Antenatal Micronutrient Supplementation Reduces Metabolic Syndrome in 6- to 8-Year-Old Children in Rural Nepal\textsuperscript{1,2}

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Abstract

Previously, we showed that antenatal micronutrient supplementation increases birth weight in a malnourished rural South Asian setting, but the long-term effects are unknown. Between 1999 and 2001, pregnant women were sector-randomized to receive from early pregnancy through 3 mo postpartum daily micronutrient supplements containing either vitamin A alone as the control or with folic acid; folic acid+iron; folic acid+iron+zinc; or a multiple micronutrient supplement that included the above nutrients plus 11 others. From 2006 to 2008, 3524 children (93\% of surviving children) were revisited between the ages of 6 and 8 y. Blood pressure, BMI, waist circumference, glycated hemoglobin, cholesterol, triglycerides, glucose, insulin, and the urinary microalbumin:creatinine ratio were assessed among children. Insulin resistance was estimated using the homeostasis model assessment (HOMA) and metabolic syndrome was defined using a modified National Cholesterol Education Program definition. None of the micronutrient supplement combinations affected blood pressure, cholesterol, triglycerides, glucose, insulin, or HOMA. There was a reduced risk of microalbuminuria (≥3.40 mg/mMol creatinine) in the folic acid [odds ratio (OR), 0.56; 95\%CI, 0.33–0.93; \(P = 0.02\)] groups and a reduced risk of metabolic syndrome in the folic acid group (OR, 0.63; CI, 0.41–0.97; \(P = 0.03\)). Maternal supplementation with folic acid or folic acid+iron+zinc reduced the risk of kidney dysfunction and, to some extent, metabolic syndrome among children at 6–8 y of age. Supplementation with multiple micronutrients had no such affect. Future follow-up studies are needed to examine long-term supplementation effects on risk of chronic diseases in adults. J. Nutr. 139: 1575–1581, 2009.

Introduction

South Asia suffers from both a high burden of undernutrition and low birth weight as well as a growing prevalence of cardiovascular disease and diabetes among adults. There has been much research into the developmental origins of health and disease in recent years; however, much of this work has been conducted in developed country populations. Observational studies have suggested that nutritional insults during pregnancy, either due to exposure to famine or manifested as lower birth weight or small size for gestational age, may predispose an individual to an increased risk of hypertension or cardiovascular disease and insulin resistance or type 2 diabetes in later life (1–6).

Micronutrient deficiencies are common among women in South Asia (7,8) and maternal supplementation trials in some studies have been shown to reduce low birth weight and preterm birth (9), but data examining the impact of such interventions beyond infancy are sparse. In our own community-based, randomized, controlled trial conducted in rural Nepal from 1999 to 2001, we assessed infant outcomes following maternal antenatal micronutrient supplementation with: 1) a control; 2) folic acid; 3) folic acid with iron; 4) folic acid with iron and zinc; or 5) a combination of these with additional vitamins and minerals. All supplements contained vitamin A and were compared with vitamin A alone as the control. The combination of folic acid +iron or the multiple micronutrient supplements reduced the risk...
of low birth weight by 16 and 14%, respectively (10). Given the plausible link between birth weight and later disease outcomes, it is reasonable to assume that micronutrient supplementation, thus may have long-term effects on child health.

Our aim, therefore, was to explore the longer term effects of micronutrient supplementation on early life markers of chronic disease. We revisited the children born to the mothers enrolled in the trial to examine survival, growth, and body composition and early markers of chronic disease. This paper examines the impact of antenatal micronutrient supplementation on cardio-metabolic risk in the offspring at 6–8 y of age in a poor, under-nourished rural South Asian population.

Materials and Methods

We conducted a community-based, cluster randomized, controlled trial of antenatal micronutrient supplementation in the rural Surkhet District of Nepal. The study area is located in the southeastern, low-lying Terai region of Nepal near the border with the Indian state of Bihar. The study procedures and initial outcomes have been described in detail previously (10,11). Briefly, the study area was divided into 426 sectors, which served as the unit of randomization. A total of 4296 women were enrolled in early pregnancy and were provided daily supplements from the time of enrollment through 3 mo postpartum with: 1) vitamin A alone as the control; 2) folic acid (400 µg); 3) folic acid with iron (60 mg); 4) folic acid with iron and zinc (30 mg); or 5) a multiple micronutrient supplement containing folic acid, iron, zinc, and an additional 11 vitamins and minerals (10 µg vitamin D as cholecalciferol, 10 mg vitamin E as d-α-tocopherol, 1.6 mg thiamine, 1.8 mg riboflavin, 20 mg niacin, 2.2 mg vitamin B-6, 2.6 µg vitamin B-12, 100 mg vitamin C, 65 µg vitamin K as phylloquinone, 2.0 mg Ca, 100 mg Mg). All supplements contained 1 mg retinol equivalents of vitamin A in the form of retinyl acetate due to earlier findings in this population that maternal vitamin A supplementation could reduce maternal mortality (12). The women gave birth to 4130 live-born infants who were weighed and measured by study staff within women’s homes soon after birth. Infants were followed through 6 mo of age to monitor morbidity and mortality.

Follow-up assessments. In 2006, children born during the antenatal micronutrient supplementation trial were revisited at their last known address to update their address and vital status information. Additional efforts were made to track children who moved or were away temporarily for schooling to optimize follow-up rates. Then, 3 field assessment teams visited the children in their homes; the first to conduct household socioeconomic interviews, to assess the child’s education, and to measure his/her blood pressure; the second to take anthropometric measurements; and the 3rd to collect early morning fasting blood and urine samples. Blood pressure was measured 4 times at 1-min intervals using an automated oscillometric device (BPM-300, BPTure). The machine was programmed to drop the first measurement and display the mean of the last 3, which was recorded by the field worker. During the second visit, anthropometrists measured the child’s weight using an electronic scale (Model 881, Seca), height using a portable stadiometer (Harpenden, Crosswell), triceps and subscapular skinfold thicknesses using precision calipers (Holtain), mid-upper arm circumference using a standard insertion tape, and waist circumference using a long insertion tape (Seca 200).

The child was then asked to fast overnight and on the following morning a phlebotomist visited the home to collect early morning venous blood and urine samples. Biopspecimens were brought to a central laboratory for processing and analysis. The microalbumin:creatinine ratio was measured in urine specimens using the DCA 2000 analyzer (Bayer Diagnostics). Whole blood was analyzed on the day of collection to measure glycated hemoglobin (HbA1c) 5 using a DCA 2000 analyzer with standard test kits. Blood was then separated into plasma aliquots, from which total and HDL cholesterol, triglycerides, and glucose were measured using a Cholestech LDX analyzer (Cholestech), which uses standard enzymatic methods. LDL cholesterol was calculated using the Friedewald equation (13). Plasma aliquots were shipped frozen to Johns Hopkins University, Baltimore, for storage and further analysis. Plasma insulin was measured using an ultrasensitive sandwich immunoassay (Alpco Diagnostics). Blood samples were collected from all assenting children; however, 33% had not fasted on the morning of the blood draw. Thus, insulin was not measured in those participants. Data for triglycerides, HDL cholesterol, and glucose were analyzed from both fasting and nonfasting children to maximize the analytic sample. This approach is justified given the documented associations between nonfasting lipid profiles and cardiovascular risk (14,15) and the utility of casual glucose measures in diagnosing type 2 diabetes (16). Moreover, the interpretation of the results did not differ when analysis was restricted to specimens that were collected in the fasting state.

Weight-for-age, height-for-age, and BMI-for-age Z-scores were calculated against the CDC 2000 growth reference using EpiInfo 3.4 (17). This growth reference was chosen to allow for comparison to the blood pressure reference curves. Blood pressure percentiles were calculated against a U.S. reference population, controlling for age, height, and sex (18) to allow comparability with other studies. Hypertension risk was defined as systolic blood pressure (SBP) ≥90th percentiles. Insulin resistance was estimated by using the homeostasis model assessment (HOMA) = (FPI × FPG)/22.5, where FPI is fasting plasma insulin concentration (mU/L) and FPG is fasting plasma glucose (mmol/L) (19).

Statistical analyses. Baseline and follow-up characteristics, including socioeconomic status indicators, maternal literacy and education, maternal smoking and alcohol consumption during pregnancy, and paternal occupation, were examined across treatment groups for comparability and any differential loss to follow-up. Differences were tested using the chi-square test. In addition, due to the relatively high number of children who had missing fasting blood data, characteristics were compared between those with complete data and those with missing data.

Dichotomous variables were created to examine the risk of high BMI (≥85th percentile of the study population), waist circumference (≥85th percentile), total cholesterol (≥4.4 mmol/L), triglycerides (≥1.7 mmol/L), HOMA (≥85th percentile), blood pressure (≥90th percentile of the reference population), microalbuminuria (microalbumin:creatinine ≥3.4 mg/mmol), and low HDL cholesterol (<0.9 mmol/L). The criteria employed here to define metabolic syndrome were chosen to be similar to the recommendations of the National Cholesterol Education Program (NCEP) ATP III guidelines for adults (20), with modifications made to use child-specific values where possible. A child was classified as “at risk” if they met ≥3 of the following criteria: 1) plasma glucose ≥85th percentile of the study population, because few of the children had a glucose concentration above the traditionally recommended cutpoint of 5.6 mmol/L (16); 2) plasma HDL cholesterol < 0.9 mmol/L, as recommended by the NCEP report on cholesterol in children and adolescents (21); 3) plasma triglycerides ≥1.7 mmol/L, as recommended by the NCEP guidelines for adults, because there is no separate recommendation for children (20); 4) SBP or DBP ≥ 90th percentile of the U.S. reference population (18); and 5) waist circumference ≥85th percentile of the study population. For comparison, the definition was also tested by including Hba1c rather than glucose as the indicator of hyperglycemia. Only children with complete data for all of the classification criteria were included in this part of the analysis.

Blood pressure, waist circumference, BMI, and Hba1c data followed a normal distribution and, therefore, the difference in means was compared across treatment groups using generalized estimation equations (GEE) with exchangeable correlation to account for the fact that communities, not individuals, had been randomized (22). Data for cholesterol, triglycerides, glucose, insulin, HOMA, and the microalbumin:creatinine ratio followed a log-normal distribution and, therefore, the difference in means was tested using the chi-square test. In addition, due to the relatively high number of children who had missing fasting blood data, characteristics were compared between those with complete data and those with missing data.

5 Abbreviations used: DBP, diastolic blood pressure; GEE, generalized estimation equation; HbA1c, glycated hemoglobin; HOMA, homeostasis model assessment; NCEP, National Cholesterol Education Program; OR, odds ratio; SBP, systolic blood pressure.
tiated to calculate the percent difference between treatment groups. Additionally, because 30% of children had a total cholesterol concentration less than the detectable limit of the assay, the odds ratio (OR) of being above compared with below the limit value was tested. Dichotomous variables were analyzed using GEE logistic regression models with exchangeable correlation (22).

A total of 3673 children aged 6–35 mo born during the antenatal supplementation trial were participants in a larger randomized trial involving iron+folic acid, zinc, iron+folic acid+zinc, or placebo, which enrolled ~25,000 children < 36 mo of age in 2001–2004 (23,24). Of the children in the current follow-up study who also participated in that child supplementation trial, the median age of enrollment was 12 mo (range, 6–35 mo) and duration of participation in the child supplementation trial was 12 mo (range, 1–25 mo). Possible confounding by that early nutritional exposure was tested by adding dummy variables for the child supplement arms, which did not change the β coefficients of the antenatal intervention terms. Additionally, interactions between the maternal antenatal and child intervention groups were tested with the primary outcomes of interest, blood pressure, insulin resistance, and metabolic syndrome, and were not significant (P > 0.05). Thus, the child intervention terms were excluded from all analytic models.

All models examining the primary outcomes were adjusted for the child’s age at the time of the follow-up, which ranged from 6–8 y, and cholesterol, triglycerides, glucose, and metabolic syndrome models were also adjusted for fasting state. Additionally, the interactions between the antenatal supplement intervention and maternal height or BMI in early pregnancy or child gender were tested and were not significant (P > 0.1 for all). Data were analyzed using Stata version 10.

The follow-up study received ethical approval from the Institutional Review Boards at Johns Hopkins School of Public Health, Baltimore, MD and at the Institute of Medicine, Kathmandu, in Nepal.

Results

Of the 3900 children surviving to 6 mo of age at the end of the original trial, 129 died between 6 mo of age and follow-up, 160 moved out of the study area, 82 could not be contacted at their homes, and 5 refused to participate. Thus, a total of 3524 children were enrolled and agreed to participate in some or all of the follow-up assessments, representing 93% of the surviving children (Fig. 1). At follow-up, children were a mean age of 7.5 y, ranging from 6.2 to 8.5 y. Losses to follow-up did not differ among groups (P = 0.52 using the chi-squared test). Household characteristics including ethnicity and caste, socioeconomic status, and education were similar among intervention groups (Table 1).

There was missing blood pressure data for 47 children and missing anthropometric data for 168 children. Blood or plasma data were missing for those who refused the blood draw (n = 177) or who were not at home at the time of the household visit despite repeated attempts (n = 175). In addition, insulin and HOMA could not be estimated in those who had not adhered to fasting instructions (n = 1161) on the morning of the blood draw, resulting in a sample size of only 2011 individuals for this measure. The children with missing or nonfasting plasma data were less likely to have originated from the hills of Nepal (i.e. of Pahadi ethnicity; 24 vs. 32%; P < 0.001), less likely to have had any schooling (64 vs. 67%; P = 0.038), and had a slightly lower BMI (13.9 vs. 14.0 kg/m²; P < 0.001) compared with children with complete data.

Cardiometabolic risk factors are summarized by treatment group in Table 2. Only triglyceride concentrations differed significantly between children in the folic acid+iron+zinc group compared with those in the control group, with a 7% (95% CI, −12, −2) reduction in children in the folic acid+iron+zinc group. Anthropometry, blood pressure, cholesterol, and measures of insulin resistance did not differ by intervention group. A total of 21.9% of children were at risk for hypertension (SBP or DBP ≥ 90th percentile of reference), mainly due to an elevation in DBP (data not shown). Only 20 children(<1%) had high fasting glucose (≥5.6 mmol/L) and no children had a high HbA1c (>7%).

Overall, 4.5% of children had a high urinary microalbuminuria: creatinine ratio (≥3.4 mg/mmol), but the prevalence in the folic acid (P = 0.025) and the folic acid+iron+zinc (P = 0.015) groups was roughly one-half that in the control (Table 3). The risk of microalbuminuria was reduced by 36% (OR, 0.64; CI, 0.45, 0.92) relative to the control group when all supplementation groups that included folic acid were combined.

Of the children with complete data on all classification criteria for metabolic syndrome (n = 3116), 522 children had a glucose measure greater than the 85th percentile, 2479 (79.5%) had low HDL cholesterol (<0.9 mmol/L), 348 (11.1%) had high triglycerides (≥1.7 mmol/L), 686 (22.0%) had high blood pressure (≥90th percentile of reference), 478 had a high waist circumference (≥83th percentile of the study population), and a total of 346 (11.1%) met the criteria for metabolic syndrome (Table 4). Because low HDL cholesterol was so common, 98%...
of children who were classified as at risk of metabolic syndrome had low HDL cholesterol plus 2 or more other factors. The risk of high glucose, high triglycerides, low HDL cholesterol, high blood pressure or high waist circumference did not differ by treatment group, yet there was a reduced risk of metabolic syndrome in the folic acid group (OR, 0.63; 95% CI, 0.41, 0.97). This was attenuated in the other treatment arms and none differed from the control group. The beneficial effect of the

**TABLE 2** Cardiometabolic risk factors among 6- to 8-y-old children by maternal supplement group

<table>
<thead>
<tr>
<th>Maternal supplement group</th>
<th>Control</th>
<th>Folic acid</th>
<th>Folic acid+iron</th>
<th>Folic acid+iron+ zinc</th>
<th>Multiple micronutrient</th>
</tr>
</thead>
<tbody>
<tr>
<td>All children, n</td>
<td>735</td>
<td>658</td>
<td>674</td>
<td>708</td>
<td>749</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>13.96 ± 1.08</td>
<td>14.01 ± 1.05</td>
<td>14.01 ± 1.06</td>
<td>14.01 ± 1.06</td>
<td>13.92 ± 1.02</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>51.23 ± 3.02</td>
<td>51.34 ± 2.94</td>
<td>51.27 ± 3.14</td>
<td>51.19 ± 2.93</td>
<td>51.22 ± 3.03</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>95.75 ± 7.98</td>
<td>95.46 ± 7.86</td>
<td>95.21 ± 8.31</td>
<td>95.02 ± 8.12</td>
<td>95.50 ± 8.51</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>64.15 ± 8.27</td>
<td>63.72 ± 8.75</td>
<td>63.68 ± 8.36</td>
<td>63.68 ± 8.66</td>
<td>64.44 ± 8.61</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.10 ± 0.27</td>
<td>5.07 ± 0.27</td>
<td>5.09 ± 0.29</td>
<td>5.11 ± 0.28</td>
<td>5.10 ± 0.25</td>
</tr>
<tr>
<td>Urinary microalbumin:creatinine, mg/mmol</td>
<td>1.24 (0.90–1.81)</td>
<td>1.24 (0.90–1.81)</td>
<td>1.24 (0.90–1.70)</td>
<td>1.24 (0.90–1.81)</td>
<td>1.24 (0.90–1.81)</td>
</tr>
<tr>
<td>Plasma total cholesterol, mmol/L</td>
<td>2.90 (&lt;2.59–3.28)</td>
<td>2.90 (&lt;2.59–3.31)</td>
<td>2.92 (&lt;2.59–3.37)</td>
<td>2.87 (&lt;2.59–3.34)</td>
<td>2.82 (&lt;2.59–3.28)</td>
</tr>
<tr>
<td>Plasma HDL cholesterol, mmol/L</td>
<td>0.67 (0.54–0.85)</td>
<td>0.70 (0.57–0.85)</td>
<td>0.72 (0.54–0.88)</td>
<td>0.70 (0.57–0.85)</td>
<td>0.70 (0.52–0.85)</td>
</tr>
<tr>
<td>Plasma LDL cholesterol, mmol/L</td>
<td>1.89 (1.60–2.15)</td>
<td>1.89 (1.60–2.12)</td>
<td>1.89 (1.63–2.17)</td>
<td>1.86 (1.63–2.20)</td>
<td>1.84 (1.58–2.09)</td>
</tr>
<tr>
<td>Plasma triglycerides, mmol/L</td>
<td>1.06 (0.79–1.38)</td>
<td>1.03 (0.79–1.32)</td>
<td>1.06 (0.80–1.40)</td>
<td>0.98 (0.73–1.38)</td>
<td>1.04 (0.77–1.36)</td>
</tr>
<tr>
<td>Child who had fasted, n</td>
<td>396</td>
<td>383</td>
<td>370</td>
<td>399</td>
<td>463</td>
</tr>
<tr>
<td>Plasma insulin, pmol/L</td>
<td>15.13 (8.5–27.64)</td>
<td>16.28 (10.7–26.46)</td>
<td>15.83 (10.03–25.07)</td>
<td>16.19 (9.08–25.95)</td>
<td>15.63 (8.43–27.58)</td>
</tr>
<tr>
<td>HOMA</td>
<td>0.43 (0.24–0.79)</td>
<td>0.47 (0.30–0.78)</td>
<td>0.45 (0.28–0.76)</td>
<td>0.47 (0.24–0.79)</td>
<td>0.45 (0.24–0.81)</td>
</tr>
</tbody>
</table>

1 Values are mean ± SD or median (IQR).
2 Data were missing for BMI (n = 741), waist circumference (n = 1800), blood pressure (n = 47), HbA1c (n = 352), total cholesterol, HDL cholesterol, triglycerides, and glucose (n = 352), LDL cholesterol (n = 1505), and the microalbumin:creatinine ratio (n = 2388).
3 963 children (30.0%) of children had a total cholesterol concentration < 2.59 mmol/L, the lower detectable limit of the assay, and thus the lower value of the IQR is reported as <2.59.
4 LDL cholesterol values could not be calculated for samples that had total or HDL cholesterol or triglyceride concentrations outside of the detectable range.
5 Among the 2011 blood samples from fasting children, data were missing for glucose (n = 1), insulin (n = 26), and HOMA (n = 71).
6 Different from control, P < 0.05. Differences for waist circumference, BMI, blood pressure, and HbA1c between each treatment and the control were tested for significance using a GEE-adjusted linear model. Relative mean differences in the urinary microalbumin:creatinine ratio, and plasma cholesterol, triglycerides, glucose, insulin, and HOMA were analyzed on the log scale using a GEE linear model with a log link. All models also controlled for the child’s age at the time of follow-up.
maternal folic acid supplementation on childhood risk of metabolic syndrome held when HbA1c rather than glucose was used to define hyperglycemia (OR, 0.57; 95% CI, 0.37, 0.87).

**Discussion**

We report here the findings of a community-based, cluster randomized, controlled trial of antenatal micronutrient supplements on early markers of chronic disease in childhood in rural Nepal. Despite a modest 40- to 65-g improvement in birth weight in the folic acid+iron and multiple micronutrient groups (10), supplementation did not affect blood pressure, plasma lipids, or insulin resistance in the children of women who participated in the antenatal micronutrient trial. We did observe a significant reduction in the risk of microalbuminuria among children whose mothers had received folic acid, with the largest effects in the folic acid and folic acid+iron-zinc groups. The OR for the risk of microalbuminuria were reduced in all intervention groups compared with the control, resulting in a 36% reduction in the risk of microalbuminuria when all supplementation groups containing folic acid were compared with the control, suggesting a protective role of folic acid on kidney function.

Microalbuminuria is a well-established marker of kidney dysfunction and cardiovascular risk (25). There is no evidence that antenatal folic acid supplementation affects fetal development of kidney function per se, although epigenetic effects of a methyl donor cannot be ruled out (26). Dietary restriction in the methyl donors of folate, vitamin B-12, and the amino acid methionine around the time of conception have been shown to induce alterations in DNA methylation, increase adiposity, promote insulin resistance, and elevate blood pressure in sheep (27). Furthermore, protein deficiency has been found to induce cardiovascular dysfunction and high blood pressure, which can be reversed with the supplementation of folate (28). Alterations in the function and morphology of the kidney are one of the key proposed pathways linking malnutrition in utero with later risk of cardiovascular disease (29). Although 1 study has linked moderate zinc restriction to perturbations in kidney development and function (30), there are limited data linking maternal deficiencies in other nutrients to renal development or function.

We also observed a likely reduction in the risk of metabolic syndrome only among the children in the folic acid group. However, there was no apparent reduction in the risk of any of the component risk factors, making it difficult to hypothesize precisely what mechanisms may have contributed to this effect. It is possible that there were common pathways linking the reduction in risk of microalbuminuria and metabolic syndrome,

<table>
<thead>
<tr>
<th>Maternal supplement group</th>
<th>Microalbuminuria, n (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>653</td>
<td>40 (6.1)</td>
</tr>
<tr>
<td>Folic acid</td>
<td>595</td>
<td>21 (3.5)</td>
</tr>
<tr>
<td>Folic acid+iron</td>
<td>609</td>
<td>29 (4.8)</td>
</tr>
<tr>
<td>Folic acid+iron+zinc</td>
<td>621</td>
<td>21 (3.4)</td>
</tr>
<tr>
<td>Multiple micronutrient</td>
<td>675</td>
<td>30 (4.4)</td>
</tr>
</tbody>
</table>

1 Microalbuminuria = urinary microalbumin:creatinine ratio ≥3.4 mg/mmol. * P < 0.05. OR and 95% CI were calculated using a GEE logistic regression model controlling for the child age at follow-up.

**TABLE 4** Risk factors associated with metabolic syndrome 6- to 8-y-old children by maternal supplement group.†,‡

<table>
<thead>
<tr>
<th>Maternal supplement group</th>
<th>Control</th>
<th>Folic acid</th>
<th>Folic acid+iron</th>
<th>Folic acid+iron+zinc</th>
<th>Multiple micronutrient</th>
</tr>
</thead>
<tbody>
<tr>
<td>High glucose (≥85th percentile)</td>
<td>143 (23.3)</td>
<td>119 (20.5)</td>
<td>127 (21.0)</td>
<td>132 (21.4)</td>
<td>165 (24.5)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>0.89 (0.65, 1.22)</td>
<td>0.91 (0.67, 1.24)</td>
<td>0.93 (0.69, 1.27)</td>
<td>1.0 (0.92, 1.48)</td>
<td></td>
</tr>
<tr>
<td>High blood pressure (SBP or DBP ≥ 90th percentile)</td>
<td>100 (15.6)</td>
<td>86 (14.8)</td>
<td>96 (15.9)</td>
<td>91 (14.8)</td>
<td>105 (15.6)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>0.87 (0.62, 1.23)</td>
<td>0.99 (0.71, 1.39)</td>
<td>0.89 (0.64, 1.24)</td>
<td>0.98 (0.69, 1.33)</td>
<td></td>
</tr>
</tbody>
</table>

1 Includes only children with complete data on all criteria for metabolic syndrome.
2 OR and 95% CI, adjusted for child age, fasting status, and for the design effect using a GEE binomial model with exchangeable correlation.
3 SBP or DBP ≥90th percentile of referent population for age, height, and sex (18).
4 Child met 3 of the above criteria.
5 Different from control, P < 0.05 (tested using a GEE logistic model controlling for the child’s age at follow-up).
possibly due to the impact of folic acid. However, when combined with other nutrients (iron, iron+zinc, or the multiple micronutrient), folic acid supplementation did not have the same effect, suggesting the possibility of nutrient interactions.

There are only a few studies of widely varying design against which to compare our results. The most direct comparison can be made with an antenatal supplementation trial comparing multiple micronutrients with iron-folic acid alone conducted in Nepal. In contrast to our results, a follow-up of children in this study showed that those in the multiple micronutrient group were >200 g heavier (95% CI, 10–390) at 2 y of age and had a small but significant 2.5-mm Hg (95% CI, 0.57, 4.35) reduction in SBP relative to the iron-folic acid control group (31). Whether those early differences are sustained remains to be examined. Our study suggests no impact of antenatal multiple micronutrient supplementation on blood pressure at 6–8 y of age. The data for other nutrients is sparse. Some observational studies in humans have noted a small negative association between maternal iron intake or hemoglobin concentration during pregnancy with the offspring’s blood pressure (32), although others have observed either no association (33,34) or a positive association (35,36). Animal studies, however, have demonstrated that iron (37,38) or zinc (30) restriction during pregnancy causes elevated offspring blood pressure and altered cardiovascular development. And, although the animal evidence suggests that folate may have a protective effect on cardiovascular dysfunction or insulin resistance (27,28), 1 study in India found that a combination of high folate and low vitamin B-12 concentrations during pregnancy were associated with a greater degree of insulin resistance in 6- to 8-y-old children (39). These data are observational, however, and thus restricted in their ability to derive causal inferences.

Nutrient-nutrient interactions may explain why the folic acid+iron and multiple micronutrient groups had attenuated effects on both microalbuminuria and metabolic syndrome, but it is difficult to speculate how these mechanisms could have operated. Interactions have been reported between iron and zinc (40,41) or folic acid and zinc (42,43). We previously reported a lack of increased serum folic acid concentration in the folic acid+iron+zinc group (44), suggesting a potential negative inhibition of zinc on folate status. It has been suggested that there may be a mutual inhibition of these 2 nutrients at the site of intestinal transport (42). Alternatively, it is possible that the differing point estimates may reflect chance variation due to their comparability to the effect ascribed to folic acid alone.

Our study was limited by high numbers of missing data on some indicators, notably in fasting insulin, glucose, and HOMA, which decreased our sample size and power to find significant differences among treatment groups. The children who had not fasted were likely to be less educated and poor. Yet the potential for bias is likely to be minimal, because we also did not observe any treatment group differences in HbA1c or nonfasting glucose, which had fewer missing data, further supporting the lack of impact of supplementation on insulin resistance. However, these children may be too young to detect insulin resistance or dyslipidemia, resulting in our null findings. Alternatively, rapid postnatal growth or the development of obesity (45), not experienced by this population, may be a necessary precondition for the development of hypertension or insulin resistance.

This study provides unique data for the effects of micronutrient supplementation during pregnancy on risk factors for chronic disease from a randomized, controlled trial with high compliance and a high rate of follow-up. Despite improvements in birth weight, survival, and anemia, the only effects of supplementation observed here were on microalbuminuria, and potentially modest effects on the risk of metabolic syndrome, but not blood pressure or insulin resistance. In particular, folic acid supplementation during pregnancy appeared to have the greatest beneficial effect on both offspring kidney function and risk of metabolic syndrome. It is possible that if this population had experienced greater exposure to the nutrition transition or if the children were older, the differences may have been more pronounced. It would be of interest to determine whether intervention group differences persist or further differences develop as these children age into young adulthood. There is still much to be learned about the effects of micronutrient nutrition during this critical period of the lifespan.

**Literature Cited**


