

## Dietary diversity scores, nutrient intakes and biomarkers vitamin B<sub>12</sub>, 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 B<sub>12</sub>, folate and Hb. Of the 355 boys and 305 girls, 19 % were classified as underweight, 57 % as vitamin B<sub>12</sub> 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 B<sub>12</sub> 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 B<sub>12</sub> 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 B<sub>12</sub> 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 increased<sup>(1)</sup>. This nutritional situation is closely associated with the dietary patterns of Indian people. Analysis<sup>(2)</sup> 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-carotene<sup>(2)</sup>. 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<sup>(3)</sup> 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 children<sup>(4,5)</sup>, adolescents<sup>(6)</sup> and adults<sup>(7)</sup>. These culminated in 2013 when the UN FAO published guidelines and questionnaires to measure individual dietary diversity scores (IDDS) from nine food groups<sup>(8)</sup>. A further refinement of a minimum dietary diversity score for women (MDD-W) from ten food groups<sup>(9)</sup> 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<sup>(10)</sup>, Philippines<sup>(11)</sup>, South Africa<sup>(12)</sup>, Kenya<sup>(13)</sup> and rural Bangladesh<sup>(14)</sup> 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<sup>(15)</sup>. 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<sup>(16)</sup>. To the best of our knowledge, only three studies with youth<sup>(15–17)</sup> 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<sup>(18)</sup>. The single study<sup>(17)</sup> 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<sub>12</sub>, folate and Hb, biomarkers of micronutrient status relevant for maternal and child health<sup>(19–21)</sup>.

The Pune Maternal Nutrition Study (PMNS), a longitudinal birth cohort study initiated in 1993<sup>(22,23)</sup> 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<sub>12</sub>, folate and Hb were measured, and at the age of 18 years dietary intake was measured with a quantitative FFQ (150 foods) at 18 years before the youth were randomised to a controlled trial of vitamin B<sub>12</sub> supplementation.

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<sub>12</sub>, 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<sup>(22,24)</sup>. 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 cardio-metabolic 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 303 girls had blood samples drawn for Hb and plasma vitamin B<sub>12</sub> 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<sup>2</sup>). Subjects were classified according to WHO BMI criteria<sup>(25,26)</sup> into underweight (<18.5 kg/m<sup>2</sup>), normal (18.5–24.9 kg/m<sup>2</sup>), overweight (25.0–29.9 kg/m<sup>2</sup>) and obese (>30.0 kg/m<sup>2</sup>). 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<sup>(22,27)</sup> 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<sup>(22)</sup>. 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<sup>(22)</sup> and national food composition databases<sup>(28)</sup>. 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<sup>(22)</sup>. Proximate analysis of energy content was by Bomb calorimetry, protein by the Kjeldahl technique, fat by Soxhlet and carbohydrate by difference. Vitamin B<sub>12</sub> and folate content



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

| Individual dietary diversity score <sup>(8)</sup> | Minimum dietary diversity score for women of reproductive age <sup>(16)</sup> |
|---------------------------------------------------|-------------------------------------------------------------------------------|
| All starchy staple foods                          | All starchy staple foods                                                      |
| Legumes (nuts and seeds)                          | Legumes (beans and peas)                                                      |
| Milk and milk products                            | Milk and milk products                                                        |
| Organ meat                                        | Nuts and seeds                                                                |
| Meat and fish                                     | Flesh foods                                                                   |
| Eggs                                              | Eggs                                                                          |
| Dark green leafy vegetables                       | Dark green leafy vegetables                                                   |
| Other vitamin A-rich fruits and vegetables        | Other vitamin A-rich fruits and vegetables                                    |
| Other fruits and vegetables                       | Other vegetables<br>Other fruits                                              |

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<sup>(8,9)</sup> (Table 1). For a food group to be counted in the dietary diversity analysis, 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<sub>12</sub> 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<sup>(29)</sup> accounting for age, sex and activity<sup>(30)</sup>. 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<sub>12</sub> 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 haemogram was measured (Beckman Coulter, T540 and AC.T diff TM Analyzers). Within an hour of collection, the remaining blood was centrifuged (2500 *g* × 15 min), and the separated plasma was used for standard biochemical measurements. Aliquots were stored at  $-70^{\circ}\text{C}$  until further analysis. Plasma vitamin B<sub>12</sub> and folate were measured by microbial assays<sup>(31,32)</sup>. 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<sub>12</sub> concentrations above 400 pmol/l were not considered for analysis. Low plasma vitamin B<sub>12</sub> was defined as  $<150$  pmol/l vitamin B<sub>12</sub>, low plasma folate as  $<7$  nmol/l<sup>(33,34)</sup> and low Hb levels  $<130$  g/l for boys and  $<120$  g/l for girls<sup>(33)</sup>.

#### Statistical analysis

All continuous data were examined for outliers and tested for normality. Variables with skewed distribution (vitamin B<sub>12</sub> 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<sub>12</sub> 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<sup>(26,35)</sup>, 25.6% of boys and 21.1% of girls ( $P$  for sex difference 0.174) were underweight, 7.6% of boys and 4.3% 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<sub>12</sub> 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.

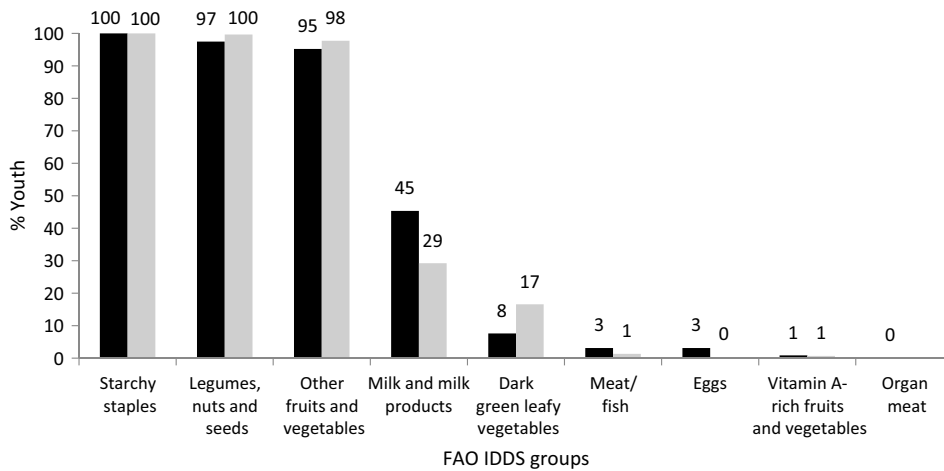
Dietary analysis of all foods consumed showed that a substantial proportion of both boys and girls had inadequate intakes of energy and protein (Table 3). These inadequacies were substantially higher for girls (53% had inadequate energy and protein intakes) than boys (33% had inadequate energy and 18% had inadequate protein intakes). Also, more girls than boys had inadequate micronutrient intakes for Fe (49 *v.* 2%) and vitamin B<sub>12</sub> (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.

**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))

| Measurements                         | Boys (n 355) |      | Girls (n 305) |      | Difference | 95 % CI      | P*     |
|--------------------------------------|--------------|------|---------------|------|------------|--------------|--------|
|                                      | Mean         | SD   | Mean          | SD   |            |              |        |
| Age (years)                          | 18.2         | 0.5  | 17.7          | 0.6  | 0.50       | 0.42, 0.59   | <0.001 |
| Weight (kg)                          | 56.7         | 10.7 | 46.1          | 7.8  | 10.67      | 9.21, 12.11  | <0.001 |
| Height (cm)                          | 169.6        | 6.7  | 156.9         | 5.8  | 12.75      | 11.78, 13.71 | <0.001 |
| BMI (kg/m <sup>2</sup> )             | 19.7         | 3.3  | 18.7          | 3.1  | 0.95       | 0.46, 1.44   | <0.001 |
|                                      | Frequency    | %    | Frequency     | %    |            |              |        |
| Underweight                          | 91           | 25.6 | 64            | 21.1 | 4.51       | -2.01, 10.87 | 0.174  |
| Normal                               | 237          | 66.8 | 227           | 74.6 | 7.82       | 0.81, 14.60  | 0.028  |
| Overweight and obese                 | 27           | 7.6  | 27            | 4.3  | 3.31       | -0.41-6.98   | 0.077  |
| Stunted (%)                          | 38/355       | 10.7 | 36/304        | 11.8 | 1.14       | -3.72, 6.07  | 0.655  |
| Vitamin B <sub>12</sub> < 150 pmol/l | 211/353      | 59.8 | 171/303       | 56.4 | 3.41       | -4.11, 10.89 | 0.377  |
| Plasma folate <7 nmol/l              | 13/353       | 3.68 | 4/303         | 1.32 | 2.36       | -0.17, 5.00  | 0.058  |
| Hb (<130 boys <120 girls, g/l)       | 49/353       | 13.9 | 91/303        | 30.0 | 16.05      | 9.80, 22.35  | 0.001  |
|                                      | Median       | IQR  | Median        | IQR  |            |              |        |
| IDDS                                 | 4            | 3-4  | 4             | 3-4  | 0.15       | 0.18, 0.27   | 0.025  |
| MDD-W                                | 6            | 5-7  | 6             | 5-7  | 0.25       | 0.04, 0.46   | 0.020  |

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,  $\chi^2$  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  $\leq 4$  IDDS food groups every day (Table 4). Youth with insufficient plasma vitamin B<sub>12</sub> 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  $\leq 4$ . Similarly, youth with low Hb concentrations, <130 g/l for boys and <120 g/l for girls, had significantly higher likelihood of having IDDS  $\leq 4$  (Table 4). Circulating folate concentrations were not associated with IDDS. Youth with insufficient plasma vitamin B<sub>12</sub> 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 B<sub>12</sub> and Hb concentrations among participants who consumed different combinations of food groups. Youth with lowest circulating vitamin B<sub>12</sub> and Hb concentrations consumed only starchy staples, legumes and other fruits and vegetables, that is, IDDS score of  $\leq 3$  ( $n = 240$ ), while those who had higher micronutrient levels additionally consumed flesh or eggs ( $n = 51$ , 13F 38M) (difference in vitamin B<sub>12</sub> -19.2 pmol/l 95 % CI -53.96, 15.59,  $P = 0.03$ ; difference in Hb -7.0 g/l, 95 % CI -12.8, -1.2,  $P = 0.01$ ). When separated by sex, these differences remained significant for boys but not in girls. Youth who consumed dark green leafy vegetables and milk or milk products but not flesh or eggs did not have significantly higher vitamin B<sub>12</sub> or Hb values. This may be as overall proportion of youth consuming nutrient-rich food groups was low. The folate values did not differ by any combination of dietary diversity food groups.



**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)

| Nutrient intake/d            | Boys (n 355)  |            |      |              |      |     |                           |            |                            |            | Girls (n 305) |            |      |              |      |      |                           |                            |  |  |
|------------------------------|---------------|------------|------|--------------|------|-----|---------------------------|------------|----------------------------|------------|---------------|------------|------|--------------|------|------|---------------------------|----------------------------|--|--|
|                              | Median intake | 25, 75 %   | RDA  | % inadequate | NAR  |     | Association with IDDS (r) | 95% CI     | Association with MDD-W (r) | 95% CI     | Median intake | 25, 75 %   | RDA  | % inadequate | NAR  |      | Association with IDDS (r) | Association with MDD-W (r) |  |  |
|                              |               |            |      |              | Mean | sd  |                           |            |                            |            |               |            |      |              | Mean | sd   |                           |                            |  |  |
| Energy (kcal)†               | 2964          | 2406, 3669 | 2730 | 33           | 1.2  | 0.4 | 0.45                      | 0.36, 0.53 | 0.49                       | 0.41, 0.57 | 2068          | 1724, 2535 | 2230 | 53           | 1.0  | 0.32 | 0.34                      | 0.24, 0.44                 |  |  |
| Protein (g)                  | 80            | 65, 99     | 60   | 18           | 1.4  | 0.5 | 0.56                      | 0.48, 0.63 | 0.57                       | 0.50, 0.64 | 53            | 45, 66     | 55   | 55           | 1.0  | 0.3  | 0.46                      | 0.37, 0.54                 |  |  |
| Fat (g)                      | 62            | 48, 81     | 30   | 3            | 2.2  | 0.9 | 0.60                      | 0.53, 0.66 | 0.63                       | 0.56, 0.69 | 47            | 37, 61     | 25   | 3            | 2.0  | 0.7  | 0.47                      | 0.38, 0.55                 |  |  |
| Fe (mg)                      | 31            | 25, 37     | 17   | 2            | 1.8  | 0.5 | 0.49                      | 0.41, 0.57 | 0.52                       | 0.44, 0.59 | 21            | 18, 26     | 21   | 49           | 1.1  | 0.3  | 0.40                      | 0.30, 0.49                 |  |  |
| Vitamin B <sub>12</sub> (µg) | 0.8           | 0.4, 1.4   | 1    | 59           | 1.1  | 1.0 | 0.54                      | 0.46, 0.61 | 0.56                       | 0.48, 0.63 | 0.5           | 0.2, 0.8   | 1    | 81           | 0.7  | 0.8  | 0.39                      | 0.29, 0.48                 |  |  |
| Folate (µg)                  | 458           | 357, 581   | 200  | 0            | 2.4  | 0.9 | 0.52                      | 0.44, 0.59 | 0.49                       | 0.41, 0.57 | 396           | 301, 478   | 200  | 4            | 2.1  | 0.8  | 0.16                      | 0.05, 0.27                 |  |  |
| Mean adequacy ratio          | -             | -          | -    | -            | 1.7  | 0.6 | 0.64                      | 0.41, 0.79 | 0.66                       | 0.60, 0.71 | -             | -          | -    | -            | 1.5  | 0.5  | 0.45                      | 0.36, 0.54                 |  |  |

RDA, Recommended Dietary Allowances by Indian Council of Medical Research, 2010; IDDS, individual dietary diversity scores; MDD-W, minimum dietary diversity scores for women.

\*  $P < 0.01$  for all correlations. NAR is intake/recommended intake by the Indian Council for Medical Research 2010<sup>(49)</sup>.

† % with inadequate intake than RDA by the Indian Council for Medical Research 2010.

‡ To convert energy values from kcal to kJ, multiply by 4.184.

On binomial regression analysis, youth with lower vitamin B<sub>12</sub> concentrations at the age of 17 years had a one and half times higher chance of low consumption of milk products (1.66, 95% CI 1.17, 2.35,  $P = 0.005$ ) and three times higher chance that they had low consumption of any flesh and egg foods every day at the age of 18 years (3.02, 95% CI 1.27, 7.19,  $P = 0.013$ ) (results not shown). These results were adjusted for sex, BMI, height, energy intake and socio-economic status at the age of 18 years. Lower Hb and folate concentrations at the age of 17 years were not associated with consumption of specific food groups

### Discussion

The key and novel finding of this retrospective analysis of prospective data provides evidence that the FAO DDS are valid predictors of vitamin B<sub>12</sub> deficiency and anaemia in youth with a high prevalence of underweight and inadequate energy intakes. In addition, it highlights that the food groups like milk and milk products were only consumed by 45% of boys and 29% of girls. The lack of vitamin A-rich vegetables, dark green leafy vegetables, fruits, eggs and flesh foods in the diet highlights that these foods should be produced and consumed in adequate quantities to ensure improved dietary quality and food security. The second key finding is the sex inequity as girls consumed less micronutrient-rich foods and more foods from relatively cheaper dietary sources such as green leafy vegetables than boys. This sex difference in diet was paralleled with more underweight girls than boys. Consumption of any food group or combination of food groups was not associated with plasma folate concentration. This information is strategic for the assessment of the impact of agriculture and the food supply on nutritional status.

To our knowledge till now, the only study that reports associations between DDS and circulating biomarkers is the one conducted among adolescent Mozambican girls (14 to 19 years old)<sup>(17)</sup>. This Mozambican study looked at associations between MDD-W with concentrations of Hb, serum ferritin, Zn and folate, and plasma retinol in both the hunger and the harvest season. They report that the only association found was where low serum Zn (the lowest 25%) in the hunger season was best predicted by an MDD-W  $\leq 3$  derived from a 24-h dietary recalls and  $\leq 5$  from a 7-d FFQ. In our study, IDDS cut-off of  $\leq 4$  FAO food groups was associated with a high prevalence of insufficient circulating micronutrient levels of vitamin B<sub>12</sub> and Hb.

Many studies<sup>(36)</sup> have measured DDS as a measure of adequate dietary intakes; however, very few have followed the FAO guidelines to calculate the DDS making it difficult for comparison. The studies we found did not directly ask the diversity questions, but like Mozambican study utilised other dietary assessment tools and variably adapted criteria to determine the count of food groups. For example, both the studies from Kenya<sup>(13)</sup> and from rural Bangladesh<sup>(14)</sup> among schoolchildren reported that the number of food groups counted for the DDS based on a minimum of 15 g/d was better associated with nutrient adequacy than the count of food groups with no requirement for a minimum quantity. A study from Nepal<sup>(37)</sup> determined the IDDS score for eight food groups (organ meat was included with meat and fish) averaged over 3 d for 500 lactating women

**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)

| Risk factor                                 | Exposed with IDDS $\leq$ 4 |       | Non-exposed with IDDS $\leq$ 4 |      | Unadjusted |            | Adjusted |            | Adjusted <i>P</i> |
|---------------------------------------------|----------------------------|-------|--------------------------------|------|------------|------------|----------|------------|-------------------|
|                                             | Frequency                  | %     | Frequency                      | %    | OR         | 95% CI     | OR       | 95% CI     |                   |
| Plasma vitamin B <sub>12</sub> < 150 pmol/l | 319/382                    | 83.5  | 190/260                        | 73.1 | 1.87       | 1.27, 2.74 | 1.89     | 1.24, 2.87 | 0.001             |
| Plasma folate < 7 nmol/l                    | 12/17                      | 70.58 | 510/639                        | 79.8 | 0.61       | 0.21, 1.78 | 0.91     | 0.27, 3.07 | 0.887             |
| Hb (boys < 130 g/l, girls < 120 g/l)        | 117/137                    | 85.4  | 392/505                        | 77.6 | 1.69       | 1.00, 2.83 | 1.76     | 1.01, 3.14 | 0.048             |

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

(17–44 years) residing in a peri-urban area with a minimum of 15 g/d. They reported that the IDDS (mean 3.9 (sd 1.0)) was associated with multiple micronutrient intakes below the estimated average requirements.

Our finding of low DDS agrees with other studies with children from China<sup>(10)</sup>, Philippines<sup>(11)</sup> and South Africa<sup>(12)</sup>, and among pregnant adolescent girls and adult women from rural Bangladesh<sup>(14)</sup> which report DDS of less than five groups. We report similar findings among rural Indian youth as the IDDS were lower than five food groups among two-thirds of youth and MDD-W less than six groups among more than one-third. Similar to the present study, diet in these developing countries has limited diversity with a predominance of starchy staples and infrequent and relatively small amounts of flesh foods, dairy products and vitamin A-rich vegetables.

Previous studies comparing adequacy of micronutrient intakes among pregnant adolescent girls and women in Bangladesh<sup>(15)</sup> observed when six rather than five food groups every day was used as the cut-off, there was better sensitivity to classify micronutrient intake inadequacies including riboflavin, vitamin B<sub>12</sub>, Ca, Zn and folate. We not only reported association of DDS with circulating micronutrients but also studied association of consumption with specific food groups. In our study, consumption of foods from nutrient-rich food groups like flesh foods, eggs and milk was associated with higher circulating micronutrient concentrations of vitamin B<sub>12</sub> and Hb and folate was not associated.

Though the median DDS was similar between sexes, there were marked differences in the fourth food group consumed by the adolescent boys and girls. Girls consumed foods from cheaper sources as green leafy vegetables, while boys consumed pricier food sources as meat and eggs. This is similar to that reported by Aurino<sup>(38)</sup> who report, from two longitudinal studies in Andhra Pradesh and Telangana, that the pro-boy gap in diet diversity widens at 15 years of age when appetites increase and boys are rapidly growing. These authors did not measure diversity scores. Food allocation especially among south Asian countries including India favours men and boys who often receive greater quantities of higher quality, nutritious food such as dairy products<sup>(39–41)</sup>. It is important to consider sex and food allocation issues while planning nutritional policies in south Asian countries to achieve the Sustainable Development Goals 2020 with a particular focus on zero hunger and sex equality<sup>(42)</sup>. DDS have been understudied in Indian settings, and this is the first to our knowledge in a rural setting with a birth cohort.

### 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<sup>(43)</sup>. The IDDS<sup>(8)</sup> as well as MDD-W<sup>(9)</sup> 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<sup>(15,16,18)</sup>. 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<sup>(44,45)</sup> and are intergenerational<sup>(39)</sup>. 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 B<sub>12</sub> 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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