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Effects of antenatal multiple micronutrient supplementation on lung function in mid-childhood: follow-up of a double-blind randomised controlled trial in Nepal

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ABSTRACT A randomised trial of prenatal multiple micronutrient supplementation in Nepalese women increased birthweight and weight at 2 years of age in offspring, compared to those born to mothers who only received iron and folic acid supplements. Further follow-up of this cohort provided an opportunity to investigate the effect of antenatal multiple micronutrients on subsequent lung function by measuring spirometry at 7–9 years of age in children born during the trial.

841 children (80% of the cohort) were seen at mean \pm SD 8.5 \pm 0.4 years. Technically successful spirometry results were obtained in 793 (94.3%) children, 50% of whom had been randomised to micronutrient supplementation. Background characteristics, including anthropometry, were similar in the two allocation groups.

Lung function was also similar, mean (95% CI) difference in z-scores (supplementation minus control) was -0.08 (-0.19 – 0.04), -0.05 (-0.17 – 0.06) and -0.04 (-0.15 – 0.07) for forced expiratory volume in 1 s (FEV₁), forced vital capacity and FEV₁/FVC, respectively. Compared with healthy white children, FEV₁ and FVC in the “healthy” Nepalese children were ~ 1 ($\sim 13\%$) z-score lower, with no difference in FEV₁/FVC.

We conclude that, compared with routine iron and folic acid, multiple micronutrient supplementation during pregnancy has no effect on spirometric lung function in Nepalese children at 8.5 years of age.



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Introduction

Micronutrient deficiency is common worldwide, especially in rural populations in low-income countries. Pregnant women with higher metabolic demands are at particular risk [1]. Impaired antenatal nutrition can affect fetal development and growth in the short term and risk of chronic disease in the longer term [2]. Birthweight is positively associated with lung function in later life [3, 4], and antenatal consumption of vitamins A and D, for example, have been implicated in the pathways that affect respiratory function and disease [5].

We previously conducted a double-blind randomised controlled trial in which pregnant women received either prenatal multiple micronutrient (MMN) supplements or iron and folic acid (control) in the second and third trimesters. Infants born in the MMN group were 77 g (95% CI 24–130 g) heavier at birth [6] and 204 g (27–381 g) heavier at 2.5 years of age, with small increases in body circumferences and lower mean blood pressure (−2.5 mmHg (95% CI 0.5–4.6)) [7]. Based on associations from a similar trial of antenatal vitamin A supplementation in humans [8], observational data between maternal diet and childhood lung function [9], and animal studies [10, 11], we hypothesised that children whose mothers had received prenatal MMN supplements would have better spirometric lung function than control children. To our knowledge only one study has investigated lung function outcomes in an antenatal micronutrient supplementation trial in childhood [8], and none have looked at MMN. Thus, we followed up the cohort at ~8 years of age (an age when the vast majority of children can perform spirometry satisfactorily) to investigate whether a prenatal micronutrient supplementation that increased birth weight was also associated with increased lung function during childhood.

Methods

The study was conducted in the central plains (Terai) of Nepal, a poor country with a high burden of respiratory disease, particularly childhood pneumonia, and high levels of indoor air pollution, mostly attributable to exposure to biomass fuels (~80% of households) [12, 13]. The study was conducted between September 2011 and December 2012 at low altitude (200 m) in a region with summer and winter temperatures of ~45°C and ~0°C, respectively, and a summer monsoon.

Details of the trial have been described previously [6]. Briefly, 1200 pregnant women attending Janakpur Zonal Hospital (Janakpur, Nepal) were recruited sequentially and randomised to receive either a daily MMN supplement (containing vitamin A 800 µg, vitamin E 10 mg, vitamin D 5 µg, vitamin B1 1.4 mg, vitamin B2 1.4 mg, niacin 18 mg, vitamin B6 1.9 mg, vitamin B12 2.6 µg, folic acid 400 µg, vitamin C 70 mg, iron 30 mg, zinc 15 mg, copper 2 mg, selenium 65 µg, and iodine 150 µg [14]) in the second and third trimesters or a control supplement of iron 60 mg and folic acid 400 µg (the standard national recommendation for pregnant women). The lower dose of iron in the MMN supplement was designed to be equivalent to the 60 mg dose in the control supplement, due to the increased absorption with vitamin C. The women took the supplements for a median (interquartile range) of 98% (91–100%) of participation days in the control group and 97% (91–100%) in the intervention group. At 32 weeks gestation, blood tests showed higher retinol and vitamin E levels and no difference in haemoglobin levels among those receiving MMN [6]. Antenatal MMN did not affect offspring cytokine or inflammatory profile [15]. Participants, their families and data collection staff remained blind to allocation.

Outcomes

Primary outcomes were forced expiratory volume in 1 s (FEV₁), forced vital capacity (FVC), FEV₁/FVC ratio and forced expiratory flow at 25–75% of FVC, expressed as standard deviation (z-)scores [16].

Procedures

Approximately half of the children were from the district capital, Janakpur, with the remainder from villages within Dhanusha or the surrounding districts. We saw 14 children whose families had moved to Kathmandu or the town of Hetauda since recruitment.

Questionnaire

Informed consent was obtained from parents or guardians. Questionnaires covering socioeconomic circumstances, household characteristics, food security and illnesses were administered to parents. Food security describes the availability to a household, region or country of enough food, both currently and in the future. Food insecurity increases the risk of malnutrition and poor growth. It was assessed using the Household Food Insecurity Access Scale (HFIAS) [17] and Household Dietary Diversity Score (HDDS) [18], which were developed by the Food and Nutrition Technical Assistance Project. The questions have been validated in many countries and are used by the Nepal Ministry of Health in demographic and health surveys. HFIAS indicates the degree of food insecurity perceived in a household over the past year, in terms of access to food, insufficient food quality and quantity. Nine questions classify a household into food secure

and mildly, moderately and severely food insecure. The HDDS was used to examine the breadth of the child's diet in the preceding week by recording food groups eaten.

Additional questions included respiratory symptoms in the past week and past year, and major (defined as warranting hospital admission) or chronic (defined as lasting for months or years) illnesses. Open questions were assessed by a clinician (D. Devakumar), who undertook further assessments if required. The International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire relevant to the age group was used to identify asthma and rhinitis [19]. The household asset score was determined from a set of predefined household assets defined by the World Health Organization (WHO), which stratified households into four categories, with more expensive items like a car or a refrigerator given the highest ranking and having none of the items as the lowest ranking [20].

Anthropometry

Measurements were made at the study office in Janakpur. Duplicate measures of height were taken using standard techniques with a Leicester stadiometer (Invicta Plastics, Leicester, UK), accurate to 0.1 cm. Weight was measured using a Tanita-418 scale (Tanita Corp., Tokyo, Japan), accurate to 0.1 kg.

Spirometry

Lung function was measured using two identical EasyOne World Spirometers (ndd Medical, Zurich, Switzerland), auto-calibrated before use and alternated fortnightly. American Thoracic Society/European Respiratory Society quality control criteria for spirometry [21], adapted for use in children [22], were used. Parents were requested to bring their children for assessment only if they were well. Three local investigators were trained to conduct spirometry tests. They explained and demonstrated the procedure to the child and parent/guardian in advance. The child performed spirometry wearing a nose clip while seated. A biological control (member of staff) was tested every fortnight to monitor any potential shift in both spirometers over time.

All spirometry tests were interpreted by a clinician (D. Devakumar) and one in 10 were over-read by a respiratory physiologist (J. Kirkby), who had provided initial spirometry training.

Air pollution

Air pollution was quantified as a potential confounding factor. Detailed air pollution methods have been described previously [23]. Personal exposure to air pollution was estimated using both gravimetric and photometric sampling of particle mass $<4\ \mu\text{m}$ in diameter in the microenvironments in which children lived. Our pilot data showed that children spent most of their time in the following locations: their bedrooms, living rooms, veranda or kitchen, school and outdoors. In many of the houses the bedroom and living room were the same and, if not, we assumed they would have similar concentrations. We sampled air from the bedroom, veranda, outdoors and school. In addition, we sampled air from kitchens when cooking was taking place and when it was not. Measurements were taken from a sub-sample of households ($n=55$), outdoor locations ($n=8$) and schools ($n=8$), repeated three times per year to capture seasonal variation. Time activity data were collected on all children, describing a normal day (school day, if they attended school). The exposure was calculated as the product of the average concentration from each location and the time in that location for each child to produce a 24-h time-weighted average. No measurements were obtained for the 14 children living outside the Terai region of Nepal.

Statistical analysis

Power estimates were based on data from rural Nepalese young adults and US data from children [24]. With a sample size of 400 in each group, with $\alpha=0.05$, the study had an 80% power to detect a difference between groups of either 2.6% or 4.0% in FEV₁ based on the US or Nepalese data, respectively. This was in keeping with a difference of ~3% observed in the adjoining district of Sarlahi [8].

Primary analysis

Lung function data were adjusted for sex, age and height using the Global Lung Function Initiative (GLI)-2012 multi-ethnic, "all-age" reference ranges [16]. As specific reference ranges do not yet exist for South Asia, the Caucasian equations (*i.e.* derived from white subjects of European origin) were used for the main group comparisons. Spirometric results were also expressed in relation to a recently derived preliminary GLI-2012 coefficient for children from South Asia [25]. The WHO child growth standards were applied to calculate z-scores for weight, height and body mass index (BMI) for age [26]. Relative leg length was calculated as the ratio of leg length to height multiplied by 100. Primary analysis was based on all children with technically acceptable spirometry results. The association between birthweight and lung function was investigated. Data were then examined by allocation group with t-tests and univariable regression models.

Secondary analysis

We conducted three secondary analyses. First, we excluded children with acute or chronic illness or prior pneumonia requiring hospitalisation (online supplementary data). Secondly, we adjusted for potential confounders in multivariable regression models. We constructed a directed acyclic graph setting out putative associations between confounding variables based on *a priori* assumptions (fig. 1). From this, we developed multivariable linear regression models adjusting for air pollution, food access and diversity, household asset score and maternal height (to augment information on diet and socioeconomic circumstances). Food security, a proxy for nutritional intake, is required for growth. Air pollution is an environmental stressor that is detrimental to lung growth and development while socioeconomic status is a distal variable in figure 1, which is thought to underpin many factors associated with lung growth. We also included covariates for maternal education and residence to offset the potential effects of differential loss to follow-up [27], and a binary covariate for which spirometer had been used as a potential source of measurement error. Finally, we considered effect modification by sex, since antenatal MMN supplementation may act differently in boys and girls, and has previously been shown to result in a greater gain in birthweight among girls [6].

Model assumptions were tested for linearity by plotting residuals against each covariate separately: for normality by creating a kernel density plot of residuals; for multi-collinearity by calculating the variance inflation factor; and for heteroscedasticity by plotting residuals against predicted values and performing Breusch–Pagan tests. The distribution of FEV₁/FVC was heteroscedastic and robust standard errors were applied to the relevant regression models. All analyses were conducted on the basis of the original intention-to-treat at enrolment of women during pregnancy using Excel (Microsoft Corp Redmond, WA, USA), Prism (GraphPad Software Inc., La Jolla, CA, USA) and Stata (StataCorp, College Station, TX, USA).

The study was approved by the Nepal Health Research Council (Kathmandu) and University College London (London, UK) research ethics board.

Results

We visited 852 families from September 2011 to December 2012, and conducted anthropometry and spirometry in 841 children. Retention rates were 81% (422 out of 526 of surviving children) for the controls and 79% (419 out of 529) for the intervention group. Figure 2 shows the trial profile. Residence and maternal education were the only variables that differed in those lost to follow-up (table 1). Mean age at follow-up was 8.4 years in the intervention group and 8.5 years in the control group. Just over 50% of children were undernourished (<-2 weight for height z-scores) and approximately one-third were stunted (<-2 height for age z-scores) and had a low BMI for their age (<-2 z-scores for BMI) [26].

There were no differences between observers for FEV₁ or FVC, or within the biological control over time. Six children were unable to perform spirometry: five had developmental delay and one had poor coordination. Spirometry data from a further 42 (5.0%) children were excluded because of poor technique, reflecting an overall failure rate of 5.7%. Table 2 shows anthropometry and lung function by allocation according to univariable and multivariable regression models. There were no anthropometric differences

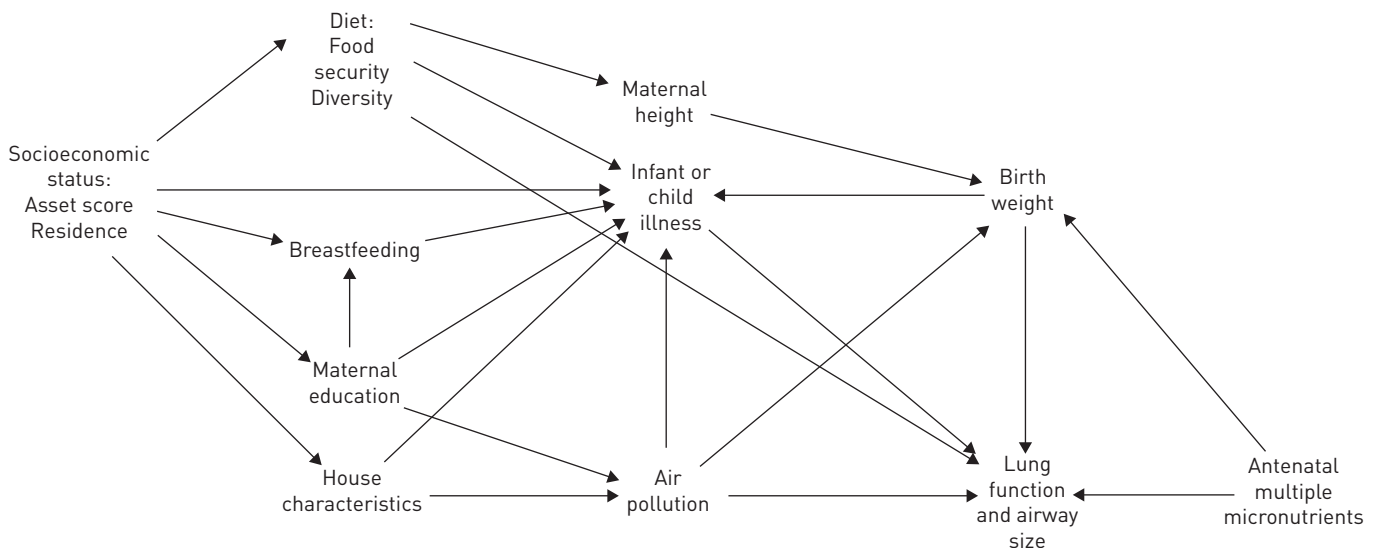


FIGURE 1 A directed acyclic graph showing associations between measured variables.

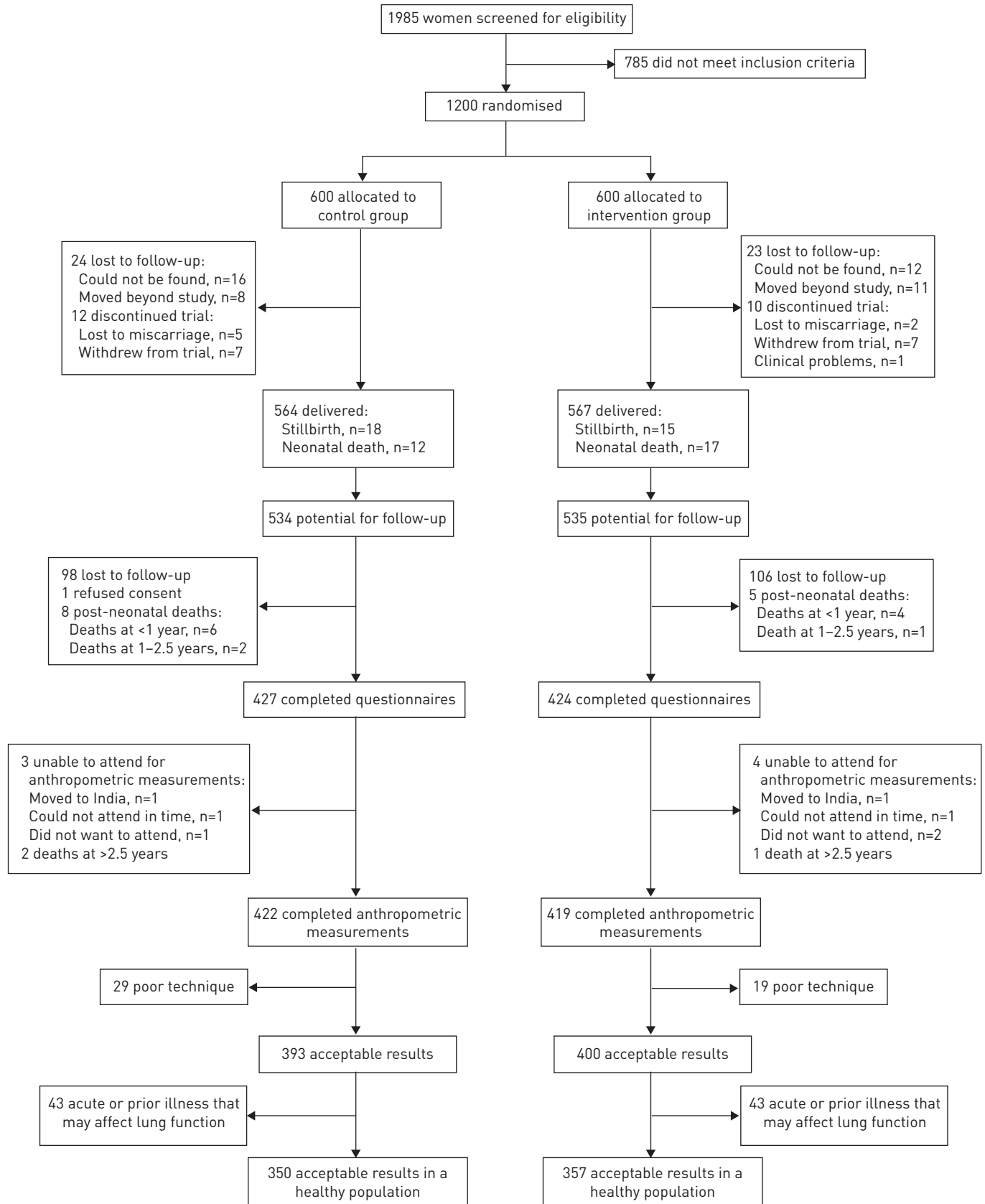


FIGURE 2 Trial profile showing the number of participants in each stage of the trial and follow-up.

TABLE 1 Subjects lost to follow-up

	8-year follow-up		Lost to follow-up	
	Control	Intervention	Before trial ended [#]	After trial ended
Subjects n	422	419	69	290
Sex				
Girl	210 (49.8)	196 (46.8)	+	152 (53.5)*
Boy	212 (50.2)	223 (53.2)	+	132 (46.5)
Residence				
Urban	199 (47.2)	197 (47.0)	47 (68.1)	184 (63.5)
Rural	223 (52.8)	222 (53.0)	22 (31.9)	106 (36.6)
Maternal education				
None	206 (48.8)	210 (50.1)	27 (39.1)	101 (34.8)
Primary	37 (8.8)	33 (7.9)	16 (23.2)	37 (12.8)
Secondary or higher	179 (42.4)	176 (42.0)	26 (37.7)	152 (52.4)
Maternal height cm	151.0±5.7	150.4±5.4	150.4±5.0	151.0±5.6
Ethnicity				
Terai Hindu	368 (87.2)	355 (84.7)	53 (76.8)	241 (83.1)
Hill Hindu	24 (5.7)	23 (5.5)	6 (8.7)	19 (6.6)
Terai Muslim	23 (5.5)	29 (6.9)	8 (11.6)	17 (5.9)
Other	7 (1.7)	12 (2.9)	2 (2.9)	13 (4.5)
Main household income				
No work	49 (11.6)	46 (11.0)	1 (1.5)	34 (11.7)
Farming	72 (17.1)	68 (16.2)	7 (10.1)	34 (11.7)
Salaried	153 (36.3)	178 (42.5)	34 (49.3)	148 (51.0)
Small business	82 (19.4)	76 (18.1)	19 (27.5)	46 (15.9)
Waged labour	53 (12.6)	43 (10.3)	5 (7.3)	18 (6.2)
Student	7 (1.7)	3 (0.7)	3 (4.4)	4 (1.4)
Out of country	6 (1.4)	5 (1.2)	0	6 (2.1)
Household asset score				
Car, TV or refrigerator	217 (51.4)	214 (51.1)	36 (52.9)*	146 (50.3)
Sewing machine, cassette player, camera, fan, bullock cart, clock, radio, iron or bicycle	147 (34.8)	138 (32.9)	21 (30.9)	95 (32.8)
None of the above	58 (13.7)	67 (16.0)	11 (16.2)	49 (16.9)
Preterm delivery[¶]	29 (6.9)	28 (6.7)	+	41 (14.1)
Birthweight kg	2.74±0.41	2.81±0.43	+	2.75±0.50

Data are presented as n (%) or mean±SD, unless otherwise stated. [#]: defined as birth of a live born infant, stillbirth or miscarriage; [¶]: <37 weeks gestation by ultrasound assessment; +: data incomplete at delivery.

between the groups, including relative leg length (intervention minus control: mean (95% CI) -0.04% (-0.19 – 0.12%)). No differences in lung function were found between allocation groups, with unadjusted differences (intervention minus control) being <0.1 z-scores for all spirometric outcomes (which at this age equates to $\sim 1\%$) [16]. Despite the shift in absolute z-scores, the magnitude of differences between groups was virtually identical when expressed according to the provisional South-Asian coefficient (table S1). Similarly, no differences were observed when using the raw data adjusted for height, sex and age (table 2 and table S1). Adjustment for confounders made little difference to the results. When analysed by sex, the lung function of girls in the intervention group had slightly lower FEV₁ than those in the control group on univariable analysis (mean (95% CI) difference: -0.18 (-0.34 – -0.02)), but this was not apparent in the multivariable model (table S2).

Although reported asthma was uncommon ($<2\%$), 95 (11.3%) children had evidence of acute or chronic illness that might have affected lung function, some of whom had multiple diagnoses. Nine (9.4%) of these children also had poor technique. There were no differences in prior medical history by allocation group. Although lung function was somewhat lower amongst those who were excluded on health grounds (data not shown), excluding such children had relatively little impact on summary results from either trial group (table 2). There was a positive association between birthweight and childhood lung function, a 1 z-score increase in birthweight was associated with a mean (95% CI) increase in FEV₁ of 0.10 (0.04–0.15) z-scores and in FVC by 0.11 (0.06–0.18) z-scores.

Spirometric outcomes for the entire group of “healthy” Nepalese children with acceptable data were close to those predicted for South-Asian children (mean±SD z-scores -0.23 ± 0.9 for FEV₁ and -0.22 ± 1.0 for FVC) whereas, as expected, they were significantly lower than those predicted for white children (mean±SD

TABLE 2 Lung function and anthropometry by allocation group in children with acceptable spirometry

	Controls	Intervention	Unadjusted difference (95% CI)	Multivariable regression [#] (95% CI)
Entire cohort	393	400		
Age years	8.5±0.4	8.4±0.4		
Weight z-score [¶]	-2.1±1.0	-2.0±1.0	0.05 (-0.09-0.19)	0.11 (-0.02-0.24)
Height z-score [¶]	-1.5±0.9	-1.5±0.9	0.03 (-0.10-0.16)	0.08 (-0.04-0.19)
BMI z-score [¶]	-1.7±0.9	-1.6±1.0	0.04 (-0.09-0.17)	0.08 (-0.05-0.21)
FEV ₁ L	1.21±0.2	1.20±0.2	-0.01 (-0.04-0.02)	-0.01 (-0.03-0.01)
FVC L	1.38±0.2	1.37±0.2	-0.01 (-0.04-0.02)	-0.01 (-0.00-0.00)
FEV ₁ /FVC	0.88±0.0	0.88±0.1	-0.00 (-0.01-0.00)	-0.00 (-0.01-0.00)
FEF _{25-75%} L	1.64±0.4	1.61±0.4	-0.03 (-0.09-0.03)	-0.03 (-0.09-0.03)
FEV ₁ z-score [*]	-1.11±0.8	-1.18±0.8	-0.08 (-0.19-0.04)	-0.06 (-0.18-0.05)
FVC z-score [*]	-1.02±0.8	-1.07±0.8	-0.05 (-0.17-0.06)	-0.04 (-0.15-0.08)
FEV ₁ /FVC z-score [*]	-0.20±0.8	-0.24±0.8	-0.04 (-0.15-0.07)	-0.04 (-0.15-0.07)
FEF _{25-75%} z-score [*]	-0.48±1.0	-0.53±1.0	-0.06 (-0.20-0.09)	-0.05 (-0.20-0.10)
Children without evidence of prior significant disease	350	357		
Age years	8.5±0.5	8.5±0.5		
Weight z-score [¶]	-2.1±0.9	-2.0±1.1	0.04 (-0.11-0.19)	0.10 (-0.03-0.23)
Height z-score [¶]	-1.5±0.9	-1.5±0.9	0.03 (-0.10-0.16)	0.08 (-0.04-0.20)
BMI z-score [¶]	-1.7±0.9	-1.6±1.0	0.03 (-0.11-0.17)	0.06 (-0.07-0.20)
FEV ₁ L	1.22±0.2	1.21±0.2	-0.01 (-0.04-0.02)	-0.01 (-0.03-0.01)
FVC L	1.38±0.2	1.37±0.2	-0.00 (-0.04-0.03)	-0.01 (-0.03-0.01)
FEV ₁ /FVC	0.89±0.0	0.88±0.0	-0.01 (-0.01-0.00)	-0.00 (-0.01-0.00)
FEF _{25-75%} L	1.67±0.4	1.63±0.4	-0.04 (-0.11-0.02)	-0.04 (-0.10-0.02)
FEV ₁ z-score [*]	-1.07±0.8	-1.16±0.8	-0.09 (-0.21-0.03)	-0.07 (-0.19-0.05)
FVC z-score [*]	-1.01±0.8	-1.05±0.8	-0.05 (-0.17-0.07)	-0.03 (-0.15-0.09)
FEV ₁ /FVC z-score [*]	-0.15±0.7	-0.22±0.7	-0.08 (-0.19-0.03)	-0.08 (-0.19-0.03)
FEF _{25-75%} z-score [*]	-0.42±1.0	-0.50±1.0	-0.08 (-0.23-0.06)	-0.08 (-0.22-0.07)

Data are presented as n or mean±SD, unless otherwise stated. Data not expressed in z-scores was also adjusted for age, sex and height. Analysis was restricted to children with technically satisfactory results. Air pollution data was not available for 14 children. BMI: body mass index; FEV₁: forced expiratory volume in 1 s; FVC: forced vital capacity; FEF_{25-75%}: forced expiratory flow at 25-75% of FVC. [#]: controlled for air pollution, dietary diversity, food security, maternal education, height, household asset score, spirometer (with the exception of anthropometry indices) and residence using robust standard errors; [¶]: z-scores calculated according to World Health Organization reference ranges [26]; ^{*}: z-scores calculated according to QUANJER *et al.* [16].

z-scores -1.15±0.8 for FEV₁ and -1.05±0.8 for FVC; 13.7% and 12.4% lower, respectively) [16]. However, the proportional reductions in both outcomes meant that the FEV₁/FVC ratio was within 1.5% of that predicted for white children irrespective of which equation was used.

Food security was generally adequate. 9% of households in the MMN group and 8% in the control group were insecure and the median 7-day dietary diversity score was nine out of 12 in both allocation groups. Other than for fever, illness rates and median number of episodes in the past week and past year were low and similar across allocation groups. We made 233 air pollution measurements in 55 households (6.6% of the cohort), eight representative outdoor locations and eight schools, totalling 2649 h of sampling across microenvironments. Table S3 shows average exposure levels in each microenvironment. Although kitchen concentrations were very high, the median kitchen exposure was zero because most children spent very little time there. The overall median 24-h time-weighted average was similar in both groups.

Discussion

Alterations in lung function among the offspring of mothers receiving nutritional supplementation would have implications in our understanding of lung development and its epigenetic influences, and for the design of nutrition recommendations and public health programmes for pregnant women in low-resource settings. However, results from this study suggest that, when compared with routine iron and folic acid supplementation, maternal MMN supplementation in the second and third trimesters of pregnancy does not increase lung function in Nepalese children at ~8 years of age. Despite challenging field conditions, spirometry was performed by 80% of children available for follow-up, achieving technically satisfactory results in 94.3%. After excluding children with a significant prior medical history and adjusting for height, age and sex, FEV₁ and FVC in healthy Nepalese children were ~13% lower than predicted for white children, but similar to those reported in South-Asian children.

To our knowledge, the effect of antenatal MMN on lung function has not been investigated previously and there is little research investigating lung function in children from low-resource settings, particularly in Nepal. Although the primary outcome of the trial was not childhood lung function, this cohort provided a rare opportunity to prospectively investigate the effect of a public health nutrient intervention during pregnancy on childhood lung function. Furthermore, since antenatal MMN has been recommended for all pregnant women [28], it is important to investigate any longer term effects they may have. Respiratory end-points are important long-term secondary trial outcomes relevant for child health that will help to shape maternal nutritional policy and, by 8 years of age, standardised, high-quality spirometric measurements can be achieved in most subjects. Animal and human evidence on antenatal micronutrients at similar doses has suggested potential long-term effects on lung function. Maternal vitamin D depletion alters lung structure and reduces lung volume in mice [10]. The evidence for effects on lung function and respiratory disease in humans is mixed [29–32]. Observational evidence from a cohort study shows an association between antenatal vitamin E intake and lung function [9]. The best evidence for long-term effects is for vitamin A, which is required for fetal lung growth, airway branching and alveolarisation [33, 34]. Supplementation in animals [11] and humans [8] has shown positive effects on lung function. In a follow-up trial of antenatal vitamin A supplementation in children at 9–13 years of age in the adjoining district of Sarlahi, the intervention group had greater mean adjusted FEV₁ and FVC (46 mL for both) [8]. These findings may differ from ours for several reasons. First, the trial design was different, being a cluster randomised trial in which married women received supplements for 3.5 years, not solely during pregnancy [35]. Peri-conceptional or early pregnancy status might be important and lung maturation continues into early childhood [2]. Secondly, the comparator group was placebo while ours was iron and folate; which is important as both are associated with long-term respiratory outcomes and antenatal iron has been positively associated with lung function in children [36]. Thirdly, while the dosages were similar, there may have been a dose effect as the Sarlahi study used 7000 µg·week⁻¹ of vitamin A, compared to 800 µg·day⁻¹ in our trial. Finally, the Sarlahi population was more disadvantaged and supplementation might only be effective in the most deficient populations.

While generally not reaching statistical significance, there was a suggestion of a weak negative association between antenatal MMN supplements and FEV₁ and FVC in girls, but not boys. Further investigation of lung function in girls may be warranted, but we do not want to over interpret the observation. If confirmed, it would raise the question of differential DNA methylation, which has been linked to antenatal diet [37] and smoking [38], and which could influence long-term outcomes.

We saw a positive association between birthweight and lung function across the entire study group. Micronutrients have been previously shown to increase birthweight [6], but our findings demonstrate that interventions that increase birth weight do not necessarily increase lung function.

Since a definitive specific GLI coefficient has yet to be established for the South-Asian population, we used the Caucasian equations to adjust for height, age and sex when comparing the groups as these are based on the largest amount of data and represent the most robust standard [16]. On testing our data against the alternative GLI ethnic-specific equations that are currently available (black, South-East Asian and other/mixed), none were found to be a perfect fit for our population [39]. As expected, due to ethnic differences in body shape and proportions, FEV₁ and FVC values were lower than would be expected for Caucasian children [16], but only slightly lower than those observed in healthy children from South Asia, whether they lived in the UK [40] or India [41]. Although a preliminary coefficient for South-Asian children has been derived recently [25], it has yet to be verified by studying more children over a wider age range and was, therefore, considered unsuitable for our primary analysis. However, as illustrated in table S2, the choice of ethnic-specific reference equations had no effect on our conclusions regarding the lack of any difference in lung function between trial groups.

Our sample size was large, with excellent follow-up rates, no evidence of bias in loss to follow-up and few exclusions due to poor spirometry technique, making the reported associations generalisable. Bronchodilators were not used, both for pragmatic reasons and because we were primarily interested in lung growth and development rather than airway responsiveness. The allocation groups were balanced and potential confounding factors documented and controlled for. Nevertheless, it is possible that any effect of MMN supplementation could have been masked by unadjusted confounding. While we controlled for air pollution in the regression models, an antenatal nutritional intervention may have no effect at such high levels. At 168 µg·m⁻³, exposure to air pollution in our sample was approximately five times higher than the WHO recommendation of a 24-h outdoor mean of 25 µg·m⁻³ for particles with a 50% cut-off aerodynamic diameter of 2.5 µm [42]. The main source of air pollution is indoor burning of biomass fuels, which has been found to adversely affect lung function in Nepalese adults [43], and is linked to respiratory infections in children [44]. While our air pollution estimates were measured directly, they were based on a subsample. It is, however, unlikely that there would have been marked differences in results had it been possible to undertake exposure estimates in all individuals.

Although morbidity was high in general, prevalence of wheeze, based on questionnaire or clinical diagnosis, was low. Recall bias may have affected responses to questions about recent illness and major illnesses may be over-reported. It is difficult to corroborate illness reports without reliable medical records. Alternatively, children from poorer households may not be taken to see medical staff when unwell, particularly those who have to travel large distances. We would, however, expect recall bias to affect both allocation groups equally.

Conclusion

Our large, well-conducted study of lung function in children in southern Nepal found lower average spirometry values than in white children, but no long-term difference in lung function or respiratory disease resulting from antenatal MMN supplementation in the second and third trimesters compared with iron and folic acid supplements. It did not support the idea that antenatal MMN supplementation alone increases childhood lung function; however, our findings do not exclude the possibility that earlier initiation, a larger dose or a longer duration of supplementation might lead to measurable differences in subsequent lung function.

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