Dietary diversity scores, nutrient intakes and biomarkers vitamin B₁₂, folate and Hb in rural youth from the Pune Maternal Nutrition Study

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Abstract
Hidden hunger is widespread in India. Individual dietary diversity score (IDDS) is a measure of the nutrient adequacy of the diet. The FAO has set guidelines for the measurement of dietary diversity: the IDDS and the minimum dietary diversity score for women (MDD-W) to assess nutritional deficiency, but validation against nutritional biomarkers is required. Using available data among rural youth (17 years) from the Pune Maternal Nutrition Study, the validity of DDS was assessed to measure deficiencies of vitamin B₁₂, folate and Hb. Of the 355 boys and 305 girls, 19% were classified as underweight, 57% as vitamin B₁₂ deficient (<150 pmol/L) and 22% as anaemic (<120/130 g/L). Cereals, legumes and ‘other-vegetables’ were the most frequently consumed foods. More boys than girls consumed milk, flesh, eggs and micronutrient-dense foods. Median IDDS of 4 (interquartile range (IQR) 3–4) and MDD-W of 6 (IQR 5–7) were low. Youth with vitamin B₁₂ deficiency had a higher likelihood of an IDDS ≤ 4 (1.89; 95% CI 1.24, 2.87) or an MDD-W ≤ 5 (1.40; 95% CI 1.02, 1.94). Youth with anaemia were more likely to have an IDDS ≤ 4 (1.76; 95% CI 1.01, 3.14) adjusted for socio-economic scores, BMI, energy intake and sex. Folate deficiency was low (3%) and was not associated with either score. Youth with lowest plasma vitamin B₁₂ and Hb infrequently or never consumed dairy products/non-vegetarian foods. These rural Indian youth were underweight, had low DDS and consumed foods low in good-quality proteins and micronutrients. Associations of DDS with circulating micronutrients indicate that DDS is a valid measure to predict vitamin B₁₂ deficiency and anaemia.

Key words: Dietary diversity scores: Rural Indian youth: Circulating micronutrients

India is experiencing the ‘dual burden’ of both chronic widespread under-nutrition and the rapidly emerging problem of over-nutrition. Both are forms of malnutrition as is hidden hunger, related to consuming foods of poor nutritional quality and therefore not meeting requirements for vitamins and minerals in the diet. On the one hand in India, in urban as well as rural settings more than half of the children in ten out of fifteen states suffer from anaemia, and on the other hand, the prevalence of overweight/obesity has also increased¹. This nutritional situation is closely associated with the dietary patterns of Indian people. Analysis² of the last, 2012, National Nutrition Monitoring Bureau survey shows that Indians continue to consume cereal-based diets which are monotonous and lack micronutrient-rich foods. The scenario is more challenging for rural Indians as their diets are inadequate in diverse foods such as green leafy vegetables, dairy products, foods that are a good source of protein and vegetables containing β-carotene³,⁴. Particularly for communities most in need, a tool to rapidly measure dietary diversity and to find out which foods and food groups are consumed or not consumed by individuals and groups within communities is essential to inform and target equitable actions. Dietary diversity scores (DDS) offer a quick and simple assessment of the macro and micronutrient adequacy of the diet. Many questionnaires for assessing DDS have been developed, but methodological differences in the classification and grouping of foods limit the comparability and generalisability of findings (⁵) and only a few studies were validated with another measure of food intake.

Multiple efforts were made to improve and harmonise measurement approaches and indicators of DDS in children⁶,⁷, adolescents⁸ and adults⁹. These culminated in 2013 when the UN FAO published guidelines and questionnaires to measure individual dietary diversity scores (IDDS) from nine food groups.⁰ A further refinement of a minimum dietary diversity score for women (MDD-W) from ten food groups¹¹ was published in 2016. The validity of DDS to assess nutritional adequacy

Abbreviations: DDS, dietary diversity score; IDDS, individual dietary diversity score; MDD-W, minimum dietary diversity score for women; PMNS, Pune Maternal Nutrition Study.

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measured by other dietary intake measures such as 24-h dietary recalls has little evidence and no reviews. Reports of small to moderate associations between micronutrient intake adequacy measured by 24-h dietary recalls and the nine FAO IDDS food groups are reported for children aged between 6 months and 12 years from China\(^{(10)}\), Philippines\(^{(11)}\), South Africa\(^{(12)}\), Kenya\(^{(13)}\) and rural Bangladesh\(^{(14)}\) where children and their mothers were assessed. A cut-off MDD-W of 6 best predicted adequate micronutrient status (by food recall) in pregnant adolescent girls and women in Bangladesh\(^{(15)}\). A study conducted in three South African towns among women aged 15–49 years reported higher food security and MDD-W in urban locations than in peri-urban or rural ones\(^{(16)}\). To the best of our knowledge, only three studies with youth\(^{(15–17)}\) have used FAO scores and included adolescent girls aged 16–18 years. No studies were found including boys of this age. In addition, there were no reports from India. Most reports have calculated DDS based on diet composition analysis from either a 24-h dietary recalls or a FFQ, and the same measurements were used to derive the DDS to demonstrate relative validity; therefore, under-, over- and misreporting were not accounted for\(^{(18)}\). The single study\(^{(17)}\) that used four objective biomarkers of circulating Hb, ferritin, Zn and folate and the only finding was that in the hunger season there was an association of low MDD-W with low serum Zn. Globally and in India, there a high prevalence of deficiency of vitamin B\(_{12}\), folate and Hb, biomarkers of micronutrient status relevant for maternal and child health\(^{(19–21)}\).

The Pune Maternal Nutrition Study (PMNS), a longitudinal birth cohort study initiated in 1993\(^{(22,23)}\) conducted in rural region of Maharashtra in India, provides an opportunity to objectively examine the validity of DDS to measure biomarkers. When the youth were aged 17 years, circulating concentrations of vitamin B\(_{12}\), folate and Hb were measured, and at the age of 18 years dietary intake was measured with a quantitative FFQ, and the same measurements were used to derive the DDS to demonstrate relative validity; therefore, under-, over- and misreporting were not accounted for\(^{(20)}\). The single study\(^{(17)}\) that used four objective biomarkers of circulating Hb, ferritin, Zn and folate and the only finding was that in the hunger season there was an association of low MDD-W with low serum Zn. Globally and in India, there a high prevalence of deficiency of vitamin B\(_{12}\), folate and Hb, biomarkers of micronutrient status relevant for maternal and child health\(^{(19–21)}\).

This analysis of the PMNS data aimed to investigate associations and validity of the consumption of food groups as defined by FAO DDS with nutrient intakes derived from the FFQ and vitamin B\(_{12}\), folate and Hb concentrations in youth living in a rural area of Pune, India.

**Methods**

**Study design**

The methodology of the longitudinal PMNS has been published previously\(^{(22–24)}\). Briefly, the study was established between 1993 and 1995 in six rural villages (total population 35 000) near the city of Pune in the state of Maharashtra, to prospectively study the influence of maternal nutrition on growth and later cardiometabolic risk of the offspring. Detailed measurements of offspring were undertaken at birth and at 6 years (2001–2003), 12 years (2006–2008), 17 years (2012–2014) and 18 years (2013–2015) in addition to regular body size measurements every 6 months. At the age of 17 years, between February 2012 and July 2012, 353 boys and 363 girls had blood samples drawn for Hb and plasma vitamin B\(_{12}\) and folate concentration measurements. At the age of 18 years, follow-up between April 2013 and September 2014, these youth completed FFQ and body size measurements at 18 years follow-up. These youth formed the sample for this study.

**Ethics**

Approval for the study was obtained from the village leaders, participants, their parents and the Ethics Committee of KEM Hospital Research Centre, Pune, India.

**Anthropometry**

At the age of 18 years, weight was measured to the nearest 0·1 kg using digital scales (Omron), and height was measured to the nearest 0·1 cm using a wall-mounted stadiometer (CMS Instruments Ltd). Anthropometric measures were recorded in duplicate and an average of both values used for analysis. Inter-observer variation studies were conducted to maintain quality; coefficients of variation for height and weight were <0.5%. BMI was calculated as the ratio of weight to height (in metres) squared (kg/m\(^2\)). Subjects were classified according to WHO BMI criteria\(^{(25–26)}\) into underweight (<18·5 kg/m\(^2\)), normal (18·5–24·9 kg/m\(^2\)), overweight (25·0–29·9 kg/m\(^2\)) and obese (>30·0 kg/m\(^2\)). Stunting was defined as height for age less than minus two standard deviations of the WHO growth reference (boys <161·6 cm, girls <150·2 cm).

**Dietary assessment**

All youth with a trained nutritionist recorded the amount and frequency of consumption over the last 12 months of 150 commonly consumed food items in a locally validated\(^{(26,27)}\) FFQ. First, the frequency for each food was recorded as never, daily, weekly, monthly or yearly. Then, within that time interval, the number of times the food was consumed was asked. Finally, the quantity consumed in each eating occasion was recorded. A variety of two- and three-dimensional food models (bowls, spoons and roti sizes pre-calibrated for volume) were provided to assist the respondent to estimate the quantity of each food. The weight of each food item consumed was determined and divided by a time factor to determine daily intake, for example, once a month divided by 30 and once a week was divided by 7\(^{(22)}\). Foods were grouped as cereals, legumes, green leafy vegetables, other vegetables, fruits, milk and milk products, non-vegetarian foods, snacks, confectionery and beverages. Detailed information concerning ingredients and the recipes for local mixed dishes were recorded.

**Determination of macro- and micronutrient intakes**

Each food item was matched by a trained nutritionist to a food (line) in a combination of local\(^{(22)}\) and national food composition databases\(^{(28)}\). The local food composition database was initiated in 1993 when macronutrient composition for cooked and commonly consumed food was measured in the Biometry and Nutrition department of the Agharkar Research Institute, Pune\(^{(22)}\). Proximate analysis of energy content was by Bomb calorimetry, protein by the Kjeldahl technique, fat by Soxlet and carbohydrate by difference. Vitamin B\(_{12}\) and folate content...
of foods were estimated using values from the national database with assumptions based on the predominant foods, ingredients and moisture gain or loss during cooking in each recipe.

Assessment of dietary diversity

Foods were grouped according to characteristics and nutrient profile predetermined by the FAO for the IDDS as an indicator of dietary adequacy and the MDD-W as an indicator of micronutrient intake adequacy\(^{89}\) (Table 1). For a food group to be counted in the dietary diversity index, the minimum average quantity was set at \(\geq 5\) g/d. Maximum score of the IDDS was 9 and for the MDD-W was 10.

Adequacy of dietary intake for selected nutrients

Nutrient adequacy ratios for the intake of energy, protein, fat, Fe, vitamin \(B_12\) and folate were calculated by dividing the daily intake of the nutrient by the recommended daily intake for that nutrient according to the Indian Council of Medical Research guidelines\(^{80}\) accounting for age, sex and activity.\(^{80}\) As a measure of overall micronutrient adequacy, mean adequacy ratio was calculated as the mean of the nutrient adequacy ratios for the intake of the micronutrients Fe, vitamin \(B_12\) and folate. For both nutrient adequacy ratio and mean adequacy ratio, a value of 1 is ideal, that is, the intake is the same as the requirement.

Biochemical measurement of circulating nutrients

Blood samples were collected into EDTA vacutainers. The haemoglobin was measured (Beckman Coulter, T540 and AC T diff TM Analyzers). Within an hour of collection, the remaining blood was centrifuged (2500 g \(\times\) 15 min), and the separated plasma was used for standard biochemical measurements. Aliquots were stored at \(-70^\circ\)C until further analysis. Plasma vitamin \(B_12\) and folate were measured by microbial assay\(^{31,32}\). The inter- and intra-assay CV were \(<5\%\) for all measurements. At the age of 17 years, seven boys and seven girls with vitamin \(B_12\) concentrations above \(400\) pmol/l were not considered for analysis. Low plasma vitamin \(B_12\) was defined as \(<150\) pmol/l vitamin \(B_12\), low plasma folate as \(<7\) nmol/l\(^{33,34}\) and low Hb levels \(<130\) g/l for boys and \(<120\) g/l for girls\(^{35}\).

Statistical analysis

All continuous data were examined for outliers and tested for normality. Variables with skewed distribution (vitamin \(B_12\) and folate concentrations) were transformed to the natural logarithm. Normally distributed variables are summarised as means and standard deviations. Continuous variables with skewed distributions are presented as medians and 25th and 75th centiles. Categorical variables are summarised as frequencies and proportions (percentages). Boys and girls were analysed separately. Binary logistic regression was used to study the association of lower and higher micronutrient concentrations at the age of 17 years (outcome variables) and DDS at the age of 18 years, sex, BMI, socio-economic status score and total daily energy intake at the age of 18 years. We also compared circulating vitamin \(B_12\) and Hb concentrations among youth consuming the three basic groups namely starchy staples, legumes and other vegetables plus, for example, dark green leafy vegetables or flesh foods by ANOVA. The statistical analysis was conducted using SPSS 22 (IBM).

Results

Blood measures at the age of 17 years and food frequency data at the age of 18 years were available for 355 boys and 303 girls. At the 18 years age of measurement point, boys were 6 months older, 10·6 kg heavier and 12·7 cm taller than girls (Table 2). Using WHO adult BMI criteria\(^{26,35}\), 25·6% of boys and 21·1% of girls (\(P = 0·077\)) were overweight and obese, and 10·7% of boys and 11·8% of girls (\(P = 0·655\)) were stunted. When we checked for micronutrient deficiencies, around half of the boys and girls had lower circulating vitamin \(B_12\) levels, 14% of boys and remarkably high, that is, 30% of girls were anaemic.

The distributions of the summed number of IDDS dietary food groups consumed were similar for both boys and girls (median 4; interquartile range 3–4) while that for MDD-W was higher (\(P = 0·020\)) among boys than girls (median 6; interquartile range 5–7). Consumption of dairy foods and fruits was very low (Fig. 1). Some youth reported that they did not consume the protein-containing foods: legumes, milk and milk products, flesh foods and eggs, and boys consumed these foods substantially more often than girls. The typical meal of these rural Maharashtrian children consists of an Indian bread (made from wheat or jowar or bajra flour) along with a savoury side dish of other vegetables/leafy greens/sprouts and a savoury dal (pulses) with boiled rice.

Dietary analysis of all foods consumed showed that a substantial proportion of both boys and girls had inadequate intakes of energy and protein (Table 3). These inadequacies were substantially higher for girls (53% had inadequate energy and protein intakes) than boys (33% had inadequate energy and 18% had inadequate protein intakes). Also, more girls than boys had inadequate micronutrient intakes for Fe (49 v. 2%) and vitamin \(B_12\) (81 v. 59%). The nutrient adequacy ratio and mean adequacy ratio \((r = 0·66)\) were positively and significantly correlated with the IDDS as well as MDD-W among boys and girls.

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Table 1. Comparison of food groupings utilised to determine dietary diversity scores

<table>
<thead>
<tr>
<th>Individual dietary diversity score(^8)</th>
<th>Minimum dietary diversity score for women of reproductive age(^8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All starchy staple foods</td>
<td>All starchy staple foods</td>
</tr>
<tr>
<td>Legumes (nuts and seeds)</td>
<td>Legumes (beans and peas)</td>
</tr>
<tr>
<td>Milk and milk products</td>
<td>Milk and milk products</td>
</tr>
<tr>
<td>Organ meat</td>
<td>Nuts and seeds</td>
</tr>
<tr>
<td>Meat and fish</td>
<td>Flesh foods</td>
</tr>
<tr>
<td>Eggs</td>
<td>Eggs</td>
</tr>
<tr>
<td>Dark green leafy vegetables</td>
<td>Dark green leafy vegetables</td>
</tr>
<tr>
<td>Other vitamin A-rich fruits and vegetables</td>
<td>Other vitamin A-rich fruits and vegetables</td>
</tr>
<tr>
<td>Other fruits and vegetables</td>
<td>Other fruits and vegetables</td>
</tr>
</tbody>
</table>

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Table 2. Characteristics of participants at the age of 18 years
(Mean values and standard deviations; frequencies and percentages; differences and 95% confidence intervals; medians and interquartile ranges (IQR))

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Boys (n=355)</th>
<th>Girls (n=305)</th>
<th>Difference</th>
<th>95% CI</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>18.2 ± 0.5</td>
<td>17.7 ± 0.6</td>
<td>0.50</td>
<td>0.42, 0.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>56.7 ± 10.7</td>
<td>46.1 ± 7.8</td>
<td>10.67</td>
<td>9.21, 12.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169.6 ± 6.7</td>
<td>156.9 ± 5.8</td>
<td>12.75</td>
<td>11.78, 13.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.7 ± 3.3</td>
<td>18.7 ± 3.1</td>
<td>0.95</td>
<td>0.46, 1.44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Frequency (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight</td>
<td>91 ± 25.6</td>
<td>64 ± 21.1</td>
<td>4.51</td>
<td>−2.01, 10.87</td>
<td>0.174</td>
</tr>
<tr>
<td>Normal</td>
<td>237 ± 66.8</td>
<td>227 ± 74.6</td>
<td>7.82</td>
<td>0.81, 14.60</td>
<td>0.028</td>
</tr>
<tr>
<td>Overweight and obese</td>
<td>27 ± 7.6</td>
<td>27 ± 4.3</td>
<td>3.31</td>
<td>−0.41, 6.92</td>
<td>0.077</td>
</tr>
<tr>
<td>Stunted (%)</td>
<td>38/355 ± 10.7</td>
<td>36/304 ± 11.8</td>
<td>1.14</td>
<td>−3.72, 6.07</td>
<td>0.655</td>
</tr>
<tr>
<td>Vitamin B₁₂ &lt; 150 pmol/l</td>
<td>211/355 ± 59.8</td>
<td>171/303 ± 56.4</td>
<td>3.41</td>
<td>−4.11, 10.89</td>
<td>0.377</td>
</tr>
<tr>
<td>Plasma folate &lt; 7 nmol/l</td>
<td>13/353 ± 3.68</td>
<td>4/303 ± 1.32</td>
<td>2.36</td>
<td>−0.17, 5.00</td>
<td>0.058</td>
</tr>
<tr>
<td>Hb (&lt;130 boys &lt;120 girls, g/l)</td>
<td>49/353 ± 13.9</td>
<td>91/303 ± 30.0</td>
<td>16.05</td>
<td>9.80, 22.35</td>
<td>0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Median IQR</th>
<th>Median IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDDS (4 ± 3)</td>
<td>4 ± 3</td>
</tr>
<tr>
<td>MDD-W (6 ± 5)</td>
<td>6 ± 5</td>
</tr>
</tbody>
</table>

IDDS, individual dietary diversity scores; MDD-W, minimum dietary diversity scores for women.
* P values for differences between boys and girls, independent t test for continuous variables, χ² test for proportions.

Fig. 1. Proportion of boys and girls consuming different dietary groups. Individual dietary diversity scores (IDDS) by FAO guidelines. ■ % Boys; ■ % Girls.

Unadjusted and adjusted binary logistic regression was used to compare the OR for youth with and without micronutrient deficiency and consuming ≤4 IDDS food groups every day (Table 4). Youth with insufficient plasma vitamin B₁₂ at the age of 17 years had almost two times higher (1.89; 95% CI 1.24-2.87; P=0.001) likelihood of having IDDS ≤4. Similarly, youth with low Hb concentrations, <130 g/l for boys and <120 g/l for girls, had significantly higher likelihood of having IDDS ≤4 (Table 4). Circulating folate concentrations were not associated with IDDS. Youth with insufficient plasma vitamin B₁₂ at the age of 17 years had higher chances of scoring MDD-W of 5 or less (1.40; 95% CI 1.02-1.94; P=0.040) (results not shown in table). Circulating folate or Hb concentrations were not associated with MDD-W. All the associations remained unchanged when adjusted for socio-economic score, BMI and energy intake at the age of 18 years and sex of the child.

We compared circulating vitamin B₁₂ and Hb concentrations among participants who consumed different combinations of food groups. Youth with lowest circulating vitamin B₁₂ and Hb concentrations consumed only starchy staples, legumes and other fruits and vegetables, that is, IDDS score of 0 (Table 4). Circulating folate concentrations were not associated with MDD-W. All the associations remained unchanged when adjusted for socio-economic score, BMI and energy intake at the age of 18 years and sex of the child.

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### Table 3. Daily nutrient intakes, nutrient adequacy ratio (NAR) and association with dietary diversity scores

(Median values and 25th, 75th percentiles; mean values and standard deviations; Pearson r values and 95% confidence intervals)

<table>
<thead>
<tr>
<th>Nutrient intake</th>
<th>Boys (n 355)</th>
<th>Girls (n 305)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Energy (kcal)†</td>
<td>2964 ± 535</td>
<td>2068 ± 535</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>80 ± 14</td>
<td>53 ± 14</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>62 ± 14</td>
<td>47 ± 14</td>
</tr>
<tr>
<td>Fe (mg)</td>
<td>31 ± 14</td>
<td>21 ± 14</td>
</tr>
<tr>
<td>Vitamin B₁₂ (μg)</td>
<td>0.8 ± 0.00</td>
<td>0.5 ± 0.00</td>
</tr>
<tr>
<td>Folate (μg)</td>
<td>458 ± 357</td>
<td>396 ± 357</td>
</tr>
</tbody>
</table>

| NAR with IDDS (%) | Median intake | RDA | % inade- | Association with MDD-W (%) | Median intake | RDA | % inade-
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>NAR with IDDS (%)</td>
<td>1.2 ± 0.4</td>
<td>0.45</td>
<td>0.45</td>
<td>0.36 ± 0.53</td>
<td>0.49</td>
<td>0.49</td>
<td>0.49</td>
</tr>
<tr>
<td>NAR with MDD-W (%)</td>
<td>1.4 ± 0.5</td>
<td>0.56</td>
<td>0.56</td>
<td>0.48 ± 0.63</td>
<td>0.57</td>
<td>0.57</td>
<td>0.57</td>
</tr>
</tbody>
</table>

*P < 0.01 for all correlations. NAR is intake/recommended intake by the Indian Council for Medical Research 2010(29). † To convert energy values from kcal to kJ, multiply by 4.184.
Diet diversity and micronutrients in youth

Table 4. Adjusted and unadjusted associations between risk factors and individual dietary diversity score (IDDS) less in 660 youth aged 18 years* 
(Frequencies and percentages; odds ratios and 95 % confidence intervals)

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Exposed with IDDS ≤ 4</th>
<th>Non-exposed with IDDS ≤ 4</th>
<th>Unadjusted</th>
<th>Adjusted</th>
<th>OR 95 % CI</th>
<th>OR 95 % CI</th>
<th>Adjusted P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma vitamin B₁₂ &lt; 150 pmol/l</td>
<td>319/382 83.5</td>
<td>190/260 73.1</td>
<td>1.87</td>
<td>1.27, 2.74</td>
<td>1.89</td>
<td>1.24, 2.87</td>
<td>0.001</td>
</tr>
<tr>
<td>Plasma folate &lt; 7 nmol/l</td>
<td>12/17  70.5</td>
<td>510/639 79.8</td>
<td>0.61</td>
<td>0.21, 1.78</td>
<td>0.91</td>
<td>0.27, 3.07</td>
<td>0.887</td>
</tr>
<tr>
<td>Hb (boys &lt; 130 g/l, girls &lt; 120 g/l)</td>
<td>117/137 85.4</td>
<td>392/505 77.6</td>
<td>1.69</td>
<td>1.00, 2.83</td>
<td>1.76</td>
<td>1.01, 3.14</td>
<td>0.048</td>
</tr>
</tbody>
</table>

* All associations adjusted for socio-economic score, BMI at the age of 18 years, energy intake at the age of 18 years and sex.

Strengths and limitations

One strength of this study is that both girls and boys were assessed and that the age of 17–18 years is an important stage of growth and development. Soon, these youths, especially the girls, will be parents, and to help break the intergenerational cycle of malnutrition, intervention should be before parenthood(43). The IDDS(8) as well as MDD-W(9) guidelines by FAO provide questions that determine if any foods from each of the nine/ten food groups were consumed in the last 24 h and the minimum quantity of at least 10 g/d. This is when a 24 HDR is used. Our dietary assessment method was a 150-item 12-month FFQ so is not directly comparable with other studies(15,16,18). We used the minimum quantity of an average of 5 g/d rather than the 10 g suggested by the FAO because of the large number (150) of food items in the FFQ and the small portions consumed and the relatively small body mass of the youth (about 50 % underweight).

One limitation is that the measure of circulating nutrients was undertaken at the age of 17 years of age, while the DDS were based on dietary records at the age of 18 years when a number of the youths were moving from the rural farm to the urban college environment, but some had been randomised to receive dietary supplementation which meant that a measure of circulating biomarkers would be affected and the allocation to treatment could not be broken for this report. However, we have shown previously that food patterns track over time(44,45) and are intergenerational(47). We have presented content validity in that we find the expected association of DDS and micronutrient intakes when determined by the same instrument. However, the real strength of our study is the internal validity of the associations of the DDS with objective measures of vitamin B₁₂ and Hb which to the best of our knowledge has not been reported in youth or a rural population before.

The way ahead and conclusion

Considering food variety and geographical, cultural, sex difference and socio-economic limitations that influence the variation in the dietary patterns and diversity, we need more population-specific studies. These studies are needed to use an objective, separate measure of DDS rather than comparing DDS and intakes calculated using the same questionnaire. More studies using the conventional FAO guidelines need to be conducted in wide age and physiological groups such as among pregnancy, during lactation and separately among both sexes to check the suitability of the DDS as a tool to measure nutrient inadequacy.
and inform and measure the effectiveness of changes in policy and action. Such studies will be helpful for agricultural and nutritional programmes and policymakers to quickly reach those most in need.

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