assessment of risks and benefits must be done in similar populations with a high prevalence of undernutrition and a high burden of diarrhoea. The extent to which our findings might apply in better nourished populations is unknown. Since micronutrient powder programmes are already being scaled up in many countries, it is imperative that further appropriately designed and powered studies be done to confirm or refute our findings. Micronutrient powder programmes need a strong monitoring and assessment component to ensure that any excess of serious adverse events, such as admissions to hospital and mortality, are detected.

Our findings also raise issues related to the process of development of policy and guidelines. Notably, the total number of participants included as evidence before development of the WHO guidelines on the global use of micronutrient powder (six efficacy studies with a total of 1447 participants) is hardly sufficient for due diligence on the use of micronutrient powder at scale and was not accompanied by an objective assessment of potential adverse effects associated with micronutrient powder over an extended period of time because of scarcity of data. Adding our study to the existing evidence (panel[7,8,11,42–46]) confirms the effect on iron-deficiency anaemia but also suggests an excess of diarrhoea among recipients. Most studies of micronutrient powder so far have involved short periods of supplementation (typically 2–3 months). Although a case has been made for longer periods of administration of micronutrient powder,[47] our data suggest that caution should be exercised until further safety studies have been done. The modest benefits reported from studies using micronutrient powder for prevention of micronutrient deficiencies must be weighed against the potential benefits of alternative strategies such as direct use of fortified foods,[48] fortified lipid spreads, and strategies to reduce maternal micronutrient deficiencies and improve iron stores at birth.[49] Further studies are needed to develop and field test effective strategies for preventive zinc supplementation or fortification in childhood.

Contributors

ZAB conceived the idea and the study design and, as principal investigator, was involved in all aspects of this study. SC led the statistical analysis. SPI, SS, AZ, TA, JK, and IA were involved in study design, implementation, analysis planning, interpretation of data. AKMZ oversaw the microbiology studies. ZAB produced the first draft of the paper with substantial input from SC and is the guarantor of the study. All authors reviewed and approved various drafts and the final paper.

Conflicts of interest

We declare that we have no conflicts of interest.

Acknowledgments

We thank the Data Monitoring Committee, consisting of Robert Black (Johns Hopkins University, Baltimore, MD, USA), Larry Moulton (Johns Hopkins University), Stanley Zlotkin (Hospital for Sick Children, Toronto, ON, Canada), and Kenneth H Brown (University of California, Davis, CA, USA) for their support. We thank all field and laboratory staff for this study and members of the community in Matiari for their participation and support. We would also like to thank the anonymous reviewers for their thoughtful and helpful review of the Article.

References

1 UNICEF, WHO, The World Bank. Levels and trends in child malnutrition. Joint child malnutrition estimates. United Nations Children's Fund, New York; World Health Organization, Geneva; The World Bank, Washington, DC: Unicef-WHO-World Bank, 2012: 35.

2 de Benoist B, McLean E , Egli I, Cogswell M, eds. Worldwide prevalence of anaemia 1993–2005: WHO global database on anaemia. 2008. http://whqlibdoc.who.int/publications/2008/9789241596657_eng.pdf (accessed March 14, 2013).

3 Wessells KR, Brown KH. Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting. *PLoS One* 2012; **7:** e50568.

4 Yakoob MY, Theodoratou E, Jabeen A, et al. Preventive zinc supplementation in developing countries: impact on mortality and morbidity due to diarrhoea, pneumonia and malaria. *BMC Public Health* 2011; **11** (suppl 3)**:** S23.

5 Imdad A, Bhutta ZA. Effect of preventive zinc supplementation on linear growth in children under 5 years of age in developing countries: a meta-analysis of studies for input to the lives saved tool. *BMC Public Health* 2011; **11** (suppl 3)**:** S22.

6 World Health Organization. Guideline: use of multiple micronutrient powders for home fortification of foods consumed by infants and children 6–23 months of age. Geneva: WHO, 2011.

7 De-Regil LM, Suchdev PS, Vist GE, Walleser S, Peña-Rosas JP. Home fortification of foods with multiple micronutrient powders for health and nutrition in children under two years of age. *Cochrane Database Syst Rev* 2011; **9:** CD008959.

8 Jack SJ, Ou K, Chea M, et al. Effect of micronutrient sprinkles on reducing anemia: a cluster-randomized effectiveness trial. *Arch Pediatr Adolesc Med* 2012; **16:** 1–9.

9 UNICEF. UNICEF and the global strategy on infant and young child feeding. Understanding the past, planning the future. 2003. http://www.unicef.org/nutrition/files/FinalReportonDistribution.pdf (accessed March 14, 2013).

10 Spottiswoode N, Fried M, Drakesmith H, Duffy PE. Implications of malaria on iron deficiency control strategies. *Adv Nutr* 2012; **3:** 570–78.

11 Suchdev PS, Ruth LJ, Woodruff BA, et al. Selling sprinkles micronutrient powder reduces anemia, iron deficiency, and vitamin A deficiency in young children in Western Kenya: a cluster-randomized controlled trial. *Am J Clin Nutr* 2012; **95:** 1223–30.

12 Soofi S, Ahmed S, Fox MP, et al. Effectiveness of community case management of severe pneumonia with oral amoxicillin in children aged 2–59 months in Matiari district, rural Pakistan: a cluster-randomised controlled trial. *Lancet* 2012; **379:** 729–37.

13 Sharieff W, Bhutta Z, Schauer C, Tomlinson G, Zlotkin S. Micronutrients (including zinc) reduce diarrhoea in children: the Pakistan sprinkles diarrhoea study. *Arch Dis Child* 2006; **91:** 573–79.

14 de Onis M, Onyango AW, Van den Broeck J, Chumlea WC, Martorell R. Measurement and standardization protocols for anthropometry used in the construction of a new international growth reference. *Food Nutr Bull* 2004; **25:** S27–36.

15 World Health Organization/UNICEF Joint Statement. Clinical management of acute diarrhoea. Geneva: WHO, 2004: 7–8.

16 Ulijaszek SJ, Kerr DA. Anthropometric measurement error and the assessment of nutritional status. *Br J Nutr* 1999; **82:** 165–77.

17 NCCLS. Document H26-A: performance goals for the internal quality control of multichannel hematology analyzers; approved standard. Wayne: NCCLS, 1996.

18 Denham E, Mohn B, Tucker L, Lun A, Cleave P, Boswell DR. Evaluation of immunoturbidimetric specific protein methods using the architect ci8200: comparison with immunonephelometry. *Ann Clin Biochem* 2007; **44:** 529–36.

19 Dupuy AM, Debarge L, Poulain M, Badiou S, Rossi M, Cristol JP. Determination of serum ferritin using immunoturbidimetry or chemiluminescent detection in comparison with radioimmunoassay a compendium of a methodological juxtaposition. *Clin Lab* 2009; **55:** 207–15.

20 Granado-Lorencio F, Herrero-Barbudo C, Blanco-Navarro I, Pérez-Sacristán B. Suitability of ultra-high performance liquid chromatography for the determination of fat-soluble nutritional status (vitamins A, E, D, and individual carotenoids). *Anal Bioanal Chem* 2010; **397:** 1389–93.

21 Smith JC, Holbrook JT, Danford DE. Analysis and evaluation of zinc and copper in human plasma and serum. *Am J Clin Nutr* 1985; **4:** 627–38.

22 Thurham DI, Mburu ASW, Mwaniki DL, Wagt AD. Micronutrients in childhood and the influence of subclinical inflammation. *Proc Nutr Soc* 2005; **64:** 502–09.

23 Panchalingam S, Antonio M, Hossain A, et al. Diagnostic microbiologic methods in the GEMS-1 case/control study. *Clin Infect Dis* 2012; **55** (suppl 4)**:** S294–302.

24 Hayes RJ, Alexander ND, Bennett S, Cousens SN. Design and analysis issues in cluster-randomized trials of interventions against infectious diseases. *Stat Methods Med Res* 2000; **9:** 95–116.

25 Macro International and National Institute of Population Studies, Pakistan Demographic and Health Survey 2006–07. 2007. http://www.measuredhs.com/pubs/pdf/FR200/FR200.pdf (accessed March 14, 2013).

26 Zlotkin S, Arthur P, Schauer C, Antwi KY, Yeung G, Piekarz A. Home-fortification with iron and zinc sprinkles or iron sprinkles alone successfully treats anemia in infants and young children. *J Nutr* 2003; **133:** 1075–80.

27 Adu-Afarwuah S, Lartey A, Brown KH, Zlotkin S, Briend A, Dewey KG. Home fortification of complementary foods with micronutrient supplements is well accepted and has positive effects on infant iron status in Ghana. *Am J Clin Nutr* 2008; **87:** 929–38.

28 Menon P, Ruel MT, Loechl CU, et al. Micronutrient Sprinkles reduce anemia among 9- to 24-mo-old children when delivered through an integrated health and nutrition program in rural Haiti. *J Nutr* 2007; **137:** 1023–30.

29 Sharieff W, Yin SA, Wu M, et al. Short-term daily or weekly administration of micronutrient Sprinkles has high compliance and does not cause iron overload in Chinese schoolchildren: a cluster-randomised trial. *Public Health Nutr* 2006; **9:** 336–44.

30 Ip H, Hyder SM, Haseen F, Rahman M, Zlotkin SH. Improved adherence and anaemia cure rates with flexible administration of micronutrient Sprinkles: a new public health approach to anaemia control. *Eur J Clin Nutr* 2009; **63:** 165–72.

31 Kounnavong S, Sunahara T, Mascie-Taylor CG, et al. Effect of daily versus weekly home fortification with multiple micronutrient powder on haemoglobin concentration of young children in a rural area, Lao People's Democratic Republic: a randomised trial. *Nutr J* 2011; **10:** 129.

32 Gera T, Sachdev HP. Effect of iron supplementation on incidence of infectious illness in children: systematic review. *BMJ* 2002; **325:** 1142.

33 Mitra AK, Akramuzzaman SM, Fuchs GJ, Rahman MM, Mahalanabis D. Long-term oral supplementation with iron is not harmful for young children in a poor community of Bangladesh. *J Nutr* 1997; **127:** 1451–55.

34 Zimmermann MB, Chassard C, Rohner F, et al. The effects of iron fortification on the gut microbiota in African children: a randomized controlled trial in Côte d'Ivoire. *Am J Clin Nutr* 2010; **92:** 1406–15.

35 Kortman GA, Boleij A, Swinkels DW, Tjalsma H. Iron availability increases the pathogenic potential of *Salmonella typhimurium* and other enteric pathogens at the intestinal epithelial interface. *PLoS One* 2012; **7:** e29968.

36 Veenemans J, Schouten LR, Ottenhof MJ, et al. Effect of preventive supplementation with zinc and other micronutrients on non-malarial morbidity in Tanzanian pre-school children: a randomized trial. *PLoS One* 2012; **7:** e41630.

37 Janda JM, Abbott SL. The genus *Aeromonas*: taxonomy, pathogenicity and infection. *Clin Microbiol Rev* 2010; **23:** 35–73.

38 Sazawal S, Black RE, Ramsan M, et al. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. *Lancet* 2006; **367:** 133–43.

39 Lemaire M, Islam QS, Shen H, et al. Iron-containing micronutrient powder provided to children with moderate-to-severe malnutrition increases hemoglobin concentrations but not the risk of infectious morbidity: a randomized, double-blind, placebo-controlled, noninferiority safety trial. *Am J Clin Nutr* 2011; **94:** 585–93.

40 Hartman-Craven B, Christofides A, O'Connor DL, Zlotkin S. Relative bioavailability of iron and folic acid from a new powdered supplement compared to a traditional tablet in pregnant women. *BMC Pregnancy Childbirth* 2009; **9:** 33.

41 Government of Pakistan. Ministry of Health. National programme for family planning and primary health care. http://www.lhwp.punjab.gov.pk/website/Home.aspx (accessed March 14, 2013).

42 Kounnavong S, Sunahara T, Mascie-Taylor CG, et al. Effect of daily versus weekly home fortification with multiple micronutrient powder on haemoglobin concentration of young children in a rural area, Lao People's Democratic Republic: a randomised trial. *Nutr J* 2011; **10:** 129.

43 Macharia-Mutie CW, Moretti D, Van den Briel N, et al. Maize porridge enriched with a micronutrient powder containing low-dose iron as NaFeEDTA but not amaranth grain flour reduces anemia and iron deficiency in Kenyan preschool children. *J Nutr* 2012; **142:** 1756–63.

44 Ogunlade AO, Kruger HS, Jerling JC, et al. Point-of-use micronutrient fortification: lessons learned in implementing a preschool-based pilot trial in South Africa. *Int J Food Sci Nutr* 2011; **62:** 1–16.

45 Osei AK, Rosenberg IH, Houser RF, Bulusu S, Mathews M, Hamer DH. Community-level micronutrient fortification of school lunch meals improved vitamin A, folate, and iron status of schoolchildren in Himalayan villages of India. *J Nutr* 2010; **140:** 1146–54.

46 Varma JL, Das S, Sankar R, Mannar MG, Levinson FJ, Hamer DH. Community-level micronutrient fortification of a food supplement in India: a controlled trial in preschool children aged 36–66 mo. *Am J Clin Nutr* 2007; **85:** 1127–33.

47 Zlotkin S. More proof that home fortification is of value in children with iron deficiency anemia. *Arch Pediatr Adolesc Med* 2012; **166:** 869–70.

48 Eichler K, Wieser S, Rüthemann I, Brügger U. Effects of micronutrient fortified milk and cereal food for infants and children: a systematic review. *BMC Public Health* 2012; **12:** 506.

49 Rabe H, Diaz-Rossello JL, Duley L, Dowswell T. Effect of timing of umbilical cord clamping and other strategies to influence placental transfusion at preterm birth on maternal and infant outcomes. *Cochrane Database Syst Rev* 2012; **8:** CD003248.