Associations of dietary diversity scores and micronutrient status in adolescent Mozambican girls

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Conflict of interest disclosures: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Abstract

Purpose: In low-income settings, dietary diversity scores (DDS) often predict the micronutrient adequacy of diets, but little is known about whether they predict levels of biochemical indicators of micronutrient status. **Methods:** In 2010, we studied two samples of non-pregnant 14 to 19-year-old girls in Central Mozambique, the first in January-February ('hunger season'; n=227) and the second in May-June (harvest season; n=223). In this paper, we examined if a low Women's Dietary Diversity Score (WDDS) predicts a low concentration of haemoglobin, serum ferritin, zinc, and folate and plasma retinol in adolescent Mozambican girls. We constructed three scores: WDDS based on 24-hour recalls, WDDS15g based on 24-hour recall and employing a 15 g limit, and 7dWDDS based on seven-day food frequency questionnaires. Logistic regression models, stratified by season, were used to estimate the odds of having a low concentration of a status indicator ($\leq 25^{th}$ percentile of the season-specific distribution or cut-off from the literature) in those with a low score compared to those with a higher score. **Results:** In January-February, after adjusting for confounders, a low (≤ 3) WDDS and a low (≤ 5) 7dWDDS were each associated with higher odds of having low serum zinc compared to having a higher score, regardless of which of the two types of cut-offs for serum zinc was used. These associations were not present in May-June. **Conclusions:** Our data from Mozambique suggest that dietary diversity is associated with serum zinc but this association seems to be limited to the hunger season.

Keywords: Dietary diversity score, micronutrient, nutritional status, adolescent girl, Mozambique, Sub-Saharan Africa

Introduction

High diversity is generally considered to be a positive feature in a diet [1], and in some low- and middle income countries, diversification of the diet is promoted through food based dietary guidelines [2]. Researchers have designed and tested various methods and indicators for collecting and processing data on dietary diversity or food variety. Food variety scores are simple counts of different food items consumed during a defined recall period, for example seven days collected via a food frequency questionnaire (FFQ) [3,4]. Dietary diversity score (DDS) is the count of food groups consumed during a recall period, which has varied from one to seven days in different studies [4-7]. Methods using consumption data from two non-consecutive days have been used for example in Mozambique [8] and Bangladesh [7]. However, much of the research on dietary diversity has focused on methods that allow collecting data from a single interview per respondent. One of the reasons for this may be that a single interview substantially reduces the workload in the field.

Research in different low- and middle-income countries has shown that DDS based on 24-hour or seven-day recall periods are positively correlated with the micronutrient adequacy of diets, as measured by mean adequacy ratios [4,9] or the mean probability of adequacy [6,7]. Correlations between DDS and mean probability of adequacy have not, however, been equally strong in all settings [6,7] and correlations between DDS and nutrient adequacy ratios or estimated usual intakes of individual nutrients have not been equally strong either [10,11].

Mozambique was one of the countries included in a five-country project on women's dietary diversity where several different DDS (called food group indicators in that project) were tested [6]. Based on the findings from the project, FAO proposed a nine-food group tool called the Women's Dietary Diversity Score (WDDS) [12]. The results for Mozambican women showed that WDDS correlated significantly with estimates of usual intake for energy, thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B_{12}, vitamin C, vitamin A, and calcium with correlation coefficients in the range of 0.16 to 0.34 but not with estimates of usual intake for iron or zinc [11].

Although in many situations DDS are useful tools for assessing the micronutrient adequacy of diets, it has not been established whether they could be used to assess the risk of micronutrient deficiencies. According to our experience, micronutrient status assessment from venous blood samples in low-income settings is challenging due to high cost and demanding logistics along with other factors such as participant refusal. Thus, it would be practical to have simple non-invasive proxy tools for assessing the risk of low micronutrient status. These type of tools, although unlikely to be suitable for diagnosis at the individual level, could be used at the population level, for

example in targeting interventions to geographical areas which are most in need, or monitoring and evaluation of programs that aim to improve the nutritional situation of vulnerable populations [12]. Indeed, previous studies in pregnant women in Southern Ethiopia [13] and breastfeeding women in Northern Kenya [14] have found that women with a low DDS had an increased risk of having vitamin A deficiency compared to women with a higher DDS. Furthermore, the Ethiopian researchers also found an association between DDS and serum zinc among pregnant women [15].

We have previously reported that anaemia, and low serum ferritin, serum zinc, and plasma retinol concentrations are prevalent among adolescent girls in Central Mozambique [16]. In this paper, our aim was to study whether having a low WDDS was associated with higher odds of having a low concentration of haemoglobin, serum ferritin, zinc, and folate, and plasma retinol concentrations compared to having a higher dietary diversity among non-pregnant adolescent girls in Central Mozambique during two distinct seasons. In addition, we discuss some methodological aspects related to using different types of dietary assessment methods for the construction of a DDS.

Materials and Methods

Data

In this paper we present secondary data analyses using the data from the ZANE Study, which was a cross-sectional observational study on the diet and nutritional status of adolescent girls in Zambézia Province. The study was registered at ClinicalTrials.gov as NCT01944891. It was approved by the Bio-ethical Committee of the Ministry of Health in Mozambique.

Details of the sampling design, a diagram of recruitment, field work methods and population characteristics are presented elsewhere [17]. In short, the data collection took place in the provincial capital city, Quelimane, and the districts of Maganja da Costa and Morrumbala. Two separate samples were recruited, one in January-February and a second one in May and the early days of June, 2010. The second sample did not include girls who were included in the first sample. January-February is considered to be the end of the hunger season. The main harvest of maize, cowpeas, and groundnuts is in April-May and thus the second sample was studied at the end of the main harvest season. In the districts, girls were recruited both from the district towns and rural areas. The total number of girls who gave their consent to participate in the study was 639. Of those, 551 (86 %) girls came to the study centres and participated in the study. All participants gave informed consent and a parental consent was obtained from those under the age of 18 years. The participants were between 14 and 19 years of age.

Background interviews and anthropometric measurements were conducted and body-mass-index-for-age (BMI-for-age) z-scores were calculated [17]. The background questionnaire asked if the participant had children and if she was currently breastfeeding. Literacy was tested by asking the girl to read out one sentence [17]. Seven-day FFQ interviews and 24-hour dietary recall interviews were conducted. The two different dietary interviews were conducted on the same day but not by the same interviewers.

The seven-day FFQ included 37 individual foods or food groups, and in addition, it had one empty line for dietary supplements, two empty lines for beverages, and two to three empty lines for items in the following food groups: “other nuts and seeds”, “other roots/tubers”, ”other vegetables”, “other fruit”, and “other foods”. The interviewer wrote the reported food on the empty line.

Pregnancy tests were performed from urine samples. Being non-pregnant was defined based on a negative urine test result (n=371), or if urine test result was not available, self-report of not being pregnant (n=79). Venous blood samples were drawn, processed, and frozen at the study centres and transported to Finland [17]. Blood haemoglobin was determined on the site using the HemoCue Hb 301 System (Hemocue AB, Sweden). Serum high-sensitivity C-reactive protein (hsCRP), ferritin, zinc and folate, and plasma retinol concentrations were analysed as described previously [16]. In short, hsCRP was analysed immunoturbidometrically (CRP Vario 6K2602) and ferritin (Ferritin 7K59) and folate (Folate 1P7) by chemiluminescent microparticle immunoassays using Architect ci8200 analyzer (Abbott Diagnostics). For analysis of plasma retinol, the samples were extracted with hexane, evaporated and dissolved in methanol. Samples were analysed by HPLC (Inertsil ODS-3 column 2.1 x 100 mm, 3µm; GL Sciences, Japan) using methanol (0.3 m/min) as the mobile phase. Five microliters were injected into the column and retinol was detected by UV-detection at 325 nm. The analyses were standardized by freshly prepared all-retinol standards in ethanol whose concentration was verified by spectrophotometry. Serum zinc concentrations were analysed by plasma emission spectrometry (ICP-OES; Thermo Jarrell Ash IRIS Advantage). The reference material was Seronorm Trace Elements Serum L-1 (Sero AS, Norway).

The analyses in this paper include all non-pregnant girls who had an analysed venous blood sample and available dietary data (n=227 in January-February, n=223 in May-June, i.e. total n=450). One participant who reported nothing but water was excluded from the analyses using the 24-hour dietary recall data. In addition, there were four girls for whom 24-hour recall was not conducted and one girl for whom FFQ data was not conducted. Thus the analyses using the 24-hour dietary recall data and the FFQ data included 445 and 449 participants, respectively.

DDS and asset score variables

For the analyses in this paper, we constructed three different DDS variables. Firstly, following the food grouping instructions by FAO [12], we constructed the Women's Dietary Diversity Score (WDDS) from the 24-hour recall data. For each of the nine food groups, the participants were coded as having a score of one if they had reported consuming a food belonging to that group during the recall period and the scores of the nine food groups were summed. Secondly, since a 15-gram minimum has also been proposed in order to avoid foods consumed in very small amounts from contributing to the total score [6], we constructed another score employing this limit (WDDS15g). This means that the food group score was given only if the girls reported eating at least 15 grams of a food belonging in that food group. Thirdly, using the FFQ data and the same food grouping as above, we constructed a score representing the dietary diversity of the previous seven days (7dWDDS). In this score, it was not possible to set a minimum gram amount since portion sizes were not asked in our FFQ. FAO has recently developed a new score called the Minimum Dietary Diversity-Women (MDD-W) [18]. MDD-W is a 10-food group indicator, meant for dietary diversity assessment in women and a cut-off of 5 is recommended. However, at the time of finalizing this paper, the detailed instructions on individual food items that are included in each food group were not yet available. Therefore, the new score could not be used here.

We created a household asset score for which we selected 20 variables from the background questionnaire data. We assigned scores from 1 to 4 to each asset item owned by each participant's household and calculated the sum of these scores for each participant. The asset items and their scoring are shown in Supplemental table 1.

Statistical analyses

We performed separate univariate and multivariate logistic regression analyses to examine associations between low DDS and low blood concentration of each nutritional status indicator. To come up with a single cut-off for each DDS variable, we calculated the 25^{th} percentiles of DDS variables with data from the two seasons combined and used them as cut-offs to divide girls into those with low DDS ($\leq 25^{th}$ percentile) and those with medium to high DDS ($>25^{th}$ percentile). For simplicity, the latter group is referred to as "high DDS". The cut-offs for low WDDS, WDDS15g and 7dWDDS were ≤ 3, ≤ 3 and ≤ 5, respectively. We calculated the proportions of consumers of different food groups in the low and high categories of the DDS variables by season and compared them with chi-squared tests. We also drew scatter plots of the total WDDS and 7dWDDS scores.

For each nutritional status variable, we used data-driven cut-offs, calculated as the 25th percentiles separately for each season and used them to divide the girls into those who had a low blood concentration and those who did not. It should be noted that by "low concentration" we do not refer to deficiency. For example, the serum concentrations of folate were mostly in the range considered adequate and thus our cut-offs for folate were relatively high.

In addition to our strategy of using data-driven cut-offs, we also performed logistic regression analyses using established cut-offs from the literature. We used cut-offs by WHO for anaemia [19], and serum ferritin [20]. For zinc, we used a cut-off by the International Zinc Nutrition Consultative Group [21]. For plasma retinol, we used the WHO cut-off for serum retinol [22]. For folate, we planned to use the cut-off by WHO [23] but the analyses could not be carried out due to small cell sizes in both seasons. For the same reason, the analyses were not carried out for retinol in January-February.

Our previous analyses showed that there were seasonal differences in the diet and the micronutrient status in this study population [16] and therefore we conducted all analyses stratified by season. We included study area, age, BMI-for-age z-score, breastfeeding, hsCRP, literacy and the asset score in all of the adjusted regression models. These potential confounders were selected from the data available based on previous literature and preliminary analyses. The study area variable was coded as having five categories (Quelimane City, the district town of Maganja da Costa, rural villages in Maganja da Costa, the district town of Morrumbala and rural villages in Morrumbala). The DDS, breastfeeding and literacy variables were dichotomous (low/high or yes/no) and age, BMI-for-age z-score, hsCRP (mg/l), and the asset score variables were continuous. Dietary supplement use was not included as a confounder as only seven girls reported using iron or other dietary supplements by the FFQ.

In the FFQs available, there were eight (0.2%) missing answers needed to create the food group scores of the 7dWDDS. Furthermore, 39 (0.4%) data points for individual asset items, one (0.2 %) total asset score, four (0.9 %) answers for current breastfeeding, and nine (2%) literacy test results were missing. These were imputed by hot deck imputation [24] in SPSS 22. The deck variables used were study area and season for the food group scores, study area and age for breastfeeding, and study area for the asset and literacy data. Cases with missing data for dietary assessment, nutritional biomarkers, hsCRP, or BMI-for-age z-score were omitted from the analyses pairwise.

In the preliminary analyses, we examined, stratified by season, the unadjusted relationships between the dependent and independent variables as well as between the different independent variables. This was done by cross-tabulation and chi-squared tests with continuous variables as quartiles, and

Mann-Whitney U-tests (data not shown). We also plotted the DDS scores against the nutritional status variables (Supplemental figures 1 to 6). One outlier was excluded from the plots for serum ferritin but not from the other analyses.

All analyses were done using the survey package [25] in R version 3.0.2 [26] and using sampling weights based on the total population sizes of the study areas. P values <0.05 were considered statistically significant.

Results

Table 1 shows descriptive statistics of the study participants. In both seasons, the median age of participants was 16 years. In January-February, the mean WDDS was 4.1 and in May-June, it was 3.7.

Consumers of food groups

Table 2 shows the proportions of consumers of different foods groups among those classified as having a low and high dietary diversity. All participants reported having consumed at least a small amount of starchy staples. Organ meats and milk or milk products were rarely reported. The food groups most commonly reported by the "low" groups were not the same for both seasons. In January-February, the most commonly reported food groups by the "low WDDS" group were "starchy staples", "dark green leafy vegetables", and "other fruit and vegetables" and during May-June they were "starchy staples", "other fruit and vegetables" and "meat and fish" (Table 2). According to the 24-hour dietary recalls, the most common foods within the food groups "starchy staples " and "other fruit and vegetables" were maize, rice, cassava and wheat; and coconut, onion and tomato, respectively.

Classification of participants according to WDDS and 7dWDDS

Figure 1 shows scatter plots of WDDS and 7dWDDS, which illustrate the total scores assigned by the two different dietary assessment methods. In January-February, out of those classified as having a low 7dWDDS, 56% were classified as having a low WDDS, and the remaining 44% (as weighted percentages) were classified as having a high WDDS. In May-June, the respective percentages were 42% and 58%. In January-February, the total percentage of girls classified in the same category

(low or high) by the WDDS and the 7dWDDS was 76% (as a weighed percentage). In May-June, the respective percentage was 56%.

The reference periods of the 24-hour dietary recall and the FFQ overlapped (for all except one participant who answered the 24-hour recall on a later day), and the empty rows in the FFQ allowed the inclusion of foods that were not listed in the form. Therefore, in theory, the 7dWDDS should include at least those food groups which are included in the WDDS. However, there were a small number of answer pairs which were incorrect in this regard. For example, some girls reported five food groups in the 24-hour dietary recall but only four food groups in the FFQ. Of all pairs of scores with overlapping recall periods, these apparently incorrect pairs of scores represented three out of 223 pairs (weighted percentage 2.0%) in January-February and three out of 220 pairs (weighted percentage 1.3%) in May-June, respectively.

Associations of dietary diversity and biochemical indicators of micronutrient status

Table 3 shows the median concentrations of the biochemical indicators of micronutrient status and the cut-offs used. Tables 4 and 5 show the associations between DDS and low concentrations of the biochemical indicators in the two seasons for the analyses using the data-driven cut-offs. In the unadjusted models, low dietary diversity, as measured by WDDS and 7dWDDS, was associated with higher odds of having a low serum zinc concentration compared to having a high dietary diversity in January-February (Table 4). These associations remained statistically significant after adjusting for study area, age, BMI-for-age z-score, breastfeeding, hsCRP, literacy and asset score. In addition, a similar association was found for WDDS15g in the adjusted model. In May-June, no associations between dietary diversity and serum zinc were observed when using the data-driven cut-offs (Table 5).

When using the literature cut-offs, the results for zinc were similar to those with data-driven cut-offs. In January-February, the unadjusted ORs for the associations between WDDS and 7dWDDS and zinc were 2.03 (95% CI: 0.95-4.33 [P value 0.07]) and 2.83 (95% CI: 1.25-6.45 [P value 0.013]), respectively. After adjusting for confounders, both were significant (OR 2.57; 95% CI: 1.01-6.50 [P value 0.048] and OR 3.01; 95% CI: 1.11-8.13 [P value 0.031], respectively). Furthermore, in May-June, after adjusting for confounders, low WDDS15g was associated with higher odds of having low haemoglobin (unadjusted OR 1.40; 95% CI: 0.71-2.74 [P value 0.33], adjusted OR 2.6; 1.09-6.18 [P value 0.033] and ferritin (unadjusted OR 0.91; 95% CI: 0.44-1.86 [P value 0.79], adjusted OR 2.6; 1.09-6.18 [P value 0.032]). When using the literature cut-offs, we found no other significant associations in the unadjusted or adjusted models in either of the seasons.

Discussion

We examined the associations between dietary diversity and low concentrations of five biochemical indicators of micronutrient status during two different seasons in non-pregnant adolescent girls in Central Mozambique. Although one type of model suggested associations with haemoglobin and serum ferritin, the most consistent finding seemed to be that in January-February, having a low dietary diversity score was associated with higher odds of low serum zinc. The associations found for zinc, were not, however, present in May-June.

Compared with earlier reports on the associations between dietary diversity and micronutrient status indicators [13-15], the strength of our study was the possibility to examine two seasons with different food consumption patterns. Indeed, our results suggest that the associations between dietary diversity and micronutrient status may vary from season to the other – we found a seasonal discrepancy in the association between dietary diversity and zinc. One of the possible reasons explaining this discrepancy is the fact that the most commonly reported food groups among those categorized as having low dietary diversity were not the same in the two seasons. For example in May-June, the meat and fish group, which is an important source of well-absorbable zinc, was commonly consumed both among those with “low” and those with “high” WDDS. In future studies, it might be useful to examine the possibilities of using WDDS (or some of its modifications) as a tool to predict the risk of low zinc status in populations. However, our study suggests that the interpretation of results from dietary diversity surveys in relation to zinc status may not be the same in different seasons. This suggests that in this population, the use of the tool might need to be restricted to a specific season.

We found that WDDS and 7dWDDS classified girls rather differently, as illustrated by the scatter plots of total scores and also the fact that a large proportion was classified differently by these two methods. Despite this, the results on associations with zinc status were similar for both recall periods (24 hours or 7 days). Therefore the results of our analyses do not allow us to make recommendations on the length of recall period in future studies.

Our results do not support those of previous studies describing positive associations between DDS and vitamin A status [13,14]. One of the possible reasons for the lack of association between dietary diversity and retinol concentrations in adolescent Mozambican girls could be the fact that only a couple of food groups were important sources of vitamin A in diets of the girls (namely mangoes and dark green leafy vegetables; for more detailed information, see our previous results [16]), and these food groups were consumed by the majority. Thus the scores may not have been able to capture much variation in the consumption of foods that are essential for vitamin A intake. The

importance of the two major sources is illustrated by the fact that in January-February, the group “other vitamin A-rich fruit and vegetables” contributed, on average, 73% (as a population proportion [27]) of the total intake of retinol activity equivalents, with the main source being mango. A majority of the girls were consumers of this food group (68% during the previous day and 95% during the previous seven days in, as weighted percentages). In May-June, dark green leafy vegetables contributed 82% of the total retinol activity equivalent intake and this food group was consumed by 42% of the girls during the previous day and 89% during the previous seven days.

Folate deficiency was rare in our study population. Thus future studies are needed to investigate whether associations of dietary diversity and folate status could be detected in populations with higher proportions of serum concentrations in the deficient range.

Reporting previous food consumption is a challenging cognitive task [28] and errors in recalls of food consumption may have contributed to over- or underreporting of food groups in the two dietary assessment methods we used. The number of apparently incorrect reports of the type where the WDDS was higher than the 7dWDDS was, however, low in this study. When looking at the proportions of consumers of individual food groups, there seems to be a discrepancy in the use of organ meat between the two dietary assessment methods with almost no reporters in the 24-h recalls but a number or reporters in the FFQs. Similarly, in May-June, the group “other vitamin A-rich fruit and vegetables” was rarely reported in the 24-hour recalls but for example pumpkin, which falls in this group, was commonly reported in the FFQ. Although it is possible that these findings are due to the different lengths of the reference periods and are in fact accurate, they may also be due to inaccurate reporting related to the interview methodologies used. The 24-hour recall is an open-ended method and, for example, some of the organ meat may have been left unreported or mistakenly reported as flesh meat, whereas in the FFQs, organ meat consumption was specifically asked, which may have improved the accuracy of reporting or even yielded in over-reporting. These observations highlight the importance of careful probing of food consumption during the interview. It is possible that for the purpose of assessing dietary diversity, the type of interview proposed by FAO [12], which involves firstly a qualitative 24-hour recall and secondly filling in a form with a list of food groups coupled with probing of the food groups not yet mentioned, is a better choice than the open-ended 24-hour recall interview without a list of food groups. This view has gained support from other researchers [29].

The study had some limitations that should be noted. The results on serum zinc should be interpreted with care, since serum zinc levels vary according to fasting status [20] and our samples were not fasting samples. Another limitation of the study was the relatively small sample size, which may have prevented us from observing some associations. It is also possible that there were

some confounding factors that we were not able to fully adjust for. For example, hsCRP may not be elevated in later stages of inflammation, when serum ferritin is still elevated. Therefore, the measurement of α-1-acid- glycoprotein might have been useful as second marker of acute phase response [30]. HIV, malaria parasitaemia and faecal helminth tests were included in our study protocol but because there were large numbers of missing test results, it was not possible to include these as possible confounders in the final regression models. We considered running the analyses in a subsample consisting of those with available test results, but the total number of subjects decreased considerably and cell sizes became too small to warrant analysis.

To conclude, dietary diversity was a predictor of low zinc status among adolescent Mozambican girls in January-February, but the association did not seem to be consistent in all seasons. Therefore, if using different DDS as tools to identify populations with a risk of low micronutrient status, it is important to have information on when best to administer the tool. In our study, the tools based on a recall period of 24 hours (WDDS) and seven days (7dWDDS) performed equally well in relation to zinc status. The use of a 15-gram minimum portion size restriction (WDDS15g) did not seem to bring additional advantage to the assessment. Associations between dietary diversity and micronutrient status may exist for biochemical indicators other than those studied here or in other population groups. Continued research in low-income settings on the associations between different types of easy-to-collect food consumption data and biochemical indicators would provide more clarity on the usefulness of dietary assessment in predicting populations' micronutrient status.

Acknowledgements

We are deeply grateful for all those who helped to make the ZANE Study possible, especially the field workers and study participants. This research was supported by the Academy of Finland, Embassy of Finland in Maputo, the Finnish Graduate School on Applied Bioscience: Bioengineering, Food & Nutrition, Environment, the Future Development Fund of the University of Helsinki, the Finnish Concordia Fund, and the Foundation for Women in Research, Finland.

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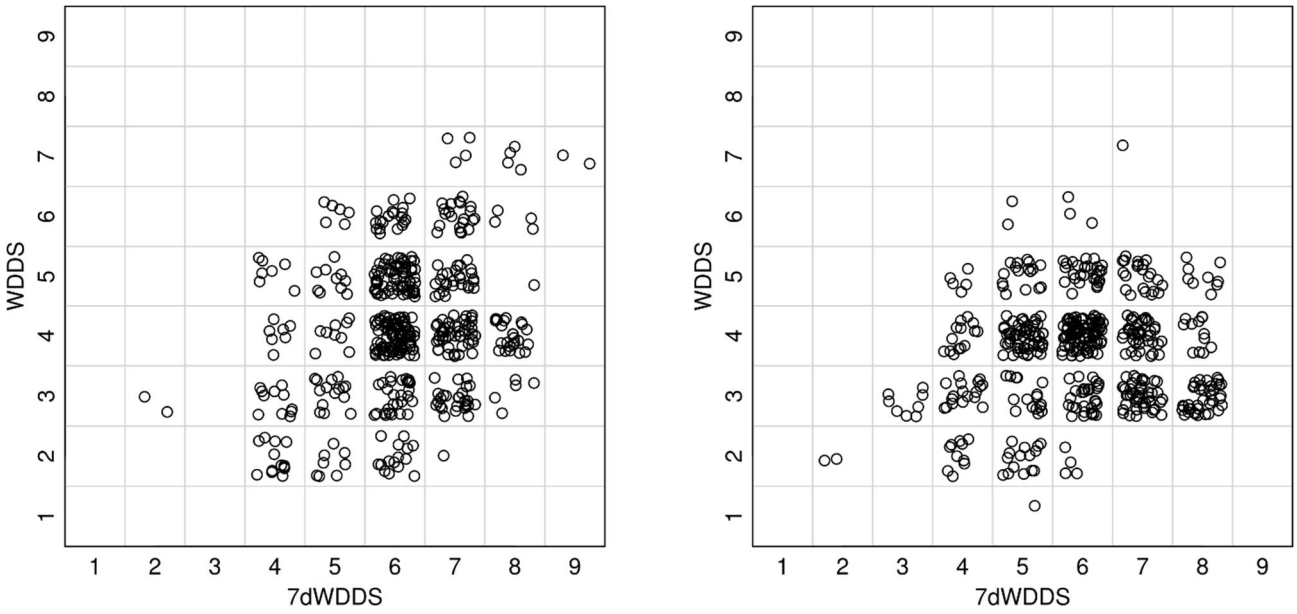


a b

Figure 1. Scatter plots of Women's Dietary Diversity Score (WDDS) and seven-day Women's Dietary Diversity Score (7dWDDS) for January-February, n=224 (a), and May-June, n=220 (b). The cut-offs for low diversity were ≤ 3 food groups reported in a 24-hour recall (WDDS) and ≤ 5 food groups reported in a seven-day food frequency questionnaire (7dWDDS).

Table 1. Descriptive statistics of non-pregnant adolescent Mozambican girls by season.

	January-February		May-June	
Variable	n		n	
Age, y, median (range)	227	16 (14,18)	223	16 (14,19)
BMI-for-age z-score	226	-0.38 ± 0.06	222	-0.45 ±0.06
WDDS	225	4.1 ± 0.08	220	3.7 ±0.07
WDDS15g	225	3.7 ± 0.07	220	3.4 ± 0.07
7dWDDS	226	6.2 ± 0.09	223	5.9 ± 0.10

Values are mean ± SEM unless stated otherwise. Sampling weights were used. WDDS, Women's Dietary Diversity Score; WDDS15g, Women's Dietary Diversity Score with a 15 g minimum; 7dWDDS, seven-day Women's Dietary Diversity Score

Table 2. Percentages of consumers of the foods groups of the WDDS and 7dWDDS in adolescent girls in Central Mozambique

	WDDS						WDDS15g						7dWDDS					
	January-February			May-June			January-February			May-June			January-February			May-June		
	low score[b]	high score	P value	low score[b]	high score	P value	low score[b]	high score	P value	low score[b]	high score	P value	low score[c]	high score	P value	low score[c]	high score	P value
Participants, n (w%)	49 (27)	176 (73)		82 (39)	138 (61)		73 (37)	152 (63)		120 (56)	100 (44)		40 (18)	186 (82)		84 (35)	139 (65)	
Food groups																		
Starchy staples, w%	100	100	N/A	100	100	N/A	100	99	N/A	100	100	N/A	100	100	N/A	100	100	N/A
Dark green leafy vegetables, w%	57	43	0.149	22	52	0.001	53	39	0.10	31	50	0.027	81	95	0.004	80	93	0.019
Other vitamin A-rich fruit and vegetables[d], w%	22	85	<0.001	0	6	N/A	29	91	<0.001	0	6	N/A	83	98	0.001	6	79	<0.001
Other fruit and vegetables[e], w%	50	94	<0.001	70	94	<0.001	55	95	<0.001	69	96	<0.001	83	100	N/A	95	100	N/A
Organ meat, w%	0	0	N/A	0	1	N/A	0	0	N/A	0	0	N/A	0	8	N/A	0	33	N/A
Meat and fish, w%	39	89	<0.001	70	86	0.027	26	75	<0.001	51	81	<0.001	78	100	N/A	86	100	N/A
Eggs, w%	0	10	N/A	0	8	N/A	4	8	0.31	0	10	N/A	2	62	<0.001	5	56	<0.001
Legumes, nuts and seeds, w%	7	37	0.001	15	85	<0.001	3	22	0.002	19	72	<0.001	18	96	<0.001	81	99	<0.001
Milk and milk products, w%	1	4	0.03	0	3	N/A	0	3	N/A	1	2	0.08	0	2	N/A	0	2	N/A

Comparisons were done using chi-squared tests. Sampling weights were used. N/A, not applicable; WDDS, Women's Dietary Diversity Score; Women's Dietary Diversity Score with a 15 g minimum; 7dWDDS, seven-day Women's Dietary Diversity Score; w%, weighted percentage

[b] Reported ≤ 3 food groups in a 24-hour recall.

[c] Reported ≤ 5 food groups in a seven-day food frequency questionnaire.

[d] For example mango (in January-February), pumpkin

[e] For example orange (in May-June), coconut, onion, and tomato

Table 3. Cut-off for different biomarkers of nutritional status used to define low blood concentration.

Variable	January-February		May-June		
	Cut-off (25th percentile)[a]	50th, 75th percentile	Cut-off (25th percentile)[a]	50th, 75th percentile	Literature cut-offs for deficiency or low status[b]
Haemoglobin, g L^{-1}	≤ 116	125, 133	≤ 111	120, 128	<120
Serum ferritin, µg L^{-1}	≤ 15.5	29.0, 43.4	≤ 12.9	21.6, 37.4	<15
Serum zinc, µmol L^{-1}	≤ 8.89	9.88, 11.0	≤ 7.92	9.40, 11.1	<9.0
Plasma retinol, µmol L^{-1}	≤ 0.79	0.92, 1.07	≤ 0.69	0.84, 0.95	≤0.70
Serum folate, nmol L^{-1}	≤ 23.6	32.2, 37.8	≤ 15.6	22.9, 30.7	<10

[a] Cut-offs used in the present analysis are the 25th percentiles of the distribution, calculated using sampling weights, in non-pregnant adolescent Mozambican girls. The 50th and 75th percentiles are presented for comparison.

[b] References [18-22]

Table 4. Cross-sectional associations of dietary diversity with biomarkers of nutritional status among non-pregnant adolescent girls in Central Mozambique, January-February 2010

	Unadjusted models				Adjusted models[a]			
Models	**n**	**OR**	**95% CI**	**P Value**	**n**	**OR**	**95% CI**	**P Value**
WDDS								
Haemoglobin	225	0.65	0.27-1.56	0.33	223	0.58	0.20-1.63	0.30
Serum ferritin	224	0.49	0.21-1.15	0.10	223	0.85	0.30-2.38	0.76
Serum zinc	223	2.36	1.10-5.03	0.028	221	3.85	1.57-9.46	0.004
Plasma retinol	224	1.34	0.61-2.97	0.47	223	1.39	0.55-3.48	0.49
Serum folate	224	0.87	0.41-1.84	0.72	223	0.98	0.31-3.09	0.97
WDDS15g								
Haemoglobin	225	0.58	0.27-1.24	0.16	223	0.46	0.19-1.11	0.09
Serum ferritin	224	0.52	0.25-1.06	0.07	223	0.70	0.30-1.63	0.41
Serum zinc	223	1.82	0.90-3.66	0.10	221	2.32	1.04-5.18	0.041
Plasma retinol	224	0.92	0.44-1.92	0.82	223	0.87	0.38-2.02	0.75
Serum folate	224	0.87	0.44-1.70	0.68	223	0.87	0.33-2.31	0.77
7dWDDS								
Haemoglobin	226	1.34	0.56-3.21	0.52	224	0.64	0.22-1.86	0.42
Serum ferritin	225	1.05	0.45-2.46	0.90	224	1.41	0.50-3.92	0.51
Serum zinc	224	3.07	1.35-6.97	0.008	222	3.47	1.25-9.60	0.017
Plasma retinol	225	1.87	0.79-4.42	0.16	224	1.77	0.64-4.88	0.27
Serum folate	225	1.38	0.62-3.09	0.43	224	1.16	0.36-3.73	0.80

WDDS, Women's Dietary Diversity Score; WDDS15g, Women's Dietary Diversity Score with a 15 g minimum; 7dWDDS, seven-day Women's Dietary Diversity Score. Separate binary logistic models showing the associations between 'low' dietary diversity and 'low' ($\leq 25^{th}$ percentile of the distribution in January-February) biomarker concentration. The reference categories were 'high' dietary diversity (≥4 for WDDS and WDDS15g and ≥6 for 7dWDDS). Sampling weights were used.

[a] Adjusted for study area, age, body-mass-index-for-age z-score, breastfeeding, high-sensitivity C-reactive protein, literacy and asset score.

Table 5. Cross-sectional associations of dietary diversity with biomarkers of nutritional status among non-pregnant adolescent girls in Central Mozambique, May-June 2010

	Unadjusted models				Adjusted models[a]			
Models	**n**	**OR**	**95% CI**	**P Value**	**n**	**OR**	**95% CI**	**P Value**
WDDS								
Haemoglobin	219	0.61	0.27-1.38	0.24	216	1.55	0.57-4.20	0.39
Serum ferritin	218	0.65	0.30-1.43	0.29	217	2.02	0.76-5.33	0.16
Serum zinc	215	1.16	0.53-2.53	0.72	214	1.07	0.46-2.52	0.87
Plasma retinol	218	1.06	0.48-2.34	0.89	215	1.05	0.40-2.75	0.91
Serum folate	218	0.96	0.47-1.97	0.92	217	1.22	0.59-2.49	0.59
WDDS15g								
Haemoglobin	219	0.83	0.38-1.78	0.63	216	1.48	0.63-3.46	0.37
Serum ferritin	218	0.66	0.31-1.40	0.28	217	1.47	0.63-3.45	0.38
Serum zinc	215	0.92	0.42-2.00	0.83	214	0.93	0.41-2.13	0.87
Plasma retinol	218	1.24	0.56-2.71	0.60	215	1.24	0.53-2.92	0.62
Serum folate	218	0.82	0.41-1.65	0.58	217	0.96	0.42-2.18	0.92
7dWDDS								
Haemoglobin	222	1.69	0.78-3.63	0.18	219	2.09	0.84-4.95	0.09
Serum ferritin	221	1.46	0.67-3.17	0.34	220	1.51	0.62-3.69	0.36
Serum zinc	218	0.71	0.31-1.61	0.41	217	0.59	0.24-1.45	0.25
Plasma retinol	221	0.87	0.38-1.95	0.73	218	0.64	0.24-1.73	0.38
Serum folate	221	1.33	0.64-2.73	0.44	220	1.13	0.44-2.90	0.80

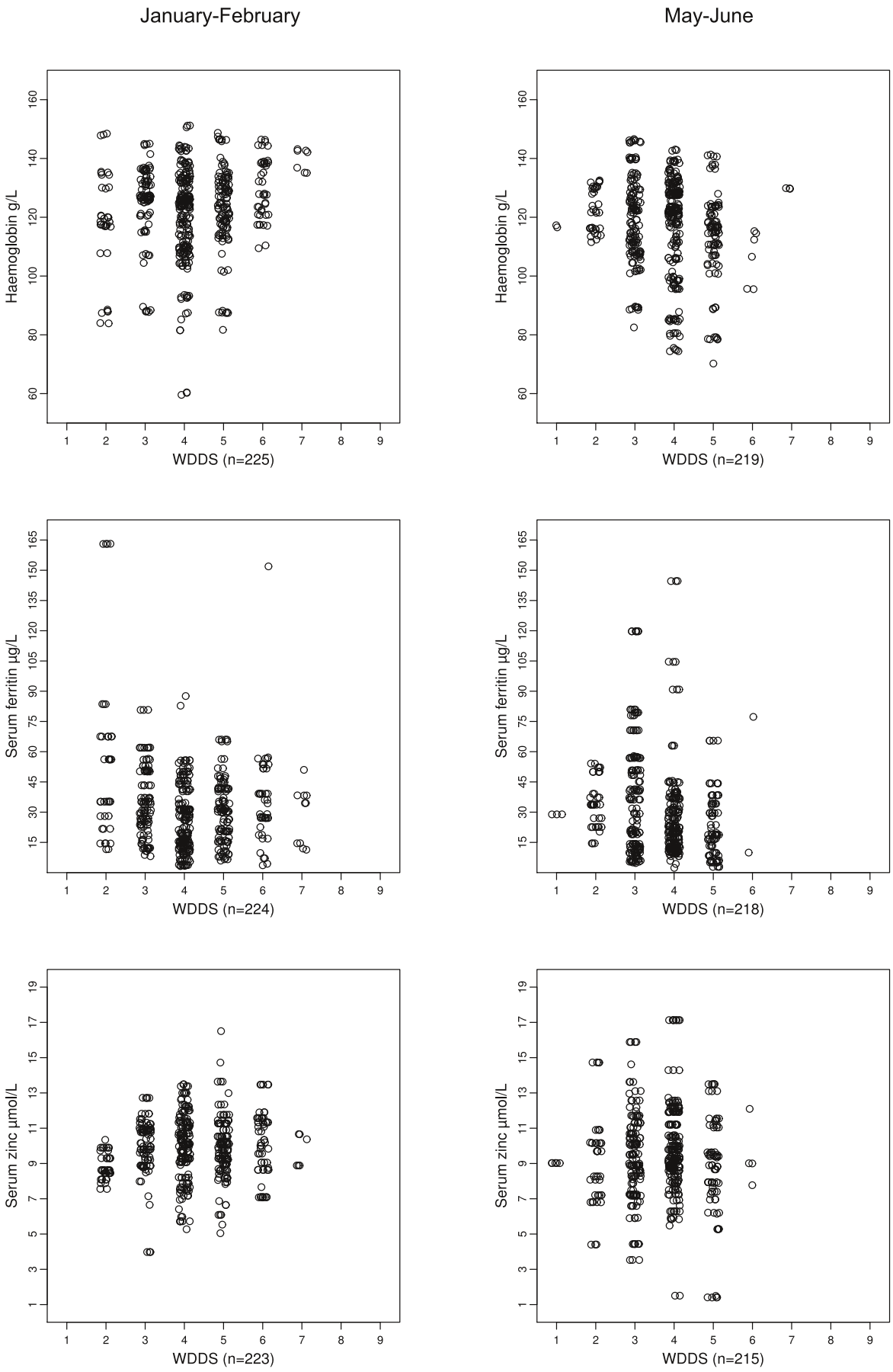
WDDS, Women's Dietary Diversity Score; WDDS15g, Women's Dietary Diversity Score with a 15 g minimum; 7dWDDS, seven-day Women's Dietary Diversity Score. Separate binary logistic models showing the associations between 'low' dietary diversity and 'low' ($\leq 25^{th}$ percentile of the distribution in May-June) biomarker concentration. The reference categories were 'high' dietary diversity (≥ 4 for WDDS and WDDS15g and ≥ 6 for 7dWDDS). Sampling weights were used.

[a] Adjusted for study area, age, body-mass-index-for-age z-score, breastfeeding, high-sensitivity C-reactive protein, literacy and asset score.

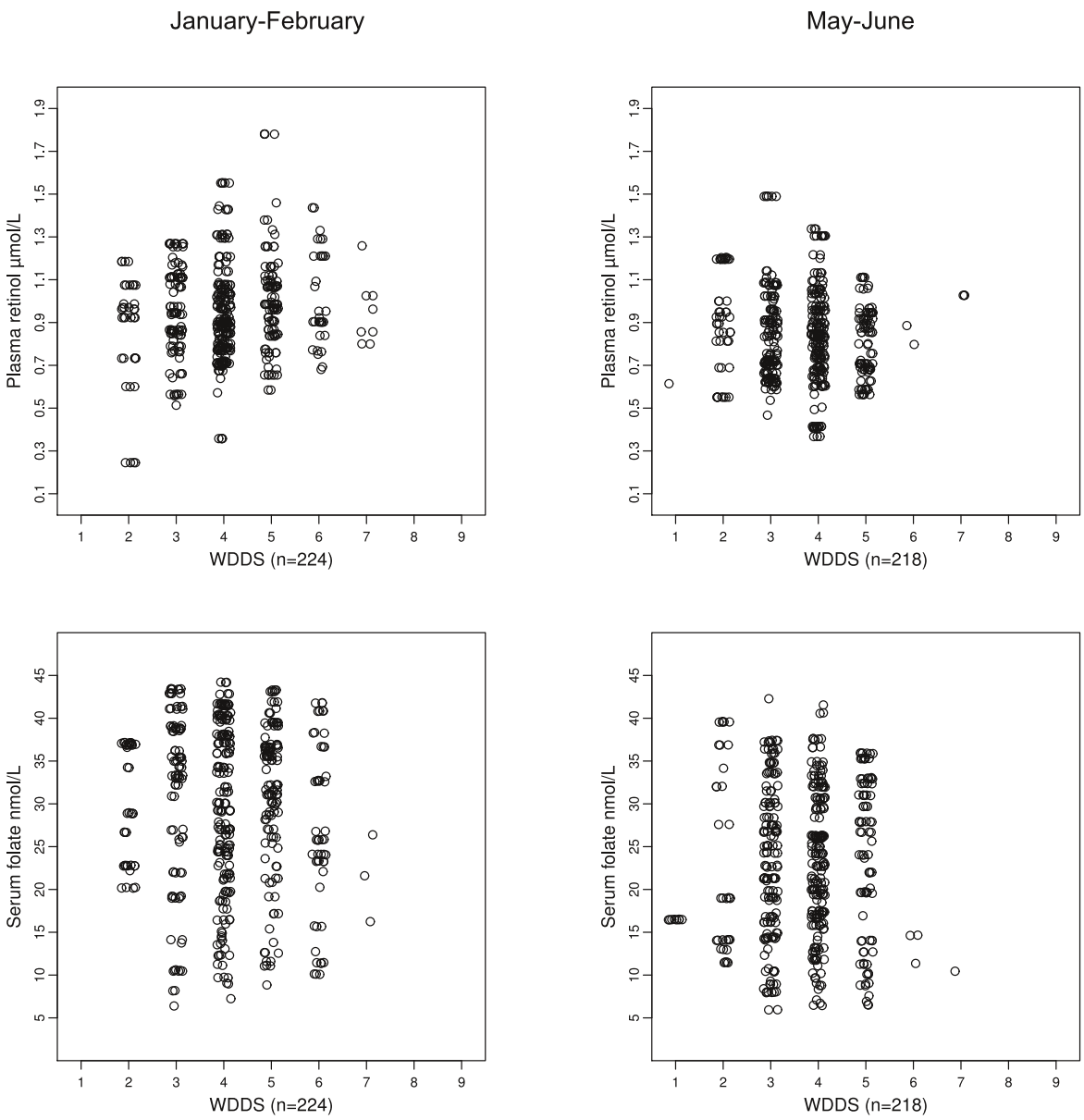
Supplemental table 1. Scores assigned to different asset items for creating an asset index.

Class	Item and score					
Source of drinking water	Well or unprotected source	0	Public tap	1	Tap	2
Type of house*	Not solid	0	Semi-solid	2	Solid	4
Type of floor material	Earth	0	Cement	2		
Sanitation facility	No toilet	0	Pit toilet	2	Flush toilet	4
Fuel for cooking	Firewood	0	Charcoal/coal	1	Electricity/gas	2
Electronic items	No radio	0	Radio	2		
	No TV	0	TV	2		
	No fridge	0	Fridge	3		
Additional items	No watch	0	Watch	1		
	No mobile phone	0	Mobile phone	3		
	No oil lamp	0	Oil lamp	2		
Furniture	No beds	0	Bed(s)	1		
	No chairs	0	Chair(s)	1		
	No tables	0	Table(s)	1		
Vehicles	No bicycle	0	Bicycle	2		
	No motorcycle	0	Motorcycle	3		
	No car	0	Car	4		
Livestock	No livestock	0	Livestock	2		
Poultry	No poultry	0	Poultry	2		
Land possession	No land	0	Land	4		

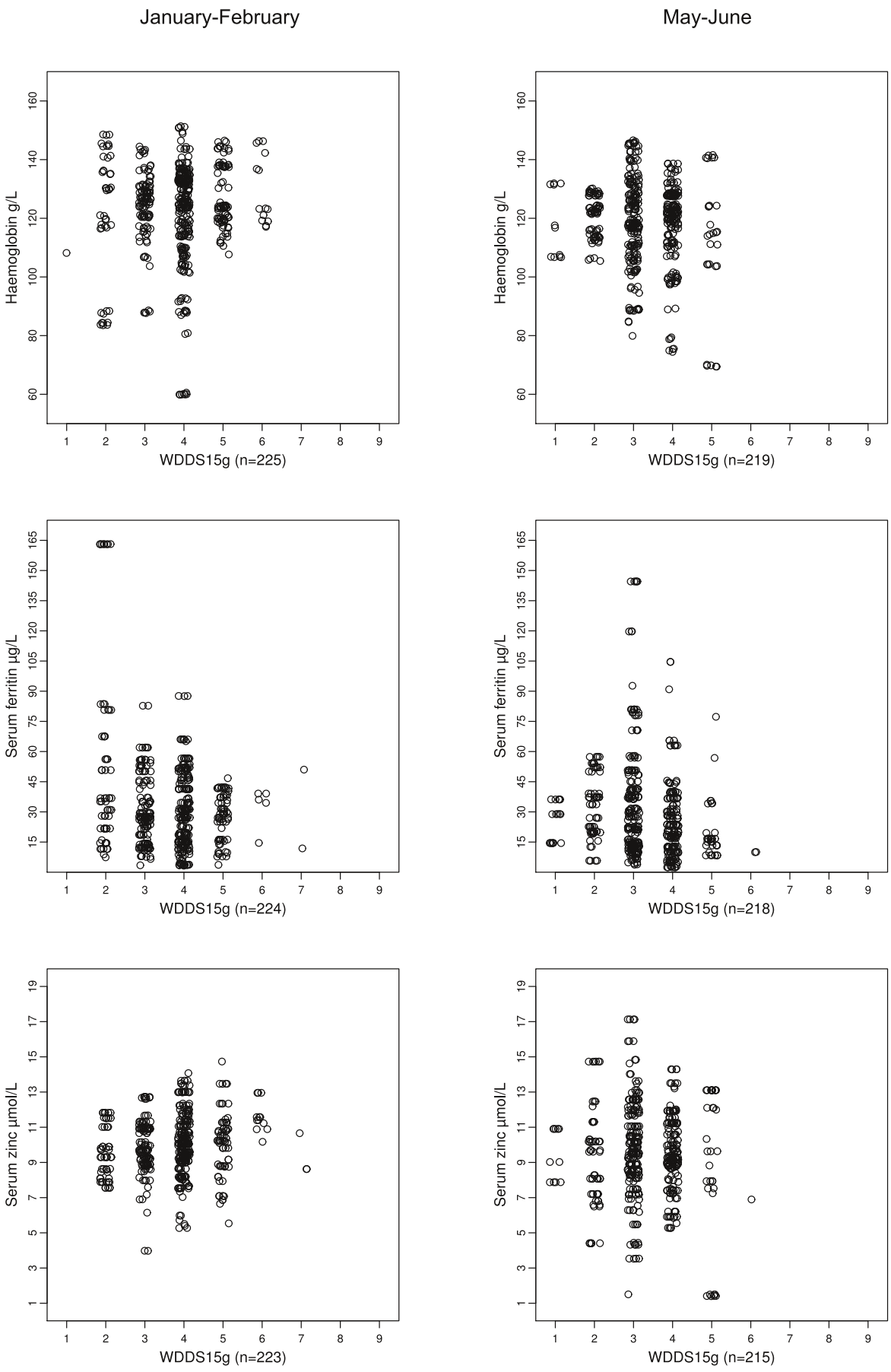
*Not solid: e.g. clay and straw; semi-sold: e.g. unburned bricks and straw; solid: e.g. concrete blocks and sheet metal.



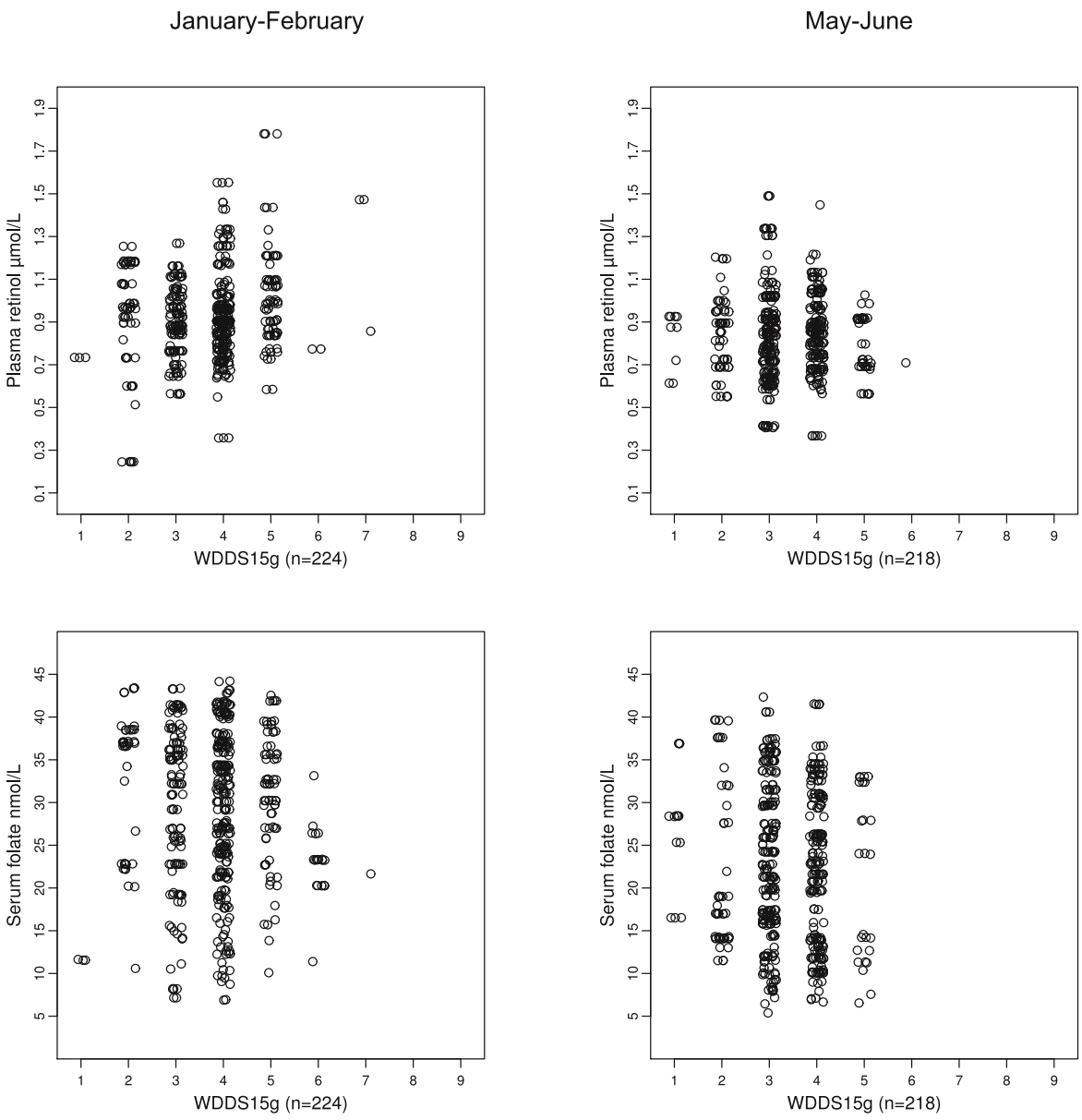
Supplemental figure 1. Scatter plots of Women's Dietary Diversity Score (WDDS) and haemoglobin, serum ferritin, and serum zinc among non-pregnant adolescent girls in Central Mozambique, January-February and May-June, 2010. One outlying ferritin value was omitted. Sampling weights were used and jitter was added on the x-axis to separate the circles.



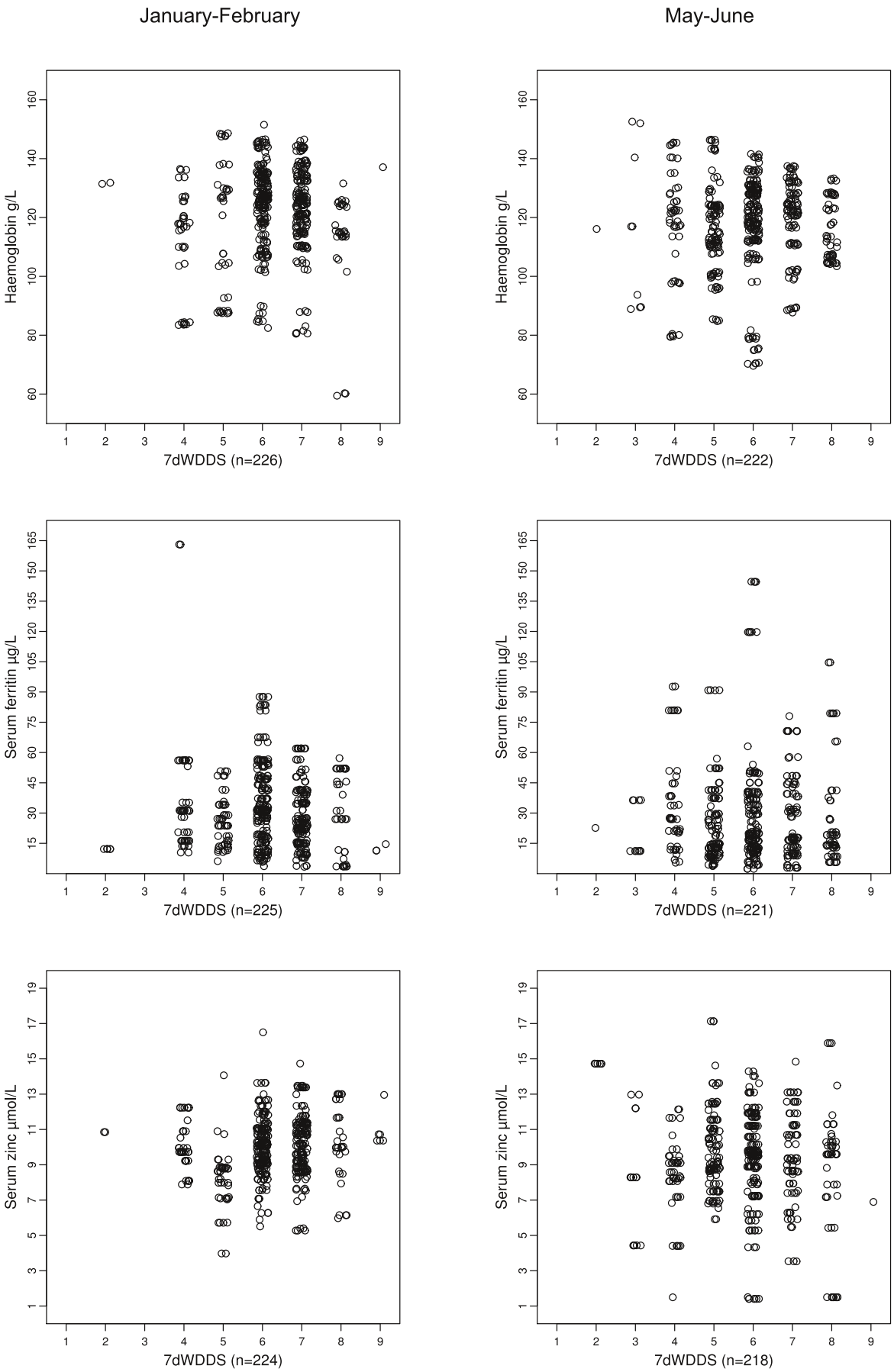
Supplemental figure 2. Scatter plots of Women's Dietary Diversity Score (WDDS) and plasma retinol and serum folate among non-pregnant adolescent girls in Central Mozambique, January-February and May-June, 2010. Sampling weights were used and jitter was added on the x-axis to separate the circles.



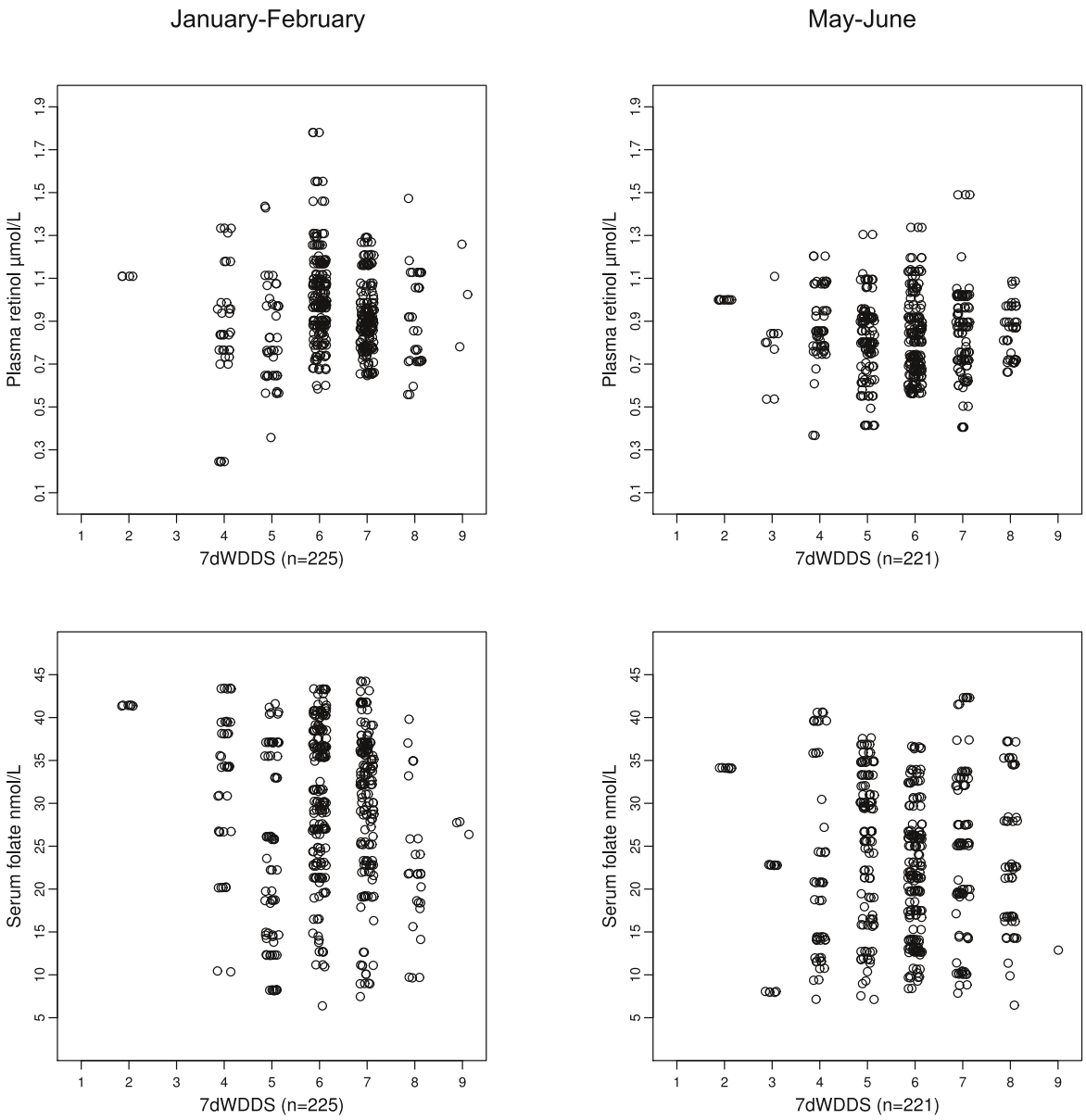
Supplemental figure 3. Scatter plots of Women's Dietary Diversity Score with a 15 g minimum (WDDS15g) and haemoglobin, serum ferritin, and serum zinc among non-pregnant adolescent girls in Central Mozambique, January-February and May-June, 2010. One outlying ferritin value was omitted. Sampling weights were used and jitter was added on the x-axis to separate the circles.



Supplemental figure 4. Scatter plots of Women's Dietary Diversity Score with a 15 g minimum (WDDS15g) and plasma retinol and serum folate among non-pregnant adolescent girls in Central Mozambique, January-February and May-June, 2010. Sampling weights were used and jitter was added on the x-axis to separate the circles.



Supplemental figure 5. Scatter plots of seven-day Women's Dietary Diversity Score (7dWDDS) and haemoglobin, serum ferritin, and serum zinc among non-pregnant adolescent girls in Central Mozambique, January-February and May-June, 2010. One outlying ferritin value was omitted. Sampling weights were used and jitter was added on the x-axis to separate the circles.



Supplemental figure 6. Scatter plots of seven-day Women's Dietary Diversity Score (7dWDDS) and plasma retinol and serum folate among non-pregnant adolescent girls in Central Mozambique, January-February and May-June, 2010. Sampling weights were used and jitter was added on the x-axis to separate the circles.