

**CHILD DIET OVER THREE SEASONS IN RURAL ZAMBIA:
ASSESSMENTS OF USUAL NUTRIENT INTAKE ADEQUACY, COMPONENTS OF
INTAKE VARIATION AND DIETARY DIVERSITY SCORE PERFORMANCE**

by

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Abstract

Inadequate dietary intakes are a key underlying cause of undernutrition, which places children at risk for impaired growth and development. Current estimates of prevalence of nutrient inadequacies are needed for the design of interventions to improve child diet. Estimates of nutrient intake variance components and validation of dietary diversity scores (DDS) among children are needed to design studies of nutrient intakes or population-level dietary adequacy, respectively. We conducted seven repeat 24-hour dietary recalls over six months among 4- to 8-year-old rural Zambian children (n=202). Participating children were enrolled in the non-intervened arm of a biofortified maize efficacy trial. We calculated observed nutrient intakes, frequencies of food consumption, usual intakes over six months, usual intakes by survey round and 7- and 10-food group DDS by survey round. Usual nutrient intakes over six months were used to estimate the prevalence of inadequacy of eleven micronutrients. We estimated within-person, between-person and seasonal components of variance in observed nutrient intakes. The performance of each DDS relative to overall nutrient intake adequacy and to usual intakes of five selected micronutrients was assessed by season. Children's diets were heavily plant based and included few animal source foods. Estimated prevalence of inadequate calcium, vitamin B12, folate and iron intakes was >99%, 76%, 57% and 25%, respectively. Mean nutrient intakes differed significantly between three agricultural seasons and season accounted for 3%–23% of total intake variance. Within- to between-person variance ratios were high due to low between-person variance. DDS were associated with overall intake adequacy, but this association was significantly weaker in the late lean season than in the late post-harvest or early lean seasons. The heavily plant-based diet of rural Zambian children places them at risk for inadequate nutrient intakes. Because nutrient intakes vary by season, future studies estimating

usual intakes should include repeat observations in multiple seasons. The 10-food group DDS is recommended over the 7-food group DDS for use as a population-level indicator of dietary adequacy.

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List of Acronyms

| | |
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| AMDR | Acceptable Macronutrient Distribution Range |
| AUC | Area under the receiver operating characteristic curve |
| BLUP | Best linear unbiased predictor |
| BMI | Body mass index |
| CV | Coefficient of variation |
| DDS | Dietary diversity score |
| DDS10 | 10-food group dietary diversity score |
| DDS7 | 7-food group dietary diversity score |
| DHS | Demographic and Health Survey |
| EAR | Estimated Average Requirement |
| FAO | Food and Agriculture Organization of the United Nations |
| IOM | Institute of Medicine |
| ISU | Iowa State University |
| IZiNCG | International Zinc Nutrition Consultative Group |
| MAR | Mean adequacy ratio |
| MDD | Minimum dietary diversity indicator |
| MDD-W | Minimum dietary diversity for women of reproductive age indicator |
| MPA | Mean probability of adequacy |
| NAR | Nutrient adequacy ratio |
| NCI | National Cancer Institute at the National Institutes of Health |
| NRC | National Research Council of the National Academy of Sciences |
| ODK | Open Data Kit survey software |
| ROC | Receiver operating characteristics curve |
| SD | Standard deviation |
| SES | Socio-economic status |
| WASH | Water, sanitation and hygiene |
| WHO | World Health Organization of the United Nations |

Chapter 1. Background and Rationale

Introduction

Undernutrition is the primary cause of impaired growth and development among children. Globally, 25% of children under five are stunted (low height for age), one third are vitamin A deficient and nearly half are anemic.¹⁻³ Anemia affects 25% of all school-age children.² In low- and middle-income countries, approximately 20% of school-age children are stunted and about 15% have low body mass index (BMI) for age.⁴ The problem of undernutrition is even higher among children in sub-Saharan Africa, where 37% of children under five years of age are stunted.³ In a review of studies of nutritional status among school-age children, Best et al. found that average reported undernutrition indicator rates in Africa were 22% for stunting, 35% for thinness, 29% for anemia, 32% for vitamin A deficiency and 54% for zinc deficiency.⁴

Inadequate dietary intakes are a key cause of undernutrition. Children, particularly those living in sub-Saharan Africa, are likely to have plant-based diets, with high consumption of cereals and starchy roots and tubers and limited consumption of animal source foods.⁵ These diets often provide nutrient intakes that are inadequate to support healthy growth and development.⁵

Vitamin and mineral intakes among school-age children in low- and middle-income countries are often inadequate to meet intake recommendations, especially for vitamin A, vitamin B12, folate, iron, calcium and zinc.⁵ Among preschool-age children, aged 2 to 5 years, undernutrition and micronutrient deficiencies are associated with increased risks of infectious disease and mortality, impaired growth and cognitive development, reduced school achievement and decreased productivity in adulthood.^{6,7} Among school-age children, aged 5 to 12 years, undernutrition has been associated with reduced muscular strength and work capacity, delays in

maturation and lower bone density.⁴ Interventions to improve nutrient intake adequacy among school-age children have shown positive effects on growth and on cognition and academic performance.^{4,5}

This dissertation is a study of diets of 4- to 8-year-old Zambian children over a six-month period. Though data specific to school-age children in Zambia are lacking, this late preschool- to early school-age population is likely to experience undernutrition and negative consequences for growth, development and cognition as a result of inadequate diets, as evidenced by the regional prevalence of undernutrition in this age group and the 45% prevalence of undernutrition among Zambian children under five.⁸ Few studies document food consumption patterns, nutrient intakes and prevalence of intake inadequacy among late preschool- to early school-age children in southern Africa , and none have been conducted in Zambia.^{5,9-11} Fewer still use dietary intake data collected over a sufficient study period to describe usual nutrient intakes in populations where food consumption is likely to vary by agricultural season.

The study was conducted in Mkushi district, Central Province, Zambia from August 2012 to April 2013. Mkushi is largely populated by smallholder farm families but also has large commercial farms that employ local residents as laborers. Agriculture provides the primary source of income for 51% of households in Mkushi.⁸ Rates of undernutrition in Central Province are above the Zambian national average. The Demographic and Health Survey (DHS) published in 2007 reported a national stunting rate of 45% among children under 5 years of age, while in Central Province, this figure was 52%.⁸ The DHS conducted one year after data collection for this dissertation found that the rate of stunting had dropped to 40% nationally and to 42.5% in Central Province.¹² A 2009 survey in Mkushi district found that 44.5% of children 2 to 5 years of age were stunted.¹³ The same survey found that 42% of 2- to 5-year-old children in Mkushi were vitamin A deficient, based on serum retinol concentrations below 20 µg/dL adjusted for

infection status, and that 30% of 2- to 5-year-old children in Mkushi were anemic. Grains provided over 50% of energy intake for 2- to 5-year-olds and mothers of children under 3 years of age in the early post-harvest season and over 65% in the late post-harvest season.¹³ These figures are indicative of repetitive diets lacking in diversity and micronutrient content. Prevalence of inadequate intake was high for calcium and vitamin B12, and there was a risk of inadequacy of iron and zinc based on assumptions of mineral bioavailability.¹³

Dietary intakes and nutrient intake adequacy among 4- to 8-year-old children in rural Zambia over a six-month period are described in Chapter 3. In Chapter 4, the seasonal, within- and between-person components of nutrient intake variance among 4- to 8-year-old children in rural Zambia are estimated, highlighting the methodological importance of seasonal variation in intakes in a developing country setting where many households rely on small-scale, subsistence agriculture. Chapter 5 presents a validation study of dietary diversity scores – counts of the number of food groups consumed by an individual over 24 hours – as population-level indicators of dietary adequacy among children in a setting where diet may change seasonally. The remainder of this chapter is a review of literature on nutrient intakes among children, components of nutrient intake variance and dietary diversity scoring. This background motivates the aims addressed in Chapters 3–5.

Nutrient intakes and intake adequacy among children

Nutrient intake estimates are needed for populations with high risk of nutrient deficiencies, such as school-age children, for the planning and design of nutrition interventions. Data specific to early primary school-age children are of particular use in the design of school-based nutrition programs. However, such data are lacking for this age group, as most studies and surveys have focused on children under five.^{4,5} Further, relatively few recent studies describing diet among

early school-age children have presented estimates of nutrient intakes; qualitative descriptions of diet based on food frequency questionnaires are more common.⁵ Surprisingly few studies take seasonality into consideration while collecting dietary intake data, although several studies have shown seasonal effects on dietary patterns and nutrient intakes.^{5,14–17} For example, agricultural patterns result in shifts in the availability of foods and in labor constraints affecting food choices, and this has been related to food consumption among children in rural Malawi.¹⁵ Among school-age children in rural Benin, changes in food consumption patterns and intakes of some nutrients occurred between the pre-harvest and post-harvest seasons.¹⁷

Components of nutrient intake variance

Day-to-day variation and other measurement error in usual nutrient intakes can cause misestimation of the prevalence of nutrient intake inadequacy or of measures of association between nutrient intakes and other variables of interest, such as markers of nutritional status or health outcomes. This section describes how these within-person components of nutrient intake variance can introduce bias to prevalence estimates or measures of association, methods used to reduce such bias, and why studies of components of nutrient intake variance are needed to inform the design of future studies that estimate nutrient intakes in similar populations.

The discussion of these methodological issues requires the establishment of several concepts used throughout this section to refer to specific components of nutrient intake variance. The nutrient intake of person i on day j , R_{ij} , can be broken into components as follows:

$$R_{ij} = T_i + D_{ij} + E_{ij} \quad (\text{Formula 1})$$

Where T_i is the true, long-term usual nutrient intake of person i

D_{ij} is the amount by which intake of person i differs from T_i on day j

E_{ij} is the amount that person i 's true intake on day j differs from the intake estimated by a 24-hour recall or food record.

Food records or 24-hour dietary recalls are tools used to estimate an individual's total nutrient intakes over one day, R_{ij} .¹⁸ Total nutrient intakes can be estimated from either tool because both record all foods consumed over a 24-hour period and details about the specific type of food, any ingredients or condiments used when it was prepared and eaten, and the amount of food consumed. Either of these methods is subject to measurement error in estimating the total nutrient intake over the day of observation. This error is shown in Formula 1 as E_{ij} . This error may be due to misestimation of amounts of foods consumed, omission of foods from the recall or record, or inaccurate data on the nutrient contents of foods.¹⁸ The size of E_{ij} is unknowable, but this term is assumed to be normally distributed with a mean of zero.

The true, or error-free nutrient intake of person i on day j is equal to $T_i + D_{ij}$. Day-to-day variation in nutrient intakes, D_{ij} , is a naturally-occurring feature of diet.¹⁹ However, in studies of dietary intakes either to assess intake adequacy or to test for associations between intake and another variable such as nutritional status or a health outcome, the intake of interest is typically the true usual intake, T_i .¹⁹ True usual intake is an individual's average intake over a long term. The length of this term may differ depending on the age group studied and outcome of interest for which nutrient intake is a potential exposure.¹⁹ When true usual intake, T_i , is the intake of interest, D_{ij} is a form of measurement error even though it is a true component of person i 's nutrient intake on day j . D_{ij} is assumed to be normally distributed with a mean of zero.

Because the intake of interest is a person's true usual intake, T_i , and the error in daily estimates of intake, E_{ij} , is unknowable, the terms D_{ij} and E_{ij} may be thought of as a single error term, ε_{ij} , around T_i such that Formula 1 becomes:

$$R_{ij} = T_i + \varepsilon_{ij} \quad (\text{Formula 2})$$

The variance in usual intakes, T_i , is the between-person variance, hereafter designated σ_b^2 . The variance in the error term, ε_{ij} , which is comprised of day-to-day variation, D_{ij} , and measurement error in the estimation of daily nutrient intake, E_{ij} , is the within-person variance, hereafter designated σ_w^2 .

Consequences of within-person variance in nutrient intakes

Within-person variance, as error around true usual intake, can introduce bias to estimates of prevalence of inadequacy or to measures of association between usual intakes and outcomes of interest — such as nutritional status markers or health outcomes — by inflating the total variance of the intake distribution. This inflation due to within-person variation causes substantial bias in estimated prevalence of nutrient inadequacy, inaccuracy in ranking of individuals by nutrient intake, attenuation of associations between nutrient intakes and outcomes of interest, and loss of power to detect differences between group mean intakes.^{20,21}

The presence of within-person variance in an intake distribution widens the distribution and results in overestimation of the proportion of the population below a given intake cutoff, such as the Estimated Average Requirement (EAR).²⁰ Using vitamin C intakes among 9- to 13-year-old Russian and US children as an example, Jahns et al. provide an illustration of the impact of within-person variance on estimates of prevalence of inadequate vitamin C intakes.²² The authors used 24-hour dietary recall data to calculate the percentage of children with intakes

below the EAR by sex for each survey. For both boys and girls in each survey, the estimated prevalence of inadequate intakes based on the unadjusted intake distribution was at least 15 percentage points higher than the prevalence estimate based on the usual intake distribution, which was adjusted to remove within-person variation.²²

In addition to biasing estimated prevalence of intake inadequacy, within-person variation increases the likelihood of misclassifying individuals relative to a cutoff such as a tertile or quartile. With a high within-person variance and small number of replicate measures, the observed mean intake has a higher likelihood of being far from the true usual intake than an observed mean intake in the case of lower within-person variance or a higher number of replicates. If the observed mean is far from the true usual intake, it may not fall on the same side of a cutoff. Therefore, the probability of correctly classifying an individual is a function of the distance between true usual intake and the cutoff, the number of dietary intake replicates and the within-person variation.²¹

A different way of expressing this effect of within-person variance is through calculation of the correlation coefficient between observed and true mean intakes, r . This unobservable correlation can be calculated as a function of the number of dietary intake replicates, d , and the within- to between-person variance ratio, σ_w^2/σ_b^2 .²³

$$r = \frac{d}{\sqrt{d + \sigma_w^2/\sigma_b^2}} \quad (\text{Formula 3})$$

As demonstrated by Nelson et al., r represents the accuracy of ranking individuals by intake distribution quantiles.²⁴ Higher correlation (r) between observed and true usual intakes results in a higher percentage of correctly classified individuals and lower percentage of grossly

misclassified individuals.^{23,24} An apparently small difference in r can have a sizeable impact on the percentage of subjects correctly classified. When ranking individuals into fifths of a distribution, an $r = 0.90$ would be expected to result in 75% correct classification into the extreme fifths, while an $r = 0.95$ should result in 83% correct classification into the extreme fifths.²⁴ Increased within-person variance results in a lower correlation between observed and true usual intakes, and, in turn, less accurate ranking of individuals by nutrient intake.

A third consequence of within-person variance inflating total variance in the distribution of nutrient intakes is the attenuation of correlation and regression coefficients in relation to other measures of interest, such as nutritional or health status markers. Inflated variance will bias these measures of association toward null. Formulas provided by Willett can be used to show the attenuation factor as a function of the within- to between-person variance ratio and number of replicates for both variables in the case of correlation and for the dependent variable in the case of regression coefficient:¹⁹

$$r_o = r_t \times \frac{1}{\sqrt{\left[1 + \frac{\left(\frac{\sigma_{w_x}^2}{\sigma_{b_x}^2}\right)}{d_x}\right] \times \left[1 + \frac{\left(\frac{\sigma_{w_y}^2}{\sigma_{b_y}^2}\right)}{d_y}\right]}} \quad (\text{Formula 4})$$

where r_o is the observed correlation coefficient and equal to the product of the true correlation, r_t , and an attenuation factor that becomes more severe with increasing variance ratios for either variable, $\sigma_{w_x}^2/\sigma_{b_x}^2$ and $\sigma_{w_y}^2/\sigma_{b_y}^2$, or less severe with increasing replicates for either variable, d_x and d_y . The estimated slope from regression analysis, b_o , is subject to increasingly severe attenuation relative to the true slope, b_t , with higher variance ratios in the dependent variable, x :¹⁹

$$b_0 = b_t \times \frac{1}{\left(1 + \frac{\left(\frac{\sigma_{w_x}^2}{\sigma_{b_x}^2}\right)}{d_x}\right)} \quad (\text{Formula 5})$$

Finally, large within-person variation in nutrient intakes decreases the power to test differences between group mean intakes, such that a null hypothesis of no difference between groups may fail to be rejected when, in truth, it should be rejected in favor of the alternative.²¹ The power to detect a difference, w , between groups means, \bar{X}_1 and \bar{X}_2 , is expressed as:

$$P(w) = Pr \left\{ \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{[\sigma_{b1}^2 + (\sigma_{w1}^2/d)]}{n_1} + \frac{[\sigma_{b2}^2 + (\sigma_{w2}^2/d)]}{n_2}}} > Z_{1-\alpha} \right\} \quad (\text{Formula 6})$$

Where σ_{b1}^2 and σ_{b2}^2 are the between-person variances, σ_{w1}^2 and σ_{w2}^2 are the within-person variances, and n_1 and n_2 are the number of individuals in groups 1 and 2, d is the number of dietary intake replicates per person, and α is the chosen significance level. Increasing the number of replicates per person reduces the loss of power due to within-person variance, though if the study aim relies only on the simple comparison of group means, increasing sample size while taking one replicate per subject would be more efficient.^{21,24}

Uses of nutrient intake variance estimates

The negative consequences of day-to-day variance in nutrient intakes for estimating usual intakes and their associations to other outcomes can be mitigated if internal or external estimates of the components of intake variance are available. Estimates of within- and between-person variance can be used to calculate the number of replicates needed obtain an accurate estimate of usual intake by averaging observed, daily intakes. They can also be used to

estimate attenuation on correlation or regression coefficients, conduct sensitivity tests on prevalence estimates, estimate usual nutrient intake distributions or predict usual nutrient intakes for use in regression models.

Use of any of these methods should further include consideration of other factors that would contribute to variance in observed food or nutrient intakes, such as day of the week, season, or validity of the intake assessment method.²⁵ Published studies provide population- and nutrient-specific estimates of such effects.^{20,25–29} In many cases these effects are small or non-detectable, but where they exist, they may increase the number or timing of replicates required to accurately rank or estimate nutrient intakes or may be addressed through study design.²⁰ Prior estimates of components of nutrient intake variance in one population can be used to plan future studies that will adequately capture important sources of variance for usual intake estimation in other populations.

Calculating required number of dietary intake replicates

Historically, a key application for estimates of nutrient intake variance components has been to inform designers of subsequent studies of the number of repeated dietary intakes measures per person required to rank individuals by intake, to minimize the degree of attenuation on measures of association, or to estimate individual usual intake with a chosen degree of accuracy.^{20,21,24} Early papers by Beaton et al., Liu et al. and Nelson et al. established these methods, based on solving the formulas 3 – 6 (above) for d , the number of replicates per person, given observed components of variance and chosen parameters for accuracy and significance.^{20,21,24} Multiple studies have applied this method to estimate d for various settings and age and sex groups.^{23,27,29–34} The number of days required to accurately rank individuals' intakes using mean observed intake is often high. For example, Nelson et al. calculated that the

number of days required to achieve accurately rank intakes (defined as correlation between observed and usual intake of $r \geq 0.9$) among adult men in the United Kingdom ranged from 4 to 35 days, depending on the nutrient of interest.²⁴ Among Dutch boys, the number of dietary recalls or food records needed to estimate usual intakes of macronutrients within 20% of true intake ranged from 4 to 28 replicates; for Filipino boys the number of replicates needed ranged from 6 to 105.²⁶

Sensitivity analyses and estimation of bias in measures of association

Work by Jahns et al. demonstrates the use of variance estimates from one survey to adjust nutrient intake distributions in another survey, but in a prior paper, the authors point out that even if a close population match is unavailable, estimates from other populations could be used in sensitivity analyses to explore the influence of within-person variance on intake distributions.^{22,35} Similar use of published components of variance estimates could be used to test the degree of potential bias on measures of association. Formulas 4 and 5 above show how the within- and between-person variance can be used to calculate an attenuation factor and estimate true correlation or regression coefficients from the observed coefficients. For this correction of bias in regression coefficients, estimates of the within- and between-person variance and number of replicates in the independent variable must be known from either study data or a comparable previous study. For correlation coefficients, these parameters must be known for both variables.¹⁹⁻²¹

Adjusting nutrient intake distributions

An increasingly important application for estimates of nutrient intake variance components is the adjustment of nutrient intake distributions to reflect usual intakes in studies. Several methods have been developed for estimating the distribution of usual nutrient intakes from

data on observed intakes collected by 24-hour recall or weighed food record.³⁶⁻⁴⁰ These methods typically rely on collection of a second recall or record in a sub-sample from which to calculate an estimate of within-person variance.^{36,37} The goal of these methods is to remove the day-to-day variability from observed intakes to obtain estimates of the usual nutrient intake distribution. This, in turn, allows more accurate estimation of the prevalence of nutrient intake adequacy. The advantage of these methods is their reliance on two recalls in a study subsample rather than collecting a large number of replicates, as was necessary in the approach described above.

The methods are all founded on a measurement error model approach, wherein an individual's day-to-day variability in intakes is considered an error term with a random distribution around their usual intake, as illustrated in Formula 2, above. Development of this measurement error model of usual nutrient intakes was initiated by the National Research Council of the National Academy of Sciences in the 1980s.⁴¹⁻⁴³ Since then, several research groups – at Iowa State University (ISU), the US National Cancer Institute (NCI), the European Food Consumption Validation project and the National Institute for Public Health and the Environment of the Netherlands – have contributed to the evolution of methods to accurately estimate usual food or nutrient intakes from observed intakes.^{40,42,44,45} Underlying the different proposed methods lies a central approach: transform the observed intakes to normal scale, estimate the within- and between-person variance components, apply a shrinkage factor calculated from the variance components to observed intakes to create intermediary values for usual intakes, and back-transform the intermediary values to original scale to form an estimated usual intake distribution. Each successive iteration of the method has addressed limitations of previous versions, including applicability to intake distributions that are non-normal, accounting for other sources of bias or variance, handling of non-consumption days, bias introduced through back-

transformation to original scale, distinction between occasional consumers and never-consumers and incorporation of intakes from supplements and fortified foods.^{36–38,40,42,44,45} The methods are made available to nutrition researchers by stand-alone software, macros or packages for use in statistical analysis software (SAS or R), or web-based application.^{45–48} Simulation studies have compared the ability of each method to overcome limitations mentioned above and accurately estimate usual intakes.^{39,49} The methods have been found to perform similarly, but with different strengths for handling different modeling scenarios, such as application using smaller sample size, smaller numbers of replicates, greater degrees of skew in intake distribution or higher within-person variance.^{39,49}

These methods generally rely on having repeat observations of food or nutrient intakes in at least a sub-sample of participants. In some cases, including when using software developed at ISU to estimate usual intake distributions, a published estimate of within-person intake variance may be used if collecting additional replicates among a subsample is infeasible.^{22,46,50} Jahns et al. test this suggestion by estimating the usual vitamin C intake distribution among Russian children using within-person variance estimates for US children, or for Russian children in a later survey. The authors found that while either external estimate of within-person variance is inferior to using data from a replicate dietary recall conducted as part of the original survey, either would be superior to not adjusting the intake distribution for within-person variance at all.²² This approach has also been demonstrated using repeat 24-hour recall data from one survey to adjust nutrient intake distributions from a previous survey in a similar population in Brazil.⁵¹ External estimates must be carefully matched by population to avoid inaccurate adjustment of the usual intake distribution, since variance components/ratios have been shown to vary by context, age and sex.^{22,31,35}

Predicting usual nutrient intakes of individuals

A recently developed means of avoiding bias due to measurement error in regression coefficients relating nutrient intakes to an outcome of interest is to apply an extension of the NCI method described above. This method, demonstrated by Kipnis et al., applies a regression calibration technique to modeling of associations between nutrient intakes and health outcomes.⁵² Rather than estimating the usual nutrient intake distribution for a population, the method instead predicts the usual nutrient intakes of study participants. These predicted usual intakes are formed based on the fixed effect and variance parameter estimates from a mixed effect measurement error model of nutrient intakes that includes observed nutrient intakes and covariates such as age, sex, food frequency data or other potential determinants of intake.⁵² The predictors can then be used in place of observed or averaged nutrient intake values in regression models of health outcomes to achieve more accurate regression coefficient estimates.⁵²

Need for population-specific variability estimates

In 1979, Beaton et al. argued that we need to know the extent to which components of variance measured in one population are applicable for estimating attenuation in correlation or regression coefficients or planning dietary intake replicates in another.²⁰ Since that early study, within- and between-person components and other effects on intake variance have been shown to vary by age, sex, cultural setting, season, and other factors such as race/ethnicity and body composition.^{23,27,31}

Several studies have shown substantive differences between age groups in the same population.^{23,24,30,31,35} Studies among Belgian, Finnish and British children found that within- to between-person variance ratios were higher among preschool- and school-age children than

among younger children and infants.^{24,30,31} Some authors observed that higher variance ratios among preschool- and school-age children compared to younger children were driven by differences in between-person variance, while within-person variance was more consistent between age groups.^{24,31} US primary school-age children have recently been found to have higher variance ratios than adolescents, and 5- to 17-year-old British children had higher variance ratios than adults.^{23,24} In contrast, Jahns et al. found differences in variance ratios between primary school-age children and adolescents among both Russian and US children but no consistent age trends.³⁵ Though variance ratios typically differ between age groups and comparison across studies reveals some consistencies in age trends, these cannot be assumed to hold in all settings.³⁵

Like age, sex is a parameter on which variance ratios have been shown to differ. Among 1-, 3- and 6-year-old Finnish children, and among 9- to 17-year-old Russian children, variance ratios were higher and between-subject variance was lower among girls than among boys.^{30,35} Other studies have found that variance ratios were similar or lacked a consistent pattern of difference between boys and girls in the same age group, but the within- and between-person variance components differed by sex.^{23,31,35} Among adults, observed variance ratios for most nutrients were higher among women than among men in studies conducted in São Paulo, Brazil, the US and the UK.^{24,27,50} In contrast to the study finding higher variance ratios among women than men in São Paulo, a study among adults in Rio de Janeiro found that variance ratios were higher among men than among women for over half of the nutrients examined, due to generally higher within-subject variances among men.³⁴ Yet the Rio de Janeiro study found the opposite among adolescents; variance ratios and within-subject variability were higher among girls.³⁴ Despite consistencies among some studies, the direction of difference cannot be assumed to be consistent. Among Canadian adults, variance ratios for most macronutrients were higher among

women, but those for the majority of micronutrients were higher among men.^{20,25} Across all age groups, many studies have found differences in nutrient intake variance components and ratios by sex, but the lack of consistency in degree and direction of difference necessitates estimation separately by sex in any population rather than assuming a particular relationship.

Variance components are likely to differ between populations according to the same factors that influence food intake, including socio-economic status, urbanization and food system such as seasonal changes in food availability.^{14,35} For example, Jahns et al. observed that higher rates of poverty among Russian children were paralleled by lower intakes of energy and most nutrients, higher between-person variability and lower variance ratios compared to US children.³⁵ Contributions to intake variance by various factors are also likely to change over time in a population with shifts in food supply and resultant consumption patterns.^{14,22,23}

Within- to between-person variance ratios may be anticipated to be lower in developing countries because of constraints on food purchasing and monotony of diets due to poverty, but several features of diet in subsistence communities challenge this assumption.^{14,19} Households of low socio-economic status may experience increased within-person intake variability due to changing constraints on food access and due to their limited ability to purchase high-value or animal source foods. On the other hand, subtle differences across households in wealth and ability to afford food may increase between-subject variability.^{14,19} Several authors have observed that estimates of variance components from low- or middle-income countries differ substantially from those reported for higher-income countries.^{14,26,50,35} In a 1987 study among 8- to 10-year-old boys across five different countries, within-person variation was similar for energy and carbohydrate intakes, but higher in Italy, the Philippines and Ghana than in the Netherlands or Finland for protein, fat, cholesterol and saturated fat intakes.²⁶

In subsistence agriculture contexts, seasonal variation in food availability has effects on food intake and may increase within-person variance.^{14,19} A seasonal component to nutrient intake variance has been hypothesized, but rarely examined. Studies assessing the contribution of season to overall variance in nutrient intakes among Japanese women and among overweight or obese US adults found that this contribution was small, though, in the case of Japanese women, significant for most nutrients.^{27,28} Nyambose et al. found that among pregnant Malawian women, mean nutrient intakes and intake variance ratios changed substantially by season.¹⁴ The results from this limited body of literature imply that the effect of season on nutrient intake variance differs by context and may be greater among populations reliant on subsistence agriculture.

Few current estimates of nutrient intake variance components that may be applicable to preschool- and early school-age children are available. Several recent studies of nutrient intake variance in this age range have been conducted in Europe and the United States.^{23,30,31} Another study provides intake variance components estimates for 8- to 10-year-old boys in Ghana, the Philippines and three European countries, but these estimates are now over thirty years old.²⁶ Estimates of nutrient intake variance among adults in low- and middle-income countries are available but are calculated from data collected in the 1990s.^{14,53,54} However, one of these older studies from a low-income country reports seasonal differences in nutrient intake variation, in this case among pregnant Malawian women.¹⁴ This body of literature provides scant information for planning future dietary intakes studies that adequately capture variance in nutrient intakes in order to estimate usual intake distributions or reduce bias in associations between nutrient intakes and outcomes of interest. This is particularly true for contexts in which seasonal changes to nutrient intakes may occur but are not currently documented.

Dietary diversity scores as indicators of dietary adequacy

Though estimates of nutrient intake variance components are needed to plan studies that can accurately assess usual nutrient intakes or control for bias resulting from measurement error in observed intakes, for some purposes, accurate estimates of usual nutrient intakes are not needed. For population-level monitoring of dietary quality or for evaluation of large-scale nutrition and food security programs, a rapid-assessment indicator of overall dietary adequacy is more appropriate. Dietary diversity scores (DDS) – counts of types of foods consumed by an individual over a set period of time – are consistently associated with measures of nutrient intake adequacy and nutritional status among young children and women of reproductive age.^{10,11,55–65} However, further validation of such scores is needed for late preschool- to early school-age children, as is assessment of whether seasonal changes in diet affect the performance of these scores for describing diet and serving as indicators of overall dietary adequacy.^{10,11,56,66}

Dietary diversity scoring provides a simple tool for assessing diet using data that can be collected and calculated with relative ease. Data on the dietary and micronutrient intakes of women and children in developing countries, which are needed for the design and evaluation of nutrition programs, are scarce due to the time and resources necessary to collect them.⁶⁷ Similarly, simple, validated, population-level indicators for assessing feeding practices are needed in efforts to improve infant and young child feeding in developing countries.⁶² A simple indicator of dietary quality such as dietary diversity scoring is useful for monitoring dietary adequacy in populations and in the evaluation and promotion of programs to improve household and individual diets.^{68,60}

Though there are clear advantages to the use of simple dietary diversity indicators, several limitations must be noted. Like other methods for describing diet, dietary diversity scoring is

subject to day-to-day and seasonal variability of intakes.⁶⁹ Simple counts of individual foods or food groups consumed cannot fully characterize nutrient adequacy of the diet, nor can these scores reveal the contributions of different foods to dietary quality without supporting descriptive analysis.⁷⁰ For example, two multi-country studies of dietary diversity scoring reported that patterns of food group consumption varied across sites.^{60,64} In contrast, among adults in rural Mali, four food groups (cereals, legumes, vegetables and green leaves) were consumed by all respondents, so variation was limited to 6 of the 10 food groups included in the score.⁶⁵ These results imply that though dietary diversity scores can be used to describe overall variety in the diet, they do not illustrate the similarity or dissimilarity of diet patterns without supporting analysis. Despite these limitations, dietary diversity indicators have continued to receive substantial attention in the scientific literature and in the monitoring of nutrition and food security programs. Critically, these scores have shown consistent associations with other measures of dietary quality and nutrition outcomes such as stunting among children under 5 years of age and body mass index among women of reproductive age.^{55,63,64}

Validity of dietary diversity scores

Multiple studies have examined the relationships between DDS and other indicators of dietary intakes and adequacy, including adherence to dietary recommendations, individual nutrient intakes, mean adequacy ratio, mean probability of adequacy, and nutrient density. These studies have observed significant associations between DDS and indicators of dietary adequacy among children under five and women of reproductive age.^{55,57,58,60–62,71}

The greatest focus in validating dietary diversity scores as measures of dietary quality has been in relation to overall micronutrient adequacy, though several studies have also shown associations between DDS and other measures of dietary quality among adults or children under

two.^{62,68,72,73,61} In a review of the dietary diversity literature, Ruel observes that DDS have consistently been shown to be associated with indicators of micronutrient intake adequacy, despite differences in reference periods, score definitions, data collection methods, and combinations of nutrients included in indicators of micronutrient intake adequacy.⁷⁴ Mean adequacy ratio (MAR), and, more recently, mean probability of adequacy (MPA), are the most commonly used indicators of overall micronutrient adequacy.

A nutrient adequacy ratio (NAR) is the ratio of an individual's nutrient intake to the estimated average requirement for that nutrient in the individual's age group. MAR is the average of NARs for multiple micronutrients. Prior to the adoption of the probability of adequacy approach, multiple studies in different settings and age groups demonstrated the positive association between DDS and MAR, or with individual nutrient adequacy ratios (NARs). Hatloy et al. provided the first influential example of this approach, finding that DDS were positively associated with MAR among 13- to 58-month-old Malian children, with correlation coefficients of 0.33 and 0.39, respectively.⁷⁵ Similar strength of correlation was found between MAR and DDS among adults in rural Mali.⁷⁰ Among 1- to 8-year-old South African children, DDS were correlated with MAR and 11 NARs.^{10,56}

Further research on the association between dietary diversity and nutrient adequacy has used the probability of adequacy approach. Probability of adequacy is the likelihood that an individual's nutrient intake is greater than or equal to their intake requirement.⁷⁶ MPA is the average of probabilities of adequacy for multiple micronutrients. Arimond et al. examined the association between MPA and DDS among women of reproductive age in five low- and middle-income countries in southern Asia and sub-Saharan Africa.⁶⁰ Several different calculations of DDS showed positive correlation with MPA and fair power to predict MPA at cutoffs of 0.5, 0.6 or 0.7 in all 5 countries studied.⁶⁰ Martin-Prevel et al. reported that among sixteen different

DDS variations calculated to describe the diets of women in urban Burkina Faso, three were acceptable predictors of MPA greater than or equal to 0.60 while the remainder were weak to moderate predictors (acceptable predictive power defined as having area under the receiver operating characteristic curve greater than 0.7).⁷⁷ Among US adults, significant, positive associations were observed between DDS and MPA, after controlling for energy intake.^{72,78} One study which used MPA to validate DDS among 2- to 4-year-old children and their caregivers in Bangladesh found that DDS showed a much stronger correlation to MPA among children ($r=0.63$) than among women ($r=0.18$).⁵⁷

Among adults – especially women of reproductive age – children under two years old and 2- to 4-year-old children, DDS have been correlated with measures of overall nutrient intake adequacy, and have shown fair strength in predicting overall intake adequacy above selected cutoffs, such as MPA greater than 0.5.^{55,57,58,60–62,71} In addition to the consistent relationship between dietary diversity and dietary adequacy, these scores have been positively associated with multiple nutrition outcomes.^{55,63,64} In developed countries, dietary diversity has been associated with nutritional status and a variety of outcomes, including risk factors for cardiovascular disease and all-cause, age-adjusted mortality.^{60,79–81} In developing countries, focus has been on associations between dietary diversity and undernutrition. In reviewing the literature on dietary diversity and child growth, multiple authors have observed that DDS have been consistently associated with nutritional status, despite the variety of methods used to calculate them.^{68,82,55} For example, in a study of height-for-age and DDS among children under two in eleven low- and middle-income countries, Arimond and Ruel found that DDS tertile was significantly associated with height-for-age z-score directly or in interaction with another factor in 10 of the 11 countries.^{68,64} That DDS are consistently related to growth status among young

children strengthens the argument that DDS are simple but effective indicators of overall dietary adequacy in the populations where they have been validated.

Need for population-specific validation of rapid dietary quality assessments

Though studies validating DDS routinely show correlations between DDS and measures of nutrient intake adequacy, the reported strength of these associations and predictive power of DDS as an indicator of nutrient adequacy above selected cutoffs vary by age group and population.^{60,62,71} Methods of calculating both DDS and nutrient intake adequacy vary from study to study, so direct comparison of these associations is limited to those studies that include multiple age groups or populations and apply consistent methods throughout. For example, in a study that validated DDS against MPA among both 2- to 4-year-old children and their female caregivers in rural Bangladesh, the energy-adjusted correlation between DDS and MPA was 0.63 among children, while the same correlation among their caregivers was much weaker, 0.18.⁵⁷ In contrast, in two related studies of groups closer in age, DDS means were similar between 1- to 3-year-old, 4- to 6-year-old and 7- to 9-year-old children in South Africa, as were energy-adjusted correlations with MAR.^{10,56} Similar differences between DDS means and correlations between DDS and MAR were observed between urban and rural children.⁵⁶

Arimond and Ruel provided cross-country comparisons of DDS performance among children under two years of age in eleven low- and middle-income countries.⁶⁸ Across all eleven countries, children with higher DDS were more likely to have consumed nutrient-rich foods, but the percentage of children with high DDS who consumed vitamin A-rich fruits and vegetables or meat, fish, eggs and poultry varied widely by country. The distribution of DDS varied greatly by country and the same value of DDS was associated with different dietary patterns in different countries.⁶⁸ Finally, in a related study, Arimond et al. assessed the performance of DDS against

MPA among women of reproductive age in five low- and middle-income countries. Both DDS and MPA varied between populations, as did patterns of food group consumption.⁶⁰ Despite these differences, unlike the study among children, strength of energy-adjusted correlation and predictive power were similar between countries.⁶⁰

Two trends emerge from this limited body of studies providing comparisons of DDS performance between age groups or settings: the utility of DDS as an indicator of dietary adequacy may differ by age group, especially between adults and children, and the diet pattern underlying a dietary diversity score varies by setting, particularly in the inclusion of nutrient-rich foods. These two factors indicate a need for age- and setting-specific validation of DDS. Though these scores are useful for within-population surveys and program evaluations once validated against a more detailed assessment of nutrient adequacy, the interpretation of cross-population comparisons is limited without documentation of the foods commonly consumed at each score level.

Two dietary diversity scores have been developed and recommended to meet the need for rapid-assessment indicators of dietary quality that can be applied consistently across surveys or evaluations conducted in different low- and middle-income countries. The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations have recommended dietary diversity scores for use among 6- to 23-month-old children and women of reproductive age, respectively.^{83,84} The intent of these scores is to provide a population-level indicator of micronutrient intake adequacy that can be used to assess population status, identify high-risk groups, advocate for nutrition policy and monitor change over time or in response to programming.^{83,84}

The WHO recommends Minimum Dietary Diversity (MDD) for use among 6- to 23-month-old children as one among a suite of indicators of infant and young child feeding quality. MDD is a

binary indicator of whether a child has consumed at least 4 out of 7 food groups in the previous 24 hours. The 7-food group score and minimum cutoff of 4 food groups as indicative of minimum dietary diversity were chosen based on the results of a multi-site validation of different DDS as indicators of adequate micronutrient density of complementary foods.⁶² Using data from nine low- and middle-income countries, the study authors tested the strength of association and predictive power of four different DDS in relation to mean micronutrient density adequacy over 10 micronutrients among 12- to 23-month-old children or over 9 micronutrients among 6- to 11-month-old infants.⁶² The 7-food group DDS was found to perform better than the 8-food group DDS, which included a group for fats and oils. Inclusion of a 10 g minimum consumption requirement increased complexity of data collection without improving score performance.⁶²

The FAO recommends the Minimum Dietary Diversity for women of reproductive age (MDD-W) as a parallel indicator to the MDD.⁸³ MDD-W uses a cutoff of 5 or more food groups consumed from a 10-food group score as an indicator of micronutrient adequacy. This score was chosen based on the second of two multi-site validation studies of DDS among women of reproductive age in low- and middle-income countries. The first multi-site study did not identify a single DDS with consistent cutoff that performed well across settings.^{60,58} However, from among the scores that performed best in that analysis, a 9-food group score was previously recommended.^{58,85} In order to identify a DDS with minimum dietary diversity cutoff that could serve as an indicator of minimum dietary diversity among women in multiple low- and middle-income-country settings, a second multi-site validation study was conducted.^{83,71} This study expanded on the work of the first study by testing additional disaggregation of food groups and including additional datasets from other low- and middle-income countries.⁷¹ Though the second multi-site validation study recommended either a 9- or 10-food group DDS with minimum consumption requirement of

15g to count toward the score, the 10-food group DDS without minimum consumption requirement is recommended.^{83,71} This choice represents a balance of score performance, ease of assessment and similarity to the MDD assessment method.

There is currently no similar, recommended DDS for use among preschool- or early school-age children. Among the body of validation studies and reviews summarized above, several have been conducted among children in this age range and in low- and middle-income countries, but these have not generated a consensus that informs recommendation of a single score for use in multiple settings.^{10,11,56,57,66} A further limitation of this body of literature is that while seasonal change in diet among children in low- and middle-income countries has been documented, few validation studies have tested whether this impacts performance of DDS for describing diet or indicating adequacy of micronutrient intakes.^{15,17,57}

Summary

Nutrition programs and policies are needed to improve the diets of children at risk of inadequate nutrient intakes, but estimates of nutrient intakes and nutrient intake adequacy needed to plan such interventions are lacking, particularly among late preschool- to early-school-age children in sub-Saharan Africa.⁵ Research is needed to describe food consumption patterns and usual nutrient intakes in these populations. Because food consumption may vary by agricultural season in these settings, studies are needed that collect dietary intake data over multiple seasons.^{15,17}

A critical challenge in the study of dietary adequacy and diet-disease relationship is the inherent day-to-day variation in nutrient intakes. Within-person variance in the observed distribution of nutrient intakes results in biased estimates of prevalence of intake adequacy and attenuated measures of association between nutrient intakes and other variables.^{19–21} Recent

methodological developments enable estimation of usual intake distributions and prediction of individual usual intakes that adjust for day-to-day variation and more accurately reflect usual intakes.^{36,40,42,45} To be useful for such purposes, estimates of components of nutrient intake variance must be representative of the population of interest. Few recent studies of nutrient intake variance have been conducted among 4- to 8-year-old children, and fewer still among children in sub-Saharan Africa.^{23,30,31} Further, few studies address the possibility of a significant seasonal component of variance among children in sub-Saharan Africa, though the study among Malawian pregnant women, in addition to limited evidence from higher-income countries, demonstrates the necessity of such analysis.^{14,27,28}

Estimating usual nutrient intakes is infeasible for many population-level surveys or program evaluations, because of the high time and resource costs of collecting dietary data with sufficient detail and replicates to estimate usual intakes accurately. For surveys to monitor dietary adequacy at the population level, to identify groups at high risk of undernutrition or estimate change associated with a nutrition policy or program, tools for the rapid assessment of dietary quality are necessary. Dietary diversity scores (DDS) have shown consistent correlation with nutrient intakes and nutritional status among women of reproductive age and children under five, making them useful rapid assessment tools in these age groups.^{55,57,58,60–64,71} Specific scores have been recommended for population-level assessment of dietary quality among 6- to 23-month-old children and women of reproductive age.^{83,84} However, few studies have validated these tools among early primary school-age children in sub-Saharan Africa.^{10,11,56} Though diets of women and children in sub-Saharan Africa have been shown to vary by season, few DDS validation studies have attempted to capture whether score performance is consistent across time.^{9,14,17} Based on their analyses of relationships between DDS and MPA among women of reproductive age in low- and middle-income countries, Arimond et al. suggest that these

relationships may vary seasonally and this variation must be considered when comparing results across site or time.⁶⁰ Further study is also needed to test whether seasonal changes in food consumption impact the performance of dietary diversity scores.

The need for studies of nutrient intakes and probability of intake adequacy, components of nutrient intake variance and seasonal validity of dietary diversity scores among children in sub-Saharan Africa will be addressed in Chapters 3–5. The following chapter presents the methods used in these studies.

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Chapter 2. Study Design

Study objectives

The goal of this dissertation is to evaluate nutritional adequacy, nutrient intake variability and dietary diversity among 4- to 8-year-old, rural Zambian children and to illustrate how knowledge of these dietary characteristics can be used to inform and guide dietary research in similar populations. This goal is achieved by describing usual nutrient intakes and identifying nutrients for which Zambian children are at highest risk of inadequacy; documenting the food intake patterns that underlie that risk; assessing the degree to which nutrient intakes vary by season; and evaluating the performance of population-level dietary diversity indicators of overall nutrient adequacy in this setting and age group. This study uses data collected within a population-based trial that tested the efficacy of pro-vitamin A biofortified maize versus conventional white maize in improving vitamin A status and reducing vitamin A deficiency among 4- to 8-year-old children. Three specific aims are addressed.

Aim 1

The first aim, addressed in Chapter 3, is to characterize diets of 4- to 8-year-old rural Zambian children over six months in terms of nutrient intakes, prevalence of inadequate intakes and nutrient contributions from commonly consumed foods. Usual intakes of energy and fourteen macro- and micronutrients and the percent of children with inadequate intakes for each nutrient are described. These results are presented overall, and by age and sex. The dietary patterns underlying the risk of inadequate intakes are illustrated by summarizing the frequencies with which key foods are consumed and the contribution of those foods to total nutrient intakes. This paper fills a gap in the literature on the current dietary patterns and

nutrient intake adequacy of children in sub-Saharan Africa by presenting a description of usual intakes over a cross-seasonal, six-month period.

Aim 2

The second aim, addressed in Chapter 4, is to describe components of variance in nutrient intakes of 4- to 8-year-old rural Zambian children over six months and by agricultural season using seven monthly 24-hour dietary recalls. The observed intakes of energy and fourteen macro- and micronutrients are described by season. This chapter presents the within-person, between-person and seasonal components of intake variance as coefficients of variation, the ratio of within- to between-person variance, and percent of total intake variance. The results reveal the importance of seasonal variation in energy and nutrient intakes and suggest ways to improve accuracy of usual nutrient intake estimation, particularly for studies of childhood dietary intake in rural sub-Saharan Africa.

Aim 3

The third aim, addressed in Chapter 5, is to validate dietary variety scores as indicators of overall nutrient intake adequacy among 4- to 8-year-old children in rural Zambia using 24-hour dietary recalls collected over a six-month period. Three hypotheses are tested. The first hypothesis is that dietary variety scores will be positively associated with the mean probability of intake adequacy. The second is that the association between dietary variety scores and mean probability of adequacy will vary by season. The third is that dietary variety measures will be positively associated with intakes of individual nutrients. Two dietary diversity scores indicating the number of food groups reported in 24-hour dietary recalls among rural Zambian children are calculated. A 10-food group score recommended for use among women of reproductive age the

by Food and Agriculture Organization (FAO) and a 7-food group score recommended for use among 6- to 23-month-old children by the World Health Organization (WHO) are used.^{1,2} The distributions of each score are presented by season, as are usual intakes of energy, vitamin A, vitamin B12, vitamin C, calcium and iron and an indicator of overall nutrient adequacy (mean probability of adequacy, described under 'Aim 3 analyses'). Percentages of children consuming foods from each food group are presented by season to illustrate seasonal shifts in dietary patterns. Validity of the dietary diversity scores is assessed relative to mean probability of adequacy and usual intakes of the five selected micronutrients through regression analyses and receiver operating characteristic curves describing the sensitivity and specificity of the scores for predicting intake adequacy.

HarvestPlus pro-vitamin A biofortified maize trial

The HarvestPlus biofortified maize efficacy trial was conducted in small towns and rural villages in Mkushi district, Central province, Zambia.³ Children living in the study area were enrolled into a three-arm trial. Children in the treatment arm were served two meals per day that included biofortified maize porridge or nshima (the local staple dish of stiff maize porridge) and children in a positive control arm were served two meals per day that included white, traditional maize porridge or nshima. Children in the non-intervened control arm received a food package at the end of the trial rather than meals. The protocol for data collection and physical, dietary, morbidity and biochemical assessments was the same in the three arms. Additional data on consumption of trial-provided meals was collected among the treatment and positive control arms.

Data collected on all participating children and their households at baseline include household roster, socio-economic and demographic descriptors, food security, household food

consumption and child diet, morbidity and anthropometry (**Table 2.1**). Blood samples were collected from children whose parents specifically consented to the procedure, and the samples were tested for malaria parasitemia, hemoglobin concentration and serum concentrations of retinol, ferritin and transferrin receptor. The food security, household food consumption, and child diet and morbidity surveys were repeated at monthly interviews over the course of the six-month trial, and were repeated in the follow-up survey, during which a second blood sample was drawn. In addition to the child and household data collected, surveys of local markets were conducted routinely to collect data on food availability and prices.

Subject Recruitment

Potential participants for the biofortified maize efficacy trial were identified in June 2012 through a mapping and census of the study area communities. Twelve communities in five health center catchment areas in the northern part of Mkushi district – Nkumbi, Masansa, Chalata, Nkolonga and Chibefwe – were included in the study area. Individual communities within each catchment area were chosen for accessibility by road and for having sufficient population density to implement the parent feeding trial, for which children needed to be in walking distance of central feeding sites. The twelve communities included two peri-urban towns (Itala and Masansa). The remaining communities are rural villages. Field staff visited villages and towns in the study area and recorded the location of all houses, schools, water pumps and other points of interest. In each household, they informed an available adult household member of the purpose of the mapping and census and requested consent to ask about children living in the household who might be eligible for the parent trial. Children identified in the census as being between 4 and 8 years old and who were not yet enrolled in school were considered eligible to participate in the parent study.

At the time of the baseline survey parents or guardians of eligible children were provided with information about the nature and purpose of the study and the burden and minimal risks involved and completed an informed consent process. Children whose guardians agreed to participation were enrolled into the efficacy trial in geographic clusters of 14 to 31 children. Each cluster was assigned by block randomization to one of three groups: white maize (n=481 children in 25 clusters), orange maize (n=543 children in 25 clusters) or non-intervened control (n=202 children in 14 clusters) (**Figure 2.1**). All children enrolled in the efficacy trial were simultaneously enrolled in this study through the informed consent process, but only children in the non-intervened group (n=202) are included in primary analyses. Because children in the white maize and orange maize groups were receiving study meals, their dietary intake data beyond baseline are not reflective of their usual diets. Baseline data on age, sex and household descriptors for children in the white and orange maize groups were used to test whether the non-intervened group is comparable to the full, efficacy trial sample.

Data Collection

Data from the biofortified maize trial used in this study include data on household socio-economic status and demographics, and child age, sex and dietary intakes (Table 2.1). Data were collected in seven survey rounds: a baseline assessment in August - October 2012, five monthly monitoring visits to participating households from September 2012 to February 2013, and a follow-up assessment in March - April 2013 (**Figure 2.2**). Data were recorded using Acer Iconia touch-screen tablets (San Jose, California) programmed with survey forms developed in Open Data Kit software (ODK, opendatakit.org, University of Washington, Seattle, Washington). All interviews were conducted by trained field interviewers (FIs) that were members of the efficacy trial field staff recruited from the study area. Data on household socio-economic status,

food security and demographics and child anthropometry, physical exam results and blood samples were collected as part of the baseline survey. Dietary intakes of the participating children and household food security were assessed at baseline and monthly thereafter through 24-hour recall interviews. The follow-up survey followed a similar data collection protocol to the baseline survey and included collection of additional data on household socio-economic status.

Baseline Data

During the baseline survey, households identified during the mapping and census as having a child between 4 and 8 years old were visited by a FI and asked to participate in the efficacy trial. If the parent or guardian of the 4- to 8-year-old child provided oral consent after being read the consent statement on the roles, rights and risks of study participation, a baseline survey was administered by the same FI and the parents were asked to bring their child to a central location for a physical assessment. The baseline survey collected data on socio-economic and demographic descriptors of the household, household food consumption over the previous seven days and household food security over the past six months. Socio-economic variables collected at baseline include ownership of durable household goods and presence of electricity in the home. Demographic data were collected through a roster of all current members of the household and their age and sex. This survey also contained modules collecting data on the participating child's health and diet, including a 24-hour dietary recall, morbidity history and health practices questionnaire.

The 24-hour dietary recall data were collected using the ODK program on Android tablets. The tablet-based 24-hour recall tool is described in detail by Caswell et al. (Appendix 1). The tool was developed to collect data in a modified multiple-pass system. The first pass was to ask the

mother or primary caregiver to recall any foods or drinks the child consumed by time of day, from when the child woke up the previous morning, through evening and overnight. In a second pass, the interviewer and respondent reviewed a picture chart to identify any missed foods. In the final pass, the interviewer asked the respondent to review the day one more time to recall any other foods or drinks the child consumed. The tablet program prompted the interviewer to record the full details on food description, portion size, added ingredients and food source the first time a food was mentioned. Portion sizes were estimated by asking the respondent to select which of a series of photos of a similar food best matched the portion the child consumed. In some cases, such as fruits and breads, the number of pieces consumed was recorded, and in rare cases, such as sugar cane, the respondent was asked to show the width and length of a food, and these were recorded in centimeters.

After completing the baseline questionnaire and 24-hour recall, a parent or guardian was asked to accompany their child to a central site in order to complete anthropometric measurements and other health assessments for the parent trial. The anthropometric measures taken included standing height, weight, mid-upper arm circumference (MUAC) and triceps skin-fold thickness. These measures were taken by a technical coordinator, FI team leader or nurse trained in the appropriate techniques. Portable stadiometers (ShorrBoards, Weigh and Measure, LLC, Olney, Maryland) were used to measure height. Height was measured three consecutive times for each child. Flat, digital scales (Seca, Model 874, Hanover, Maryland) were used to measure weight. MUAC was measured three times with an insertion tape, using the child's left arm, midway between the elbow and shoulder. Triceps skinfold thickness was measured with Holtain calipers, midway between the elbow and shoulder of the left arm. Anthropometric measurements were taken with the child wearing light clothing and no shoes.

Monitoring & Follow-up Visits

Throughout the feeding trial, at monthly intervals, a FI visited each participating household for a monitoring visit. The FI administered a questionnaire which included a 24-hour recall of foods consumed by the participating child. The FI also collected data on child morbidity and health practices and household food consumption and food security to support parent study analyses. These survey instruments were replicates of those administered during the baseline survey.

In March - April 2013, a follow-up survey was conducted among all children participating in the efficacy trial. The follow-up survey was a repeat of the baseline procedures, except that a different survey on socio-economic factors was included to collect additional data on housing materials, fuel sources and sanitation facilities. For the diet study, the 24-hour recall and additional socio-economic status data will be used from among the data collected at monitoring visits and follow-up.

Child diet study

This dissertation on child dietary adequacy, variability and diversity used the 24-hour dietary recall data collected monthly over the course of the biofortified maize efficacy trial. Supporting information on child age and sex, and household socio-economic and demographic descriptors collected in the baseline and follow-up surveys of the efficacy trial were also used. Because the larger trial provided regular meals to participating children enrolled in the orange and white maize groups, this study included only the children who were enrolled into the non-intervened arm that did not receive meals during the trial but participated in the same data collection protocol. Table 2.1 provides a summary of the data collected by the efficacy trial and shows which data were used in the child diet study. All data analysis was conducted in SAS 9.4 (SAS Institute, Cary, NC). Statistical significance is defined as $p < 0.05$ throughout.

Study sample

The biofortified maize efficacy trial enrolled 1226 children, aged 4 to 8 years, living in Mkushi district, Zambia and not yet enrolled in school. As described in detail above, children enrolled in the efficacy trial were assigned by geographic cluster to a biofortified maize treatment group, a traditional, white maize positive control group or a non-intervened control group. Data from all baseline, monthly monitoring and follow-up survey rounds collected among the 202 children in the non-intervened group were included as the dataset for the child diet study. The 24-hour recall was completed for 172 – 200 children in each round.

To ensure that the non-intervened group is as representative of the population of 4- to 8-year-old children and their households in rural Zambia as is the efficacy trial full sample, the non-intervened group was compared to the orange maize and white maize groups in terms of child age and sex, household asset score, head of household literacy and head of household education (**Table 2.2**). No significant differences between groups were observed.

Calculation of observed nutrient intakes

The method for calculating observed nutrient intakes from the tablet 24-hour recall data is described in detail by Caswell et al. (Appendix 1). Briefly, tablet variables describing each food reported in the 24-hour recall were consolidated into a series of codes that identified specific mixed or unmixed foods, portion sizes, recipes for mixed foods, and food composition codes for unmixed foods and ingredients. These codes served as links to reference tables containing the relative weights of ingredients in recipes for mixed dishes, the portion weights in grams associated with each food type and portion size depicted in the portion photo book or by number of pieces consumed, and the nutrient contents of each unmixed food or ingredient.

Using these codes and reference tables, the weight of each unmixed food or ingredient consumed was multiplied by its matching food composition data to arrive at nutrients consumed from each food or drink. These were summed over all foods and drinks reported in the recall to estimate total observed nutrient intakes.

Exploratory data analyses and descriptive statistics

Nutrient intakes

Estimated 24-hour nutrient intakes from food and drink were explored by survey round using descriptive statistics, histograms with kernel density plots and box plots. Nutrient intake distributions were generally found to be right-skewed with seasonal shifts in intakes. Reported use of iron, vitamin or other supplements was explored to determine whether nutrient intakes from supplements should be considered in addition to dietary intakes in calculation of total intakes for use in analyses. Supplement use was seldom reported. Iron supplement use was reported 6 times among all children in all rounds, vitamin supplement use was reported 8 times and other supplement use was reported 3 times. Based on these use rates and limited data on the nutrient contents of the supplements, intakes from supplements were not included in estimates of nutrient intakes.

Means with standard deviations, medians with inner quartile ranges, frequency distributions or histograms were generated to describe child and household factors. These included age, sex, head of household education and literacy, head of household primary occupation, number of children less than 15 years old in the household, rural versus peri-urban residence and descriptors of asset ownership, housing quality and water and sanitation access. Indicator variables summarizing asset ownership, housing quality and water and sanitation access (WASH) were formed by summing the responses to all questions in the indicator category. For example,

the responses (1 = Yes, 0 = No) to all questions on whether the household owned items such as a bicycle, mobile phone, automobile, hammermill, etc. were summed to create an asset score. For the housing and WASH indicators, responses to items such as the building material used to make the house walls or the household's main source of drinking water were re-ordered into ascending economic value of the resource prior to summing across variables.

Potential covariates to nutrient intakes

In preparation for later analyses, univariate continuous and discrete distributions were described and bivariate correlations between potential covariates and observed nutrient intakes were explored using Pearson correlation coefficients, ANOVA or t-tests. Potential covariates in initial exploratory data analysis included: child age, sex, age*sex group, whether the 24-hour recall period fell on a market, feast, fast or weekend day, whether the child resided in a rural or peri-urban cluster, years of schooling completed by the head of household, number of children less than 15 years old living in the household, and indicators of asset ownership, housing quality and water and sanitation facilities. A series of socio-economic status (SES) scores was also created using principle components analysis, but these were not found to show any advantage over the asset, housing and WASH scores in explaining variance in energy intakes so were not pursued further.

Aim 1 analyses

Usual nutrient intake distribution estimation

When assessing nutrient intakes and their adequacy, the measurement of interest is usual intake, that is, the average daily intake over time, rather than intake on a single day or simple average over several days. Several methods to estimate the usual intake distribution by

removing within-person variance have been developed by the National Research Council / Institute of Medicine (NRC/IOM), Iowa State University, the National Cancer Institute (NCI) and other research groups.⁴⁻⁹ The methods all follow a common basic framework in which nutrient intakes are transformed to approximate a normal distribution, adjusted using a shrinkage factor based on the within- and between-subject components of variance of the transformed data, and back-transformed to describe the estimated usual intake distribution in the original unit of measurement.^{4,6,7} A more detailed description of this approach and rationale for selecting methods developed by NCI over those developed by other research groups for use in this dissertation are described in Appendix 2.

The NCI method for estimating usual nutrient intake distributions is carried out using macros to be run in SAS statistical analysis software (SAS Institute, Cary, NC).¹⁰ Two macros are used to estimate usual nutrient intake distributions. The first macro fits non-linear mixed models, producing parameter estimates for the distribution of nutrient intakes and predicted values of intakes for each child. The parameter estimates include the overall intercept, regression coefficients for covariates such as age, sex or socio-economic indicators that are specified by the user, variance of an individual-level random intercept and error variance. The second macro uses the parameter estimates and predicted values to run simulation procedures, drawing 100 simulated random samples for each child from a distribution with a mean of their predicted value and variance equal to the random intercept variance estimated by the first macro. The simulated samples are then pooled into a single distribution. The percentiles of this distribution are the percentiles of the estimated usual intake distribution.

The NCI macros were used to estimate usual intakes of energy, protein, carbohydrates, fat, iron, calcium, zinc, vitamin A, thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12 and vitamin C. A covariate controlling for difference in intake on market days was included in models run by

the first macro. The control for market day was included in the nutrient intake models because observed energy and nutrient intakes were found to be lower on market days than on non-market days in exploratory analyses. The first macro was run separately by age and sex groups in order to control for differences between groups in both mean intakes and intake variance. The estimates of means and variances from the subgroup models were then combined in the second macro when modeling usual nutrient intake distributions of the full sample. Use of the NCI macros to estimate usual nutrient intake distributions is described in detail in Appendix 2.

Prevalence of nutrient intake inadequacy

The probability of adequacy (PA) approach is recommended by the IOM as a method for estimating the percentage of individuals at risk for inadequate nutrient intakes based on the distribution of nutrient requirements and the distribution of usual nutrient intakes.^{11,12} At the individual level, PA is equal to the probability that the intake requirement for an individual falls below their usual intake on the requirement distribution. Probability of inadequacy is equal to 1 minus the probability of adequacy. Prevalence of nutrient intake inadequacy is equal to the group average probability of inadequacy. It can be calculated by plotting the moments of the group's nutrient intake distribution of nutrient intakes against the nutrient requirement distribution for the life stage group of interest.¹² Prevalence of inadequacy for eleven micronutrients was calculated using the estimated usual nutrient intake distributions described above. Further detail on these calculations can be found in Appendix 2.

For most nutrients -- those with approximately normal requirement distributions -- the Estimated Average Requirement (EAR) and standard deviation (SD) or coefficient of variation (CV) can be used to describe the distribution and calculate probability of adequacy for a particular intake as the proportion of the population with intake requirements at or below that

intake.^{13,14} An important nutrient for which intake requirements cannot be accurately described by an approximately normal distribution is iron.^{11–13} Tables describing percentiles of the iron requirement distribution are used to determine the probability of adequacy for ranges of intakes.^{13,15}

Nutrient requirement distributions described by the IOM were used in calculations of probability of adequacy for most nutrients.^{16–19} For iron and zinc, different requirements were used to account for low bioavailability of these minerals in plant-based diets such as are typical in rural Zambia. The percentile values of the IOM iron requirement distribution for 4- to 8-year-old children were adjusted to reflect low iron bioavailability in a high phytate, low meat diet, as recommended by the World Health Organization (WHO).²⁰ The rural Zambian diet is characterized by high intakes of phytate-containing cereals, vegetables and legumes and low intakes of meat, poultry and fish (Chapter 3, Appendix 1).^{21,22} To estimate probability of zinc intake adequacy, the EAR and SD of the requirement distribution provided by the International Zinc Nutrition Consultative Group (IZiNCG) were used.²³ The IZiNCG values reflect low zinc bioavailability associated with unrefined, cereal-based diets.²³ The use of nutrient intake requirements from the IOM, WHO and IZiNCG is discussed in further detail in Appendix 2.

Food consumption patterns and sources of nutrient intakes

To describe food consumption patterns and sources of nutrient intakes, all foods reported in the repeat 24-hour recalls were broken down into a list of single, unmixed foods and individual ingredients of mixed foods. These disaggregated foods were then coded into fifty-two food types according to similarity and local consumption patterns. Foods were first classified by major food groups (vegetables, fruits, meats, etc.). Within each major food group, commonly consumed foods were retained as individual food types, and infrequently consumed foods were

aggregated by similarity. A count of the number of times a child consumed each food type during each 24-hour recall was calculated and these counts were averaged over all included recalls to describe the average number of times each food type was consumed per day. The average quantity consumed, in grams dry weight, was calculated as the average serving size among all instances of a food type being consumed, omitting all non-consumption days, and including one observation for each time a food was eaten, in some cases multiple observations from the same day.

To describe sources of nutrient intakes, the percent of total intake of each nutrient from each food type was calculated in three steps. First, the total amount of each nutrient available in all foods of a given type consumed in each 24-hour recall was determined. Second, the total amount of each available nutrient from a given food type was divided by the total observed intake from the same 24-hour recall to obtain the percent of total nutrient intake from each food type during each of the seven 24-hour recalls. Finally, these percentages were averaged across all included 24-hour recalls.

Aim 2 analyses

Transformation of variables

Because all distributions of observed nutrient intakes were skewed to the right and exploratory models of nutrient intakes had non-normally distributed residuals, all nutrient intakes were Box-Cox transformed prior to fitting models for Aim 2. Three Box-Cox transformations were fit using SAS PROC TRANSREG. Each used a mean model of intake as dependent on age, sex and season. The three models varied in the shift parameter applied: 1, 2 or 0.01. A shift parameter was applied because for vitamin C, vitamin A and vitamin B12 there were 2, 1 and 175 instances, respectively, of observed intakes of zero. For each nutrient, these transformations were carried

out using the distribution of intakes over all rounds. To determine the best choice among the three Box-Cox transformations, models were fit using the three sets of transformed data and the originally scaled data. The models were run for each nutrient and all included intake as the outcome and age, sex, season, market day and asset score as fixed effects. Plots of scaled residuals by predicted value and Q-Q plots of standardized residuals were examined for departure from model assumptions of homogeneity of residual variance and normal distribution of residuals. A shift parameter of 1 was chosen for clarity, ease of back transformation and most nearly normal and homogeneous distributions of residuals.

Previous authors typically have used logarithmic transformation to create distributions better approximating a normal curve, then reported results for both transformed and non-transformed data.²⁴⁻²⁹ In most cases, use of transformed data resulted in slightly lower variance ratios or lower numbers of replicates required for most nutrients to meet given criteria for accuracy.²⁴⁻²⁷ In consideration of convention in this body of literature, final components of variance models were also fit with untransformed data and the effect on estimates of within- to between-person variance reported.

Nutrient intakes by season

Three seasons were defined for use in Aim 2 and Aim 3 analyses: late post-harvest season (Season 1), August–October; early lean season (Season 2), November–January; and late lean season (Season 3), February–April. This definition of three seasons was chosen to approximately evenly divide the study period while aligning with agricultural seasons. The numbers of included observation days in each season were n=263 in the late post-harvest season, n=506 in the early lean season and n=302 in the late lean season.

Longitudinal models were fit to test for differences in mean observed energy and nutrient intakes by season, while controlling for covariance among repeated measures (SAS PROC MIXED). ESTIMATE and CONTRAST statements were used in the modeling procedure to request estimates of overall and seasonal means and 95% confidence intervals, as well as pair-wise contrasts between the seasons. All models took the form:

$$intake' = \beta_0 + \beta_1 season1 + \beta_2 season2 + \varepsilon$$

where *intake'* is the Box-Cox transformed energy or nutrient intake. For each energy or nutrient intake, this mean model was fit three times using different models of the covariance structure: unstructured, Toeplitz or first-order autoregressive (AR1). The fit of the three models was compared using AIC. Because the unstructured covariance model most often provided the best fit among the three covariance model options over energy and all nutrients, it was used for all final models of nutrient intakes by season. Means and confidence intervals were back-transformed for presentation of the results.

The same modeling procedure was repeated with control for total energy intake as a supplemental analysis. Models were fit and reported as described above, but with a fixed effect for centered total energy intake added to the model.

Determinants of energy and nutrient intakes

Potential factors were chosen based on a review of significant determinants of nutrient intakes or contributors to intake variance in previous papers as well as findings of association with energy or nutrient intakes in exploratory data analyses.^{25,27-34} Models were fit sequentially by level of clustering of the covariates. After fitting a model with a child-level random intercept, covariates were added one clustering level at a time and non-significant covariates were removed before proceeding to the next level. When there was evidence of collinearity between

two covariates, as determined by correlation analysis and impact on regression coefficients, the variable with stronger associations among most nutrients was retained and the other variable dropped. Recall-level covariates tested in model development included season, interviewer, day of the week, whether the recall period was a market day, and where the recall fell in the sequence of repeated recalls. Child-level covariates tested included age, sex and age*sex interaction. Household-level covariates included head of household literacy, years of schooling completed by the head of household, and asset ownership, housing quality and WASH scores. Rural residence was considered as a cluster-level covariate. Covariates were reordered or centered for ease of interpretation of model results.

The final models of nutrient intakes included fixed effects for interviewer, season, market day, child age, household asset score and rural residence. Because convergence errors were encountered when including random intercepts to account for correlation within households and clusters, the final model was also fit with a household-level random intercept in place of the child-level random intercept to determine whether this altered estimates of regression coefficients and their standard errors enough to affect interpretation. Minor changes to regression coefficients and standard errors were observed but were not substantial enough to change conclusions.

Because all models were run using Box-Cox transformed data, the regression coefficients were not readily interpretable. In order to present the size of fixed effects in models of energy and nutrient intakes in the original scale of intake measurement, a series of group means were estimated (PROC MIXED, ESTIMATE statement), back-transformed and compared to determine effect sizes. Estimates were generated for a reference group mean intake during late lean season on non-market days among children with mean age (5.48 years) living in households with the median asset score of 3 in a rural village. For each of the fixed effects, a comparison group

mean was estimated by varying the fixed effect by one unit and holding all other fixed effects constant to the reference mean values. The difference between each back-transformed comparison mean and the back-transformed reference mean was used to show the size of the associated fixed effect in original scale.

Components of variance models

Models to estimate within-person, between-person and seasonal components of variance were fit using SAS PROC MIXED. All models were run using both untransformed and Box-Cox transformed data in order to compare estimates, but only the results from the transformed data models were presented in full. For energy and each nutrient, a null model was first fit with a child-level random intercept and no other fixed or random terms. Adjusted versions of this model were then tested with fixed effects for potential covariates including season, interviewer, age, household asset score and rural versus peri-urban residence. Season, interviewer and market day were selected for use in the final model. These fixed effects were chosen for being: 1) critical to the aim of describing seasonal effects on nutrient intakes; 2) consistent across energy and nutrient intakes in significance and size of effect; and/or 3) informative for future study design or protocol development. The results of these models were presented as the within- and between-person coefficients of variation and the ratio of within- to between-person variances.

A second series of models of energy and nutrient intakes were then fit with a child-level random intercept and random slope term for season. These models were fit with and without adjustment for interviewer and market day. The results of these models were presented as the within- to between-person variance ratios and the percent of total variance attributable to

within-person, seasonal and between-person effects in order to illustrate the impact of season on intake variance.

Finally, a supplemental set of models estimating coefficients of variance separately by age, by sex and by season were fit to look for differences between subgroups. Age groups were defined as children under five years old or five years and older. Three season subgroups were formed according to the season definitions described above. The null model with child-level intercept was run for each set of subgroups. An adjusted model controlling for season, interviewer and market day was fit for each age and sex subgroup, and an adjusted model controlling for interviewer and market day was fit for each season.

Aim 3 analyses

Dietary diversity scores

Two different dietary diversity scores (DDS) were evaluated in Aim 3, the 7-food group score recommended by the WHO as an indicator for minimum dietary diversity among 6- to 23-month-old children and the 10-food group score recommended by the FAO as parallel indicator for use among women of reproductive age.^{1,2} These scores represent as close to a consensus as exists in the current literature on the number and definition of food groups for assessing dietary diversity. As the study population of 4- to 8-year-old children falls between the two age groups for which there are recommendations for assessing dietary diversity, we evaluated which of these scores is more effective for use in this age group in rural Zambia. For brevity, we refer to the 7-food group score recommended by the WHO for use among 6- to 23-month-old children as DDS7 and the 10-food group score recommended by the FAO for use among women as DDS10.

Each of the dietary diversity scores was calculated for each 24-hour recall collected among children in the non-intervened group over the course of the study. Prior to tallying food groups consumed, mixed dishes were disaggregated into individual ingredients. Each unmixed food or individual ingredient was assigned a code for the food group to which it belongs according to each scoring system. Guidance provided by the WHO and FAO was used to assign local foods to the correct food groups.^{1,35} The seven food groups used in the WHO score and the ten food groups used in the FAO score are shown in **Table 2.3**. First, each individual food reported within the 24-hour recalls was coded according to both the DDS7 and the DDS10 food groups. Then the number of food groups consumed by each child in each 24-hour recall was tallied to calculate a DDS7 and a DDS10 score. A set of binary marker variables was created to mark which food groups each child had consumed in each 24-hour recall. The combinations of these variables were used to determine the most common combination of food groups consumed. These food group marker variables were also aggregated by season, creating binary markers of whether a child had consumed any foods from each food group during each season. These seasonal markers were used to describe the percent of children consuming foods from each food group in each season.

Prediction of usual energy and nutrient intakes and usual dietary diversity

As has been discussed in Chapter 1, day-to-day variation in food and nutrient intakes is a form of error when the measure of interest is usual intakes. This error biases measures of association between two variables toward null. To account for this error in energy and nutrient intakes and in dietary diversity scores while examining seasonal effects, techniques previously described for cross-sectional analyses of dietary data were adapted for use in longitudinal models. These methods are described briefly below and in detail in Appendix 2.

The regression models in Chapter 5 (Aim 3) use an extension of the NCI method for estimating usual nutrient intakes described above, in combination with methods recommended for validation of DDS against MPA or usual nutrient intakes.^{14,36} Kipnis et al. demonstrate an extension of the NCI method for use when modeling an association between usual food or nutrient intakes and another variable such as nutritional status or a health outcome measure.³⁶ This method uses the same procedure for fitting non-linear mixed models of nutrient intakes. The extension of the method is to use the parameter estimates and predicted values from the intake model to predict the usual intakes of individuals. When the measurement error model of usual intake is linear, as is true in these analyses, the predictor recommended in the approach described by Kipnis et al. takes the form of the best linear unbiased predictor (BLUP) of usual intake.³⁶ The BLUP of usual intake for an individual is a weighted average of the individual's predicted value from the intake model and the overall mean intake. These two values are weighted by a term that incorporates the within- and between-person variances and number of nutrient intake observations.

Where Kipnis et al. demonstrated the use of BLUPs of usual intake as independent variables in a model of a health outcome, this analysis uses BLUPs of usual intake as dependent variables in relation to DDS or as the basis for calculating a more complex indicator of overall nutrient adequacy, mean probability of adequacy (MPA, described below).³⁶ Joseph and Carriquiry address this simultaneous use of BLUPs of usual intake and of DDS for minimizing bias due to day-to-day variation in cross-sectional models of the association between DDS and usual intakes or MPA.¹⁴ Because the analysis of seasonal difference in the association between DDS and usual intake or MPA required a longitudinal model, a further recommendation from Joseph and Carriquiry – that the use of BLUPs of both the DDS and usual intake can be used to reduce bias in simple linear regression approaches – was followed.¹⁴ The authors recommend that for MPA,

the BLUPs of usual nutrient intakes be used to calculate the probability of adequacy for each of the nutrients included, in order to reflect overall probability of adequacy of usual intakes.¹⁴

Drawing on the work by Joseph and Carriquiry and using the NCI SAS macros as demonstrated by Kipnis et al., BLUPs of usual intakes of energy and 11 micronutrients were calculated, as well as BLUPs of DDS7 and DDS10. These BLUPs were used in the regression models described below.

Mean probability of adequacy

Mean probability of adequacy (MPA) is a measure of overall micronutrient adequacy recommended by the Institute of Medicine (IOM).¹² The probability of usual intake adequacy of 11 micronutrients – calcium, iron, zinc, vitamin A, thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12 and vitamin C – was averaged to create an estimate of MPA in each round for each child. As described in Appendix 2, an individual's probability of adequacy is calculated as the percent of the nutrient requirement distribution that falls below their usual intake. EARs and standard deviations (SDs) of requirement distributions from the IOM were used to calculate probability of adequacy for most nutrients, the EAR and SD from IZiNCG were used for zinc, and percentiles of the iron requirement distribution published by the IOM were used after being adjusted as recommended by the WHO to reflect low bioavailability.^{16–20,37} The probability of iron intake adequacy was calculated as the average of the two requirement distribution percentiles between which a child's usual intake fell.

Seasonal impacts on diet and on associations between DDS and nutrient adequacy or intakes

To describe seasonal changes in DDS7, DDS10, MPA and usual intakes of vitamin A, vitamin B12, vitamin C, calcium and iron by season, one-way ANOVA were fit by season for each outcome,

estimating overall and seasonal means and conducting pair-wise tests for difference between seasons (SAS PROC GLM with ESTIMATE and CONTRAST statements). To visually describe the relationship between each DDS and MPA in each season, the dataset was divided into seasonal subgroups and within each subgroup, the mean MPA was calculated and plotted at each value of DDS. For these plots, observed DDS and the MPA calculated from BLUPs of usual intakes were used.

To describe the linear relationship between each DDS score and each outcome measure of nutrient adequacy or intake (MPA and usual intakes of vitamin A, vitamin B12, vitamin C, calcium or iron), as well as determine whether this relationship differed by season, a series of longitudinal models was fit, using generalized estimating equations to account for within-person correlation among repeat measures (SAS PROC GENMOD). In the first set of models, each measure of nutrient adequacy or intake was modeled as an outcome of DDS7 or DDS10. In the second set of models, fixed effects for season and the interaction between season and DDS were added. In the final set of models, fixed effects for child age, housing quality score and rural residence were added. These covariates were selected from other potential covariates of nutrient adequacy or intake including sex, asset score, head of household education and head of household literacy. A final set of models was fit to determine whether including covariates impacted conclusions about the relationships among DDS, MPA or nutrient intake and season. The final set of covariates was selected by running a full model of all potential covariates and dropping those that were not significant in two or more among the set of models. The results of these models did not impact conclusions about the relationships of interest so were not presented.

Sensitivity to influential clusters of observations, where a cluster is defined as the repeat measures from one child, was tested by re-running the first and second sets of models excluding

clusters with clustered Cook's D value greater than 0.02. This cutoff was determined by visual examination of clustered Cook's D plots. Exclusion of influential clusters did not change estimates of regression coefficients or their standard errors to a degree that would alter interpretation of the model results.

The effectiveness of observed DDS7 and DDS10 for predicting MPA > 0.75 was tested using receiver operating characteristics (ROC) curves. We fit the ROC curves separately by season using a random sub-sample of one record per child from each season (late post-harvest season n=187; early lean season n=186; late lean season n=178 in late lean). Though previous papers have used multiple MPA cutoffs, including lower values of 0.5 or 0.6, to test the predictive power of DDS, our range of MPA values did not permit analysis at lower values.³⁸⁻⁴⁰ We tested cutoffs of 0.7, 0.75 and 0.8 and chose a cutoff of 0.75 for similarity to other papers and because it falls near the middle of the MPA range for our sample.⁴⁰ SAS PROC LOGISTIC with ROC and ROCCONTRAST statements, run separately by seasonal sub-samples, was used to generate the sensitivity and 1 – specificity plot points for the ROC curves and estimate the area under the curve (AUC) value for each DDS in each season. The plot points were exported to Excel to create plots of with ROC curves for all three seasons on a single plot for each DDS.

Ethical considerations

The biofortified maize efficacy trial protocol was approved by the Institutional Review Board of the Johns Hopkins Bloomberg School of Public Health (Baltimore, Maryland) and the Ethics Review Committee of the Tropical Disease Research Centre (Ndola, Zambia). Protocols for all data collection procedures that supported this dissertation research and assessment of child diet were included in the parent trial documentation reviewed by both institutions. Any child aged 4 to 8 years, living in the study area and not yet enrolled in school was eligible to

participate. Identification of eligible children was done during mapping and census of the study area by interviewing household members present in each home. Verbal consent to participate in the efficacy trial was requested of each eligible child's parent or guardian after they were read a statement on the nature of the trial, risks of participation and rights of participants.

The burden on participants for the collection data for this diet study was the cost of their time. Respondents to diet or socio-economic surveys were at minimal risk of social discomfort if reporting experiences of hardship to the interviewer. All data were kept confidential, and interviews were conducted with only the respondent, participating child and field staff member present, unless the respondent admitted observers from his/her household.

At all times throughout data collection and analysis, all participant data were stored in password protected devices or digital locations. All participant data were recorded on password-locked tablet computers. When uploaded from the tablets, data were stored on a secure server in the efficacy trial field office and in access-limited cloud storage. Individually identifiable information were collected and used only for the purposes of identifying eligible participants and ensuring their continued correct identification in a population where some households move seasonally for agricultural work. De-identified data were used for analyses.

Mkushi, Central province, Zambia is a rural, agricultural district with high prevalence of child undernutrition, making this an appropriate population for this research on child diet. In Central province, 52% of children under five years of age are stunted, indicating a high prevalence of micronutrient deficiency.⁴¹ Documentation of the extent of micronutrient inadequacy, impact of season on dietary intakes, and methodological considerations for assessments of child dietary quality are needed to inform future research and programs to reduce child undernutrition in this and similar contexts.

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Tables and figures

Table 2.1. Data domains and timing of collection in the efficacy trial of pro-vitamin A biofortified maize (Mkushi, Zambia, 2012 – 2013).

Data used in the child diet study are indicated in bold.

| | Mapping and census | Baseline | Monthly monitoring | Follow-up |
|---|-----------------------|---|-----------------------|-----------|
| Enrollment data | | | | |
| Child ID | X | | | |
| Child birthdate | X | | | |
| Child eligibility for efficacy trial* | X | | | |
| Consent to participate | | X | | |
| Household ID | X | | | |
| Cluster ID | X | | | |
| Household data | | | | |
| Roster of household members | | X | | |
| Asset ownership | | X | | |
| Housing materials | | | | X |
| Water and sanitation access | | | | X |
| Food security & food consumption | | x | x | x |
| Child data | | | | |
| 24-hour dietary recall | | X | X | X |
| Morbidity history | | x | x | x |
| Health practices | | x | x | |
| Weight | | x | | x |
| Height | | x | | x |
| Mid-upper arm circumference | | x | | x |
| Triceps skin fold thickness | | x | | x |
| Serum retinol | | x | | x |
| Serum β -carotene and other carotenoids | | x | | x |
| Serum ferritin & transferrin receptor | | x | | x |
| AGP & CRP acute phase proteins | | x | | x |
| Hemoglobin | | x | | x |
| Malaria rapid diagnostic test | | x | | x |
| Malaria parasitemia | | x | | x |
| Dark adaptometry | | x | | x |
| Feeding trial monitoring | | | | |
| β -carotene content of maize meal | | sampled and tested over course of feeding trial | | |
| β -carotene content of prepared nshima | | sampled and tested over course of feeding trial | | |
| Attendance at meals | | collected daily over course of feeding trial | | |
| Amount of food consumed | | collected daily over course of feeding trial | | |

*Living within study area, born between 2004 and 2008 and not yet enrolled in school

Table 2.2. Comparison of child and household characteristics between non-intervened group and orange and white maize treatment groups in the pro-vitamin A biofortified maize efficacy trial, Mkushi, Zambia, 2012 – 2013*

| | Non-intervened group | White maize group | Orange maize group | p-value |
|--|-----------------------------|--------------------------|---------------------------|----------------|
| Children | | | | |
| Age (years) | 5.5 (1.2) | 5.6 (1.2) | 5.7 (1.3) | 0.2 |
| Male | 55% (112) | 51% (243) | 50% (270) | 0.4 |
| Households | | | | |
| Head of household completed primary school | 78% (105) | 81% (261) | 78% (273) | 0.5 |
| Head of household completed secondary school | 19% (25) | 24% (76) | 19% (65) | 0.2 |
| Head of household can read and write | 81% (127) | 84% (297) | 82% (317) | 0.6 |
| Asset score | 2.5 (1.5) | 2.6 (1.7) | 2.5 (1.5) | 0.5 |

*Values in table are % (n) or mean (standard deviation)

**p-values are for chi-squared tests of frequency distributions or analysis of variance tests for difference in group means

Table 2.3. Food groups in the World Health Organization’s Minimum Dietary Diversity indicator and in the Food and Agriculture Organizations Minimum Dietary Diversity for Women of Reproductive Age indicator^{1,2}

| MDD* | MDD-W** |
|--------------------------------------|---|
| Grains, roots and tubers | Grains, white roots and tubers, and plantains |
| Legumes and nuts | Pulses (beans, peas and lentils) |
| | Nuts and seeds |
| Dairy products | Dairy |
| Flesh foods | Meat, poultry and fish |
| Eggs | Eggs |
| Vitamin A-rich fruits and vegetables | Dark green leafy vegetables |
| | Other vitamin A-rich fruits and vegetables |
| Other fruits and vegetables | Other vegetables |
| | Other fruits |

*Minimum Dietary Diversity indicator

**Minimum Dietary Diversity for Women of Reproductive Age indicator

Figure 2.1. Enrollment into orange maize treatment, white maize positive control or non-intervened control groups in the efficacy trial of pro-vitamin A biofortified maize, Mkushi, Zambia, 2012 – 2013.

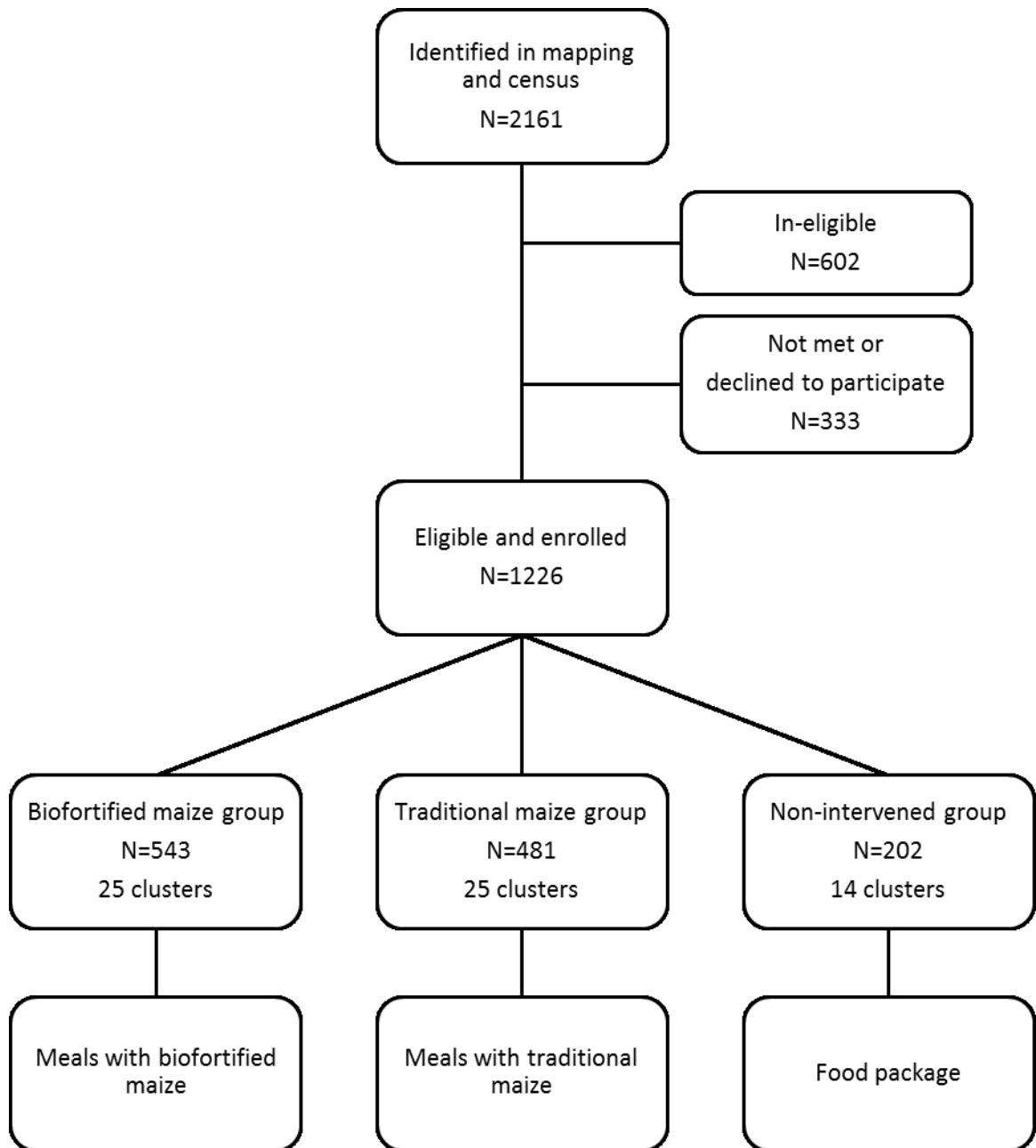
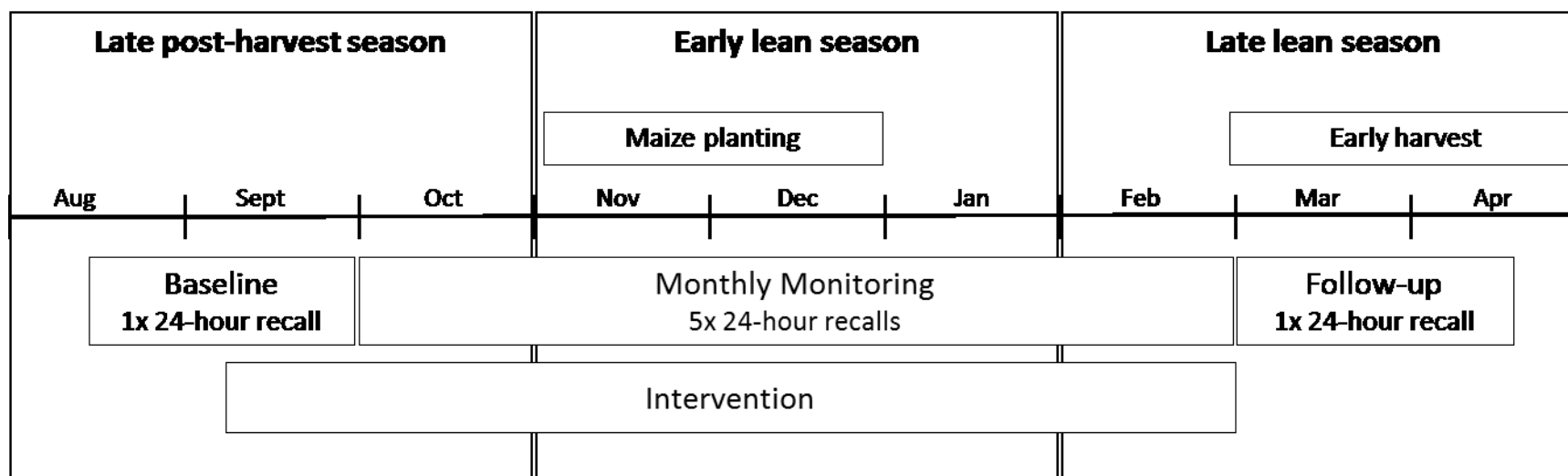


Figure 2.2. Calendar of data collection, delivery of intervention and agricultural seasons in the efficacy trial of pro-vitamin A biofortified maize, Mkushi, Zambia, 2012 – 2013.



Chapter 3. Aim 1: Usual Nutrient Intake Adequacy among Young, Rural Zambian Children

Abstract

Inadequate nutrient intakes place children at risk for impaired growth and development. We described the overall diet and usual intakes of energy and macro- and micronutrients among 4- to 8-year-old Zambian children, as well as the percent of children with inadequate intakes for each nutrient. Children 4–8 years old, not yet enrolled in school and living in Mkushi district, Central Province, Zambia were enrolled into a 3-arm, cluster-randomized efficacy trial of pro-vitamin A biofortified maize versus conventional maize intake on vitamin A status. Children in the non-intervened control arm were included in this analysis (n=202). Detailed dietary intake data were collected by tablet-based 24-hour recall on monthly basis over the six-month trial period. Observed nutrient intakes – calculated from food quantities consumed and a local food composition table – were used to estimate usual nutrient intake distributions. Prevalence of inadequacy was estimated by comparing the usual nutrient intake distribution to the nutrient requirement distribution. Foods reported in the 24-hour recalls were categorized into 52 food types for description of frequency and quantity of consumption. Data from ≥ 5 recalls were available for 181 children (90%). Median usual energy intake was 1535 kcal/day. Estimated prevalences of inadequacy of vitamin A, vitamin C, zinc, thiamin, riboflavin, niacin and vitamin B6 were low (0.1%–2.2%). Estimated prevalences of inadequate intakes of iron, folate, vitamin B12 and calcium were 25%, 57%, 76% and >99%, respectively. Commonly consumed foods included maize, vegetable oil, tomatoes, rape leaves and small fish (>0.6 servings per day), whereas meat, eggs or dairy were rarely eaten (<0.2 servings per day). These findings suggest

that the heavily plant-based diet of rural Zambian children is inadequate in intakes of calcium, folate, vitamin B12 and iron.

Introduction

Inadequate nutrient intakes are a key cause of impaired growth and other facets of undernutrition among children. In low- and middle-income countries, 1 in 5 school-age children are stunted (low height for age) and 15% have low body mass index (BMI) for age.¹ In Africa, 22% of school-age children are stunted and 35% have low BMI for age.¹ Vitamin and mineral intakes among this age-group are often inadequate to meet intake recommendations, especially for vitamin A, vitamin B12, folate, iron, calcium and zinc.² Among African school-age children, 32% are deficient in vitamin A and 54% are deficient in zinc.¹ These estimates indicate an ongoing state of undernutrition among children in this age group, with serious short- and long-term consequences for their health and development. Detailed assessments of nutrient intake adequacy and important sources of nutrients are needed to plan effective solutions to this public health problem.

Late preschool- and early school-age children need adequate nutrient intakes to maintain growth and development. Undernutrition and micronutrient deficiencies among young children have been found to impair growth, increase morbidity and mortality risk, reduce school achievement and decrease productivity in adulthood.³ School-age children with low BMI are at increased risk for reduced muscular strength and work capacity, delays in maturation and lower bone density in adulthood.¹ Though substantial impairment of linear growth is known to occur early in life, studies among undernourished school-age children have shown that linear growth deficits continue to accrue in contexts where dietary intakes are insufficient to fuel catch-up or maintenance growth.^{4,5} Zanzibari primary school boys, already short for their age at baseline,

fell nearly 2 cm further below the reference median height-for-age over one year of observation.⁴

Several micronutrients, including iron, zinc, vitamin B12, and folate, are critical for brain function, and deficiencies may impair cognitive development in school-age children.^{6,7} Brain development spurts that occur in this age range may increase this vulnerability.⁸ Among rural Kenyan children participating in a school-based feeding trial, higher intakes of available iron, available zinc, and riboflavin and vitamin B12 were associated with greater increases in scores for problem-solving, concentration and memory.⁹

Detailed dietary intake assessments are needed for planning effective strategies to prevent these compromises to child growth and development and for informing ongoing research into the causes and consequences of undernutrition among school-age children. Data specific to school-age children are of particular use in the design of school-based nutrition programs. However, few recent studies document nutrient intakes or prevalence of intake inadequacy among early school-age children in sub-Saharan Africa.^{2,10-16}

A significant limitation of studies that have examined food and nutrient intakes among sub-Saharan African school-age children is that most assess diet at a single timepoint.^{10,12,13,15,16} Assessment at one point in time may restrict validity in representing usual intake, often the goal in assessing dietary adequacy and its relation to malnutrition and disease. Thus, especially where dietary intakes change seasonally, longer-term assessments are needed to estimate usual intakes. Food consumption patterns have been shown to vary by season among 6- to 8-year-old Beninese children and among preschoolers in rural Ghana and Malawi.^{14,17} Intakes of protein, fat and several micronutrients have been found to differ significantly between harvest and late post-harvest seasons among preschoolers in rural Zambia.^{18,19} Rather than estimating usual

intakes, the dietary studies we identified among early school-age children in sub-Saharan Africa reported intakes observed during a single day or estimated a simple average of intakes over two to three days in a short period of time.^{10,11,13-15,17} Estimation of usual intakes reduces day-to-day variation in nutrient intakes, enabling more accurate estimates of the prevalence of inadequacy.²⁰ Multiple 24-hour recalls or weighed dietary records for at least a subset of study participants are needed to accurately estimate usual nutrient intakes, whereas most studies tend to measure only one day's intake.¹⁰⁻¹³ Finally, few of the studies used the probability approach for estimating prevalence of intake inadequacy based on a requirements distribution, as recommended by the Institute of Medicine.²¹

This study provides a detailed description of food and nutrient intakes and risk of intake inadequacy among 4- to 8-year-old children in rural Zambia, based on seven 24-hour dietary recalls evenly spaced on random days of the week over a multi-season, six-month period. Though data specific to Zambian school-age children are lacking, this population is likely to experience dietary inadequacies leading to undernutrition. Undernutrition and nutrient deficiencies are prevalent among school-age children in other countries in sub-Saharan Africa, and 46% of Zambian children under five are stunted.^{1,22} A six-month period of repeated dietary intake assessment is novel in the literature, allowing for description of children's usual nutrient intakes over three agro-ecological seasons. The objectives of the study are to describe usual diets and nutrient intakes of 4- to 8-year-old Zambian children over a period of six months; to estimate the likelihood of intake adequacy of 14 nutrients and energy; and to describe major sources of each nutrient.

Methods

Study context

Data for this study were collected within an efficacy trial of pro-vitamin A biofortified maize, described elsewhere.²³ Children aged 4 to 8 years, not yet enrolled in school and living in villages or towns in the northern half of Mkushi district, Central Province, Zambia were enrolled into geographic clusters of ~15 to 30 children. Mkushi is a rural, agricultural district with a mix of small-holder and commercial farming. Eligible children were identified by a door-to-door census conducted in all towns and villages in northern Mkushi with sufficient estimated population density to form clusters with a diameter of one kilometer or less. Clusters were assigned by block randomization to a treatment group receiving meals containing biofortified maize (n=25 clusters), a control group receiving meals containing traditional, unfortified maize (n=25 clusters) or a non-intervened group (n=14 clusters). In the non-intervened group, children were not provided any food during the study but their families received a food package equivalent to the intervention's food value at the end of the six-month trial. This analysis is restricted to evaluating ambient dietary patterns of children enrolled into the non-intervened arm of the efficacy trial.

This study was approved by the Institutional Review Board at the Johns Hopkins Bloomberg School of Public Health (Baltimore, USA) and the Ethics Review Committee of the Tropical Disease Research Centre (Ndola, Zambia). Oral, informed consent of each child's primary caregiver was obtained at enrollment.

Data collection

Data were collected on a monthly basis over the six-month period during which the efficacy trial was conducted (August 2012–April 2013), yielding a total of seven survey rounds over three agro-ecological seasons (late post-harvest, early lean and late lean seasons). Household socio-economic and demographic data were collected by questionnaire administered to the primary caregiver of the enrolled child during the baseline survey. Dietary intake data were collected in all seven monthly survey rounds, using a tablet-based 24-hour recall previously described by Caswell et al (Appendix 1).²⁴ Data collection was scheduled to capture dietary intakes on six days of the week, excluding Saturdays. Data were not collected on Sundays due to high rates of religious service attendance in this population. Clusters were randomly assigned to a day of week for the first survey round and subsequent scheduling ensured that each cluster was visited at least once on all six days of the week over the following survey rounds.

The primary caregiver, accompanied by the child, was asked to recall all foods and drinks the child consumed between waking the previous day and waking the day of the interview. For most foods, the respondent was asked to describe the amount the child consumed by indicating the closest match among five portions of a similar food shown in a photo booklet. For other foods, the respondent was asked to report the number of food units the child consumed. The tablet tool used a modified multi-pass method, first prompting the respondent to describe foods as they were consumed sequentially through the day, with time-of-day specific prompts to aid recall. In the second pass, a picture chart memory aid was used to check for missed foods. In the final pass, the interviewer asked the respondent to review the child's day, probing for any occasions on which the child may have consumed foods not yet recalled. All food description

details -- including detailed description, added ingredients, portion size and where the child obtained the food -- were collected for each recalled food in the first pass the food was recalled.

Data analysis

All data analysis was done using SAS 9.4 (Cary, NC). Simple descriptive statistics were used to report distributions of household-level descriptors and child age and sex.

Observed nutrient intakes

Observed nutrient intakes were calculated from the 24-hour recall data using a standard database of recipes and a food composition table of local foods, as described previously.²⁴ We used a database of standard local recipes (unpublished) developed by HarvestPlus, an initiative within the CGIAR Research Program on Agriculture for Nutrition and Health, during a 2009 survey of dietary intakes among women and children under five. Where needed, we modified recipes by removing ingredients or substituting similar main ingredients, retaining relative proportions of the other ingredients. We also conducted a focus group with local women to collect additional recipe data. We compiled a local food composition table based primarily on a food composition table developed by HarvestPlus in their 2009 survey, adding food composition data from the Zambia Food Composition Tables published by the Zambian National Food and Nutrition Commission, the U.S. Department of Agriculture National Nutrient Database for Standard Reference, and several other regional and international food composition tables.²⁵⁻²⁹ Portion weights were estimated based on the weight of food in the portion size photograph selected by the respondent and a density adjustment, or by a weight per unit for foods recorded by number of units consumed. Weights of ingredients in mixed foods were calculated by multiplying the portion weight by each ingredient's fraction in the standard recipe. Nutrient contents of foods were calculated by multiplying ingredient or unmixed food weights by

nutrient contents from the food composition table, and the nutrient contents of all foods reported in the 24-hour recall were summed to estimate observed intake of each nutrient.

Usual nutrient intake distributions

Our objective in this analysis was to describe usual, daily intake over time, rather than intake on a single day or simple average over several days. Because nutrient intakes can vary widely from day to day, even where diets are monotonous, intake on a single day can be expected to yield an inaccurate estimate of usual intake.²⁰ This day-to-day variation inflates the variance of the intake distribution and creates bias in estimates of the prevalence of inadequacy. Therefore, appropriate statistical models to reduce the excess variance are required to describe the distribution of usual nutrient intakes.²⁰

We used SAS macros provided by the National Cancer Institute (NCI) to estimate the usual nutrient intake distributions of energy and 14 macro- and micronutrients.³⁰ In these and subsequent food and nutrient intake analyses, we used the observed intake data from all recalls for which the enrolled child was not reported ill on the day covered by the dietary recall interview. We estimated usual intake distributions over all included children, by age group (under five years v. five years or older) and by sex.

The SAS macros from NCI estimate the usual intake distributions of single nutrients consumed on a daily basis using methods described by Tooze et al.³¹ In the first phase of the estimation procedure, a non-linear mixed model was fit (SAS PROC NLMIXED) using observed intake data that have been transformed to approximate a normal distribution using a Box-Cox procedure. For each nutrient, we fit non-linear mixed models of transformed, observed intake separately by age and sex group. The model included an individual-level random term and a covariate controlling for whether the recall day was a market day. Dietary intakes have been shown in

previous studies to vary by day of the week.³² The NCI SAS macros include a built-in option for modeling this source of variation. We used this option to control for market day because, in exploratory analyses, we found a trend toward lower intakes of energy and nutrients on market days. The first phase of the usual intake estimation procedure produced two output datasets: parameter estimates from the non-linear mixed model and predicted values. The parameter estimates included the regression coefficients for the intercept and covariates and the variances of the random term and the residual. The predicted values were each individual's mean observed intake multiplied by a shrinkage factor that incorporates the within- and between-person (residual and random term) variance estimates, plus each of the regression coefficients multiplied by the individual's values for the covariates. Rather than including a linear term for age and for sex in a single model, we fit the models separately by age and sex group, allowing the regression coefficients and variance terms to differ between groups.

In the second phase of the usual intake estimation procedure, a Monte Carlo simulation was run using the parameter estimates and predicted values generated in the first phase modeling procedure. A simulated draw of 100 random samples per participant was taken from a distribution with a mean of the participant's predicted value and variance equal to the random term variance from the mixed model. In this simulation, we assigned market days a weight of 1 out of 7 days per week. The simulated samples were pooled into a single distribution, of which the percentiles were the estimated percentiles of the usual intake distribution. Because these values were still in transformed terms, the final step was a back-transformation procedure to state the percentiles of the usual intake distribution in the original units of nutrient intake.

This usual intake estimation procedure was followed for energy and all macro- and micronutrients with the exception of vitamin B12. Because vitamin B12 is found only in fortified or animal source foods which are consumed infrequently in this population, the observed intake

of vitamin B12 was zero on many of the recall days in our study. For such cases of zero-inflated intake data, the NCI macros include an additional step in the first phase of the modeling procedure, estimating usual nutrient intake as the usual amount consumed on consumption days multiplied by the probability of consuming the nutrient on any single day.³³ In addition to the non-linear mixed model of intake quantities, a model was fit for the probability of consumption of a food or nutrient on a single day. The modeling procedure for this probability model was similar to that for the amount model described above, and paralleling the modeling procedure for other nutrients, we modeled the probability and amount of vitamin B12 intake separately by age and sex group. The two-part probability and amount model may be fit assuming that the probability and amount of consumption are correlated or that they are uncorrelated. We found that the individual-level random terms for the amount and probability models did not significantly covary and model fit diagnostics were slightly better for the uncorrelated two-part model, so it was selected as the final model. The second phase of estimating the usual nutrient intake distribution by Monte Carlo simulation was carried out as described above.

Similar modeling procedures were used to estimate the percent of energy from protein, carbohydrates and fat. NCI provides SAS macros for estimating the distribution of the ratio of two nutrient intakes, as described by Freedman et al.³⁴ In this case, bivariate models of protein and energy, carbohydrates and energy and fat and energy were fit, with the same covariates and sub-group modeling as described above. The Monte Carlo simulation procedure was run for each macronutrient and energy based on the parameter estimates and predicted values from the bivariate models. Percent energy from each macronutrient was calculated using the 100 simulated values per participant prior to estimating the moments of the usual ratio distribution based on the simulated values for the ratio.

Prevalence of inadequacy

We calculated prevalence of inadequacy overall, and separately by sex and by age group using the usual intake distributions produced by the NCI macros. For most micronutrients with normal requirement distributions, we used the Estimated Average Requirement (EAR) and coefficient of variation (CV) published by the Institute of Medicine (IOM) to calculate the probability of inadequacy.³⁵ The prevalence of zinc inadequacy was calculated using the EAR and CV for diets with low zinc bioavailability provided by the International Zinc Nutrition Consultative Group (iZiNCG).³⁶ We calculated probability of inadequacy using the PROBNORM statement in SAS, as described by Murphy et al.³⁷ However, instead of using observed individual intakes, we compared midpoints between each percentile of the usual intake distribution to the requirement distribution. The PROBNORM statement was used to return the area to the left of the midpoint under the requirement distribution curve. This value was subtracted from 1 to obtain the probability of inadequacy for the midpoint. The probabilities of inadequacy for all 100 percentile midpoints were averaged to arrive at the prevalence of inadequacy for each nutrient.

To calculate prevalence of inadequacy of iron, we adjusted the percentile values of the iron requirement distribution published by the IOM to reflect the 10% iron bioavailability of a high phytate, low meat diet estimated by the World Health Organization (WHO).^{35,38} The probability of inadequacy for each midpoint between percentiles of the usual intake distribution was assigned as the average of the requirement distribution percentiles above and below the midpoint value. For example, an intake of 8.88 mg iron was assigned a probability of inadequacy of 35% because it falls between the 60th and 70th percentiles of the requirement distribution. To describe adequacy of macronutrient intakes, we calculated the percent of

children with intakes above and below the Acceptable Macronutrient Distribution Ranges (AMDR) recommended by the IOM.³⁵

Food consumption patterns and contribution to nutrient intakes

To describe food consumption patterns and contributions to nutrient intakes, we grouped unmixed foods and disaggregated ingredients of mixed dishes into fifty-two food types. To determine the food types used in this analysis, we first classified foods by major food groups (vegetables, fruits, meats, etc.). Within each major food group, commonly consumed foods were retained as individual food types, and infrequently consumed foods were aggregated by similarity. For example, among vegetables, tomatoes are frequently consumed so were assigned their own food type code, but green beans are infrequently consumed so were assigned to the aggregate food type for other non-leafy vegetables.

A count of the number of times a child consumed each food type during each 24-hour recall was calculated and averaged over all included recalls to describe the average number of times foods of each type were consumed per day. The average quantity consumed, in grams dry weight, was calculated as the average serving size among all instances of a food type being consumed.

For energy and each nutrient, we calculated the percent of total intake provided by each of the fifty-two types of foods. We first summed the contents of all foods of a given food type consumed in each 24-hour recall. This sum was divided by the total observed intake from the same 24-hour recall to obtain the percent of total intake provided by each food. These percentages were averaged across all included 24-hour recalls to describe the usual sources of each nutrient.

Results

The biofortified maize trial enrolled 1226 children, of which 202 children were assigned to the non-intervened group. Two children were excluded because they only completed one round of dietary data collection and were reported ill in that round, resulting in a sample size for these analyses of 200 children. The mean age at baseline of included children was 5.5 years (± 1.2), and 55.5% of the children were male (**Table 3.1**). Among heads of households with one or more included child, 81% could read and write, 41% had completed 9–12 years of schooling, and 42% had completed 3–8 years of schooling. Salary or wage employment was the most commonly reported primary occupation (n=61, 39%), followed by self-employment (n=43, 27%) and farming (n=37, 24%). Sixty-five percent of households (n=101) reported owning a mobile phone, 54% reported owning a bicycle, and 1% reported having electricity in the home.

The total number of children per round for whom a recall was completed ranged from 172 to 200. The number of children reported ill in each round ranged from 17 to 54, leaving 125 to 177 included recalls per survey round. Thus, all estimates of child dietary intake relate to apparently healthy days based on caregiver's report that the child was not ill.

Median usual energy intake was 1535 kcal/day (**Table 3.2**). Children under five had lower energy intakes than children five years and older, and boys had higher energy intakes than girls. Differences between groups in carbohydrate intake parallel those of energy intake, though the magnitude of difference is small. Intakes of protein and fat were similar across groups. Most micronutrient intakes were similar across groups, with the exceptions of iron and vitamin C, which were higher among older children than among younger children.

Children in this study were at highest risk of inadequate intakes for iron, folate, vitamin B12 and calcium (**Table 3.3**). The estimated prevalence of inadequate calcium intake was virtually 100%.

The estimated prevalence of inadequate intakes of iron, folate and vitamin B12 was 25%, 57% and 76%, respectively. The prevalence of inadequate nutrient intake was less than 3% for each of zinc, vitamin A, thiamin, riboflavin, niacin and vitamin B6 and vitamin C. Estimates of prevalence of inadequacy were similar between the two age groups and between boys and girls, except for folate and vitamin B12, which were slightly higher among older children than among younger children, and for iron, which was higher among younger children. Most children were within the AMDR for protein and 76% of children were within the AMDR for fat (**Table 3.4**). For nearly one quarter of children, fat intakes were below the recommended range, and a similar proportion of children had high carbohydrate intakes. The percentage of children falling outside the AMDRs for protein and fat showed little difference between sexes or between age groups. Boys and children under five were less likely than girls and children five years and older to have high carbohydrate intakes.

The most commonly consumed foods among 4- to 8-year-old Zambian children over the six months of data collection were vegetable oil, tomatoes, maize, onions, small fish and rape leaves (**Table 3.5**). Vegetable oil, tomatoes and maize were each consumed an average of 3 times per day, and main ingredients in common side dishes, such as rape leaves, small fish, pumpkin leaves and beans were consumed 0.3 to 0.6 times per day, on average. Foods consumed in the largest quantities included maize, mango, fritters or scones, bread, other fruits, and cassava. The average serving size of maize, the staple grain, was 82 grams dry weight per serving. The average quantity consumed per serving of many main ingredients in side dishes was between 20 and 40 grams raw, dry weight per serving.

Maize was the main contributor to intakes of energy, protein, carbohydrate, iron, zinc, and most of the B vitamins (**Tables 3.6a – 3.6d**). Commonly consumed side dish ingredients were important sources of nutrients. Vegetable oil accounted for 51% of fat intakes. Small fish were

the main source calcium and vitamin B12, and were among the top five contributors to intakes of energy, protein, fat, and all micronutrients except vitamin C and folate. Rape leaves were an important source of vitamin A, vitamin C and calcium in the diets of children.

Discussion

Using dietary recalls collected over six months, we have described the usual nutrient intakes of apparently healthy rural Zambian children, identified nutrients with high prevalence of inadequate intake and described key foods and food sources of each nutrient. The overall median energy intake in this study population was similar to those reported by other studies of young school-aged children in Mexico and in peri-urban Kenya.^{12,39,40} The median energy intake recently reported for first graders (median age 7.0 years) in rural Kenya was similar to that for children over 5 years old reported here.¹⁵ Our estimate of usual energy intakes among 4- to 4.9-year-old children in Mkushi district, Zambia, 1452 kcal/d, is slightly lower than the usual energy intake of 1527 kcal/d reported by Hotz et al. for 4- to 5-year-old children in Mkushi and Nyimba districts, in Eastern Province, Zambia in 2009.¹⁸ This difference may be attributable to the fact that the authors observed lower intakes in Mkushi than in Nyimba, or to differences in timing of data collection.¹⁸ A second paper from the same 2009 survey reported that usual energy intakes were lower in the late post-harvest season (October-December) than in the harvest / early post-harvest season (May – June).⁴¹ Our data collection period, August – April, covers the late post-harvest, lean and early harvest seasons, so did not capture the full harvest season when intakes may have increased.

The median energy intakes by age group exceeded energy requirements estimated by the FAO and WHO.⁴² Though one quarter of children in this study had high carbohydrate intakes, nearly all children consumed fat and protein within the Acceptable Macronutrient Distribution Ranges

for these macronutrients.³⁵ We observed higher protein and fat intakes than those previously reported for preschool children in Mkushi and Nyimba districts of Zambia, presumably due to differences between age groups surveyed.⁴¹ Risk of protein-energy malnutrition in this study population appears low, though additional factors such as high prevalence of parasitic infection and high physical activity levels may increase energy requirements above those cited.⁴²

Though macronutrient intakes appear adequate, we found that children in this population are at high risk of inadequate calcium and vitamin B12 intakes, with additional risks of iron and folate inadequacy. These results are corroborated by other studies conducted in the region. Gewa et al. found similar probability of inadequate iron intake among rural Kenyan first graders, and lower but still substantial risks of calcium, vitamin B12 and folate inadequacy.¹⁵ However, they also found much higher risks of inadequacy of other micronutrients, such as zinc, vitamin A and vitamin C, which we did not observe among Zambian children.¹⁵ In another study of nutrient intakes among school-age children in a rural area of Kenya, Semproli et al. reported low potassium and calcium intakes among 5- to 8-year-olds.¹³ Steyn et al. found that 1- to 9-year-old South African children, like the children in our study, were at risk for inadequate intakes of calcium and folate, as well as a lesser risk of iron inadequacy.¹¹ Unlike this study, Steyn et al. also observed risks for inadequate intakes of niacin.¹¹ In a previous survey in Mkushi and Nyimba districts of Zambia, highest risks of nutrient inadequacy among 4- to 5-year-olds were observed for calcium, iron, zinc and vitamin B12, and risks of folate inadequacy were lower than those we observed.¹⁸ The discrepancies observed between these studies and ours may be due to differences in context, age group or methods. Most of the previous studies used single-day or averaged intakes rather than modeling usual nutrient intakes.^{11,13,15} Two used the Recommended Nutrient Intakes from the WHO rather than the Dietary Reference Intakes from the IOM used here.^{11,13} Finally, different methods and assumptions were used when accounting

for the bioavailability of iron and zinc.^{11,13,15,18} That inadequate calcium intakes emerge as a consistent problem despite varying methods suggests that this is likely a substantial public health nutrition problem among school-age children across the region.

Our findings of inadequate iron, folate, vitamin B12 and calcium intakes in this population are also supported by studies conducted in the region on biomarkers of nutrient status and dietary supplies of calcium. Our results agree with the 29% prevalence of iron deficiency among African school-age children reported in a review by Best et al., and with the 58% prevalence of anemia among Zambian children under five, of which about 20% would respond to iron supplementation.^{1,43} Data on the prevalence of vitamin B12 and folate deficiencies are limited and inconsistent. Biomarker data from the school-based feeding trial in rural Kenya showed that 38% of primary school children in the trial had low plasma vitamin B12, though only 1% had low plasma folate.⁴⁴ A more recent study in Cameroon reported that 8% of preschool-age children had low plasma folate and 30% had low plasma vitamin B12.⁴⁵ However, using dietary assessment, the same study found that 39% of non-breastfeeding preschool-age children had inadequate folate intakes, which more closely matches our findings.⁴⁵ Estimates of calcium deficiency are difficult to obtain, given a lack of reliable biomarkers of deficiency. Estimates of calcium intake inadequacy based on national food supplies and demographic data have recently been calculated, with a calcium deficiency risk of 80% in Africa and, like that found in this analysis, a calcium deficiency risk of 100% in Zambia.⁴⁶

Inadequate intakes of vitamin B12, iron and folate imply serious risks to children in this population. Insufficiencies of these nutrients are associated with risk of impaired cognitive function, and with anemia and its consequences.^{7,47} Deficiencies of vitamin B12 and folate during gestation and childhood have been associated with lasting deficits in cognitive development, and micronutrient supplementation trials among school-age children have shown

positive effects on memory.^{6,7} Dutch children who consumed a macrobiotic, vegan diet and were consequently vitamin B12 deficient in early childhood had lower scores on tests of problem-solving, spatial reasoning and memory in adolescence than did peers who had consumed an omnivorous diet.⁴⁸ In the school-based feeding trial conducted in rural Kenya, children who received snacks containing meat showed greater gains on tests of problem-solving ability than did children who received snacks containing milk or vegetables or who did not receive the snack intervention.⁴⁹ Similarly, children in the trial with higher iron intakes had greater increases in problem-solving ability scores and children with higher vitamin B12 intakes had greater increases in scores on a test of attention and recall.⁹ Deficiencies of iron, vitamin B12 and folate are among the nutritional causes of anemia, which can cause fatigue, cognitive impairment and reduced work capacity.⁴⁷ The observed inadequate intakes of calcium may compromise bone growth and accrual of bone mineral density. Particularly if calcium inadequacy extends into puberty, adolescents may not achieve optimal bone mass during the pubertal growth spurt.⁵⁰

The risks of micronutrient inadequacy we observed in this population are a consequence of the monotonous, predominantly plant-based diet. In low- and middle-income countries, studies of dietary intakes among school-age children have shown that their diets are mainly plant-based, with high consumption of cereals and starchy roots and tubers and limited consumption of animal source foods.^{2,17} This is consistent with our finding that maize contributed over half of total calories consumed by 4- to 8-year-old, rural Zambian children and most of the foods consumed frequently and in largest quantities were plant foods. Despite this finding, several foods that are occasionally consumed and widely available in the study area are good sources of the nutrients for which kids are most deficient. Small, whole fish are good sources of calcium, iron and vitamin B12 and were consumed at a rate of 0.66 servings per day. They were among

the top sources of energy, protein and fat, and several macronutrients, including providing 41% of vitamin B12 intakes and 29% of calcium intakes. However, they were consumed in small portions, averaging 29 grams raw weight per serving. Beans, groundnuts and dark green leafy vegetables are good sources of folate that were commonly consumed but in small portions. Each of these nutrient-rich foods is served as a side dish or sauce for the staple dish of stiff maize porridge. Because nutrient-rich vegetables, beans and fish are widely available but underutilized, dietary diversification programs, including nutrition education and rural development programs to improve household access to nutrient-rich foods, should be evaluated for impact and feasibility in rural Zambia. However, the amount of calcium available in Zambia's national food supply is insufficient to meet the dietary requirements of its population, so dietary diversification strategies alone will not be sufficient for reducing the prevalence of calcium inadequacy.^{46,51} Therefore diversification of food production, fortification and other national strategies merit exploration.

We found that children in this population consumed maize several times per day and in large quantities. Maize provides over half of total energy intakes, and is the primary source of carbohydrates and several micronutrients. Given its importance in the diet, fortification of maize with calcium has been suggested as a strategy for decreasing the nation-wide risk of calcium inadequacy in Zambia.⁴⁶ Maize fortification with other micronutrients has also been under policy consideration in Zambia.⁵² Unfortunately, rural Zambians commonly consume maize milled at small-scale, local hammer mills, and the logistics of coordinating an effective national fortification policy through such mills is logistically and financially infeasible.⁵²

Though national fortification is not currently considered an effective strategy for improving micronutrient intakes among rural Zambians, household-level, or point-of-use fortification, such

as the addition of multiple micronutrient sprinkles to maize prepared for children, may be a more appropriate strategy. Multiple micronutrient sprinkles added to food at point-of-use have been shown to reduce anemia and iron deficiency among children under two and preschoolers and could also be considered for school-age children.⁵³⁻⁵⁵ In an effectiveness trial of multiple micronutrient powders distributed by community marketing in rural Kenya, Suchdev et al. found that intervention villages had greater reductions in iron and vitamin A deficiencies among 6 – 35 month old children.⁵⁶ Community-based marketing of point-of-use fortification products may be similarly successful among rural Zambian 4- to 8-year-old children, but more evidence is needed to assess this possibility.

Our study has several important strengths. We collected 24-hour dietary recall data on a monthly basis over six months, yielding up to seven recalls per child and capturing data on dietary intakes over three agricultural seasons. Further, we used these repeat measures to estimate usual nutrient intake distributions prior to assessing probability of inadequacy. These strengths distinguish this study from previous assessments of diet among school-age children in sub-Saharan Africa that were based on data collected at one or two time points and used observed or average intakes rather than usual intakes. However, our data do not cover the full year, and dietary intakes in harvest season might have influenced our estimates had they been available. Modeling usual intake distributions reduces the variance inflation due to day-to-day variation in nutrient intakes and thereby reduces bias in estimating prevalence of inadequacy.

Weaknesses of this study include: the use of portion size photos in the 24-hour recall rather than using food models; use of a standardized local recipes table based on recipe from a prior survey rather than collecting household recipes; and limited sample size and study area and sampling design chosen for a food-based intervention rather than a representative survey. We describe diet and nutrient intakes among apparently healthy children, which may affect

comparison of our results to other population studies of diet. We present absolute iron and zinc intakes, not adjusted for bioavailability. Though we used iron and zinc requirements set by the IOM and iZiNCG to reflect low bioavailability diets, we may still be underestimating the prevalence of inadequacy of these nutrients if iron and zinc bioavailability from the rural Zambian diet are lower than those assumed in the EARs.

Conclusion

The heavily plant-based diet of 4- to 8-year-old rural Zambian children places them at risk for anemia, impaired cognitive development and reduced bone growth due to deficiencies of iron, folate, vitamin B12 and calcium. Foods providing these nutrients, such as small, whole fish, beans and leafy vegetables, are consumed infrequently and in small quantities. Further research into strategies to improve the year-round availability, affordability and provision of micronutrient-rich foods to children in this population is urgently needed to safeguard their health, growth and development.

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Tables

Table 3.1. Baseline characteristics of children (n=200) and households (n=156) participating in the non-intervened arm of a biofortified maize efficacy trial, Mkushi, Zambia, 2012–2013

| | N | Percent |
|---------------------------------------|-----|---------|
| Child Characteristics | | |
| Male | 110 | 55.0 |
| 4 – 4.9 years | 74 | 37.0 |
| 5 – 5.9 years | 67 | 33.2 |
| 6 – 6.9 years | 33 | 16.5 |
| 7+ years | 26 | 12.9 |
| Household Characteristics | | |
| Literate head of household | 127 | 81.4 |
| <u>Head of household's occupation</u> | | |
| Salary or wage employment | 61 | 39.1 |
| Self-employed | 43 | 27.6 |
| Farming | 37 | 23.7 |
| Other | 15 | 9.6 |
| <u>Asset ownership</u> | | |
| Bicycle | 84 | 53.9 |
| Motor vehicle | 5 | 3.2 |
| Mobile phone | 101 | 64.7 |
| Radio | 112 | 71.8 |
| Television | 47 | 30.1 |
| Electricity in the home | 2 | 1.3 |

Table 3.2. Usual nutrient intakes over six months [median (inter-quartile range)], by age and sex, among 4- to 8-year-old children (n=200) participating in the non-intervened arm of a biofortified maize efficacy trial, Mkushi, Zambia, 2012–2013

| Nutrient | Overall | 4–4.9y | 5–8y | Boys | Girls |
|--|----------------------|----------------------|----------------------|----------------------|----------------------|
| Energy (kcal/d) | 1535 (1235, 1802) | 1452 (783, 1604) | 2009 (1529, 2598) | 1581 (1485, 1682) | 1484 (832, 1972) |
| Carbohydrates (g/d) | 244 (228, 259) | 242 (227, 254) | 246 (229, 263) | 247 (236, 259) | 237 (216, 260) |
| Percent calories from carbohydrates(%) | 63.0 (60.9, 65.1) | 60.9 (58, 63.8) | 63.3 (61.5, 65.3) | 63.0 (61.3, 64.7) | 63.0 (60.4, 65.5) |
| Protein (g/d) | 46 (43, 50) | 46 (43, 49) | 47 (43, 50) | 47 (44, 50) | 45 (41, 50) |
| Percent calories from protein (%) | 11.9 (11.3, 12.5) | 11.8 (11.1, 12.7) | 11.9 (11.4, 12.5) | 12 (11.4, 12.5) | 11.8 (11.2, 12.6) |
| Fat (g/d) | 47 (41, 54) | 48 (42, 55) | 47 (40, 54) | 48 (41, 55) | 47 (41, 53) |
| Percent calories from fat (%) | 27.4 (25.3, 29.5) | 28.1 (25.4, 30.5) | 27 (25.2, 28.9) | 27.3 (25.4, 29.4) | 27.4 (25.1, 29.7) |
| Calcium (mg/d) | 261 (236, 286) | 259 (233, 287) | 261 (238, 285) | 262 (243, 283) | 257 (226, 291) |
| Iron (mg/d) | 10.4 (9.7, 11.1) | 9.7 (8.9, 10.6) | 10.7 (10.1, 11.2) | 10.4 (9.7, 11.1) | 10.5 (9.7, 11.1) |
| Zinc (mg/d) | 6.4 (5.7, 7.1) | 6.2 (5.5, 6.8) | 6.6 (5.9, 7.3) | 6.6 (6, 7.3) | 6.1 (5.4, 6.9) |
| Vitamin A (µg RAE*/d) | 490 (439, 533) | 490 (473, 491) | 491 (431, 557) | 490 (466, 508) | 483 (412, 558) |
| Thiamin (mg/d) | 0.8 (0.7, 0.9) | 0.8 (0.7, 0.9) | 0.8 (0.7, 0.9) | 0.8 (0.8, 0.9) | 0.8 (0.7, 0.9) |
| Riboflavin (mg/d) | 0.7 (0.7, 0.8) | 0.7 (0.7, 0.8) | 0.7 (0.7, 0.8) | 0.7 (0.7, 0.8) | 0.7 (0.7, 0.8) |
| Niacin (mg/d) | 12.6 (11.6, 13.8) | 12.5 (11.8, 13.3) | 12.8 (11.4, 14.2) | 12.8 (12, 13.7) | 12.4 (10.9, 13.9) |
| Vitamin B6 (mg/d) | 1.4 (1.2, 1.7) | 1.3 (1.1, 1.6) | 1.5 (1.3, 1.7) | 1.5 (1.3, 1.7) | 1.2 (1, 1.5) |
| Folate (µg/d) | 155 (139, 170) | 157 (142, 173) | 153 (137, 169) | 155 (142, 169) | 153 (133, 174) |
| Vitamin B12 (µg/d) | 0.8 (0.7, 1) | 0.9 (0.7, 1) | 0.8 (0.7, 1) | 0.9 (0.8, 1) | 0.8 (0.6, 1) |
| Vitamin C (mg/d) | 76 (68, 81) | 63 (53, 75) | 78 (74, 82) | 77 (71, 82) | 73 (61, 80) |

*Retinol Activity Equivalent

Table 3.3. Prevalence of nutrient intake inadequacy (%), by age and sex, among 4- to 8-year-old children (n=200) participating in the non-intervened arm of a biofortified maize efficacy trial, Mkushi, Zambia, 2012–2013

| Nutrient | Overall | 4–4.9y | 5–8y | Boys | Girls |
|-----------------|----------------|---------------|-------------|-------------|--------------|
| Calcium | >99.9 | >99.9 | >99.9 | >99.9 | >99.9 |
| Iron | 24.8 | 29.1 | 22 | 24.2 | 25.2 |
| Zinc | 1.8 | 2.8 | 1.2 | 0.3 | 3.2 |
| Vitamin A | 2.2 | 3.0 | 1.9 | 1.0 | 3.5 |
| Thiamin | 2.1 | 3.5 | 1.4 | 0.2 | 4.2 |
| Riboflavin | 1.4 | 1.2 | 1.3 | 0.3 | 2.1 |
| Niacin | 0.5 | <0.1 | 0.6 | <0.1 | 0.7 |
| Vitamin B6 | 0.3 | 0.7 | <0.1 | <0.1 | 0.9 |
| Folate | 57.3 | 53.5 | 59.5 | 57.1 | 57.5 |
| Vitamin B12 | 76.0 | 72.8 | 77.9 | 75.0 | 77.3 |
| Vitamin C | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |

Table 3.4. Prevalence of macronutrient intakes (%) above and below Acceptable Macronutrient Distribution Ranges*, by age and sex, among 4- to 8-year-old children (n=200) participating in the non-intervened arm of a biofortified maize efficacy trial, Mkushi, Zambia, 2012–2013

| | Carbohydrates | | Protein | | Fat | |
|----------|---------------|------------|------------|------------|------------|------------|
| | Below AMDR | Above AMDR | Below AMDR | Above AMDR | Below AMDR | Above AMDR |
| Overall | 0 | 26 | 2 | 0 | 23 | 1 |
| 4 – 4.9y | 0 | 17 | 4 | 0 | 23 | 2 |
| 5 – 8y | 0 | 28 | 1 | 0 | 22 | 0 |
| Boys | 0 | 22 | 1 | 0 | 21 | 1 |
| Girls | 0 | 29 | 3 | 0 | 24 | 1 |

*Acceptable Macronutrient Distribution Range (AMDR) reference values from the Institute of Medicine³⁵

Table 3.5. Number of servings per day and quantity consumed per serving of twenty-five most frequently consumed foods among 4- to 8-year-old children participating in the non-intervened arm of a biofortified maize efficacy trial (n=1071 observation days among 200 children), Mkushi, Zambia, 2012–2013

| Food | Number of servings per day (mean ± s.d.**) | Quantity consumed per serving (g raw weight) (mean ± s.d.) |
|-----------------------------------|---|---|
| Vegetable oil | 3.14 ± 1.33 | 8 ± 7.7 |
| Tomato | 3.1 ± 1.32 | 25 ± 13.7 |
| Maize | 2.66 ± 0.82 | 82 ± 33.1 |
| Onion | 1.95 ± 1.70 | 7 ± 4.4 |
| Small fish | 0.66 ± 0.76 | 29 ± 12.9 |
| Rape leaves | 0.61 ± 0.77 | 26 ± 13.8 |
| Sugar | 0.56 ± 0.65 | 21 ± 13.9 |
| Pumpkin leaves | 0.35 ± 0.62 | 31 ± 14.3 |
| Beans | 0.31 ± 0.61 | 38 ± 18.9 |
| Tea | 0.26 ± 0.47 | 12 ± 39.5 |
| Tilapia or bream fish | 0.22 ± 0.50 | 36 ± 25.8 |
| Mango | 0.22 ± 0.51 | 396 ± 207 |
| Bread | 0.18 ± 0.41 | 82 ± 41.1 |
| Other dark green leafy vegetables | 0.17 ± 0.47 | 27 ± 16.1 |
| Fritters, donuts or scones | 0.16 ± 0.38 | 106 ± 48.2 |
| Milk | 0.16 ± 0.41 | 34 ± 31.1 |
| Rice | 0.15 ± 0.37 | 69 ± 28.2 |
| Chicken | 0.13 ± 0.42 | 22 ± 11.6 |
| Eggs | 0.12 ± 0.36 | 58 ± 26.4 |
| Eggplant | 0.12 ± 0.37 | 32 ± 14.0 |
| Cabbage | 0.11 ± 0.39 | 23 ± 10.8 |
| Groundnuts | 0.11 ± 0.34 | 49 ± 47.7 |
| Insects | 0.10 ± 0.38 | 24 ± 22.7 |
| Other fruit | 0.08 ± 0.31 | 112 ± 82.3 |
| Cassava | 0.08 ± 0.34 | 82 ± 40.0 |

**s.d. Standard deviation

Table 3.6a. Foods contributing to intakes of energy, protein, fat and carbohydrates* among 4- to 8-year-old children (n=200) participating in the non-intervened arm of a biofortified maize efficacy trial, Mkushi, Zambia, 2012–2013

| Energy | | Protein | | Fat | | Carbohydrates | |
|--------------------|------------------------------|--------------------|------------------------------|---------------------|------------------------------|--------------------|------------------------------|
| Food | Mean percent of total intake | Food | Mean percent of total intake | Food | Mean percent of total intake | Food | Mean percent of total intake |
| Maize | 52 | Maize | 42 | Vegetable oil | 51 | Maize | 69 |
| Vegetable oil | 14 | Small fish | 21 | Maize | 21 | Mango | 5 |
| Small fish | 5 | Tilapia fish | 7 | Small fish | 8 | Sugar | 4 |
| Fritters or scones | 4 | Beans | 5 | Fritters or scones | 6 | Bread | 3 |
| Mango | 3 | Bread | 3 | Groundnuts | 3 | Fritters or scones | 3 |
| Sugar | 3 | Fritters or scones | 2 | Butter or margarine | 2 | Rice | 3 |
| Bread | 3 | Eggs | 2 | Bread | 1 | Beans | 2 |
| Rice | 2 | Groundnuts | 2 | Eggs | 1 | Tomato | 1 |
| Beans | 2 | Insects | 2 | | | Sweet potato | 1 |
| Groundnuts | 1 | Tomato | 2 | | | Cassava | 1 |
| Tilapia fish | 1 | Chicken | 2 | | | | |
| | | Rice | 2 | | | | |
| | | Rape leaves | 1 | | | | |
| | | Other large fish** | 1 | | | | |
| | | Mango | 1 | | | | |

*Only foods contributing, on average, at least 1% of total intakes are listed

** Any large fish other than tilapia species

Table 3.6b. Foods contributing to intakes of iron, calcium, and zinc* among 4- to 8-year-old children (n=200) participating in the non-intervened arm of a biofortified maize efficacy trial, Mkushi, Zambia, 2012–2013

| Iron | | Calcium | | Zinc | |
|--------------------------|------------------------------|--------------------------|------------------------------|--------------------|------------------------------|
| Food | Mean percent of total intake | Food | Mean percent of total intake | Food | Mean percent of total intake |
| Maize | 50 | Small fish | 29 | Maize | 60 |
| Small fish | 7 | Rape leaves | 10 | Small fish | 7 |
| Beans | 6 | Bread | 8 | Mango | 4 |
| Bread | 6 | Maize | 7 | Beans | 4 |
| Insects | 3 | Beans | 7 | Tomato | 3 |
| Rape leaves | 3 | Fritters or scones | 5 | Bread | 2 |
| Tomato | 3 | Tomato | 4 | Rice | 2 |
| Pumpkin leaves | 3 | Mango | 4 | Groundnuts | 2 |
| Fritters or scones | 2 | Other leafy vegetables** | 4 | Fritters or scones | 1 |
| Mango | 2 | Pumpkin leaves | 3 | Eggs | 1 |
| Other leafy vegetables** | 1 | Sweet potato | 2 | Tilapia fish | 1 |
| Eggs | 1 | Milk | 2 | Rape leaves | 1 |
| Tilapia fish | 1 | Eggs | 2 | | |
| Groundnuts | 1 | Onion | 2 | | |
| Other fruit† | 1 | Groundnuts | 1 | | |
| Rice | 1 | Tilapia fish | 1 | | |
| | | Other fruit† | 1 | | |

*Only foods contributing, on average, at least 1% of total intakes are listed

** Any leafy vegetables other than rape leaves, cabbage or pumpkin leaves

† Any fruit other than mango, banana or citrus fruit

Table 3.6c. Foods contributing to intakes of vitamin A, vitamin C, folate and vitamin B12* among 4- to 8-year-old children (n=200) participating in the non-intervened arm of a biofortified maize efficacy trial, Mkushi, Zambia, 2012–2013

| Vitamin A | | Vitamin C | | Folate | | Vitamin B12 | |
|-------------------------------|------------------------------|--------------------------|------------------------------|--------------------------|------------------------------|-------------------------------|------------------------------|
| Food | Mean percent of total intake | Food | Mean percent of total intake | Food | Mean percent of total intake | Food | Mean percent of total intake |
| Rape leaves | 21 | Tomato | 32 | Maize | 29 | Small fish | 41 |
| Sugar | 21 | Rape leaves | 24 | Beans | 11 | Tilapia fish | 15 |
| Small fish | 19 | Mango | 14 | Bread | 8 | Eggs | 7 |
| Tomato | 12 | Other leafy vegetables** | 6 | Tomato | 7 | Insects | 5 |
| Mango | 6 | Pumpkin leaves | 4 | Mango | 6 | Chicken | 4 |
| Pumpkin leaves | 5 | Onion | 3 | Fritters or scones | 4 | Milk | 3 |
| Other leafy vegetables** | 4 | Other fruit [†] | 3 | Small fish | 3 | Other large fish [§] | 3 |
| Eggs | 3 | Cabbage | 3 | Groundnuts | 3 | Other meat | 3 |
| Sweet potato | 2 | Cassava | 2 | Rape leaves | 3 | Beef | 2 |
| Butter or margarine | 2 | | | Other leafy vegetables** | 3 | Fritters or scones | 1 |
| Other fruit [†] | 2 | | | Tilapia fish | 3 | | |
| Other vegetables [‡] | 1 | | | Pumpkin leaves | 2 | | |
| | | | | Eggs | 2 | | |
| | | | | Other fruit [†] | 1 | | |
| | | | | Onion | 1 | | |
| | | | | Sweet potato | 1 | | |

*Only foods contributing, on average, at least 1% of total intakes are listed

**Any leafy vegetables other than rape leaves, cabbage or pumpkin leaves

[†]Any fruit other than mango, banana or citrus fruit

[‡]Any vegetables other than leafy vegetables, root vegetables, tomato, eggplant, mushrooms, or okra

[§]Any large fish other than tilapia species

^{||}Any meat other than beef, liver or offals

Table 3.6d. Foods contributing to intakes of thiamin, riboflavin, niacin and vitamin B6* among 4- to 8-year-old children (n=200) participating in the non-intervened arm of a biofortified maize efficacy trial, Mkushi, Zambia, 2012–2013

| Thiamin | | Riboflavin | | Niacin | | Vitamin B6 | |
|--------------------|------------------------------|--------------------------|------------------------------|--------------------|------------------------------|----------------|------------------------------|
| Food | Mean percent of total intake | Food | Mean percent of total intake | Food | Mean percent of total intake | Food | Mean percent of total intake |
| Maize | 55 | Maize | 42 | Maize | 44 | Maize | 56 |
| Bread | 8 | Small fish | 9 | Small fish | 16 | Mango | 6 |
| Mango | 4 | Bread | 6 | Bread | 5 | Small fish | 5 |
| Small fish | 4 | Mango | 5 | Tilapia fish | 5 | Tomato | 4 |
| Fritters or scones | 4 | Eggs | 4 | Tomato | 5 | Rape leaves | 4 |
| Beans | 3 | Fritters or scones | 4 | Mango | 3 | Tilapia fish | 2 |
| Tomato | 3 | Other leafy vegetables** | 3 | Groundnuts | 3 | Other fruit† | 2 |
| Rape leaves | 2 | Rape leaves | 3 | Fritters or scones | 2 | Pumpkin leaves | 2 |
| Groundnuts | 2 | Tomato | 2 | Chicken | 2 | Sweet potato | 2 |
| Pumpkin leaves | 1 | Beans | 2 | Rice | 2 | Beans | 2 |
| Sweet potato | 1 | Pumpkin leaves | 2 | Rape leaves | 2 | Bread | 2 |
| Biscuits | 1 | Tilapia fish | 2 | | | Rice | 2 |
| Rice | 1 | Mushrooms | 2 | | | Banana | 1 |
| | | Sweet potato | 1 | | | Onion | 1 |
| | | Milk | 1 | | | | |

*Only foods contributing, on average, at least 1% of total intakes are listed

**Any leafy vegetables other than rape leaves, cabbage or pumpkin leaves

†Any fruit other than mango, banana or citrus fruit

Chapter 4. Aim 2: Within-person, Between-person and Seasonal Components of Nutrient Intake Variance among 4- to 8-year-old Rural Zambian Children

Abstract

Precise estimates of usual nutrient intakes are required to assess risks of inadequacy or associations between intakes and health outcomes. Estimates of the components of nutrient intake variance can be used to determine the number of dietary assessment replicates needed to precisely estimate usual intakes or to correct total variation for the day-to-day component in studies without replicates. Season is a potential additional source of variation in nutrient intakes. We described seasonal variation in nutrient intakes, determinants of intakes and the major components of intake variance among 4- to 8-year-old children in rural Mkushi District, Zambia (n=200). Up to seven 24-hour dietary recalls were obtained from each participating child at monthly intervals over a six-month period covering the late post-harvest (August–October), early lean (November–January) and late lean (February–April) seasons. Nutrient intakes varied significantly by season. For energy and most nutrients, intakes were highest in the early lean season and lower in the late post-harvest and late lean seasons. Season and recall on a market day had the strongest effects on nutrient intakes among covariates examined. Unadjusted within- to between-person variance ratios ranged from 4.5 to 31.3. In components of variance models, season accounted for 3% to 20% of nutrient intake variance. Particularly in rural settings in low- and middle-income countries, where availability of locally-grown, nutrient-rich foods may vary seasonally, studies should include replicates across seasons to more precisely estimate long-term usual intakes.

Introduction

Precise estimates of dietary intakes are required to identify risks of inadequate nutrient intakes and to study associations between diet and health. Typically, the dietary exposure of interest in epidemiological studies is usual, or long-term average, intake of foods or nutrients. However, because an individual's nutrient intakes vary from day to day, intake on a single day, as measured by 24-hour recall or food record, will typically not reflect usual intake.¹⁻³ Observed nutrient intakes should be adjusted to account for within-person variation to reduce bias in estimates of the prevalence of inadequate intakes, misclassification of individuals by nutrient intake, loss of power to detect differences in group mean intakes and attenuation of measures of association between nutrient intakes and other variables of interest.¹⁻⁴ To minimize these problems, estimates of within- and between-person variance derived from repeated observations can be used to calculate the number of dietary intake replicates needed to achieve a chosen degree of precision, to adjust nutrient intake distributions to account for variance inflation, or to conduct sensitivity analyses to test the degree of bias in prevalence estimates or attenuation in measures of association.^{1,2,4-8} Studies with a single assessment of dietary intakes may use previously published values for intake variance in a similar population to explore the effect of reduced variability on reducing bias in estimated prevalence of inadequacy or to conduct sensitivity analyses on such estimates.^{7,9}

To be useful for such purposes, estimates of components of nutrient intake variance must be representative of the population under study. Within-person variability and the ratio of within- to between-person variance have been shown to differ by age, sex, body composition, country, and season and to change over time.^{1,4,5,7,9-18} Such differences highlight the importance of deriving within-study-population estimates of these components of variance. Recent studies have documented within- and between-person nutrient intake variance among children and

adolescents in high- and middle-income countries, but few estimates are available for preschool- or primary school-age children in sub-Saharan Africa.^{5,10,11,14,19} Older studies from sub-Saharan Africa were conducted among pregnant women, or among boys of primary school age.^{16,17} Further, evidence on the seasonal component of nutrient intake variance is needed to better describe usual nutrient intakes and plan future studies among children in sub-Saharan Africa. It is currently not known how precisely nutrient intakes assessed at one point during the year reflect usual intakes year-round. Particularly in rural sub-Saharan Africa, where diet is heavily determined by local food production, dietary assessments over multiple seasons should help reduce measurement error and resultant bias in estimates of nutrient intakes and prevalence of inadequacy. Seasonal variations in food consumption patterns have been documented among 3- to 6-year-old children in Ghana and Malawi and among 6- to 8-year-old children in rural Benin.^{20,21} A seasonal effect on nutrient intakes observed among pregnant Malawian women, in addition to evidence from high-income countries, demonstrates the necessity for study of this component of nutrient intake variance and how it may impact estimates of usual intakes.^{12,17,18}

In this paper, we examine the sources of nutrient intake variance among 4- to 8-year-old children in rural Zambia, with particular focus on the role of seasonality.

Methods

Study context

The data used in these analyses were collected within an efficacy trial of pro-vitamin A biofortified maize conducted in Mkushi district, Central Province, Zambia, in 2012–2013. The efficacy trial is described in detail by Palmer et al.²² The study area included towns and villages in this rural, agricultural district that were accessible by road year-round. We conducted a

mapping and census of every household in the study area to identify those with a child between 4 and 8 years old. Children between 4 and 8 years old, not yet enrolled in school and living within ~500km of a local site established by the study for meal provision were eligible to participate in the study. Oral informed consent was obtained from respondents to the mapping and census and from the primary caregiver of each child enrolled in the study.

The efficacy trial enrolled children into 64 geographic clusters. Clusters were assigned by block randomization to one of three groups: a treatment group receiving meals containing biofortified maize (n=543 children in 25 clusters), a control group receiving meals containing traditional maize (n=481 children in 25 clusters), and a non-intervened control group (n=202 in 14 clusters). Children in the treatment and control groups received breakfast and lunch six days per week for six months. Children in the non-intervened group participated in data collection activities over the same six-month trial period and received a package of food at the conclusion of the trial rather than regular meals. The purpose of the non-intervened group was to provide comparison data on dietary intakes and food security in the study population, absent the effects of a feeding trial. Only data from the non-intervened group are used in this analysis.

Data collection

Data were collected through surveys at the efficacy trial baseline (August–September 2012), at five monthly monitoring visits over the course of the six-month intervention, and at follow-up (March–April 2013). Birthdates of participating children and household descriptors, including a roster of all household members, education and employment of the head of household, and asset ownership, were collected at baseline. Twenty-four-hour dietary recalls were conducted in all survey rounds, for a total of up to seven recalls per child.

Dietary intake data were collected by tablet-based 24-hour recall, as previously described by Caswell et al (Appendix 1).²³ The primary caregiver of each enrolled child was asked to describe all foods and beverages the child consumed between waking the previous morning and waking the morning of the interview. Prompts in the 24-hour recall tablet program instructed the interviewer to move chronologically through the day, starting with asking the caregiver to recall all details of foods or beverages the child consumed shortly after waking the previous morning. The respondent was asked to recall the type of food, the ingredients used in mixed dishes, the portion consumed and where the child obtained the food. Photo booklets showing ranges of portion sizes for each of thirteen commonly consumed foods were used for portion estimation of most foods. For some foods, such as fruit or bread, the number of pieces consumed was recorded. After discussing the foods consumed during each time of the day, the interviewer reviewed a picture chart with the respondent to identify any mixed foods. Picture charts were provided to respondents two days before the recall interview, and respondents were asked to place tick marks by the foods the child consumed during the day before the interview. Finally, the interviewer did a final pass of probes, asking the mother to review the child's day one more time to recall whether there were any more foods that had been consumed.

Observed nutrient intakes were calculated from each 24-hour recall by multiplying all ingredient or unmixed food weights by their nutrient contents and summing each nutrient over all foods consumed. Portion sizes of each food were converted to grams based on the gram weight of the portion shown in the selected photo, with adjustment for relative density if the photographed food differed from the recalled food. Mixed foods were separated into ingredients using a database of standard recipes provided by HarvestPlus (unpublished) from their previous survey of dietary intakes among women and preschoolers in the same study area.²⁴ The total portion size was multiplied by the fraction of each ingredient in the recipe to

obtain weights of each ingredient. Weights of ingredients and of individual (unmixed) foods were multiplied by their nutrient contents as listed in a local food composition table compiled primarily from two Zambian food composition tables, one by HarvestPlus (unpublished) and one published by the National Food and Nutrition Commission of Zambia.²⁵ As needed, we also incorporated food composition data from the U.S. Department of Agriculture's National Nutrient Database for Standard Reference, the Food and Agriculture Organization's INFOODS Food Composition Tables for Biodiversity 2.0 and additional regional and global food composition tables.^{26–30}

Data analysis

All data calculation and analysis was conducted in SAS 9.4 (Cary, NC). Descriptive statistics were used to assess the distributions of household and child characteristics. Observed nutrient intakes were Box-Cox transformed prior to regression-based analyses to improve model fit and adherence to assumption of a normal distribution of residuals. Results presented as means, confidence limits or effect sizes were back-transformed to the original scale of nutrient measurement. In all analyses we defined statistical significance as $p < 0.05$.

We modeled nutrient intakes by season, dividing the study period into three seasons of approximately equal length: late post-harvest season (August through October), early lean or planting season (November through January) and late lean season (February through April). Seasonal means were estimated and tested for significant differences using a longitudinal model with season as the fixed effect and an unstructured covariance model to control for correlation among repeat measures.

We developed a full model to identify recall-, child-, household- and cluster-level factors that were significantly related to observed nutrient intakes. We considered season, where recall fell

in the sequence of repeated recalls, day of the week, interviewer and whether the recall period fell on a market day as potential recall-level factors; age and sex as potential child-level factors; head of household literacy and education, housing score and asset score as potential household-level factors; and urban versus rural residence as a potential cluster-level factor. Potential factors were chosen based on a review of significant determinants of nutrient intakes or contributors to intake variance in previous papers.^{1,9-12,15,16,18,31} Null models with random terms for child, household and cluster were first fit. Because models would not converge without error with multiple random intercepts, we proceeded with a null model including a child-level random intercept only. Fixed effects were then added one level at a time, starting with recall-level factors and concluding with cluster-level factors. At each level, all potential covariates were tested and the covariates showing no or very few significant associations with intake over energy and all 14 nutrients were dropped. When there was evidence of collinearity between two variables, as determined by correlation analysis and impact on regression coefficients, the variable with stronger associations among most nutrients was retained and the other variable dropped. Following the standard established by Beaton et al., we examined nutrient intake data for interviewer effects.^{1,15} We determined that three among the team of fifteen interviewers appeared to be under-reporters. As has been done by previous authors, we therefore controlled for interviewer in all analyses.¹² We also tested the full models of nutrient intakes excluding records collected by the under-reporting interviewers. Because this did not substantially change model results, we did not exclude their records from further analyses. The final model for intake of each nutrient includes a child-level random intercept and fixed effects for interviewer, market day, season, asset score and rural residence.

To describe effect sizes, we estimated the intercept as a reference mean intake during the late lean season, on a non-market day, among children with mean age, median asset score and rural

residence, interviewed by the reference interviewer. We then estimated a comparison value for late post-harvest season, early lean season, market day, age, asset score and urban residence by varying each fixed effect by one unit, holding all other fixed effects at the reference value. We back-transformed the reference mean and comparison values to original scale and expressed the difference between each comparison value and the reference mean as the change in nutrient intake associated with a one unit change in the fixed effect.

To estimate components of energy and nutrient intake variance, we fit two sets of unadjusted and adjusted models: one set with a child-level random intercept and a second set with a child-level random intercept and random slope for seasonal effect. The first set of adjusted models included fixed effects for season, market day and interviewer, and the second set contained fixed effects for market day and interviewer. To determine whether variance components differ by age, sex or season, we also fit the unadjusted and adjusted models with child-level random intercepts separately by age, sex and season subgroups. The adjusted models by season included fixed effects for market day and interviewer only. The variance components models were fit with Box-Cox transformed data. Running the same models with untransformed data generally produced slightly higher estimates of within- to between-person variance, as has been reported by previous authors.⁹ Here, we report results from the models using transformed data for consistency between analyses and because model assumptions were better met with transformed data.

Results

Individual recalls for which the child was reported ill are excluded from analyses (n=208), for a final sample size of 1071 observations among 200 children. Therefore results reflect nutrient intakes and nutrient intake variance among apparently healthy children. The mean age was 5.5

± 1.2 years at baseline and 55% were male. Heads of household had completed 8.1 years of formal schooling, on average, and most could read and write. The mean asset score was 2.5 out of 15 items included in the survey module on ownership of durable goods. The mean housing quality scoring, summing responses to questions on housing building materials, home size and sources of energy for light and cooking, was 22.4 out of an observed range of 14 to 40. Half of included households were located in regional towns and half were located in rural villages.

Nutrient intakes by season

Intakes of energy and all 14 nutrients examined showed seasonal differences, though the magnitude of difference varied (**Table 4.2**). Mean energy intake was over 100 kcal/d higher in early lean season than it was in the post-harvest or late lean seasons. This pattern was driven primarily by changes in carbohydrate intakes; though protein and fat intakes followed the same trend, the magnitude of difference between seasons was small. A similar rise during the early lean season of intakes of B vitamins was observed. For three nutrients, iron, zinc and vitamin C, intakes peaked in early lean season and dropped their lowest levels in late lean season. Vitamin B12 and calcium intakes rose non-significantly between late post-harvest and early lean seasons and fell in late lean season. A notable exception to this general trend of rising in early lean season and falling in late lean season was intake of vitamin A, which decreased over the three seasons, though the difference in intakes in post-harvest and early lean seasons was marginally significant ($p=0.054$). Though some seasonal differences in mean intake remained significant when adjusting for total energy intake, the trend weakened substantially (**Supplemental Table 4.1**). Vitamin A remained the note-worthy exception, retaining a clear downward trend over the three seasons, and vitamin C retained a strong peak in intakes in the early lean season.

Determinants of nutrient intakes

The final model of factors associated with nutrient intakes included season, whether the recall period was a market day, child age, household asset score and urban versus rural residence (**Table 4.3**). The seasonal effect on nutrient intakes shown in Table 4.2 remained significant for energy and 13 out of 14 nutrients when included in the full, adjusted models. For fat intakes, the seasonal effect was no longer significant when controlling for other variables related to intakes. Intakes of energy, protein, carbohydrates, zinc, thiamin, vitamin B6 and vitamin C were significantly lower on market days than on non-market days, with energy intakes reduced by 113 kcal on market days. Increasing age was positively associated with higher intakes of four micronutrients, though the effect size was small. Energy, fat and four micronutrient intakes were positively associated with household asset ownership. Energy intake increased by 34 kcal/d with each additional asset owned by the household, and fat intake increase by 2 g/d. Intakes of fat and vitamin B12 were higher among children living in towns than among children living in rural villages. In contrast, children in rural villages had higher intakes of iron, zinc, vitamin A, thiamin, riboflavin and vitamin B6.

Components of variance in nutrient intakes

Within- and between-person coefficients of variation (CVs) and the within- to between-person variance ratios based on unadjusted and adjusted models of nutrient intakes are presented in **Table 4.4**. These estimates of the components of variance enable comparison of the amount of variance attributable to day-to-day variation in individuals' diets to the amount attributable to differences between individuals, and they enable estimation of numbers of required replicates or correction for bias in analyses based on observed intakes. Unadjusted variance ratios ranged from 4.5 for vitamin B6 to 31.3 for vitamin C. Adjusting for season, interviewer and market day

resulted in a slightly lower estimate of the variance ratio in most cases. The variance ratios for vitamin A and vitamin B6 increased slightly, and the variance ratio for vitamin C decreased substantially. For the majority of nutrients, the within-person CV was lower and the between-person CV was slightly higher when estimated by the adjusted model compared to the unadjusted model. The number of observation days required to achieve a correlation of $r=0.8$ between observed and usual nutrient intakes ranged from 9 for vitamin B6 to 56 for vitamin C in the unadjusted model, or 9 to 39 in the adjusted model. With three observation days, an estimated correlation of 0.3 to 0.63 between observed and usual intakes would be observed. Though CVs and variance ratios differed between children under five years old ($n=405$ observations) and children aged five years and older ($n=666$ observations), no strong trend for direction of difference was observed in either the unadjusted or adjusted models (**Supplemental Table 4.2**). In both adjusted and unadjusted models, variance ratios were much higher among boys ($n=593$ observations) than among girls ($n=478$ observations), primarily related to higher between-person CVs among girls (**Supplemental Table 4.3**). Variance ratio, within-person CVs and between-person CVs varied by season (late post-harvest season: $n=263$ observations; early lean season: $n=506$ observations; late lean season: $n=302$ observations), but without strong trends in direction of difference (**Supplemental Tables 4.4a & 4.4b**).

The contribution of a seasonal effect to total variance in energy and nutrient intakes is described in **Table 4.5**. In unadjusted models, the percent of variance attributed to season ranged from 1.1% for vitamin A to 20.2% for riboflavin. In most cases, adjustment for interviewer and market day resulted in a slightly increased estimate of the percent of variance attributable to season, so that variance attributed to season ranges from 2.9% for fat to 23.0% for riboflavin. With a random effect for season in the model, estimates of between-person variance as a percent of total variance were low, ranging from 0% to 15.2%. Further, the model including a seasonal

random effect shown in Table 4.5 produced much higher estimates of the within- to between-person variance ratio than were seen in Table 4.4.

Discussion

We used seven repeat 24-hour dietary recalls, collected over six months among 200 apparently healthy, rural Zambian children to describe seasonal changes in energy and nutrient intakes, to identify factors associated with those intakes, to estimate within- and between-person components of intake variance and to test whether components of intake variance differed by age group, sex or season.

We found significant changes in intakes by season, with highest intakes for energy and most nutrients occurring in the early lean season (November – January). This relationship remained highly significant in analyses controlling for market day, interviewer, child age, household asset ownership and urban residence. The relationship also held for several nutrients when controlling for energy intake, indicating that the seasonal shift in intakes is not just in food quantity, but also in nutrient composition of the diet. Seasonal differences in food and nutrient intakes have been observed among children in other sub-Saharan African countries. Among 6- to 8-year-old children in rural Benin, food consumption patterns differed significantly between the post-harvest and pre-harvest seasons, and intakes of vitamin C and fat were found to be higher in the post-harvest season. Other nutrient intakes were not shown to differ significantly by season, though this may be due to the study's small sample size and choice of micronutrients examined.²¹ A study of 6- to 17-year-old Kenyan school children that examined nutrient intakes in the food shortage and harvest seasons found that intakes of energy, calcium, vitamin A, folate and vitamin C were significantly lower in the food shortage season.³² Seasonal differences in

foods consumed were also observed among 3- to 6-year-old children in rural Ghana and Malawi.²⁰

Several factors, including agricultural work, holidays and local food availability, may contribute to the seasonal difference in energy and nutrient intakes among children in our study area. The early lean season, in which intakes of most nutrients were highest, corresponds to the maize planting season. This is traditionally thought of as the early lean season, but is also a period of increased agricultural labor. Changes in family activities and cooking patterns in response to agricultural labor demands may contribute to the observed changes in intake. The Food Reserve Agency of Zambia buys maize in the harvest season and re-releases it onto the market throughout the year to stabilize prices.^{33,34} This may mitigate household grain shortages that could occur during the planting season, enabling households to increase food intakes among all members, including children who may not be directly involved in agricultural labor. The Christmas holiday also falls during this season, and though our data did not show an effect of feast days on dietary intakes or a sharp increase in intakes in the days surrounding the holiday (data not shown), intakes of more or richer foods on occasions throughout the holiday season in this predominantly Christian population may provide an additional explanation for the observed trend.

Local food availability was considered one of the drivers of seasonal food consumption changes among Ghanaian and Malawian children, and this may be an important factor in seasonal nutrient intakes of Zambia children as well.²⁰ The early lean season corresponds with mango season, and other seasonally available horticultural, wild or semi-cultivated crops may be contributing to nutrient intakes, as well. This hypothesis is supported by the substantial increase in vitamin C intakes in early lean season. Seasonal availability of local vegetable crops

may also explain the decreasing trend in vitamin A intakes over the study period, particularly as nearly all vitamin A is obtained from plant sources in this population (Chapter 3).

Our findings of significant seasonal differences in nutrient intakes among Zambian children imply that collecting dietary data at only one time point in populations with seasonal changes in food availability or consumption patterns may result in misrepresentation of longer term usual intakes. Had we measured nutrient intake only in the late post-harvest season, we would have estimated mean energy intakes at over 100 kcal/d lower than if we had measured only in the early lean season. The size and importance of this seasonal difference varies by nutrient. Seasonal differences in some nutrients, such as thiamin, riboflavin or vitamin B6, were significant but modest. In contrast, vitamins A and C show strong seasonal trends. Both of these nutrients are obtained primarily from locally grown plant sources (Chapter 3). In such cases, local agriculture has an important, seasonal impact on nutrient intakes that may be missed if dietary data collection does not cover multiple seasons in order to capture different periods of local food availability.

Compared to other studies that have examined seasonal variance in nutrient intakes, we found a high percentage of total variance attributable to season. Among children in Mkushi, the seasonal effect contributed 2.9%–23% of total intake variances in adjusted models of variance components. In contrast, season contributed less than 2% of total intake variances among overweight and obese US adults and less than 3% of total intake variances among Japanese women.^{12,18} This result reinforces the importance of accounting for season in studies of nutrient intake, particularly among children in low- and middle-income countries.

In addition to a seasonal effect, we examined child age, market day, household socio-economic status and urban residence as predictors of energy and nutrient intakes. Though nutrient

intakes are expected to increase with age and prior studies on nutrient intake variance have identified differences in mean intakes by age, the age range in our study may be too narrow to discern an age effect on intakes of most nutrients.^{4,10} Several studies have estimated the amount of intake variance attributable to day of the week and found mixed results among different populations.^{1,12,15,16,18} Other studies have controlled for day of the week using a fixed effect in components of variance models.^{10,11} We did not find a day of the week effect among young Zambian children, but did find a strong and consistent relationship between market days and lower energy and nutrient intakes.^{15,16} Caregivers may be away from children or the home more on market day, which may change household cooking and eating patterns, or caregivers may observe and recall less of what children eat. In a study of Kenyan school-age children, Gewa et al. found that mothers underestimated energy and nutrient intakes in 24-hour recalls of children's food consumption because a large proportion of foods consumed out of the home were missed.³² Though children participating in our study were younger than those in the Kenyan survey and not yet enrolled in school, mothers may similarly not observe food intakes on market days when they are away from the home.

In this population, household socio-economic status showed little relationship to child nutrient intakes. This could be due to low variability in socio-economic status in the study population. Two Mexican national surveys of preschool- and school-age children that identified a clear, inverse relationship between socio-economic status and nutrient intakes or risk of inadequacy presumably captured much more variation in socio-economic status.^{35,36} Limited local availability of more nutritious foods or exchange of inexpensive foods for preferred but nutritionally similar foods with increasing purchasing power may also help explain the weak relationship between socio-economic status and energy and nutrient intakes. Children in urban areas have previously been reported to have higher energy and nutrient intakes than children in

rural areas, but our results were mixed.^{35,36} Though children living in towns in the study area had higher fat and vitamin B12 intakes and non-significantly higher caloric intakes than children living in rural villages, they had lower intakes of several micronutrients, particularly vitamin A. As with socio-economic status, living in an urban area may be associated with food substitutions that do not increase micronutrient intakes.

In unadjusted and adjusted analyses, and in analyses by age and sex subgroups, we found that our ranges of within- to between-person variance ratios were higher than those reported in several previous papers on nutrient intake variance among children, though they were similar to those reported in two older studies conducted in the US and the UK.^{4,9-11,17,31,37} In adjusted components of variance models, the within- to between-person variance ratio ranged from 4.7 for vitamin B6 to 21.9 for vitamin C. The variance ratio for energy intake was 5.4. In contrast, a recent study by Ollberding et al. describing components of variance in nutrient intakes among US 6- to 11-year-olds, found that the variance ratio ranged from 2.3 to 6.7 for boys and 2.6 to 4.1 for girls, and was 2.8 for energy intake in both sexes.¹¹ Among Belgian 4- to 6.5-year-olds, Huybrechts et al. reported variance ratios ranging from 1.0 to 2.9.¹⁰ Earlier studies of nutrient intake variance among children have reported similarly low within- to between-person variance ratios.^{4,9,37}

Potential explanations for these contrasting results arise in examination of the within- and between-person CVs. Compared to other studies conducted among children or in southern Africa that report values for within-person CVs, our results are similar.^{9,10,16,17} Our range of within-person CVs in adjusted models was 17.6% - 37.4%, excluding a high value of 66.2% for vitamin B12. The range of within-person CVs for 4- to 6.5-year-old Belgian girls was 2.6%–36.0% or, for boys, 18.1%–34%.¹⁰ Our within-person CVs are also similar in range to those reported for 8- to 9-year-old boys in Ghana, the Philippines, Finland, the Netherlands and Italy,

and 9- to 17-year-olds in the US and Russia.^{9,16} However, our range of within-person CVs is much lower than that reported for pregnant Malawian women.¹⁷ In contrast, our values for between-person CVs are generally lower than those reported for children in other regions or for Malawian women.^{9-11,17} While the between-person CVs estimated from our adjusted models of nutrient intakes range from 5.0%–21.5%, those reported for Belgian 4- to 6.5-year-olds range from 11.1% to 192.1% and those for US and Russian 9- to 17-year-olds from 21.5% to 52.0%.^{9,10} Between-person CVs among pregnant Malawian women ranged from 18% to 81%.¹⁷

The high within- to between-person variance ratios we observed among 4- to 8-year-old Zambian children appear to be driven by low between-person variance rather than by high within-person variance. One explanation for this result is that children in this population have quite similar diets, compared to the variety found in other populations, particularly high-income countries. This is a population with a monotonous diet, with meals typically consisting of stiff maize porridge accompanied by a small range of vegetable, fish or meat dishes. In the full models of nutrient intakes, we found that socio-economic status and urban residence had only minor associations with nutrient intakes. This is likely due to homogeneity in both nutrient intakes and asset scores in the study population drawn from a single, rural district that does not have a wide range of household socio-economic status, dietary patterns or food access.

A second possible explanation for the low between-person variance is the tablet-based 24-hour recall tool, used for the first time in this study.²³ The tablet-based tool used photos for portion size estimation rather than food models and scales, as the latter could not be transported to participants' homes by field interviewers travelling on motorbikes. Though portion size estimates were adjusted for food density, constraining participants to indicate one of five depicted portion sizes rather than indicating portion by food models may have contributed to the low between-person variance. Authors of two studies conducted in sub-Saharan Africa

concluded that estimation of portion size using photographs performed acceptably for estimation of food intakes at the group level, but they noted that the method is subject to estimation error.^{38,39} Among teenage Mozambiquan girls, Korkalo et al. found that portion sizes of foods similar to those consumed in Mkushi were, on average, underestimated by 5% when portion size photos were used for estimating the amount of foods consumed in a prior meal.³⁸ Huybregts et al. found that when women in rural Burkina Faso used photos as a reference to estimate quantities of foods consumed the previous day, the average estimation error by type of food ranged from -34 g for couscous to 12 g for liquid sauce.³⁹

Within- to between-person variance ratios were found to vary by season. Though the impact of season as a fixed effect on nutrient intakes or as a variance component was consistent across nutrients, we did not observe a consistent difference in within- to between-person ratios by seasonal subgroup. Among pregnant Malawian women, within- to between-person variance ratios tended to be highest in the post-harvest season compared to the harvest and pre-harvest seasons, though the pattern was not strong.¹⁷ The seasonal changes without pattern observed in this study may be spurious shifts in within- and between-person variance, or may be a limitation of analytic sample size when dividing the full sample into three seasonal subgroups. More important are the very consistent changes in mean intakes by season and the contribution of season to total intake variance over six months.

With the advent of statistical methods and programs for estimation of usual energy and nutrient intake distributions, such as those from the National Cancer Institute, Iowa State University or the European Food Consumption Validation Consortium, published estimates of within- and between-person components of nutrient intake variance take on purposes beyond calculating number of dietary replicates needed or adjusting measures of association as laid out in early papers by Beaton et al. and Liu et al.^{1,2,8,15,40,41} As has been demonstrated and discussed by

Jahns et al. and Morimoto et al., external estimates of variance components may be used to adjust distributions of nutrient intakes in surveys with only one intake measure, or to conduct sensitivity analyses on the effect of different variance component estimates on the usual nutrient intake distribution.^{6,7} However, without variance component estimates and study designs that account for a seasonal component of variance, these applications may introduce inaccuracy in the estimation of long-term usual nutrient intakes. In rural Zambia, mean energy and nutrient intakes varied systematically by season and the season effect contributed as much as 23% of total intake variance. Even studies with long data collection periods that capture seasonal variation as part of within-person variance may miss a systematic seasonal effect on mean intakes or misattribute seasonal variance to another component of variance if this is not specifically incorporated into analyses. Ollberding et al. and Stote et al. provide examples of studies controlling for a potential seasonal effect on mean intakes.^{11,12} Our study indicates a need for dietary data collection across multiple seasons to accurately estimate usual intakes, particularly in populations where diet is influenced by seasonal harvests of local horticultural and wild or semi-cultivated crops.

Conclusion

Energy and nutrient intakes changed significantly by season among apparently healthy, 4- to 8-year-old children in rural Zambia, and this seasonal effect contributed substantially to total nutrient intake variance. These findings indicate a need for repeat dietary intake measures across multiple seasons to precisely estimate usual nutrient intakes in settings like rural Zambia where diets shift according to seasonal availability of local crops. Between-person variance in nutrient intakes was low. Most critically, this evidence of seasonal effects on mean energy and

nutrient intakes and intake variances should be used to plan future studies that adequately account for likely seasonal effects in similar populations.

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Tables

Table 4.1. Child and household characteristics of participants in the non-intervened arm of a pro-vitamin A maize efficacy trial in Mkushi, Zambia, 2012–2013

| | Mean \pm standard deviation or N (percent) |
|---|--|
| Child characteristics | |
| Age (years) | 5.5 \pm 1.2 |
| Age under 5 years | 74 (37%) |
| Male | 110 (55%) |
| Household characteristics | |
| Head of household can read and write | 127 (85%) |
| Years of schooling completed by head of household | 8.1 \pm 2.7 |
| Asset score | 2.5 \pm 1.5 |
| Housing score | 22.4 \pm 4.6 |
| Rural residence | 79 (50%) |

Table 4.2. Nutrient intakes by season among 4- to 8-year-old children (n=200) participating in the non-intervened arm of a biofortified maize efficacy trial, Mkushi, Zambia, 2012–2013

| Nutrient | Nutrient intake by season [mean (95% confidence interval)] | | |
|-----------------------|--|--------------------------------|--------------------------------|
| | Late post-harvest season (Aug–Oct) | Early lean season (Nov–Jan) | Late lean season (Feb–Apr) |
| Energy (kcal/d) | 1441 (1373, 1512) ^a | 1578 (1528, 1629) ^b | 1457 (1406, 1508) ^a |
| Protein (g/d) | 44 (42, 47) ^a | 46 (44, 48) ^a | 43 (41, 45) ^b |
| Fat (g/d) | 42 (39, 45) ^a | 45 (43, 48) ^b | 44 (41, 46) ^{ab} |
| Carbohydrates (g/d) | 224 (213, 234) ^a | 250 (242, 259) ^b | 226 (218, 233) ^a |
| Calcium (mg/d) | 241 (222, 260) ^a | 249 (234, 265) ^a | 197 (183, 211) ^b |
| Iron (mg/d) | 9.2 (8.7, 9.7) ^a | 9.9 (9.5, 10.4) ^b | 8.5 (8.1, 8.9) ^c |
| Zinc (mg/d) | 5.9 (5.6, 6.2) ^a | 6.3 (6.0, 6.6) ^b | 5.3 (5.1, 5.6) ^c |
| Vitamin A (µg RAE*/d) | 481 (437, 528) ^a | 430 (401, 460) ^a | 318 (289, 348) ^b |
| Thiamin (mg/d) | 0.74 (0.69, 0.78) ^a | 0.82 (0.78, 0.87) ^b | 0.74 (0.7, 0.78) ^a |
| Riboflavin (mg/d) | 0.61 (0.58, 0.65) ^a | 0.71 (0.68, 0.74) ^b | 0.6 (0.57, 0.64) ^a |
| Niacin (mg/d) | 10.5 (9.7, 11.4) ^a | 11.7 (11.0, 12.4) ^b | 10.4 (9.7, 11.1) ^a |
| Vitamin B6 (mg/d) | 1.2 (1.1, 1.3) ^a | 1.3 (1.2, 1.4) ^b | 1.1 (1.1, 1.2) ^a |
| Folate (µg/d) | 119 (110, 129) ^a | 142 (135, 150) ^b | 128 (120, 136) ^a |
| Vitamin B12 (µg/d) | 0.6 (0.53, 0.67) ^a | 0.61 (0.56, 0.67) ^a | 0.47 (0.41, 0.53) ^b |
| Vitamin C (mg/d) | 33 (30, 36) ^a | 52 (46, 58) ^b | 27 (24, 30) ^c |

*RAE: Retinol Activity Equivalent

^{a,b,c} difference in letters indicates significant difference between seasonal means (p<0.05)

Table 4.3. Linear regression model results as change in mean energy or nutrient intake with one unit change in fixed effect from reference mean*, among 4- to 8-year-old children (n=200) participating in the non-intervened arm of a biofortified maize efficacy trial, Mkushi, Zambia, 2012–2013

| Nutrient | Change from reference mean with one unit change in fixed effect | | | | | | |
|-----------------------|---|---------------------|------------------|-------------------|------------|-------------|-------------|
| | Reference mean | Late harvest season | post-lean season | Early lean season | Market day | Age (years) | Asset score |
| Energy (kcal/d) | 1477 | -20 | 115** | -113*** | 12 | 34** | 46 |
| Protein (g/d) | 44 | 3*** | 4** | -5** | 0 | 1 | 1 |
| Fat (g/d) | 44 | -1 | 3 | -3 | 0 | 2** | 6** |
| Carbohydrates (g/d) | 229 | -6 | 21** | -19** | 4 | 2 | -4 |
| Calcium (mg/d) | 205 | 53** | 55** | -22 | -1 | 5 | -18 |
| Iron (mg/d) | 9.4 | 0.8*** | 1.5** | -0.8 | 0.3*** | 0 | -0.6*** |
| Zinc (mg/d) | 5.9 | 0.7** | 1.1** | -0.7** | 0.2*** | 0 | -0.5*** |
| Vitamin A (µg RAE†/d) | 356 | 180** | 111** | -53 | 4 | 1 | -60** |
| Thiamin (mg/d) | 0.82 | 0.06*** | 0.11** | -0.08*** | 0.03*** | 0.01 | -0.09** |
| Riboflavin (mg/d) | 0.66 | 0.05 | 0.13** | -0.05 | 0.02 | 0.02** | -0.05*** |
| Niacin (mg/d) | 11 | 1.2*** | 1.9** | -1.3 | 0 | 0.4** | -0.4 |
| Vitamin B6 (mg/d) | 1.29 | 0.2** | 0.3** | -0.2** | 0.1** | 0 | -0.2** |
| Folate (µg/d) | 129 | -4 | 18** | -10 | 1 | 4** | 6 |
| Vitamin B12 (µg/d) | 0.46 | 0.15** | 0.16** | -0.07 | -0.02 | 0.03** | 0.12** |
| Vitamin C (mg/d) | 30 | 8*** | 26** | -6*** | 1 | 0 | -4 |

*Reference mean is the mean intake in the late lean season on non-market days, among children with mean age and median asset score living in rural village. Model additionally controls for interviewer.

**Significantly different from reference mean at $p < 0.01$

*** Significantly different from reference mean at $p < 0.05$

†RAE: Retinol Activity Equivalents

Table 4.4. Within- and between-person coefficients of variation in energy and nutrient intakes, variance ratios, number of observations (D) required to achieve a correlation of $r=0.8^*$ and r value achieved with three observations, among 4- to 8-year-old participants in the non-intervened arm of a pro-vitamin A maize efficacy trial in Mkushi, Zambia, 2012–2013

| Nutrient | Unadjusted | | | | | Adjusted** | | | | |
|-----------------------|---------------------|---------------------|-------------------------|-----------|-----------|---------------------|---------------------|-------------------------|-----------|-----------|
| | CV _w (%) | CV _b (%) | σ_w^2/σ_b^2 | D (r=0.8) | r (3-day) | CV _w (%) | CV _b (%) | σ_w^2/σ_b^2 | D (r=0.8) | r (3-day) |
| Energy (kcal/d) | 19.3 | 7.5 | 6.6 | 12 | 0.56 | 18.0 | 7.7 | 5.4 | 10 | 0.60 |
| Protein (g/d) | 21.9 | 7.2 | 9.3 | 17 | 0.49 | 21.3 | 7.4 | 8.3 | 15 | 0.52 |
| Fat (g/d) | 20.3 | 8.8 | 5.3 | 10 | 0.60 | 19.2 | 8.8 | 4.7 | 9 | 0.62 |
| Carbohydrates (g/d) | 20.3 | 6.6 | 9.3 | 17 | 0.49 | 19.0 | 7.0 | 7.4 | 14 | 0.54 |
| Calcium (mg/d) | 28.4 | 7.2 | 15.6 | 28 | 0.40 | 30.0 | 8.3 | 13.1 | 24 | 0.43 |
| Iron (mg/d) | 19.7 | 5.0 | 15.5 | 28 | 0.40 | 19.2 | 5.0 | 14.6 | 26 | 0.41 |
| Zinc (mg/d) | 21.2 | 7.9 | 7.2 | 13 | 0.54 | 20.3 | 8.1 | 6.2 | 12 | 0.57 |
| Vitamin A (µg RAE†/d) | 34.8 | 9.3 | 14.0 | 25 | 0.42 | 37.4 | 9.7 | 15.0 | 27 | 0.41 |
| Thiamin (mg/d) | 30.7 | 12.6 | 5.9 | 11 | 0.58 | 29.0 | 12.3 | 5.6 | 10 | 0.59 |
| Riboflavin (mg/d) | 27.3 | 6.9 | 15.5 | 28 | 0.40 | 26.4 | 7.9 | 11.2 | 20 | 0.46 |
| Niacin (mg/d) | 25.2 | 6.6 | 14.4 | 26 | 0.41 | 24.5 | 6.9 | 12.5 | 23 | 0.44 |
| Vitamin B6 (mg/d) | 28.2 | 13.2 | 4.5 | 9 | 0.63 | 26.9 | 12.5 | 4.7 | 9 | 0.63 |
| Folate (µg/d) | 18.1 | 5.4 | 11.4 | 21 | 0.46 | 17.6 | 5.5 | 10.1 | 19 | 0.48 |
| Vitamin B12 (µg/d) | 63.2 | 19.6 | 10.4 | 19 | 0.47 | 66.2 | 21.5 | 9.4 | 17 | 0.49 |
| Vitamin C (mg/d) | 30.7 | 5.5 | 31.3 | 56 | 0.30 | 31.7 | 6.8 | 21.9 | 39 | 0.35 |

* The correlation, r , between observed and usual intakes

**Model contains fixed effects for season, interviewer and market day

†Retinol Activity Equivalents

Table 4.5. Within-person, between-person and seasonal variance components as percent of total variance in energy and nutrient intakes, and within- to between-person variance ratios, among 4- to 8-year-old participants in the non-intervened arm of a pro-vitamin A maize efficacy trial in Mkushi, Zambia, 2012–2013

| Nutrient | Unadjusted | | | | Adjusted* | | | |
|---|---|----------|---------------|-------------------------|---|----------|---------------|-------------------------|
| | Variance components (% of total variance) | | | | Variance components (% of total variance) | | | |
| | Between-person | Seasonal | Within-person | σ_w^2/σ_b^2 | Between-person | Seasonal | Within-person | σ_w^2/σ_b^2 |
| Energy (kcal/d) | 11.0 | 8.3 | 80.7 | 7.3 | 12.1 | 6.2 | 71.3 | 5.9 |
| Protein (g/d) | 8.6 | 4.2 | 87.2 | 10.1 | 8.4 | 5.5 | 80.9 | 9.7 |
| Fat (g/d) | 15.2 | 2.9 | 81.9 | 5.4 | 16.1 | 2.9 | 76.4 | 4.8 |
| Carbohydrates (g/d) | 6.1 | 12.7 | 81.2 | 13.3 | 7.5 | 8.0 | 70.9 | 9.4 |
| Calcium (mg/d) | 3.4 | 9.0 | 87.6 | 25.5 | 3.0 | 11.6 | 83.2 | 27.5 |
| Iron (mg/d) | 4.5 | 4.3 | 91.1 | 20.1 | 4.1 | 4.6 | 85.0 | 20.8 |
| Zinc (mg/d) | 10.2 | 5.5 | 84.3 | 8.3 | 10.7 | 3.7 | 77.7 | 7.3 |
| Vitamin A ($\mu\text{g RAE}^{**}/\text{d}$) | 6.4 | 1.1 | 92.5 | 14.4 | 4.9 | 3.5 | 88.5 | 17.9 |
| Thiamin (mg/d) | 9.4 | 15.6 | 75.0 | 8.0 | 8.4 | 17.5 | 65.1 | 7.7 |
| Riboflavin (mg/d) | 0.3 | 20.2 | 79.5 | 232.8 | 1.1 | 23.0 | 69.1 | 65.5 |
| Niacin (mg/d) | 3.3 | 10.0 | 86.7 | 26.3 | 2.6 | 14.3 | 78.6 | 30.7 |
| Vitamin B6 (mg/d) | 12.7 | 14.0 | 73.3 | 5.8 | 9.0 | 17.3 | 63.3 | 7.0 |
| Folate ($\mu\text{g}/\text{d}$) | 5.0 | 10.3 | 84.6 | 16.8 | 6.1 | 9.1 | 80.6 | 13.2 |
| Vitamin B12 ($\mu\text{g}/\text{d}$) | 8.1 | 2.9 | 89.1 | 11 | 7.9 | 4.1 | 87.0 | 11.0 |
| Vitamin C (mg/d) | 0.0 | 11.2 | 88.8 | -- | 0.0 | 9.4 | 84.8 | -- |

*Model contains fixed effects for interviewer and market day

**Retinol Activity Equivalents

Supplemental Tables

Supplemental Table 4.1. Nutrient intakes by season, adjusting for energy intake, among 4- to 8-year-old children (n=200) participating in the non-intervened arm of a biofortified maize efficacy trial, Mkushi, Zambia, 2012–2013

| Nutrient | Nutrient intake by season [mean (95% confidence interval)] | | |
|-----------------------|--|--------------------------------|--------------------------------|
| | Late post-harvest season (Aug–Oct) | Early lean season (Nov–Jan) | Late lean season (Feb–Apr) |
| Protein (g/d) | 46 (44, 47) ^a | 44 (43, 45) ^a | 44 (42, 45) ^a |
| Fat (g/d) | 43 (42, 45) ^{ab} | 43 (41, 44) ^a | 45 (43, 46) ^b |
| Carbohydrates (g/d) | 228 (224, 233) ^a | 238 (235, 241) ^b | 232 (228, 235) ^a |
| Calcium (mg/d) | 249 (232, 266) ^a | 238 (225, 252) ^a | 201 (189, 214) ^b |
| Iron (mg/d) | 9.2 (8.8, 9.6) ^{ab} | 9.5 (9.1, 9.9) ^a | 8.7 (8.4, 9.1) ^b |
| Zinc (mg/d) | 5.9 (5.7, 6.1) ^a | 6 (5.8, 6.3) ^a | 5.4 (5.2, 5.6) ^b |
| Vitamin A (µg RAE*/d) | 500 (460, 543) ^a | 407 (381, 433) ^b | 325 (298, 354) ^c |
| Thiamin (mg/d) | 0.75 (0.71, 0.78) ^a | 0.78 (0.75, 0.81) ^a | 0.75 (0.72, 0.79) ^a |
| Riboflavin (mg/d) | 0.63 (0.6, 0.65) ^a | 0.68 (0.66, 0.71) ^b | 0.62 (0.59, 0.65) ^a |
| Niacin (mg/d) | 10.7 (10, 11.5) ^a | 11.1 (10.5, 11.7) ^a | 10.5 (9.9, 11.2) ^a |
| Vitamin B6 (mg/d) | 1.2 (1.1, 1.3) ^{ab} | 1.3 (1.2, 1.3) ^a | 1.1 (1.1, 1.2) ^b |
| Folate (µg/d) | 121 (114, 128) ^a | 135 (129, 140) ^b | 132 (125, 139) ^b |
| Vitamin B12 (µg/d) | 0.61 (0.54, 0.68) ^a | 0.58 (0.53, 0.63) ^a | 0.47 (0.42, 0.53) ^b |
| Vitamin C (mg/d) | 35 (31, 38) ^a | 48 (43, 54) ^b | 28 (25, 31) ^c |

*RAE: Retinol Activity Equivalent

^{a,b,c} difference in letters indicates significant difference between seasonal means (p<0.05)

Supplemental Table 4.2. Within- and between-person coefficients of variation in energy and nutrient intakes and variance ratios by age group, among 4- to 8-year-old participants in the non-intervened arm of a pro-vitamin A maize efficacy trial in Mkushi, Zambia, 2012–2013

| Nutrient | Unadjusted | | | | | | Adjusted* | | | | | |
|------------------------|-----------------|-----------------|-------------------------|-------------------|-----------------|-------------------------|-----------------|-----------------|-------------------------|-------------------|-----------------|-------------------------|
| | Under 5 years | | | 5 years and older | | | Under 5 years | | | 5 years and older | | |
| | CV _w | CV _b | σ_w^2/σ_b^2 | CV _w | CV _b | σ_w^2/σ_b^2 | CV _w | CV _b | σ_w^2/σ_b^2 | CV _w | CV _b | σ_w^2/σ_b^2 |
| Energy (kcal/d) | 19.0 | 6.9 | 7.5 | 19.5 | 7.9 | 6.1 | 18.4 | 7.7 | 5.7 | 17.7 | 7.9 | 5.1 |
| Protein (g/d) | 20.8 | 7.6 | 7.4 | 22.6 | 7.0 | 10.4 | 20.9 | 8.7 | 5.8 | 21.4 | 6.6 | 10.5 |
| Fat (g/d) | 20.0 | 8.5 | 5.5 | 20.6 | 9.0 | 5.2 | 19.5 | 8.9 | 4.8 | 19.0 | 8.6 | 4.9 |
| Carbohydrates (g/d) | 20.0 | 6.5 | 9.6 | 20.4 | 6.7 | 9.2 | 19.4 | 7.0 | 7.6 | 18.5 | 7.2 | 6.6 |
| Calcium (mg/d) | 27.8 | 8.1 | 11.9 | 28.7 | 6.8 | 17.7 | 30.1 | 9.7 | 9.6 | 29.6 | 7.4 | 15.9 |
| Iron (mg/d) | 19.1 | 6.3 | 9.3 | 20.1 | 3.5 | 33.0 | 18.9 | 6.8 | 7.7 | 19.3 | 3.3 | 34.1 |
| Zinc (mg/d) | 21.2 | 8.2 | 6.6 | 21.2 | 7.4 | 8.1 | 21.2 | 9.4 | 5.1 | 19.6 | 7.4 | 7.1 |
| Vitamin A (µg RAE**/d) | 33.2 | 8.4 | 15.6 | 35.8 | 9.9 | 13.0 | 36.9 | 9.4 | 15.4 | 37.0 | 10.6 | 12.3 |
| Thiamin (mg/d) | 30.0 | 12.9 | 5.4 | 31.0 | 12.3 | 6.4 | 29.9 | 13.1 | 5.2 | 28.3 | 11.6 | 5.9 |
| Riboflavin (mg/d) | 25.8 | 6.5 | 15.7 | 28.2 | 7.2 | 15.1 | 25.5 | 7.2 | 12.5 | 26.9 | 8.0 | 11.4 |
| Niacin (mg/d) | 24.9 | 5.2 | 23.2 | 25.3 | 7.4 | 11.7 | 24.2 | 5.5 | 19.7 | 24.8 | 7.5 | 11.1 |
| Vitamin B6 (mg/d) | 27.8 | 15.6 | 3.2 | 28.4 | 11.3 | 6.3 | 27.7 | 15.6 | 3.2 | 25.9 | 10.3 | 6.3 |
| Folate (µg/d) | 18.0 | 4.7 | 14.7 | 18.2 | 5.8 | 9.8 | 17.8 | 5.2 | 11.6 | 17.5 | 5.7 | 9.5 |
| Vitamin B12 (µg/d) | 58.6 | 17.9 | 10.7 | 66.2 | 20.4 | 10.5 | 62.4 | 21.9 | 8.1 | 67.4 | 21.1 | 10.2 |
| Vitamin C (mg/d) | 31.0 | 7.0 | 19.7 | 30.6 | 3.3 | 85.6 | 31.7 | 8.5 | 14.0 | 31.9 | 4.5 | 49.7 |

*Model contains fixed effects for season, interviewer and market day

**Retinol Activity Equivalents

Supplemental Table 4.3. Within- and between-person coefficients of variation in energy and nutrient intakes and variance ratios by sex, among 4- to 8-year-old participants in the non-intervened arm of a pro-vitamin A maize efficacy trial in Mkushi, Zambia, 2012–2013

| Nutrient | Unadjusted | | | | | | Adjusted* | | | | | |
|------------------------|-----------------|-----------------|-------------------------|-----------------|-----------------|-------------------------|-----------------|-----------------|-------------------------|-----------------|-----------------|-------------------------|
| | Boys | | | Girls | | | Boys | | | Girls | | |
| | CV _w | CV _b | σ_w^2/σ_b^2 | CV _w | CV _b | σ_w^2/σ_b^2 | CV _w | CV _b | σ_w^2/σ_b^2 | CV _w | CV _b | σ_w^2/σ_b^2 |
| Energy (kcal/d) | 19.4 | 6.6 | 8.5 | 19.1 | 8.6 | 5.0 | 18.0 | 7.4 | 6.0 | 17.8 | 8.7 | 4.2 |
| Protein (g/d) | 21.6 | 5.3 | 16.8 | 22.2 | 9.2 | 5.8 | 21.2 | 5.8 | 13.5 | 21.3 | 9.2 | 5.3 |
| Fat (g/d) | 20.8 | 9.1 | 5.3 | 19.8 | 8.6 | 5.2 | 19.8 | 9.2 | 4.7 | 18.3 | 8.8 | 4.3 |
| Carbohydrates (g/d) | 20.3 | 4.6 | 20.0 | 20.1 | 8.8 | 5.2 | 18.5 | 5.8 | 10.4 | 19.0 | 8.7 | 4.7 |
| Calcium (mg/d) | 29.2 | 5.6 | 27.4 | 27.4 | 9.1 | 9.1 | 30.0 | 5.7 | 27.7 | 29.8 | 10.9 | 7.5 |
| Iron (mg/d) | 19.5 | 4.6 | 17.9 | 20.0 | 5.6 | 12.8 | 19.1 | 5.2 | 13.7 | 19.2 | 5.7 | 11.4 |
| Zinc (mg/d) | 21.6 | 6.5 | 11.1 | 20.5 | 9.2 | 4.9 | 20.5 | 7.4 | 7.7 | 19.8 | 8.8 | 5.0 |
| Vitamin A (µg RAE**/d) | 35.5 | 7.5 | 22.5 | 33.9 | 11.4 | 8.9 | 35.8 | 7.4 | 23.4 | 38.5 | 11.7 | 10.8 |
| Thiamin (mg/d) | 31.3 | 9.8 | 10.2 | 29.7 | 15.7 | 3.6 | 29.1 | 10.0 | 8.4 | 28.3 | 15.2 | 3.5 |
| Riboflavin (mg/d) | 27.1 | 5.6 | 23.3 | 27.6 | 8.5 | 10.6 | 25.8 | 6.9 | 14.0 | 26.9 | 9.5 | 8.0 |
| Niacin (mg/d) | 24.8 | 5.2 | 23.1 | 25.6 | 8.3 | 9.5 | 24.5 | 6.1 | 16.3 | 24.7 | 8.1 | 9.4 |
| Vitamin B6 (mg/d) | 27.6 | 11.5 | 5.8 | 28.9 | 15.2 | 3.6 | 25.8 | 10.8 | 5.6 | 27.9 | 14.1 | 3.9 |
| Folate (µg/d) | 18.5 | 4.5 | 16.5 | 17.7 | 6.3 | 7.9 | 17.8 | 5.0 | 12.6 | 17.2 | 6.3 | 7.4 |
| Vitamin B12 (µg/d) | 64.2 | 7.6 | 70.8 | 61.7 | 29.3 | 4.4 | 72.2 | 10.3 | 48.9 | 61.7 | 29.4 | 4.4 |
| Vitamin C (mg/d) | 31.1 | 4.3 | 52.7 | 30.2 | 6.8 | 19.8 | 29.4 | 6.2 | 22.5 | 34.0 | 7.6 | 20.3 |

*Model contains fixed effects for season, interviewer and market day

**Retinol Activity Equivalents

Supplemental Table 4.4a. Within- and between-person coefficients of variation in energy, macronutrient and mineral intakes and variance ratios by season, among 4- to 8-year-old participants in the non-intervened arm of a pro-vitamin A maize efficacy trial in Mkushi, Zambia, 2012–2013

| Nutrient | Unadjusted | | | | | | | | | Adjusted* | | | | | | | | |
|---------------------|------------------------------------|-----------------|-------------------------|-----------------------------|-----------------|-------------------------|----------------------------|-----------------|-------------------------|------------------------------------|-----------------|-------------------------|-----------------------------|-----------------|-------------------------|----------------------------|-----------------|-------------------------|
| | Late post-harvest season (Aug–Oct) | | | Early lean season (Nov–Jan) | | | Late lean season (Feb–Apr) | | | Late post-harvest season (Aug–Oct) | | | Early lean season (Nov–Jan) | | | Late lean season (Feb–Apr) | | |
| | CV _w | CV _b | σ_w^2/σ_b^2 | CV _w | CV _b | σ_w^2/σ_b^2 | CV _w | CV _b | σ_w^2/σ_b^2 | CV _w | CV _b | σ_w^2/σ_b^2 | CV _w | CV _b | σ_w^2/σ_b^2 | CV _w | CV _b | σ_w^2/σ_b^2 |
| Energy (kcal/d) | 19.6 | 13.5 | 2.1 | 18.4 | 7.8 | 5.5 | 16.9 | 6.9 | 6.1 | 18.3 | 11.0 | 2.8 | 18.2 | 8.2 | 4.9 | 14.6 | 7.5 | 3.8 |
| Protein (g/d) | 23.5 | 10.7 | 4.8 | 21.0 | 7.4 | 8.0 | 19.9 | 7.7 | 6.7 | 21.9 | 8.3 | 7.0 | 20.5 | 7.1 | 8.2 | 18.7 | 7.9 | 5.7 |
| Fat (g/d) | 20.9 | 13.4 | 2.4 | 19.8 | 8.4 | 5.6 | 18.9 | 8.5 | 4.9 | 18.7 | 11.0 | 2.9 | 20.0 | 9.3 | 4.6 | 16.2 | 9.4 | 3.0 |
| Carbohydrates (g/d) | 19.3 | 13.9 | 1.9 | 19.5 | 7.9 | 6.1 | 17.0 | 6.3 | 7.3 | 18.7 | 11.4 | 2.7 | 18.9 | 7.7 | 6.1 | 15.3 | 6.4 | 5.7 |
| Calcium (mg/d) | 29.8 | 6.2 | 22.8 | 26.1 | 10.2 | 6.5 | 28.3 | 6.1 | 21.7 | 26.0 | 10.1 | 6.7 | 25.3 | 11.3 | 5.0 | 30.4 | 4.4 | 48.4 |
| Iron (mg/d) | 20.3 | -- | -- | 20.4 | 6.8 | 9.1 | 16.0 | 6.6 | 5.8 | 17.4 | -- | -- | 20.0 | 5.6 | 13.0 | 14.3 | 7.1 | 4.0 |
| Zinc (mg/d) | 19.6 | 5.9 | 10.9 | 22.1 | 9.8 | 5.1 | 18.1 | 8.0 | 5.1 | 17.8 | 5.4 | 10.9 | 20.4 | 8.5 | 5.7 | 16.8 | 8.1 | 4.3 |

*Model contains fixed effects for interviewer and market day

Supplemental Table 4.4b. Within- and between-person coefficients of variation in vitamin intakes and variance ratios by season, among 4- to 8-year-old participants in the non-intervened arm of a pro-vitamin A maize efficacy trial in Mkushi, Zambia, 2012–2013

| Nutrient | Unadjusted | | | | | | | | | Adjusted* | | | | | | | | |
|------------------------|------------------------------------|-----------------|-------------------------|-----------------------------|-----------------|-------------------------|----------------------------|-----------------|-------------------------|------------------------------------|-----------------|-------------------------|-----------------------------|-----------------|-------------------------|----------------------------|-----------------|-------------------------|
| | Late post-harvest season (Aug–Oct) | | | Early lean season (Nov–Jan) | | | Late lean season (Feb–Apr) | | | Late post-harvest season (Aug–Oct) | | | Early lean season (Nov–Jan) | | | Late lean season (Feb–Apr) | | |
| | CV _w | CV _b | σ_w^2/σ_b^2 | CV _w | CV _b | σ_w^2/σ_b^2 | CV _w | CV _b | σ_w^2/σ_b^2 | CV _w | CV _b | σ_w^2/σ_b^2 | CV _w | CV _b | σ_w^2/σ_b^2 | CV _w | CV _b | σ_w^2/σ_b^2 |
| Vitamin A (µg RAE**/d) | 35.0 | 5.6 | 39.6 | 32.4 | 9.8 | 11.1 | 38.1 | -- | -- | 36.0 | -- | -- | 29.9 | 9.2 | 10.6 | 38.5 | -- | -- |
| Thiamin (mg/d) | 29.5 | 15.1 | 3.8 | 27.8 | 17.6 | 2.5 | 30.1 | 13.3 | 5.2 | 21.9 | 13.8 | 2.5 | 26.7 | 18.2 | 2.1 | 26.5 | 12.1 | 4.8 |
| Riboflavin (mg/d) | 28.8 | 11.0 | 6.9 | 23.6 | 11.3 | 4.4 | 25.6 | 11.5 | 5.0 | 22.3 | 12.8 | 3.0 | 22.0 | 12.3 | 3.2 | 24.2 | 12.1 | 4.0 |
| Niacin (mg/d) | 25.9 | 10.0 | 6.8 | 23.0 | 10.0 | 5.3 | 25.3 | 4.2 | 35.6 | 22.2 | 10.7 | 4.3 | 21.5 | 11.3 | 3.6 | 23.9 | 6.6 | 12.9 |
| Vitamin B6 (mg/d) | 29.9 | 2.6 | 129.6 | 25.3 | 18.9 | 1.8 | 27.0 | 13.3 | 4.1 | 19.2 | 9.6 | 4.1 | 23.4 | 17.9 | 1.7 | 24.3 | 13.3 | 3.3 |
| Folate (µg/d) | 18.4 | 7.3 | 6.3 | 17.1 | 7.1 | 5.8 | 17.6 | 4.7 | 14.2 | 17.6 | 6.9 | 6.4 | 17.1 | 7.3 | 5.5 | 16.0 | 5.8 | 7.6 |
| Vitamin B12 (µg/d) | 56.7 | 29.1 | 3.8 | 59.1 | 15.9 | 13.8 | 71.1 | 30.0 | 5.6 | 58.0 | 32.5 | 3.2 | 53.0 | 15.1 | 12.3 | 66.4 | 28.3 | 5.5 |
| Vitamin C (mg/d) | 26.6 | -- | -- | 29.7 | 10.7 | 7.8 | 29.5 | -- | -- | 25.2 | -- | -- | 28.0 | 9.4 | 8.9 | 29.9 | -- | -- |

*Model contains fixed effects for interviewer and market day

**Retinol Activity Equivalent

Chapter 5. Aim 3: Dietary Diversity across Seasons among Young, Rural Zambian Children: Effects on Dietary Diversity Scores and Their Association with Micronutrient Adequacy

Abstract

Dietary diversity scores (DDS) are used for rapid assessment of dietary quality in monitoring and program evaluation. DDS have been recommended by the Food and Agricultural Organization (FAO) and the World Health Organization (WHO) for use among women of reproductive age and children 6–23 months of age, respectively, but there is no recommended score for use among preschool- or school-age children. In settings where diet changes seasonally, mean DDS and the association between DDS and overall nutrient adequacy may also vary by season, but few studies have examined this possibility. We conducted 24-hour dietary recalls among 4- to 8-year-old children in rural Zambia (n=200) over a six-month period that covered three seasons. We assessed dietary diversity by the 10-food group DDS (DDS10) recommended by the FAO and the 7-food group DDS (DDS7) recommended by the WHO. We evaluated the relationship of each measure to overall micronutrient adequacy and to usual intakes of five micronutrients, the performance of each score for predicting micronutrient adequacy and whether these relationships or predictive power varied by season. Mean scores over six months were 4.11 ± 0.03 for DDS7 and 4.39 ± 0.03 for DDS10. Both scores were significantly higher in the early lean season (November–January) than in the late post-harvest (August–October) or late lean (February–April) seasons, but DDS10 better reflected seasonal change in dietary patterns. Across seasons, a one-unit change in DDS7 was associated with a 1–6 percentage point change in mean probability of adequacy (MPA), an indicator of overall micronutrient adequacy. A one-unit change in DDS10 was associated with a 1–10 percentage point change in MPA. At a cutoff

of 4 out of 7 food groups, DDS7 predicted MPA > 75% with 71%–99% sensitivity and 5%–75% specificity. At a cutoff of 5 out of 10 food groups DDS10 predicted MPA > 75% with 58%–65% sensitivity and 30%–69% specificity. We conclude that the DDS10 recommended by the FAO is a better indicator of dietary quality among rural Zambian children and recommend that studies plan for seasonal impacts on DDS and their association with overall nutrient adequacy.

Introduction

Dietary diversity scores (DDS) have emerged as a popular tool for the rapid assessment of dietary quality, particularly for nutrition and food security monitoring and program evaluation.

For example, a food frequency module enabling calculation of dietary diversity scores for women and children under five has been included in Demographic and Health Surveys since 2003 (DHS Phase 5), and the United States Agency for International Development includes individual dietary diversity scoring as an evaluation indicator for Food for Peace programs.^{1,2}

Validation studies conducted in low- and middle-income countries have consistently shown that DDS are associated with nutrient intake adequacy and nutritional status among women and young children.^{3–15} This literature has also refined how DDS are scored, particularly in number and definition of food groups and incorporation of a minimum consumption quantity.^{3–6,9,10,12}

However, few studies have assessed whether seasonal changes in diet affect the performance of DDS as indicators of dietary adequacy. Shifting food consumption patterns may change the strength of association between DDS and nutrient intake adequacy.

The Food and Agricultural Organization (FAO) and the World Health Organization (WHO) of the United Nations (UN) have released guidance on the measurement of dietary diversity among women of reproductive age and among children 6–23 months of age, respectively.^{16,17} The recommended survey technique for assessing both scores is an open recall in which the

interviewer marks foods consumed on a preset list as the respondent lists the foods that were eaten during the previous day.^{16,18} Either score could also be calculated from survey methods such as a food frequency questionnaire or 24-hour dietary recall interview.

The Minimum Dietary Diversity for Women of Reproductive Age (MDD-W) indicator is a 10-food group score recommended by the FAO based on multi-site studies validating different scoring formulas against mean probability of adequacy (MPA), an indicator of overall micronutrient intake adequacy.^{7,16,19} Minimum dietary diversity using MDD-W is defined as consumption of at least 5 of the 10 food groups.^{16,19} The MDD-W is an update to the FAO's previously recommended Women's Dietary Diversity Score (WDDS), a 9-food group score.^{16,20} The MDD-W is based on an extension of the research that formed the WDDS and has the added feature of a binary cutoff for minimum dietary diversity.^{9,16,19}

The WHO recommends a minimum dietary diversity measure as one among a suite of indicators to assess quality of infant and young child feeding.¹⁷ The recommended dietary diversity score is based on seven food groups, and the cutoff for minimum dietary diversity for a child 6–23 months of age is defined as receiving foods from at least 4 of the 7 groups. This dietary diversity indicator was validated against micronutrient density adequacy, using dietary intake data from nine different low- and middle-income countries.¹²

The DDS recommended by the FAO and the WHO have emerged as points of consensus on scores that are valid and practicable for rapid assessment of population-level dietary quality among women of reproductive age and children aged 6–23 months. Similar DDS have been validated against nutritional status or nutrient intake adequacy among children over 2 years old, however, there is no current consensus or recommendation on a best score for this age group.^{3–5,11,14,21,22} In a review of dietary diversity validation studies conducted in low- and middle-income

countries among children under 5 years old, Ruel observed that dietary diversity was consistently positively associated with nutrient adequacy and with child growth, despite differences in number and definition of food groups and in minimum consumption requirements used when calculating DDS.³ A small number of DDS validation studies have been conducted among early school-age children. DDS were associated with nutrient adequacy among 1- to 9-year-old South African children and rural Kenyan primary-school children.^{4,5,11}

Most DDS studies use dietary assessments made at a single time point, so cannot reflect whether the validity of DDS relative to nutrient intake adequacy may be subject to seasonal fluctuations in dietary patterns. We have previously reported that nutrient intakes, especially vitamin A and vitamin C, fluctuate seasonally among our sample of 4- to 8-year-old rural Zambian children, even when adjusting for total energy intakes (Chapter 4). Food consumption patterns have been found to differ between lean and harvest seasons among 6- to 8-year-old children in northern Benin and 3- to 6-year-old children in rural Malawi and Ghana.^{23,24} Food consumption patterns changed between the beginning and end of the lean season among women in rural Burkina Faso, and a 9-food group DDS that was significantly related to body mass index (BMI) prior to the lean season was not significantly related to BMI at the end of the lean season.²⁵

The FAO and the WHO have recommended DDS that are valid and practicable as indicators of dietary adequacy among women and among 6- to 23-month-old children. DDS have been similarly validated among preschool- and early school-age children but a consensus on scoring method has not emerged. Diets of children in this age group have been shown to change seasonally, but the impacts of such seasonal shifts on the relationship between DDS and nutrient adequacy have not been tested. In this paper, we describe diets of apparently healthy, 4- to 8-year-old rural Zambian children over three agricultural seasons using the MDD-

W recommended by the FAO and the MDD indicator recommended by the WHO. For simplicity, we refer to the 10-food group MDD-W as DDS10 and 7-food group MDD as DDS7. Our objectives are to describe the relationship between each DDS and nutrient adequacy and intakes, to determine whether that relationship varies by season and to evaluate each DDS as a predictor of overall nutrient adequacy in each of three seasons.

Methods

Study context

Our study uses dietary data collected during a feeding trial to test the efficacy of pro-vitamin A biofortified maize for improving vitamin A status and reducing vitamin A deficiency among 4- to 8-year-old children in Mkushi district, in Zambia's Central province. Mkushi is a rural district with an agricultural economy of mixed large-scale and smallholder farms. Eligible children between 4 and 8 years of age, not yet enrolled in school and living in rural towns or villages within walking distance of central feeding sites were identified through a mapping and census of the study area. The efficacy trial enrolled 1226 children who were block randomized by geographic cluster into one of three groups: a treatment group receiving meals containing biofortified maize (n=543 children in 25 clusters), a positive control group receiving meals with traditional Zambian maize (n=481 children in 25 clusters) or a non-intervened control group which received an equivalent food package at the end of the trial (n=202 children in 14 clusters). This study includes only the children assigned to the non-intervened control group for whom at least one complete 24-hour recall without report of illness was completed during the six-month trial period (n=200). Two children who completed a 24-hour recall only during the baseline survey and were reported ill during the recall period were excluded.

All parents of children enrolled in the study provided verbal informed consent. The study was approved by the Institutional Review Board at the Johns Hopkins Bloomberg School of Public Health (Baltimore, USA) and the Ethics Review Committee of the Tropical Disease Research Centre (Ndola, Zambia).

Data collection

Data were collected monthly over the six-month trial period (August 2012–March 2013). The baseline survey included modules on household membership, socio-economic status and food security and modules on child health and diet. Child diet was assessed by 24-hour recall interview with the child's primary caregiver. Details of the 24-hour recall method and tablet-based data collection tool have been described previously (Appendix 1).²⁶ The recall used a modified multi-pass approach to collect detailed descriptions of all foods consumed by the child from when they woke up the previous morning to waking the morning of the interview. Portion sizes were estimated using a photo booklet showing different portions of commonly consumed foods or, in the case of foods such as fruit or buns, the number of units consumed. The child health and diet module and the household food security module were repeated monthly. The endline survey included the child health and diet module and a module collecting additional household socio-economic data.

Data analysis

Data from 24-hour recalls in which the child was reported ill during the recall period (n= 155 observations) were excluded from all analyses, for a total sample of 1071 observations among 200 children. All results are reflective of diet among apparently healthy children. Food groups for DDS7 and DDS10 were defined according to guidelines from the WHO and FAO, respectively

(Table 5.1).^{16,17} Ingredients in mixed dishes or unmixed foods consumed in any quantity were matched to the food groups for each score, and the number of food groups consumed were tallied to create observed DDS7 and DDS10 scores for each 24-hour recall.

Observed nutrient intakes were calculated from the 24-hour recall data using a local recipe database and food composition table. The recipes database was compiled by HarvestPlus for use in a 2009 survey including 24-hour dietary recall in the same district of Zambia (personal communication, HarvestPlus). The food composition table was based on a table compiled from existing tables by HarvestPlus for the same 2009 survey, with additional data from global and regional food composition tables and the US Department of Agriculture National Nutrient Database for Standard Reference.²⁷⁻³² Further details on the calculation of observed nutrient intakes are described by Caswell et al (Appendix 1).²⁶

For both foods and nutrients, due to high day-to-day variation in diet, a single day's measurement is not a precise estimator of usual intake.³³ Such errors can bias measures of association toward null.³³ In order to reduce this impact of measurement error on regression analyses of the associations between DDS and MPA or nutrient intakes, we calculated best linear unbiased predictors (BLUPs) of usual DDS and nutrient intakes. The use of BLUPs to predict usual intakes for use in the calculation of MPA has been demonstrated in several prior validation studies of DDS, and the value of BLUPs of usual DDS and nutrient intakes for reducing bias in cross-sectional models of MPA has been described by Joseph and Carriquiry.^{6-8,19} BLUPs of usual DDS7, DDS10 and intakes of energy and 14 macro- and micronutrients in each round were predicted using SAS macros published by the National Cancer Institute.^{34,35} The BLUPs (\hat{X}_i) were predicted without use of covariates, following the formula

$$\hat{X}_i = \hat{w}_i \bar{X}_i + (1 - \hat{w}_i) \bar{X} \quad (\text{Formula 1})$$

$$\text{where } \hat{w}_i = \frac{\hat{\sigma}_b^2}{\hat{\sigma}_b^2 + (\hat{\sigma}_w^2/n_i)},$$

\bar{X}_i is the mean of observed values for subject i , \bar{X} is the overall mean, $\hat{\sigma}_b^2$ is the estimated between-subject variance, $\hat{\sigma}_w^2$ is the estimated within-subject variance and n_i is the number of 24-hour recalls completed for subject i .³⁵ For each round, the BLUPs of DDS and nutrient intakes were predicted using observed values from the round of interest and from the adjacent round(s). For example, to calculate usual values at baseline, observed values from baseline and the first monthly monitoring survey were used. To calculate usual values in the first monthly monitoring round, data from that round, baseline and the second monitoring round were used. MPA, a measure of overall micronutrient adequacy, was calculated as recommended by the Institute of Medicine (IOM).³⁶ An individual's probability of adequacy for a given nutrient is the probability that their requirement falls at or below their intake of that nutrient [Pr(requirement \leq intake)], calculated as the area under the requirement distribution curve that falls below their usual intake (SAS PROBNORM function). We averaged the probability of usual intake adequacy of 11 micronutrients – iron, calcium, zinc, vitamin A, thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12 and vitamin C – to create an estimate of MPA in each round for each child. These nutrients were chosen for completeness of available nutrient composition data and public health importance.

For nutrients that have a normal requirement distribution, probability of adequacy is calculated using the estimated average requirement (EAR), equal to the mean of the requirement distribution, and its standard deviation (SD). We used EARs and SDs from the IOM for calculating probability of adequacy for all micronutrients other than zinc and iron. For zinc, we used the EAR and SD estimated by the International Zinc Nutrition Consultative Group (IZiNCG) for diets with low zinc bioavailability.³⁷ For iron, we adjusted the percentiles of the requirement

distribution from the IOM to reflect a 10% zinc bioavailability diet characterized by high phytate and low meat intakes, as recommended by the WHO and demonstrated in prior studies.^{5,6,8,9,38,39}

We calculated the probability of iron intake adequacy as the average of the two requirement distribution percentiles between which a child's usual intake fell.

Descriptive statistics were used to estimate the frequency or continuous distributions of child and household characteristics. We defined three seasons, which divide the trial period into three roughly equal periods and correspond to the agricultural calendar in Mkushi: the late post-harvest season (August–October), the early lean or planting season (November–January) and the late lean season (February–April). One-way ANOVA of observed DDS, usual nutrient intakes and MPA by season were fit to estimate overall and seasonal means and test for significant differences by season (SAS PROC GLM). To describe seasonal changes in dietary patterns, we calculated the number and percent of children consuming foods from each food group on at least one recall day within each season and the most common combinations of food groups.

To explore the relationship between DDS and MPA, we first plotted MPA by observed DDS7 and by observed DDS10, in seasonal subgroups. We then fit a series of longitudinal models with MPA or usual intake of iron, calcium, vitamin A, vitamin B12 or vitamin C as the outcome and either DDS7 or DDS10 as the key predictor. Model 1 included DDS as the only predictor. In Model 2, we added indicators for season and interactions between season and DDS. Both Models 1 & 2 were also fit including a fixed effect for usual energy intake. Further models were tested with potential covariates including child age and sex and descriptors of household socio-economic status, but most additional terms were non-significant and inclusion did not meaningfully change results for DDS, season or DDS by season interaction. Models were fit using generalized estimating equations (SAS PROC GENMOD) to account for correlation among repeat measures.

To assess the performance of each DDS as a predictor of MPA, we generated receiver operating characteristic (ROC) curves and calculated the area under each curve (SAS PROC LOGISTIC). We fit the ROC curves separately by season using a random sub-sample of one record per child from each season (late post-harvest season n=187; early lean season n=186; late lean season n=178 in late lean).

Results

The mean age at baseline among included children was 5.5 ± 1.2 years, and 55% (n=110) were male. Mean DDS7 over all rounds of observation was 4.11 ± 0.03 food groups, and mean DDS10 was 4.39 ± 0.03 food groups (**Table 5.2**). Mean MPA over all rounds was 0.75 ± 0.001 and mean energy intake was 1537 ± 3 kcal per day. Both DDS7 and DDS10 showed small but significant variations by season, with dietary diversity by either score being lowest in the late post-harvest season and highest in the early lean season. MPA and usual intakes of energy, vitamin B12, iron and calcium showed similar seasonal trends. Usual vitamin A intake also varied significantly by season, but in a decreasing trend over the three seasons.

All children consumed starchy staple foods during each season, and nearly all consumed other vegetables (**Table 5.3**). The most commonly observed combination of DDS7 food groups was grains, roots and tubers, flesh foods, vitamin A rich fruits and vegetables and other fruits and vegetables (n=352 observation days, 32.9%). The most commonly observed combination of DDS10 food groups was grains, white roots and tubers and plantains; meat, poultry and fish; dark green leafy vegetables; and other vegetables (n=232 observation days, 21.7%).

Seasonal variation in dietary patterns was observed based on the percent of children consuming foods from each food group. Among the DDS7 food groups, dairy products, flesh foods, eggs, and vitamin A rich fruits and vegetables were consumed by more children in the early lean

season than they were in the other seasons. Consumption of legumes and nuts was lower in the late post-harvest season than in the early or late lean seasons. Examination of the DDS10 food groups shows that the percent of children consuming dark green leafy vegetables was fairly stable across seasons, but consumption of other vitamin A-rich fruits and vegetables was considerably more common in the early lean season than in the other seasons, as was consumption of other fruits.

Positive trends in MPA by observed values of DDS7 and DDS10 are seen in **Figure 1**. For both scores, mean MPA is lowest for a given DDS value in the late lean season, and, over most of the observed DDS range, highest in the early lean season. The increase in MPA with each one food group increase in DDS is least in the late lean season for both scores and is similar in the late post-harvest and early lean seasons. Plots of usual micronutrient intakes by DDS vary substantially by micronutrient and show strong seasonal differences (**Supplemental Figures 1 & 2**). However, as was found for MPA, usual nutrient intakes associated with each level of DDS are consistently lowest in the late lean season.

Usual DDS7 and DDS10 were significantly associated with MPA in both uncontrolled models and models adjusting for season (**Table 5.4a**). For both scores, including terms for season and interaction between season and DDS revealed significant seasonal influences on MPA and the relationship between DDS and MPA. In models controlling for energy but not for season, neither score retained a significant association with MPA (**Supplemental Table 5.1a**). In seasonal models controlling for energy, the association between DDS10 and MPA remained significant and positive in the late post-harvest and early lean seasons and null in the late lean season (**Supplemental Table 5.1a**). The energy-adjusted association between DDS7 and MPA was significant only in the early lean season.

Associations between DDS and usual intakes varied by nutrient and by season (**Tables 5.4a & 5.4b**). In unadjusted models for both DDS, associations with usual intakes of calcium, vitamin B12 and vitamin C were positive and associations with usual intakes of iron and vitamin A were negative or null. For all nutrients there was at least one significant interaction between DDS and season, but the direction and significance of these seasonal shifts in association differed between nutrients. Only calcium remained positively, significantly associated with both DDS in all seasons, though the strength of association varied. The association with iron intake was positive and consistent across seasons for DDS7, but was significant only in the late post-harvest season for DDS10. Vitamin A was positively associated with DDS7 in the late post-harvest and early lean seasons and with DDS10 in the early lean season only. For both scores, vitamin C showed null or negative associations in the late post-harvest and late lean seasons but positive associations in the early lean season. Iron intakes were positively associated with DDS10 in the early and late lean seasons, but had varying negative or null associations with DDS7 in all three seasons. Unlike MPA, associations between DDS and usual nutrient intakes usually remained significant when controlling for usual energy intake (**Supplemental Tables 5.1a & 5.1b**).

Performance of observed DDS7 or DDS10 as a predictor of MPA > 0.75 was moderate, with AUC values by season ranging from 0.63 to 0.77 for DDS7 and from 0.66 to 0.72 for DDS10 (**Figure 2**). Previous DDS validation studies have used a cutoff of AUC \geq 0.7 to indicate usefulness of an indicator, a guideline which both scores meet only in the late lean season.^{9,19} At the WHO-recommended cutoff of 4 food groups for minimum dietary diversity using DDS7, sensitivity and specificity for predicting MPA > 0.75 were 99% and 5%, respectively, in the late post-harvest season, 79% and 32% in the early lean season and 71% and 76% in the late lean season.¹⁷ At the FAO-recommended cutoff of 5 of the DDS10 food groups, sensitivity and specificity for predicting MPA > 0.75 were 86% and 30%, respectively, in the late post-harvest season, 58% and

69% in the early lean season, and 71% and 65% in the late lean season.¹⁶ In some seasons, lowering either score's cutoff for minimum dietary diversity by one food group produced a higher sum of sensitivity plus specificity, but at considerable loss of sensitivity for specificity. The most balanced combination of high sensitivity with high specificity occurred at the recommended cutoffs for each score. In each round, 66% - 88% of children met the WHO cutoff for minimum dietary diversity, and 32% - 53% met the FAO minimum dietary diversity cutoff.

Discussion

We used dietary intake data collected over seven months among 200 apparently healthy, rural Zambian children aged 4–8 years to demonstrate the performance of two recommended dietary diversity scores in this age group and setting and across three agricultural seasons. We used the Minimum Dietary Diversity indicator recommended by the WHO for assessing diets of 6- to 23-month-old children (DDS7) and the Minimum Dietary Diversity for Women of Reproductive Age indicator recommended by the FAO (DDS10). We found that both scores captured the typical dietary pattern of children in this population but that DDS10 was more effective for detecting seasonal shifts in dietary patterns. In longitudinal models, both scores were positively associated with overall nutrient adequacy, but had mixed relationships with usual nutrient intakes. For both scores, seasonal differences in the relationship to overall nutrient adequacy were observed, with the smallest change in MPA per one-food group change in DDS occurring in the late lean season. The strength of association with MPA was similar for the two scores, but the DDS10 score produced slightly stronger associations and these were more robust when controlling for energy intake. Both scores are acceptable for use as indicators of nutrient intake adequacy at the population level, but neither should be used as an indicator of usual intakes of

individual nutrients without population-specific modification and validation. DDS10 more often had higher predictive power and was a more consistent indicator across seasons.

Children consumed an average of 4 food groups per day whether the food groups were defined for DDS7 or DDS10. A typical Zambian meal consists of stiff maize porridge (nshima) with a savory side dish of leafy greens or fish. The savory side dishes are usually prepared with tomato and onion, resulting in high consumption of these vegetables. This dietary pattern is captured by both scores in the most commonly observed combinations of food groups. The mean scores of 4.1 using DDS7 and 4.4 using DDS10 we observed among 4- to 8-year-old children are similar to those reported in recent studies among similar age groups in other low- and middle-income countries.^{4-6,22,40}

Though the mean scores on DDS7 or DDS10 are similar to those observed elsewhere, the dietary pattern of four commonly consumed food groups we observed may not be comparable to 4-food group dietary patterns in other settings. In our Zambian study, other vegetables (DDS10) or other fruits and vegetables (DDS7) was the second most commonly consumed food group after grains, roots and tubers. In South Africa, Steyn et al. found that dairy was the second most common food group after starchy staples. Multi-site studies of DDS have analyzed dietary patterns and reported that the same dietary diversity score is associated with different dietary patterns in different countries.^{12,41}

Examination of the food groups consumed and counted toward each score not only reveals different dietary patterns between settings, but also illustrates differences in score performance in capturing seasonal dietary patterns. The most commonly consumed food groups from DDS7 – grains, roots and tubers; flesh foods; vitamin A rich fruits and vegetables; and other fruits and vegetables – largely overlap with the commonly consumed food groups as defined for DDS10.

The dark green leafy vegetables group in DDS10 is a subset of the vitamin A rich fruits and vegetables group in DDS7 and is the most commonly consumed type of food in this DDS7 group, so the two food groups capture similar patterns in the late post-harvest and late lean seasons. However, in the early lean season, differentiation in DDS10 between dark green leafy vegetables and other vitamin A rich fruits and vegetables captures changes in child diet associated with seasonally available fruits and vegetables, particularly mangoes. During the early lean season, there is a 7 point increase in the percent of children consuming vitamin A rich fruits and vegetables as defined by DDS7 and a 47 point increase in the percent of children consuming other vitamin A rich fruits and vegetables as defined by DDS10. Thus, while the two scores similarly tally the most commonly consumed foods in this population – maize, dark green leafy vegetables, tomatoes, onions and fish (Chapter 3) – greater differentiation between food groups in DDS10 enables identification of seasonal trends in dietary patterns.

We found that both DDS7 and DDS10 were significantly and positively associated with MPA. These findings are similar to the conclusions of previous reviews that DDS scores are consistently, positively related to nutrient adequacy or nutritional status among children despite variations in scoring methods.^{21,41,42} In unadjusted analyses, a one-food group increase in DDS7 or DDS10 was associated with increases in MPA of 0.04 or 0.05, respectively. Among Bangladeshi preschoolers, a 1-food group increase in a 9-food group score was associated with 0.1 unit increase in MPA in an unadjusted model.⁶ The associations we observed between DDS and MPA had larger coefficients than those found among Kenyan first graders, among whom a 1-food group increase in an 8-food group DDS was associated with an increase in MPA of 0.004.⁵ Comparisons of our findings on associations between DDS and usual micronutrient intakes to those reported by other recent studies are mixed. We found null or negative associations between DDS and usual vitamin A intakes, in contrast to a 0.05 $\mu\text{mol/L}$ change in serum retinol

concentration with a one-unit change in 10-food group DDS among women in rural Kenya, a correlation of $r=0.37$ between a 9-food group score and vitamin A intake adequacy among Filipino preschoolers and a correlation of $r=0.17$ between vitamin A adequacy and a 9-food group DDS among South African 4- to 9-year-olds.^{11,22,43} The study in the Philippines found that DDS was not significantly correlated with probabilities of calcium or vitamin B12 adequacy, though we found positive associations between DDS and these nutrients, as did the study in South Africa ($r=0.347$ for calcium, $r=0.112$ for vitamin B12).^{11,22} The studies in South Africa and the Philippines found positive correlations between DDS and vitamin C and iron intake adequacy.^{11,22} We found positive associations with between DDS and vitamin C intakes, but null or negative associations with iron intakes.

The discrepancies between our study and others that have examined associations between DDS and micronutrient intakes or adequacy may be due to dietary patterns specific to our population. That associations with usual iron intake are null or negative in unadjusted models may indicate substitution of foods lower in iron for iron-rich foods as more food groups are included in the diet. We have previously reported that the primary source of iron in this population is maize (Chapter 3). Iron is otherwise obtained in small quantities across a range of other foods. The even distribution of iron across foods in children's diets, as well as the absence of fortified or naturally high-iron foods, may explain the lack of positive association between DDS and usual iron intake. In contrast, small fish are the largest source of both calcium and vitamin B12 (Chapter 3) and are in the fourth most commonly consumed food group by either DDS, hence the increase in intakes as this food group is added to the diet. While important sources of vitamin B12 and calcium are likely to be added to the diet as diversity increases, dark green leafy vegetables are the key source of vitamin A and are consumed on one or more recall days by at least 84% of children in each season. The addition of food groups beyond the three

most common food groups may create a substitution effect away from this primary source of vitamin A. Though the next most common food group consumed – flesh foods (DDS7) or meat, poultry and fish (DDS10) – includes sources of vitamin A, these are likely be consumed in smaller quantities than dark leafy greens. Finally, the two largest sources of vitamin C in this population are dark leafy greens and tomatoes (Chapter 3). These are captured in the second and third most commonly consumed food groups. That DDS10 shows a much stronger correlation to vitamin C than does DDS7 may be due to the differentiation between other vegetables and other fruits, which enables DDS10 to reflect the addition of the next most important sources of vitamin C, mangoes and other fruit, to the diet.

We observed seasonal variation in mean DDS7, DDS10 and MPA. Shifts in usual nutrient intakes by season were significant and, for some nutrients, substantial. Though these seasonal changes in means are mostly small, they did result in significant seasonal differences in the strength of association between DDS and MPA or usual nutrient intakes. For both DDS7 and DDS10, association with MPA was weakest in the late lean season, dropping from a coefficient of 0.05 or 0.06 in the early lean season to a coefficient of 0.01. Both mean MPA and mean energy intakes were lowest in the late lean season. The flattening of the association between DDS and MPA illustrated in Figure 1 and in the regression coefficients in Table 5.4a may be reflective of an overall decrease in food quantities in the late lean season. Even as DDS increased in this season, portions may have been smaller, resulting in smaller increases to nutrient intake adequacy. This is supported by results from the supplemental analyses, in which positive associations between DDS10 and MPA remained significant when controlling for energy intake in the late post-harvest and early lean seasons but were not significant in the late lean season. The energy-controlled association between DDS7 and MPA was significant only in the early lean season. Associations with vitamin A and vitamin C were strongest in the early lean season, likely driven by the

seasonal availability of mangoes and other local fruits and vegetables described above. Few other studies have explicitly examined the effect of season on the relationship between DDS and MPA to provide comparison to our results. A study conducted among 2- to 4-year-old Bangladeshi children found no significant relationships between MPA and season.⁶

In addition to a linear association between DDS and MPA that varied by season, both DDS7 and DDS10 were fair predictors of MPA > 0.75 and predictive power, as indicated by AUC, varied by season. DDS10 performed more consistently across seasons, but for both scores, AUC was highest in the late lean season. In the late lean season, both scores produced the best predictive power in terms of balanced sensitivity and specificity at the cutoff recommended as an indicator of minimum dietary diversity by the WHO or the FAO. The AUC values we observed in the late post-harvest and early lean seasons were lower than those reported by other studies, though the difference may be due in part to our use of MPA > 0.75 as a cutoff. Steyn et al. reported AUC values between 0.8 and 0.9 when evaluating 6-, 9-, 13- or 21-food group DDS as predictors of mean adequacy ratio among 1- to 9-year-old South African children.⁴ The multi-site study informing development of the WHO minimum dietary diversity indicator found that DDS performed better at identifying low dietary quality (mean micronutrient density adequacy (MMDA) < 50%) than at identifying higher dietary quality (MMDA \geq 75%).¹² The range of MPA observed in this population was not low enough to evaluate DDS as a predictor of MPA > 0.5. We have previously reported low between-subject variance in nutrient intakes among children in our study sample (Chapter 4). Given this similarity of diet among children, there was a narrow range of MPA across which to test the association and predictive power of DDS7 and DDS10. Our sample of 200 children in a rural district of Zambia does not capture a wide range of dietary patterns and intakes, and this is a potential limitation. However, the dietary recalls

repeated at regular intervals over six months provided a rich, long-term dietary dataset enabling seasonal analyses and are a key strength of this study.

Conclusion

We conclude that of the two scores currently recommended by UN organizations for the assessment of dietary diversity at the population level, the 10-food group score recommended by the FAO for use among women of reproductive age is a better choice for assessing diets of apparently healthy, 4- to 8-year-old Zambian children than is the 7-food group score recommended by the WHO for use among 6- to 23-month-old children. As the same data collection method is used for both scores, these gains can be achieved without additional survey time or costs. Surveys or program evaluations using either of these scores should account for potential seasonal impacts on both mean score values and strength as an indicator of overall micronutrient adequacy. This is particularly the case in rural, low- and middle-income-country settings where diets may change seasonally. Further studies are needed to validate and recommend dietary diversity scores as indicators of nutrient intake adequacy among preschool- and school-age children, and these should include evaluation of seasonal effects.

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Tables & Figures

Table 5.1. Food groups in the World Health Organization’s Minimum Dietary Diversity indicator and in the Food and Agriculture Organization’s Minimum Dietary Diversity for Women of Reproductive Age indicator^{16,17}

| MDD* | MDD-W** |
|--------------------------------------|---|
| Grains, roots and tubers | Grains, white roots and tubers, and plantains |
| Legumes and nuts | Pulses (beans, peas and lentils) |
| | Nuts and seeds |
| Dairy products | Dairy |
| Flesh foods | Meat, poultry and fish |
| Eggs | Eggs |
| Vitamin A-rich fruits and vegetables | Dark green leafy vegetables |
| | Other vitamin A-rich fruits and vegetables |
| Other fruits and vegetables | Other vegetables |
| | Other fruits |

*Minimum Dietary Diversity indicator, referred to as DDS7 in text

**Minimum Dietary Diversity for Women of Reproductive Age indicator, referred to as DDS10 in text

Table 5.2. Dietary diversity score, usual nutrient intakes and mean probability of nutrient intake adequacy (mean \pm standard error), overall and by season, among 4- to 8-year-old children participating in the non-intervened arm of a provitamin A maize efficacy trial in Mkushi, Zambia, 2012 – 2013

| | Overall | Late post-harvest season (Aug–Oct) | Early lean season (Nov–Jan) | Late lean season (Feb–Apr) |
|---|------------------|---|------------------------------------|-----------------------------------|
| 7-food group DDS* | 4.11 \pm 0.03 | 3.99 \pm 0.05 ^a | 4.18 \pm 0.04 ^b | 4.15 \pm 0.05 ^c |
| 10-food group DDS* | 4.39 \pm 0.03 | 4.19 \pm 0.06 ^a | 4.55 \pm 0.05 ^b | 4.43 \pm 0.06 ^b |
| MPA** | 0.75 \pm 0.001 | 0.75 \pm 0.002 ^a | 0.77 \pm 0.002 ^b | 0.72 \pm 0.002 ^c |
| Energy intake (kcal/d) | 1537 \pm 3 | 1511 \pm 6 ^a | 1603 \pm 5 ^b | 1497 \pm 6 ^a |
| Vitamin A intake (μ g RAE [†] /d) | 485 \pm 1 | 553 \pm 2 ^a | 506 \pm 1 ^b | 395 \pm 2 ^c |
| Vitamin B12 intake (μ g/d) | 0.82 \pm 0.005 | 0.88 \pm 0.009 ^a | 0.87 \pm 0.007 ^a | 0.72 \pm 0.009 ^b |
| Vitamin C intake (mg/d) | 66 \pm 0.6 | 55 \pm 1.1 ^a | 97 \pm 0.8 ^b | 46 \pm 1.1 ^c |
| Iron intake (mg/d) | 10.3 \pm 0.02 | 10.8 \pm 0.04 ^a | 10.7 \pm 0.03 ^b | 9.3 \pm 0.04 ^c |
| Calcium intake (mg/d) | 260 \pm 1 | 273 \pm 1 ^a | 277 \pm 1 ^b | 229 \pm 1 ^c |

*Dietary diversity score

**Mean probability of adequacy, the average probability of adequacy [Pr(requirement \leq intake)] across 11 micronutrients

†RAE: Retinol Activity Equivalent

^{a,b,c}: Different letters indicate significant difference between seasons tested using one-way ANOVA ($p \leq 0.01$).

Table 5.3. Percent of children consuming each food group on at least one dietary recall day during each season*, among 4- to 8-year-old children participating in the non-intervened arm of a provitamin A maize efficacy trial in Mkushi, Zambia, 2012 – 2013

| | Late post-harvest season (Aug–Oct) | Early lean season (Nov–Jan) | Late lean season (Feb–Apr) |
|---|---|--|---------------------------------------|
| 7-food group dietary diversity score | | | |
| Grains, roots and tubers | 100 | 100 | 100 |
| Legumes and nuts | 39 | 56 | 56 |
| Dairy products | 13 | 30 | 17 |
| Flesh foods | 84 | 94 | 85 |
| Eggs | 16 | 27 | 15 |
| Vitamin A-rich fruits and vegetables | 89 | 96 | 91 |
| Other fruits and vegetables | 99 | 100 | 100 |
| 10-food group dietary diversity score | | | |
| Grains, white roots and tubers, and plantains | 100 | 100 | 100 |
| Pulses (beans, peas and lentils) | 25 | 45 | 52 |
| Nuts and seeds | 18 | 23 | 13 |
| Dairy | 13 | 30 | 17 |
| Meat, poultry and fish | 85 | 94 | 85 |
| Eggs | 16 | 27 | 15 |
| Dark green leafy vegetables | 88 | 84 | 89 |
| Other vitamin A-rich fruits and vegetables | 13 | 60 | 23 |
| Other vegetables | 99 | 100 | 100 |
| Other fruits | 11 | 27 | 17 |

*In late post-harvest season n= 263 recalls among 187 children; early lean season n=506 recalls among 186 children; late lean season n=302 recalls among 178 children

Table 5.4a. Regression coefficients from longitudinal models of mean probability of adequacy and usual calcium and iron intakes on dietary diversity score and season, among 4- to 8-year-old children participating in the non-intervened arm of a biofortified maize efficacy trial, Mkushi, Zambia, 2012 - 2013

| | DDS7* | | DDS10** | |
|-------------------------------------|---------------------|---------------------|---------------------|---------------------|
| | Model 1 | Model 2 | Model 1 | Model 2 |
| Mean probability of adequacy | | | | |
| Intercept | 0.58 [‡] | 0.67 [‡] | 0.55 [‡] | 0.68 [‡] |
| Dietary diversity score (DDS) | 0.04 [‡] | 0.01 [†] | 0.05 [‡] | 0.01 [†] |
| Late post-harvest season | | -0.16 [‡] | | -0.35 [‡] |
| Early lean season | | -0.14 [‡] | | -0.19 [‡] |
| Late lean season | | -- | | -- |
| DDS*Late post-harvest season | | 0.05 [‡] | | 0.09 [‡] |
| DDS*Early lean season | | 0.05 [‡] | | 0.06 [‡] |
| DDS*Late lean season | | -- | | -- |
| Iron intake (mg/d) | | | | |
| Intercept | 11.52 [‡] | 8.04 [‡] | 9.62 [‡] | 7.97 [‡] |
| DDS | -0.29 [†] | 0.29 | 0.16 | 0.29 [‡] |
| Late post-harvest season | | 3.28 [‡] | | 2.85 [‡] |
| Early lean season | | 1.79 | | 0.12 |
| Late lean season | | -- | | -- |
| DDS*Late post-harvest season | | -0.41 [‡] | | -0.29 [†] |
| DDS*Early lean season | | -0.08 | | 0.29 |
| DDS*Late lean season | | -- | | -- |
| Calcium intake (mg/d) | | | | |
| Intercept | 228.62 [‡] | 183.09 [‡] | 202.05 [‡] | 192.82 [‡] |
| DDS | 8.25 [†] | 10.99 [‡] | 13.70 [‡] | 8.11 [‡] |
| Late post-harvest season | | 14.45 | | -47.55 |
| Early lean season | | -7.75 | | -23.62 |
| Late lean season | | -- | | -- |
| DDS*Late post-harvest season | | 7.99 | | 22.09 [‡] |
| DDS*Early lean season | | 13.49 [†] | | 15.86 [‡] |
| DDS*Late lean season | | -- | | -- |

*7-food group dietary diversity score

**10-food group dietary diversity score

†p<0.05

‡p<0.01

Table 5.4b. Regression coefficients from longitudinal models of usual vitamin A, vitamin B12 and vitamin C intakes on dietary diversity score and season, among 4- to 8-year-old children participating in the non-intervened arm of a biofortified maize efficacy trial, Mkushi, Zambia, 2012 – 2013

| | DDS7* | | DDS10** | |
|--|---------------------|---------------------|---------------------|---------------------|
| | Model 1 | Model 2 | Model 1 | Model 2 |
| Vitamin A intake (µg RAE[¶]/d) | | | | |
| Intercept | 619.35 [‡] | 387.06 [‡] | 573.91 [†] | 376.68 [‡] |
| Dietary diversity score (DDS) | -32.24 [‡] | 1.93 | -19.78 [†] | 4.15 |
| Late post-harvest season | | 102.01 [‡] | | 133.77 [‡] |
| Early lean season | | -9.5 | | -17.22 |
| Late lean season | | -- | | -- |
| DDS*Late post-harvest season | | 14.00 [†] | | 5.89 |
| DDS*Early lean season | | 29.07 [‡] | | 28.5 [‡] |
| DDS*Late lean season | | -- | | -- |
| Vitamin B12 intake (µg/d) | | | | |
| Intercept | 0.40 [‡] | 0.30 | 0.56 [‡] | 0.54 [‡] |
| DDS | 0.10 [‡] | 0.10 [‡] | 0.06 [‡] | 0.04 |
| Late post-harvest season | | -0.24 | | -0.95 [†] |
| Early lean season | | -0.07 | | -0.08 |
| Late lean season | | -- | | -- |
| DDS*Late post-harvest season | | 0.10 | | 0.26 [‡] |
| DDS*Early lean season | | 0.05 | | 0.05 |
| DDS*Late lean season | | -- | | -- |
| Vitamin C intake (mg/d) | | | | |
| Intercept | 30.16 [†] | 64.62 [‡] | -72.62 [‡] | 53.35 [‡] |
| DDS | 10.24 [‡] | -4.38 [†] | 32.78 [‡] | -1.57 |
| Late post-harvest season | | 13.99 | | 1.63 |
| Early lean season | | -3.78 | | -83.64 [‡] |
| Late lean season | | -- | | -- |
| DDS*Late post-harvest season | | -1.37 | | 1.68 |
| DDS*Early lean season | | 13.03 [‡] | | 29.83 [‡] |
| DDS7*Late lean season | | -- | | -- |

*7-food group dietary diversity score

**10-food group dietary diversity score

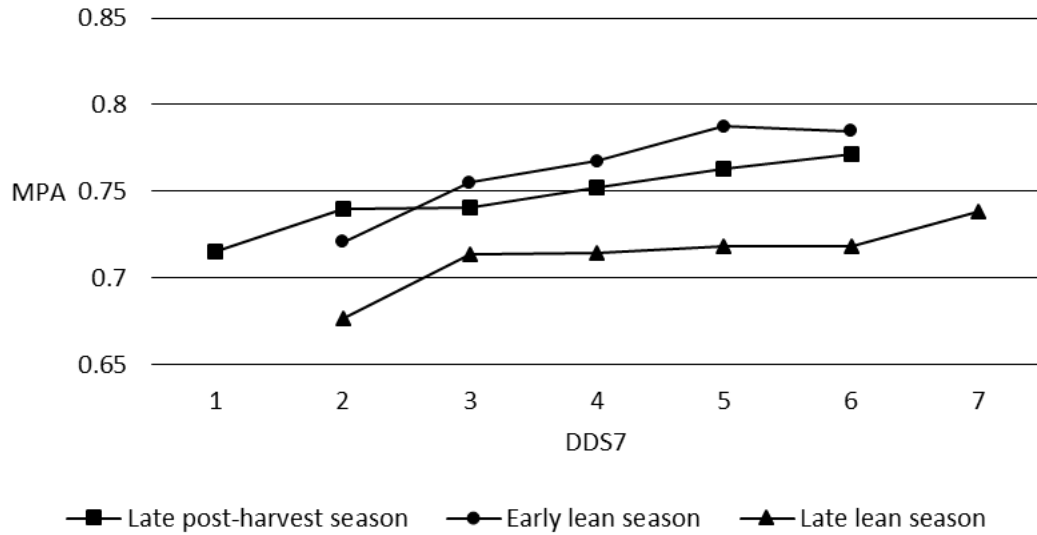
†p<0.05

‡p<0.01

¶ Retinol Activity Equivalent

Figure 5.1. Mean probability of adequacy by observed dietary diversity score, by season, among 4- to 8-year-old children participating in the non-intervened arm of a biofortified maize efficacy trial, Mkushi, Zambia, 2012 - 2013

1a. Mean probability of adequacy (MPA) by 7-food group dietary diversity score (DDS7)



1b. Mean probability of adequacy (MPA) by 10-food group dietary diversity score (DDS10)

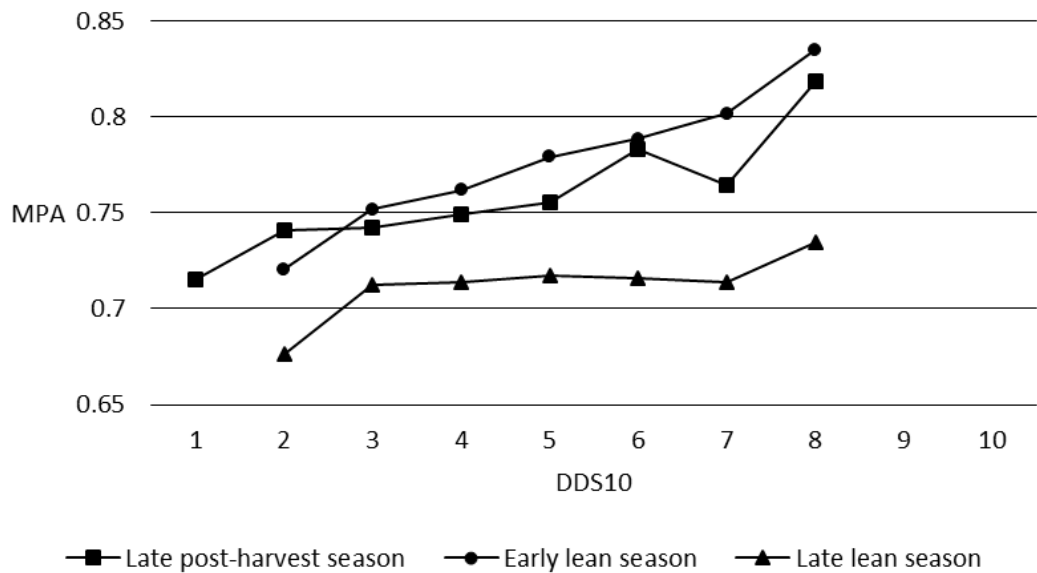
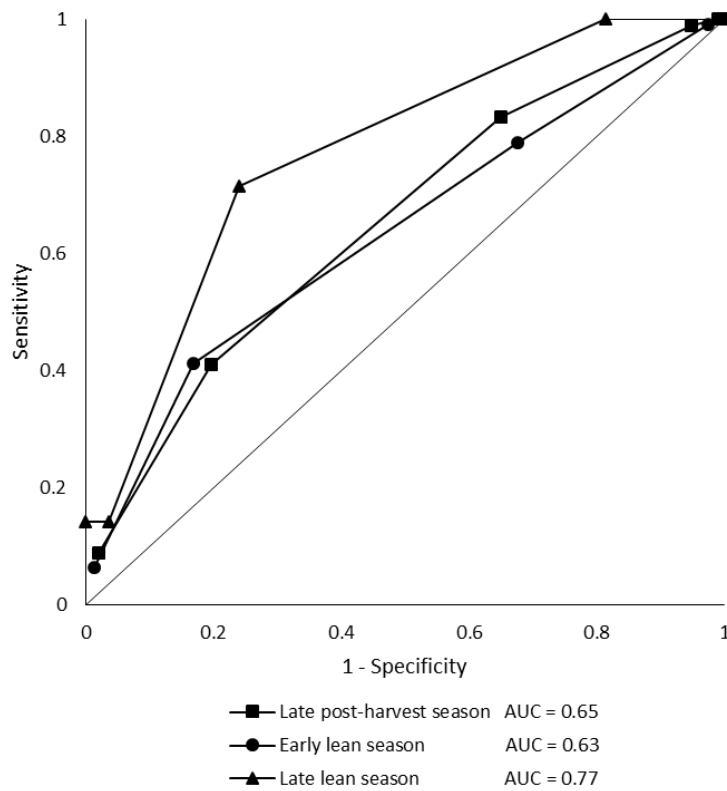


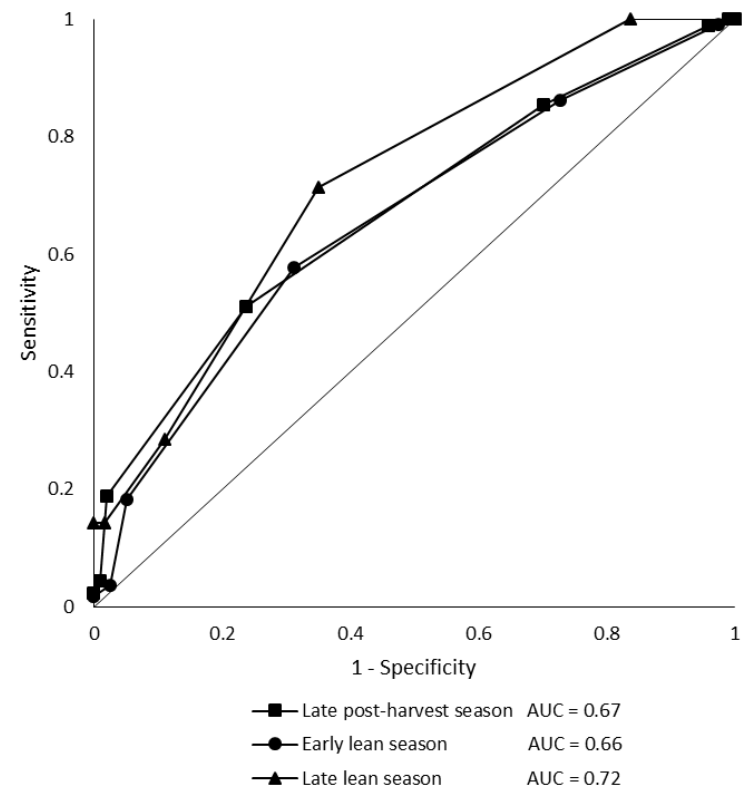
Figure 5.2. Receiver operating characteristics curves for observed 7- or 10-food group dietary diversity score as predictor of mean probability of adequacy > 0.75, by season, among 4- to 8-year-old children participating in the non-intervened arm of a biofortified maize efficacy trial, Mkushi, Zambia, 2012 - 2013

Figure 2a. DDS7 as predictor of MPA > 0.75, by season



DDS7: 7-food group dietary diversity score
 DDS10: 10-food group dietary diversity score

Figure 2b. DDS10 as predictor of MPA > 0.75, by season



MPA: Mean probability of adequacy
 AUC: Area under the radio operating characteristics curve

Supplemental Table 5.1a. Regression coefficients from energy-adjusted longitudinal models of mean probability of adequacy and usual calcium and iron intakes on dietary diversity score and season, among 4- to 8-year-old children participating in the non-intervened arm of a biofortified maize efficacy trial, Mkushi, Zambia, 2012 - 2013

| | DDS7* | | DDS10** | |
|-------------------------------------|---------------------|---------------------|---------------------|---------------------|
| | Model 1 | Model 2 | Model 1 | Model 2 |
| Mean probability of adequacy | | | | |
| Intercept | 0.36 [†] | 0.42 [†] | 0.34 [†] | 0.45 [†] |
| Dietary diversity score (DDS) | 0.00 | 0.00 | 0.01 | 0.00 |
| Energy intake (kcal/d) | 0.0002 [‡] | 0.0002 [‡] | 0.0002 [‡] | 0.0002 [‡] |
| Late post-harvest season | | 0.00 | | -0.08 |
| Early lean season | | -0.11 [†] | | -0.14 [†] |
| Late lean season | | -- | | -- |
| DDS*Late post-harvest season | | 0.01 | | 0.03 [†] |
| DDS*Early lean season | | 0.03 [†] | | 0.04 [†] |
| DDS*Late lean season | | -- | | -- |
| Iron intake (mg/d) | | | | |
| Intercept | 8.16 [†] | 5.24 [†] | 6.59 [†] | 5.42 [†] |
| DDS | -0.89 [†] | 0.15 | -0.41 [†] | 0.18 |
| Energy intake (kcal/d) | 0.004 [†] | 0.002 [†] | 0.004 [†] | 0.002 [†] |
| Late post-harvest season | | 5.09 [†] | | 5.75 [†] |
| Early lean season | | 2.17 [†] | | 0.7 |
| Late lean season | | -- | | -- |
| DDS*Late post-harvest season | | -0.88 [†] | | -0.98 [†] |
| DDS*Early lean season | | -0.23 | | 0.11 |
| DDS*Late lean season | | -- | | -- |
| Calcium intake (mg/d) | | | | |
| Intercept | 127.77 [†] | 117.08 [†] | 108.71 [†] | 130.60 [†] |
| DDS | -9.82 [†] | 7.62 [†] | -3.97 | 5.35 [†] |
| Energy intake (kcal/d) | 0.11 [†] | 0.05 [†] | 0.11 [†] | 0.05 [†] |
| Late post-harvest season | | 57.06 [†] | | 23.25 |
| Early lean season | | 1.21 | | -9.4 |
| Late lean season | | -- | | -- |
| DDS*Late post-harvest season | | -2.91 | | 5.18 |
| DDS*Early lean season | | 9.95 [†] | | 11.55 [†] |
| DDS*Late lean season | | -- | | -- |

*7-food group dietary diversity score

**10-food group dietary diversity score

†p<0.05

‡p<0.01

Supplemental Table 5.1b. Regression coefficients from energy-adjusted longitudinal models of usual vitamin A, vitamin B12 and vitamin C intakes on dietary diversity score and season, among 4- to 8-year-old children participating in the non-intervened arm of a biofortified maize efficacy trial, Mkushi, Zambia, 2012 – 2013

| | DDS7* | | DDS10** | |
|--|---------------------|---------------------|----------------------|---------------------|
| | Model 1 | Model 2 | Model 1 | Model 2 |
| Vitamin A intake (µg RAE[¶]/d) | | | | |
| Intercept | 467.53 [‡] | 336.75 [‡] | 425.68 [‡] | 330.01 [‡] |
| Dietary diversity score (DDS) | -59.45 [‡] | -0.63 | -47.83 [‡] | 2.08 |
| Energy intake (kcal/d) | 0.17 [‡] | 0.04 [‡] | 0.18 [‡] | 0.04 [‡] |
| Late post-harvest season | | 134.49 [‡] | | 186.87 [‡] |
| Early lean season | | -2.68 | | -6.56 |
| Late lean season | | -- | | -- |
| DDS*Late post-harvest season | | 5.7 | | -6.79 |
| DDS*Early lean season | | 26.37 [‡] | | 25.27 [‡] |
| DDS*Late lean season | | -- | | -- |
| Vitamin B12 intake (µg/d) | | | | |
| Intercept | -0.09 | -0.22 | 0.06 | -0.02 |
| DDS | 0.01 | 0.07 | -0.03 | 0.02 |
| Energy intake (kcal/d) | 0.0006 [‡] | 0.0004 [‡] | 0.0006 [‡] | 0.0004 [‡] |
| Late post-harvest season | | 0.1 | | -0.31 |
| Early lean season | | 0.00 | | 0.05 |
| Late lean season | | -- | | -- |
| DDS*Late post-harvest season | | 0.02 | | 0.11 |
| DDS*Early lean season | | 0.03 | | 0.01 |
| DDS*Late lean season | | -- | | -- |
| Vitamin C intake (mg/d) | | | | |
| Intercept | -86.18 [‡] | 5.93 | -166.47 [‡] | 10.69 |
| DDS | -10.6 [‡] | -7.37 [‡] | 15.02 [‡] | -3.46 [‡] |
| Energy intake (kcal/d) | 0.13 [‡] | 0.05 [‡] | 0.11 [‡] | 0.03 [‡] |
| Late post-harvest season | | 51.87 [‡] | | 50.17 [‡] |
| Early lean season | | 4.19 | | -73.9 [‡] |
| Late lean season | | -- | | -- |
| DDS*Late post-harvest season | | -11.06 [‡] | | -9.91 [‡] |
| DDS*Early lean season | | 9.88 [‡] | | 26.88 [‡] |
| DDS*Late lean season | | -- | | -- |

*7-food group dietary diversity score

**10-food group dietary diversity score

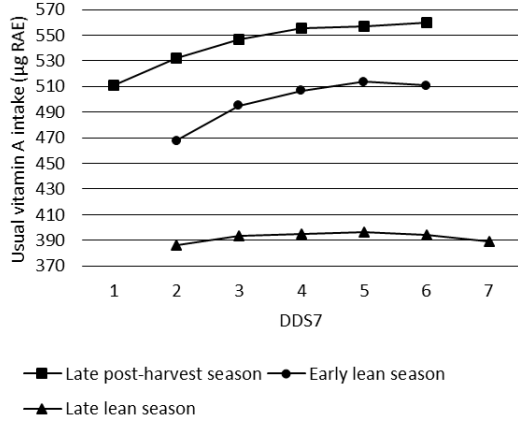
[†]p<0.05

[‡]p<0.01

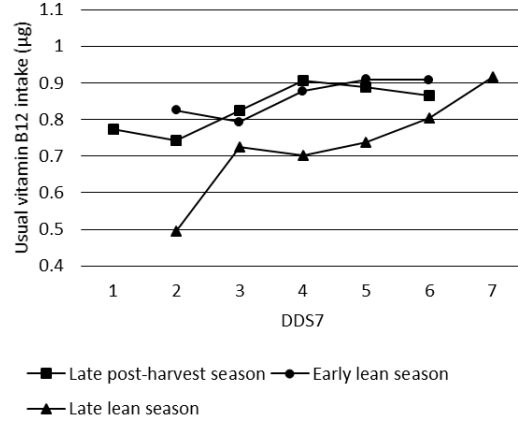
[¶] Retinol Activity Equivalent

Supplemental Figure 5.1. Usual nutrient intakes by 7-food group dietary diversity score (DDS7), by season, among 4- to 8-year-old children participating in the non-intervened arm of a biofortified maize efficacy trial, Mkushi, Zambia, 2012 - 2013

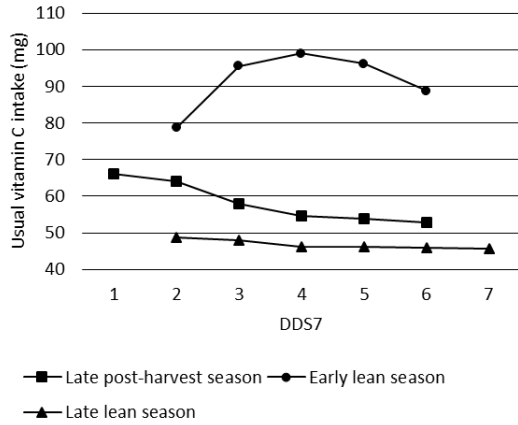
1a. Usual vitamin A intake by DDS7



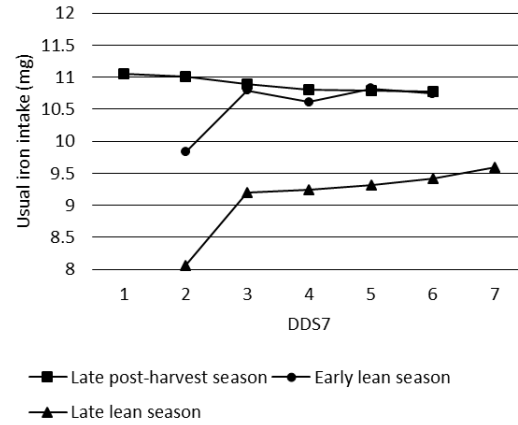
1b. Usual vitamin B12 intake by DDS7



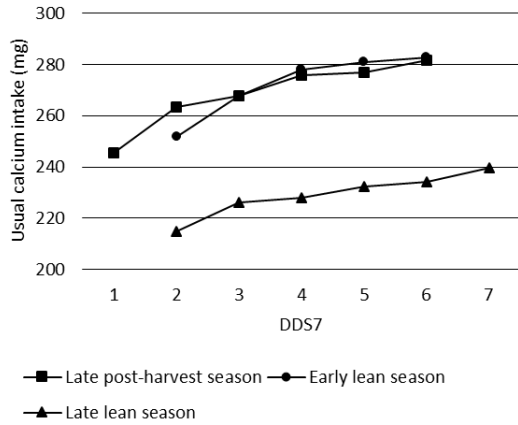
1c. Usual vitamin C intake by DDS7



1d. Usual iron intake by DDS7

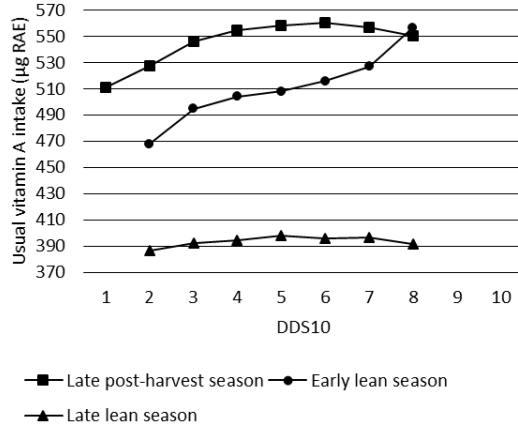


1e. Usual calcium intake by DDS7

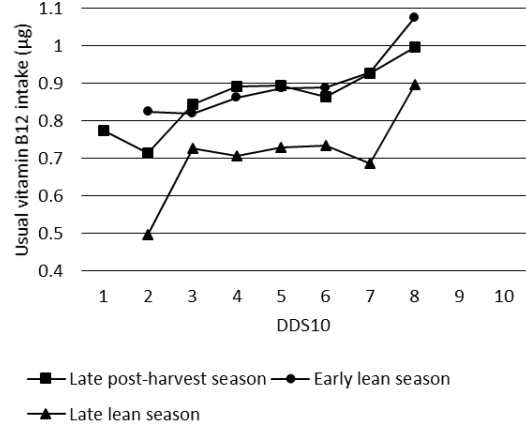


Supplemental Figure 5.2. Usual nutrient intakes by 10-food group dietary diversity score (DDS10), by season, among 4- to 8-year-old children participating in the non-intervened arm of a biofortified maize efficacy trial, Mkushi, Zambia, 2012 - 2013

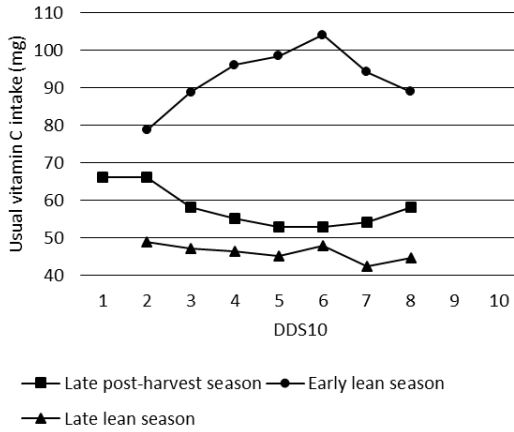
2a. Usual vitamin A intake by DDS10



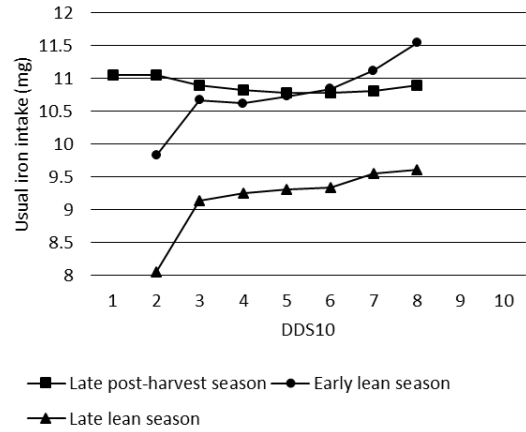
2b. Usual vitamin B12 intake by DDS10



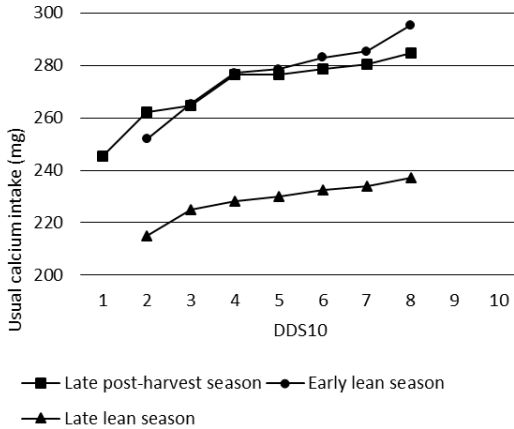
2c. Usual vitamin C intake by DDS10



2d. Usual iron intake by DDS10



2e. Usual calcium intake by DDS10



Chapter 6. Conclusions

Adequate intakes of micronutrients are necessary for healthy physical and cognitive growth and development among preschool- and early primary school-age children, but undernutrition in this age group is common, particularly in low- and middle-income countries.¹⁻³ Globally, 27% of children under five have impaired height growth, one third are vitamin A deficient and nearly half are anemic.⁴⁻⁶ Among school-age children in low- and middle-income countries, 20% are stunted, 15% have low body mass index for age, and 25% are anemic.^{5,1} Data on the diets of children in this age group are needed: to identify which nutrients are consumed in inadequate amounts; to plan nutrition interventions to improve intake adequacy; and to monitor population-level changes in dietary quality. Data on nutrient intake variance are needed to plan future studies in similar populations.

We used data collected in an efficacy trial of pro-vitamin A biofortified maize to study the diets of 4- to 8-year-old children in rural Zambia. Children in this age range living in Mkushi district, Central province, Zambia, were enrolled in the efficacy trial by geographic cluster. Clusters were assigned by block randomization to a treatment group that received meals of biofortified maize, a positive control group that received meals of regular Zambian maize and a non-intervened group that received an equivalent food package at the end of the trial. The trial protocol included seven 24-hour recalls. Recalls were conducted at baseline and then monthly over the course of the six-month trial. Of 1226 children enrolled in the efficacy trial, the 202 children assigned to the non-intervened group were included in this diet study, to study typical diets unaltered by the meals provided to the treatment and positive control groups. We used data from the 24-hour recalls conducted among this sub-sample to study the adequacy, variability and diversity of child diet over six months covering three agricultural seasons.

We met three aims in this study. First, we described the usual nutrient intakes and dietary patterns of 4- to 8-year-old Zambian children, identifying nutrients for which they are at highest risk of inadequate intakes and documenting the underlying food consumption patterns. Second, we quantified the within-person, between-person and seasonal components of nutrient intake variance. Finally, we tested the performance of two recommended dietary diversity scores as indicators of overall nutrient intake adequacy and usual intakes of five key micronutrients during three seasons. This chapter presents the conclusions of each aim, the strengths and limitations of the study, program and policy implications and directions for future research.

Conclusions from Aim 1

Inadequate intakes of calcium, vitamin B12, iron and folate are common among 4- to 8-year-old rural Zambian children. These inadequate intakes occur in association with a diet of mostly plant-based foods with limited consumption of fortified or animal source foods.

In our study of usual nutrient intakes and food consumption patterns among 4- to 8-year-old Zambian children, we found that usual intakes of energy and macronutrients were adequate for most children in the study. The median usual energy intake was 1535 kcal/d and though 26% of children had carbohydrate intakes above the Acceptable Macronutrient Distribution Range (AMDR), only 2% fell below the AMDR for protein intakes and 7% fell below the AMDR for fat intakes.⁷ Median usual energy intakes we observed were similar to those reported for this age group in other low- and middle-income countries and in a prior study conducted in Zambia, and energy intakes exceeded estimated energy requirements for this age group.⁸⁻¹²

Prevalence of inadequate usual micronutrient intakes were highest for calcium, vitamin B12, iron and folate. Nearly 100% of children had inadequate calcium intakes. Rates of inadequate intakes of vitamin B12, folate and iron were 76%, 57% and 25%, respectively. Several previous

studies in sub-Saharan Africa, including an earlier survey of 2- to 5-year-old children in Mkushi and Nyimba districts in Zambia, have also reported high prevalence of calcium inadequacy.¹³⁻¹⁶

Further, an analysis of the amount of calcium available in the Zambian national food supply indicates a very high, population-level risk of calcium intake inadequacy.¹⁷ Vitamin B12

inadequacy is also a risk among children in sub-Saharan Africa, though results for vitamin B12 inadequacy in dietary surveys are not as consistent as those found for calcium.^{13,16} High

prevalence of vitamin B12 deficiency as determined by plasma vitamin B12 concentrations has been reported among Kenyan school-age children and preschool-age children in Cameroon.^{18,19}

Similar results for prevalence of inadequate iron intake have also been reported.^{13,16}

Children consumed mostly plant-based foods, and intakes of animal sources foods or fortified foods were limited. Maize provided over half of children's total energy intake, on average, and was the biggest contributor to intakes of protein, carbohydrates and several micronutrients. Of the 10 most commonly consumed foods, 6 were plant foods commonly grown in Mkushi. Small fish were the most commonly consumed animal source food. Eggs, milk and chicken were infrequently consumed.

Our analysis of usual nutrient intakes and prevalence of nutrient intake inadequacy contributes to a limited body of recent studies with estimates of energy and nutrient intakes or nutrient

intake adequacy among early school-age children in sub-Saharan Africa.^{8,13-15,20-22} Our estimates of high prevalence of calcium, vitamin B12 and iron inadequacy, coupled with

descriptions of commonly consumed foods and key sources of each nutrient, illustrate how a

heavily plant-based diet results in nutritional risks to children in this population. Calcium

inadequacy may impair bone growth or result in low bone mineral density.²³ Vitamin B12, folate

and iron deficiencies are associated with anemia and with risk of impaired cognitive function,

and supplementation trials have shown beneficial impact on measures of cognitive

performance.²⁴⁻²⁷ These deficiencies are also nutritional causes of anemia, which causes fatigue, cognitive impairment and reduced work capacity.²⁸

Conclusions from Aim 2

Mean energy and nutrient intakes varied by season and season contributed substantially to total nutrient intake variance. Within- to between-person variance ratios were high in comparison to those observed in other populations due to low between-person variance.

Documentation of the components of nutrient intake variance is useful to nutrition researchers for planning future diet studies to accurately estimate usual nutrient intakes in similar populations. To test the importance of planning for seasonal impacts on nutrient intakes or nutrient intake variance when assessing diet in low- and middle-income countries, we estimated mean nutrient intakes by season, associations between nutrient intakes and season and child- and household-level factors, and seasonal, within- and between-person components of nutrient intake variance.

Nutrient intakes varied significantly by season, and season contributed 3%–23% of total nutrient intake variance. For most nutrients, intakes were higher in the early lean season than in the late post-harvest or late lean seasons. Food and nutrient intakes have been similarly found to vary seasonally among 6- to 8-year-old children in rural Benin, 6- to 17-year-old children in Kenya and 3- to 6-year-old children in rural Ghana and Malawi.^{21,27,29} The contribution of season to total nutrient intake variance was much larger in this population than in other populations for which it has been reported. Season was found to account for less than 2% of variance among overweight and obese US adults or less than 3% of variance among Japanese women.^{30,31} Nyambose et al. reported that within- to between-subject variance ratios differed by season among pregnant Malawian women, and for most nutrients were highest in the post-harvest

season.³² Our findings and those of previous studies on seasonal differences in food and nutrient intakes or within- to between-person intake variance ratios support a recommendation that seasonal effects should be planned for in studies of dietary intakes in sub-Saharan Africa. In addition to our findings of seasonal changes in nutrient intakes and intake variance, we found that within- to between-person variance ratios among rural Zambian children were much higher than those reported for other populations, with the exception of two older studies among children in the US and the UK.^{32–38} However, the within-person coefficients of variance we observed were comparable to those reported for children in other countries or among other age groups in sub-Saharan Africa.^{35,36,39} In contrast, the between-person coefficients of nutrient intake variance were much lower than those reported elsewhere.^{32,35–37} The high within- to between-person variance ratios observed in this population are driven by relative homogeneity of nutrient intakes between children. This may be a feature of diet shared with other populations that do not have a wide range of socio-economic status, dietary patterns or food access and is an important consideration in planning sampling and analyses in future studies.

Conclusions from Aim 3

Both 10-food group and 7-food group dietary diversity scores were significantly associated with overall nutrient intake adequacy among 4- to 8-year old rural Zambian children. The strength of association varied by season. The 10-food group score is recommended over the 7-food group score as a population-level indicator of dietary adequacy.

For population-level monitoring of dietary quality, identification of groups at high risk of micronutrient inadequacies or evaluation of large-scale nutrition and food security programs, estimating usual food and nutrient intakes is often infeasible due to high time and resource costs of conducting 24-hour recalls or food records. Rapid assessments of dietary quality are

more feasible. Dietary diversity scores have been recommended for such assessments among infants and young children 6 to 23 months of age and among women of reproductive age.^{40,41} Though these and similar scores have been validated against nutrient intakes or nutritional status among children under five years old and women of reproductive age, few studies have validated dietary diversity scores as indicators of nutrient adequacy among early school-age children.^{13,15,42–53} Even fewer studies have compared the performance of these scores in different seasons, though food and nutrient intakes among children in sub-Saharan Africa have been shown to vary seasonally.^{21,29,43,54}

We used two recommended dietary diversity scores – the Minimum Dietary Diversity indicator for 6- to 23-month-old children recommended by the World Health Organization, and the Minimum Dietary Diversity for Women of Reproductive Age indicator recommended by the Food and Agriculture Organization – to describe dietary diversity among rural Zambian children in three seasons. The score for use among 6- to 23-month-old children is based on seven food groups and is hereafter referred to as DDS7. The score for use among women is based on ten food groups and is hereafter referred to as DDS10. We estimated the association of each score to mean probability of nutrient intake adequacy (MPA) and usual intakes of five key micronutrients in three agricultural seasons. We also plotted radio operating characteristics curves to test the performance of each score as a predictor of $MPA > 0.75$ in each season.

Using either score, children were found, on average, to consume foods from 4 food groups each day. This is similar to results reported for preschool- and early school-age children in other low- and middle-income countries.^{13,15,43,51,55} Though mean values of DDS7 and DDS10 were similar, examination of the percent of children consuming foods from each food group in each season revealed seasonal changes in dietary patterns detectable with DDS10 but not with DDS7. The greater disaggregation of food groups in DDS10 captured changes in fruit and vegetable

consumption. Mean DDS7, DDS10, usual nutrient intakes and MPA differed significantly by season.

Both scores were significantly, positively associated with MPA, though the strength of this association varied by season. The finding of association with MPA is consistent with results from validation studies using each score among the recommended age group, though few other studies have examined this association over multiple seasons.^{48,50} Across the three seasons, DDS10 had stronger correlations with MPA and higher and more consistent power for predicting MPA over 0.75. Similar to our findings comparing the two DDS, other authors have concluded that dietary diversity scores with greater disaggregation of food groups perform better than those with fewer, more consolidated food groups.^{47,50} However, the ability of either score to predict whether an individual's MPA was greater than 0.75 was fair to poor, so neither is recommended as an individual-level indicator of dietary quality. This result is in keeping with the recommended use of either score as a population-level assessment and not as a measure of the dietary adequacy of individuals.^{40,41,48,50} Associations with nutrient intakes were mixed, and based on our results neither score can be recommended as an indicator of intakes of individual nutrients.

Strengths and limitations

A key strength of this study is the number and timing of repeat 24-hour dietary recalls completed for each of 200 included children. We collected up to seven recalls per child, evenly spaced over six months. This sample size and the replicates over three agricultural seasons allowed us to estimate usual nutrient intakes over six months as well as detect seasonal changes in diet over that time period. This ability to describe changes over time is unusual among dietary intake studies, which often collect fewer replicates spaced much closer together in time.

A second important strength of this study is our estimation of usual nutrient intakes for describing prevalence of inadequate intakes in Chapter 3 and when evaluating the relationship between DDS and intakes or intake adequacy in Chapter 5. Other recent studies reporting nutrient intakes or nutrient intake adequacy among early school-age children in sub-Saharan Africa reported observed intakes from a single day or average intake over 2–3 days.^{8,13,14,20,22} In contrast, we used SAS macros provided by the National Cancer Institute (NCI) for estimation of usual intake distributions.⁵⁶ This likely reduced bias in our estimates of prevalence of nutrient intake inadequacy.^{57–59} The NCI method has been shown to produce more accurate estimates of usual intakes than does the average of observed intakes.⁵⁷ Two simulation studies have further found that NCI performs comparably well to methods for usual intake estimation from other research groups.^{59,60} Finally, use of the NCI procedures enabled us to estimate individual usual nutrients in order to reduce bias in our estimates of association between DDS and usual nutrient intakes or nutrient intake adequacy in Chapter 5.^{56,61}

Though our collection of 24-hour dietary recalls over six months is a strength of this study, our lack of data on diet over a full year or over multiple years is a limitation. A full year of dietary data would have allowed us to characterize food and nutrient intakes, intake variance and DDS performance in the main harvest and post-harvest seasons. We have attributed the temporal changes we observed in nutrient intakes to season. This is highly plausible based on the agricultural calendar and availability of local fruit and vegetable crops. However, we cannot eliminate the possibility that the temporal changes are due to some other, unmeasured time-varying effect on food supplies or diet. Our findings of seasonal variation remain informative for future studies. We have demonstrated the need to plan for seasonal or other temporal effects on diet in study design in rural, low- and middle-income-country settings, where such effects

may not be the same as those we observed in rural Zambia but may occur in population-specific ways that impact usual intake estimation.

A final, potential limitation of our study is the low between-person nutrient intake variance captured in our 24-hour recall data. The design of the efficacy trial under which our data were collected provided repeat dietary measures which are a key strength, but also meant our sample is geographically limited and more homogeneous in nutrient intakes than other populations.^{32,35-37} Low between-person variance in observed intakes may have resulted in estimated usual nutrient intake distributions with inaccurately low variance. When within-person variance is large relative to between-person variance, the usual intake estimation approach shrinks intakes more toward the overall mean and further from within-person means, resulting in a very narrow estimated usual nutrient intake distribution.⁶² Failure to adjust for day-to-day variation in intakes has well-established effects of introducing bias to estimated prevalence of inadequacy or measures of association, but with such low between-person variance, it is possible we have over-corrected for within-person variance and introduced contrary forms of bias.^{63,64}

The low between-person variance we observed may be a feature of nutrient intakes among children in our study area, but it may also be due to features of our tablet-based 24-hour recall tool and associated interview protocol. Because interviewers traveled to participants' homes by motorcycle, they could not carry a set of food models and scales for portion size estimation. Instead, we used booklets with a range of typical, local foods photographed in different portions and asked respondents to indicate which photo best matched the quantity their child consumed (Appendix 1).⁶⁵ Two prior studies conducted in sub-Saharan Africa have found that estimation of portion size using photographs was acceptable for estimation of food intakes at the group level, though the method is subject to estimation error.^{66,67} A second limitation associated with

use of the tablet-based 24-hour recall tool is that we could not collect household recipes given the capabilities of the survey application used.⁶⁵ Instead, we used a set of standardized recipes created by a previous 24-hour recall survey in the same district of Zambia.¹⁶ The use of portion size photos and standardized recipes may have introduced error that reduced the between-subject variance in nutrient intakes.

Program and policy implications

The main findings of this dissertation, that children are at risk of inadequate intakes of calcium, vitamin B12, iron and folate in association with a predominantly plant-based diet, and that nutrient intakes and dietary diversity vary by season, have implications for nutrition-related programs and policy in Zambia and similar contexts. The results inform policies and programs regarding food fortification, nutrition education, and agricultural and economic development. They are also relevant to nutrition monitoring and program evaluation.

Zambia currently mandates fortification of sugar with vitamin A.⁶⁸ We found that, based on assumed levels of fortificant in sugar and estimated sugar intakes, sugar was providing 21% of vitamin A intakes among 4- to 8-year-old children in Mkushi (Chapter 3). Most remaining vitamin A intake was in the form of pro-vitamin A carotenoids from plant foods. Further, we found a low prevalence of inadequate vitamin A intakes. These findings should be considered along with the results of the efficacy trial of biofortified maize, which found a 12% prevalence of vitamin A deficiency (defined as serum retinol concentration $< 0.70 \mu\text{mol/L}$) and other recent studies of vitamin A status among Zambian children in the ongoing evaluation of Zambia's vitamin A fortification policy.^{11,69,70}

Adoption of a maize fortification policy has been under consideration in Zambia for several years.^{68,71,72} Given the importance of maize in the diets of Zambian children, as demonstrated in

Chapter 3, this is an attractive vehicle for fortification with the nutrients for which children may still be at risk of inadequacy. However, the national maize fortification program is currently stalled due to multiple logistical barriers.⁷¹ An alternative strategy for reducing rates of inadequate micronutrient intakes among children in Zambia is point-of-use fortification. Point-of-use fortification products, such as micronutrient powders that can be added to maize or other foods during preparation in the household, could be distributed through the existing network of community health centers and combined with nutritional education for mothers. Though not investigated here, in-home fortification has been considered among several options for addressing micronutrient deficiencies among women and children in Zambia.⁶⁸ Our results on usual micronutrient intakes, prevalence of inadequacy and food consumption patterns could be used to guide development of such policies when targeting young children.

In addition to low intakes of calcium, vitamin B12, iron and folate, we found that children in Mkushi had low dietary diversity (Chapter 5). Two types of program or policy strategies may be considered to improve dietary diversity and nutrient intakes. First, nutrition education could be offered encouraging mothers to increase the variety of foods provided to children in this age group and to offer larger portions of the nutrient-rich relishes already commonly consumed. With support from the US Agency for International Development and other aid organizations, delivery of nutrition counseling in conjunction with other health care facility services is being tested in Kitwe District, Zambia ahead of national scale-up.⁷³ However, the affordability and availability of nutrient-rich foods may present a substantial obstacle to caregivers' adoption of child feeding recommendations.

Improving the availability and accessibility of nutrient-rich foods would help address these risks of dietary inadequacy among children. Interventions promoting household production of nutrient-rich foods have been shown to improve dietary diversity scores or other measures of

dietary quality among women and children.⁷⁴ Economic policy could target improved food distribution and import/export networks. Given the lack of calcium in the national food supply as assessed through food balance sheets, broader economic strategies, in addition to national fortification policies, may be particularly important for bringing dairy products and other high-calcium foods to local markets.^{17,75}

Two key findings have implications for the conduct of surveys used in national nutrition monitoring or program evaluation. First, our findings of seasonal change in nutrient intakes and dietary diversity imply that seasonal effects should be considered when planning surveys (Chapters 3 & 5). For national nutrition monitoring surveys, this may include strategies such as staggering data collection over multiple seasons in each geographic area or, if that is infeasible, noting potential confounding of seasonal and geographic effects when comparing results from geographic areas surveyed in different seasons. Similarly, in the case of program evaluation, pre- and post-intervention data collection could be conducted in the same season, or a concurrent control group could be included in data collection in order to isolate seasonal and program effects. Second, when dietary diversity scoring is used in monitoring or evaluation surveys, we recommend the 10-food group DDS from the FAO over the 7-food group DDS from the WHO.^{40,41}

Implications for future research

This dissertation contributes to the public health literature on child nutrition in low- and middle-income countries by providing detailed studies of the adequacy, variability and diversity of diets among 4- to 8-year-old children in rural Zambia. We have identified high rates of calcium, vitamin B12, iron and folate intake inadequacy and connected those to a plant-based dietary pattern. We have estimated the components of nutrient intake variance, highlighting the

importance of seasonal variation in intakes and informing the design of future studies to accurately assess nutrient intakes in similar populations. Finally, we have described the performance of two dietary diversity scores for population-level assessment of dietary adequacy, pointing out the importance of planning for seasonal variation when using such scores. The findings in each chapter have implications for future research, and the dissertation as a whole highlights how plant-based diets typical in rural, low- and middle-income-country settings present risks of nutrient inadequacy among children and suggests methodological implications for nutritional assessment.

The predominantly plant-based diet of rural Zambian children places them at high risk of inadequate intakes of calcium, vitamin B12, iron and folate, nutrients that are more bioavailable or present in higher quantities in fortified or animal source foods.⁷ In Chapter 3, we showed that children consume mostly plant foods, and few foods which are industrially processed to enable fortification. Though both maize and oil are frequently consumed, neither is currently fortified in Zambia.^{71,76} Further study of these deficiencies is needed in Zambia and the region to describe these nutritional risks to child health, growth and development. The very low intakes of calcium and high prevalence of deficiency we observed, coupled with studies showing a lack of calcium availability in the national food supply, indicate that calcium deficiency may be a public health problem across age groups and warrants urgent investigation, though such investigation is limited by the availability of calcium status measures that can be applied in population-based surveys.^{17,77} More broadly, testing and adoption of strategies to increase intakes of calcium, iron, vitamin B12 and folate are needed to reduce the potential negative consequences of impaired cognitive development, academic performance, bone growth and density and physical work capacity associated with these deficiencies among school-age children.²³⁻²⁸ Our results can be used to inform the design of such interventions, which could

include point-of-use or industrial fortification or economic and agricultural development programs to increase dietary diversity.

In contrast to high-income countries for which components of nutrient intake variance have been more widely and recently reported, we found that season contributed substantially to intake variance and between-person variance was low relative to both within-person variance and between-person variance in other populations.^{30,31,33,35–37} Our findings on seasonal change in nutrient intakes indicate a need to plan for similar, temporal effects on diet in both data collection and analysis of future studies of diet in rural, low- and middle-income-country settings. Though software programs or packages from the National Cancer Institute, Iowa State University and elsewhere provide tools for estimating usual nutrient intakes from repeat dietary observations, modeling that accounts for seasonal effects is not possible if they are not captured with repeat observations that cover multiple seasons.^{56,57,78–80} We also demonstrated that low between-person variance in nutrient intakes may be observed in settings where dietary patterns or their determinants, such as socio-economic status, have little variance within the study sample. Such low between-person variance presents a potential limitation for modeling of usual nutrient intakes or detection of associations between nutrient intakes and other factors.⁶² Sample selection and analysis plans in future studies should be designed with these potential limitations in mind. Our estimates of within- and between-subject components of nutrient intake variance could be used to calculate correlation or regression coefficient attenuation factors that might be obtained with different numbers of replicates.

In our examination of two recommended dietary diversity scores, we concluded that among 4- to 8-year-old Zambian children, the FAO's Minimum Dietary Diversity for Women of Reproductive Age indicator (DDS10) performed better for assessing dietary adequacy than the WHO's Minimum Dietary Diversity indicator for 6- to 23-month-old children (DDS7). The

disaggregation into 10 food groups used in the FAO's score enabled detection of seasonal differences in dietary pattern, and associations to overall nutrient adequacy were stronger. We further concluded that differences in score performance by season should be examined in future DDS validation studies and planned for where DDS are used in monitoring or program evaluation. Future validation and development of recommended scores for population-level assessment of dietary adequacy among preschool- and school-age children is needed. Validation studies in low- and middle-income countries, particularly in rural areas, should include assessment over multiple seasons. Based on our results, it would be possible to over- or under-estimate the strength of association between DDS and overall nutrient adequacy if dietary data for the validation study are collected in only one season.

Summary

Two main conclusions regarding the diets of 4- to 8-year-old children in rural Zambia emerge through this dissertation research. First, the predominantly plant-based diet with minimal inclusion of fortified foods puts children at risk of deficiencies of calcium, vitamin B12, iron and folate. Such inadequacies have serious consequences for child development in this age range and merit urgent action to improve dietary intakes. Second, children's nutrient intakes are subject to seasonal shifts, which are partially attributable to seasonal availability of locally produced fruits and vegetables. For studies of food and nutrient intakes or surveys of dietary diversity, seasonal changes in diet have important implications that must be considered in data collection and analysis.

Children need adequate diets for healthy growth and development, so research is needed to accurately identify where their diets are inadequate and then design and test interventions to address those inadequacies. This dissertation identifies shortfalls in the diets of Zambian

children, provides methodological insight on the importance of season in assessing diet in similar populations, and guides the future use and development of rapid dietary assessments that can be used to track whether programs or policies to improve the diets of children are effective. Future research should test interventions to reduce calcium, vitamin B12, iron and folate inadequacy among Zambian children, identify nutrients for which intervention is needed among children in other settings, and provide increasingly effective tools for tracking progress toward the goal of adequate diets for all children.

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Appendix 1. Assessing Child Nutrient Intakes Using a Tablet-based 24-hour Recall Tool in Rural Zambia

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Abstract

Background: Detailed dietary intake data in low-income populations are needed for research and program evaluation. However, collection of such data by paper-based 24-hour recall imposes substantial demands for staff time and expertise, training, materials, and data entry.

Objective: To describe our development and use of a tablet-based 24-hour recall tool for conducting dietary intake surveys in remote settings.

Methods: We designed a 24-hour recall tool using Open Data Kit software on an Android tablet platform. The tool contains a list of local foods, questions on portion size, cooking method, ingredients and food source, and prompts to guide interviewers. We used this tool to interview caregivers on dietary intakes of children participating in an efficacy trial of provitamin A biofortified maize conducted in Mkushi, a rural district in central Zambia. Participants were children aged 4-8 years, not yet enrolled in school (n=938). Dietary intake data were converted to nutrient intakes using local food composition and recipe tables.

Results: We developed a tablet-based 24-hour recall tool and used it to collect dietary data among 928 children. The majority of foods consumed were maize, leafy vegetable or small fish dishes. Median daily energy intake was 6125 kilojoules (1464 kilocalories).

Conclusions: Food and nutrient intakes assessed using the tablet-based tool were consistent with those reported in prior research. The tool was easily used by interviewers without prior nutrition training or computing experience. Challenges remain to improve programming, but the tool is an innovation that enables efficient collection of 24-hour recall data in remote settings.

Introduction

Twenty-four hour recalls are a frequently used method for collecting detailed dietary intake data in international nutrition research. However, this method is resource-intensive, requiring substantial staff time, training and expertise. New technologies may substantially reduce these demands, making 24-hour recall data more accessible. For example, tablets equipped with survey software can now be used to record data in the field, eliminating the need for paper forms and data entry. A number of computer- or web-based programs have been designed for self- or interviewer-administered 24-hour recalls in high-income countries.¹ Mobile devices such as tablets and personal digital assistants have been shown to be effective, well-accepted and cost-saving tools for direct entry of survey data, even in resource-poor settings.²⁻⁵ However, to our knowledge, tablets have not previously been used for the collection of detailed dietary intake data.

We developed a tablet-based 24-hour recall tool for use at our research site in Mkushi District, Central Province, Zambia. Mkushi is a rural, agricultural district, with high rates of poverty and food insecurity and low population density. The district has had limited prior exposure to research or development projects, and locally hired interviewing staff had no specialized training in nutrition. We planned to conduct interviews at participants' homes, most of which were located 30-90 minutes from our office by motorcycle, with limited access to electricity and no internet connectivity. We therefore required a data collection tool that could guide

interviewers through a complex recall protocol with minimal training in nutrition and limited prior computer experience. It was also essential that this tool be available on a portable device, with long battery life and offline capabilities.

In this paper, we describe the development and use of our tablet-based tool to conduct 24-hour dietary recalls in remote field settings. We present results of a dietary intakes survey conducted in Zambia as an illustrative case, describe successes and challenges in tool development and use, and discuss future directions for this innovation in dietary data collection.

Methods

Study context

This work was undertaken as part of a large-scale cluster-randomized trial of provitamin A biofortified maize (registered as NCT01695148 at clinicaltrials.gov), described by Palmer et al.⁶ We identified children aged ~4-8 years and not yet enrolled in school by a census of all households in villages or towns accessible by vehicle year-round. Households were grouped by proximity to form clusters of ~15-30 children. Each cluster was randomly assigned to the treatment group (n=25 clusters), which received daily meals containing provitamin A carotenoid biofortified maize, the control group (n=25 clusters), which received meals containing conventional white maize, or the non-intervened group (n=14 clusters), which received an equivalent food package at the end of the trial. We enrolled 1226 children at baseline (August – September 2012) and collected data on household socio-economic status and children's diet, morbidity, height and weight. Diet and morbidity surveys were repeated on a monthly basis until the endline assessment in March 2013, yielding up to seven 24-hour recalls per child. In this paper, we present results of the baseline dietary survey only and exclude children who were enrolled after feeding had begun in their cluster. By applying this exclusion criterion, we

describe the performance of the tablet-based tool in a free-living, non-intervened context, since participation in the intervention may have influenced nutrient intake estimates.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Institutional Review Board of the Johns Hopkins Bloomberg School of Public Health (Baltimore, Maryland, USA) and the Ethics Review Committee of the Tropical Disease Research Centre (Ndola, Zambia). Verbal informed consent was obtained from all participants, witnessed, and formally recorded.

Development of the 24-hour recall tool

We selected Acer Iconia tablets (Acer Inc., Taipei, Taiwan) running the Android operating system version 3.2.1 (android.com) for data collection due to their low cost (~USD 200), durability, screen size, and GPS functionality. The 24-hour recall was programmed using Open Data Kit version 1.1.7 (opendatakit.org), a free software for data collection forms. The tool was designed to walk interviewers through the recall protocol step-by-step, with prompts specific to each step and pre-coded response choices on each screen.

Local foods list and food identification framework

We generated a list of local foods by combining foods listed in recipe and food composition tables created by HarvestPlus, a Challenge Program of the CGIAR, for use in Mkushi (unpublished) with additional foods listed in the Zambian Food Composition Tables published by the National Food and Nutrition Commission.⁷ Local fishes and wild or semi-cultivated vegetables and fruits were included in the food list based on inclusion in the food composition tables and discussion with local staff. Through group interviews with local staff, we refined the list to foods, preparation methods and local names used in the study area. We then organized

the list into a food identification framework based on local food consumption practices (**Figure A1.1**).

The framework enabled each food to be recorded without use of scrolling on the tablet screen or using a searchable list (**Figure A1.2a**). We chose to avoid scrolling in order to speed data entry and prevent errors, and the current version of ODK did not support searchable lists. We programmed one question to correspond to each category or subcategory in the food selection framework, linking these using conditional logic to create branching food selection pathways. Using this strategy, all foods in the list could be uniquely identified in two to five rapid-select questions on the tablet. For some foods, the final selection stage described the size, ripeness or freshness of the food or its primary ingredient. An option to record 'other' and then type a food description was available at each selection stage, if the food being recorded was not in the pre-programmed list. An option to record 'don't know' was also available when the respondent could not provide further detail.

Collection of additional food description data

For mixed dishes, the tablet prompted interviewers to ask and record the ingredients used in the preparation of the dish (**Figure A1.2b**). The tablet program displayed a list of likely ingredients matched to the food type, from which interviewers could select as many as were mentioned by respondents. We created each list of likely ingredients based on combinations from the recipes database or input from local staff. Each list of likely ingredients also included 'other' and 'don't know' options, where the 'other' option prompted interviewers to type in a description of the added ingredient for later re-coding. A standard question recorded the cooking method for foods other than raw fruits or commercially prepared snacks.

To estimate the quantity consumed, the tablet displayed one of three coding options depending on the type of food: a) photographs of approximate portion sizes, b) number of units, or c) the width and length of the food item. For most foods, interviewers were prompted to show respondents a specific set of portion size photos marked A – E in an accompanying photo book, described below. They then recorded the response in a variable corresponding to the photo book page for later link to gram weight estimates (**Figure A1.2c**).

A final screen prompted interviewers to ask and record where the child obtained the food: from home, a neighbor, the market, etc.

Tablet-based interview structure

The full set of food description questions was repeated for each food within a series of built-in prompts guiding interviewers to review the previous day sequentially with the caregiver and child. Interviewers first asked about foods consumed when the child woke up in the morning, then about foods consumed mid-morning, and so on through the day and night. The tablet then prompted interviewers to review a picture chart interview aid, described below, to check for any missed foods, and to do a final review of the day as a last check. At each stage in the interview process, the tablet displayed prompts to help interviewers guide respondents in recalling children's activities and any foods consumed (e.g., to consider the child's activities, or where the child went and what he or she might have eaten there). A final series of questions collected data on whether the child was ill, whether the day was a special occasion or market day, whether the child's food intake was different for any other reason, and whether the child took any vitamin, mineral or other supplements.

Interview aids

We used two visual aids in the 24-hour recall protocol. The first was a picture chart showing line drawings of twenty common local foods. Caregivers received these two days before the scheduled interview and were asked to place a tick mark next to each food the child consumed during the day prior to the recall. Interviewers reviewed charts with respondents to probe for any foods missed by the previous prompts. If any previously undescribed foods were identified during the picture chart review, these were recorded using the series of food description questions, discussed above.

The second visual aid was a thirteen-page portion size booklet, each page showing five photos of a different type of dish (e.g., nshima, leafy vegetable relish, bean relish, fruit drink, or groundnuts). The photos depicted a range of portion sizes, plated on the same dish and photographed with a woman's hands shown holding the plate as a visual reference for size. We recruited six local mothers with young children to guide the development of standard portion sizes. Using typically prepared foods from a local restaurant, we asked each mother to serve onto a plate the amount she perceived as a small portion for a 5- to 6-year-old child. We then weighed the portions. We repeated this procedure for medium and large portions of each food. To create the photo booklet page for each food, we weighed out and photographed five serving sizes representing the minimum portion, median small, medium and large portions and maximum portion.

Survey implementation

We had trained staff on tablet operation and use of ODK-based forms during an initial phase of mapping and census-taking in the study area. As a result, interviewers were adept at tablet use prior to training on the dietary data collection. Training for the 24-hour recall lasted

approximately one week, during which we covered recall interview techniques and all aspects of the tablet-based 24-hour recall form. Staff practiced interview techniques and tablet data entry using hypothetical examples and by interviewing each other. We concluded training with a drill for interviewers to collect data from mothers of young children in a neighborhood adjacent to our study area. The latter were supervised by research staff to check quality of the interview technique and data entry.

Two days prior to the interview, field supervisors visited households to inform caregivers of their upcoming interview. Supervisors provided the household with a picture chart, pencil, and standard-sized bowl, along with instructions for the use of these materials in noting children's dietary intake on the subsequent day. Interviewers asked the primary caregiver of the participating child to recall the child's food intakes, following the prompts and food description questions described above. As often as possible, the child was present to respond to questions from the caregiver or interviewer.

Interviewers traveled to scheduled visits by motorcycle, carrying tablets, portion size photo booklets, a small notepad, and pen in backpacks. Tablets could be held in hand or on the lap while conducting the interview, and screens were legible for use when conducting interviews outdoors. We randomly selected 10% of interviews each month for quality control. Senior staff observed these visits to ensure that interviewers adhered to the recall protocol. We also used timestamps embedded in the ODK questionnaire to calculate interview length. Interviewers who had two or more records with outlying interview times were flagged for additional supervision. Data were uploaded from the tablets to a computer and back-up drive on a daily basis. Data were converted from the extensible markup language (.xml) format stored on the tablets to comma separated value (.csv) format using ODK Briefcase software, a free program for

uploading and organizing data collected in ODK without use of internet-based data aggregation tools (<http://opendatakit.org/use/briefcase/>). Data in .csv format were cleaned and backed up to secure cloud storage. File formatting was done in Microsoft SQL Server 2012 (Microsoft Corporation, Redmond, WA). All further data formatting and analysis was conducted in SAS 9.3 (SAS Institute Inc., Cary, NC).

Calculation of nutrient intake estimates

Recipes database

A set of standardized Zambian recipes developed by HarvestPlus was our primary source for data on ingredient proportions for mixed foods (unpublished). This database describes recipes as sets of ingredients whose proportions are expressed as the ratio of the ingredient uncooked weight to the recipe final weight.⁸ Food composition data for use with these recipes retain the water, dry matter, and macronutrient contents from the raw food, and all micronutrients are scaled using nutrient retention factors to account for cooking losses.

For mixed foods lacking a recipe in the HarvestPlus database, we modified existing recipes or added new recipes through a separate data collection protocol. Where a mixed food differed from an existing recipe only in the main ingredient, we modified the recipe providing the closest match by substituting the key ingredient while retaining the original ingredient proportions. To create a modified recipe omitting one or more of the ingredients in the original recipe, we adjusted the fractions of the remaining ingredients such that the ratios between ingredients – including water added or lost in cooking – stayed consistent with the original recipe.

Due to differences in data collection, recipe data from HarvestPlus were unavailable for several types of recipes, e.g., meat relishes and bread or rolls with spreads. Further, some combinations

of ingredients were reported in the 24-hour recall data which were not included in the HarvestPlus recipe database. In order to create recipes for these items, we collected recipe data using a focus group of six women from our study area and a protocol similar to that described by Hotz et al.⁸ For each recipe or mixed food item, the women used raw ingredients to demonstrate how much of each they would use to prepare the recipe. The weight of each ingredient was measured in grams. To estimate post-cooking recipe weights, we applied yield factors to each ingredient and summed the resulting weights.⁹ The recipe fractions for each ingredient, for each participant, were calculated as the raw weight divided by the post-cooking recipe weight. We averaged the fractions for each ingredient across all participants to create the recipe.

Food composition database

Our primary source of food composition data was a table developed for Zambia by HarvestPlus (unpublished). For any foods not included in this database, we used data from the U.S. Department of Agriculture (USDA) National Nutrient Database for Standard Reference Release 26, Zambia Food Composition Tables from the National Food and Nutrition Commission, FAO/INFOODS Food Composition Database for Biodiversity, Food Composition Tables for Mozambique, the FAO Food Composition Table For Use in Africa or a Food Composition Table for Central and Eastern Uganda.^{7,8,10-13} We used the Zambia Food Composition Tables, Agroforestry database, FishBase, or searches of other sources to identify the scientific names of local foods, in order to locate them in regional and global food composition tables.^{7,14-18} Where food composition data for a specific food were unavailable, we used data from the closest available matching food. For foods missing food composition data for some nutrients, values for those nutrients were imputed as the average among similar foods for which data

were available. When food composition data for cooked foods were missing, we used nutrient retention factors to impute the nutrient contents of the cooked food based on the nutrient composition of the raw food.¹⁹

Linking tablet data to recipe and food composition databases

In order to link the data as recorded on the tablet to recipe and food composition databases, we created a series of variables, each of which reduced a set of related tablet variables to a single code. Lookup codes combine data from the food identification questions and serve as a key between each recorded food and a unique item in the foods list. The variables marking which ingredients were added to the food, combined with the lookup code, connect each record to a unique recipe code in the recipes database and its associated food codes in the food composition table. Finally, the portion size estimates based on the photos, or number or size of food units consumed, were converted to gram weights.

Food and recipe coding

To form lookup codes, we linked the responses to the sequential food identification questions into a ten-digit code associated with a unique item in the pre-programmed list of foods (**Figure A1.1**). Formation of the lookup code matches the branching food selection framework programmed into the tablet. The first two digits are the response to the initial food selection question and indicate which food identification variable should supply the third and fourth digits, and so on, up to ten digits for five food selection stages. For foods identified in fewer than five stages, trailing zeroes maintain consistent code length and format. Lookup codes for which the respondent could not recall specific food descriptors, such as whether a chicken drumstick was consumed skin-on, were recoded to the most commonly reported food in the same sub-

category. Lookup codes for which the interviewer selected 'other' and entered a description were reassigned to a matching, existing code or assigned a new code.

Lookup codes for unmixed food items were associated directly with a food code for a unique entry in the food composition table. For mixed foods, we matched each record to the unique code in the recipes database with the same combination of lookup code and added ingredient variables. In turn, the recipe code linked each record to a database listing the individual ingredients with their food codes and proportions in the recipe. For example, the combination of the lookup code for cabbage relish and added ingredient variables for tomato, onion and oil is uniquely associated with the recipe code 9180860, which links to an entry in the recipe database containing the food codes for oil and cooked cabbage, tomatoes and onions and their proportions in the recipe. When respondents did not know the ingredients used in a mixed dish, the records were recoded to the most commonly reported recipe with the same lookup code.

Portion size estimation

We combined the tablet variables containing portion size data into a single portion code. For foods estimated by portion size photos, each portion code is associated with the gram weight of the food shown in the corresponding photo. We adjusted for any difference in density between the food recorded and the food shown in the photo to arrive at a final gram weight estimate. To make this adjustment, we used data from the FAO/INFOODS density database, food composition tables, or data collected for this purpose at the study site.^{10,20} For foods such as fruit or bread slices which were recorded as number of pieces consumed, we multiplied the number of pieces by the estimated gram weight of a single piece. The estimates of gram weight per piece are based on data from the USDA National Nutrient Database for Standard Reference, locally collected food weights, or additional references.¹⁰ For the small number of foods

estimated by length and width in centimeters (e.g., raw sugar cane), the portion gram weight is estimated according to cylindrical volume and an estimate of density. When respondents could not report the amount consumed, we estimated portion size as the median reported portion size among all other records of the same food. Finally, for mixed foods, we estimated the gram weight of each ingredient by multiplying the total portion weight by its recipe fraction.

Statistical methods

To describe dietary patterns in terms of foods consumed, we calculated the number of times each lookup code appeared at baseline, aggregating similar foods. For nutrient intake estimates, we multiplied the nutrient contents per gram from the food composition table by the estimated gram weight of each ingredient or unmixed food consumed. We summed each nutrient across all foods consumed by each child and then generated descriptive statistics on the distribution of total intakes of each nutrient across all children.

Results

Of the 1226 children enrolled in the trial, n=938 were enrolled prior to the start of the feeding intervention and are included in the present analysis. Of these, the tablet-based 24 hour recall was completed among 928 children. The average age at baseline was 5.7 years (s.d. 1.2) and 51% were male (**Table A1.1**). The prevalence of stunting was 26% and that of underweight was 14%. Socio-economic descriptors of participating households are presented in Table A1.1.

Fourteen categories of foods were consumed by at least 100 children during the baseline 24-hour recall period (**Table A1.2**). Each of the remaining categories of foods made up 5% or less of the total number of foods reported and was consumed by less than 10% of included children.

The staple dish, white maize nshima, accounts for 30% of all foods recorded and was consumed

by 99% of children (n=922). Among these children, white maize nshima was consumed an average of 2.2 times per day. Leafy vegetable relish and small fish relish make up the next thirty percent of foods recorded. Leafy vegetable relish was consumed by 86% of children (n=801), and 54% consumed small fish relish (n=500).

The median energy intake over the previous 24 hours was 6146 kilojoules (1469 kilocalories, **Table A1.3**). Across age and sex groups, median energy intake ranged from 93% to 117% of the estimated energy requirement.²³ Median daily intakes for most micronutrients exceeded the estimated average requirement (EAR).²⁴⁻²⁷ The median intake of calcium, 263 mg, was well below the EAR for this age group of 800 mg/day.²⁸ The median intake of folate was 117 µg, compared to the EAR of 160 µg/d.²⁶

Of 6,777 records of foods consumed by included children, 21 foods were incompletely described because the respondents did not know one or more descriptive details. For 262 food records, the option to record a description for a food not found in the pre-coded food list was used. Of these 'other' foods, 101 foods were local foods not included in the pre-coded list. In the remaining cases, the interviewer did not locate the item in the pre-coded list or did not know the English name. For 5 foods, the recipe was unknown to the respondent. Portion sizes were unknown for 116 foods and missing for 44 foods.

Discussion

In this paper, we have described the development and use of a tablet-based tool to collect 24-hour dietary recalls in a resource-limited setting. This tool, created in ODK survey software on the Android platform, is, to our knowledge, the first use of tablet computers for detailed dietary intake data collection. Estimates of energy intakes from our baseline assessment of 4- to 8-year-old children are consistent with estimated energy requirements for boys and girls in this age

range.²³ Our findings are also in line with data collected in the same area in 2009 using a paper-based multi-pass method, showing a monotonous diet composed primarily of maize-based porridge and a limited range of side dishes containing leafy green vegetables or small fish.²⁹

Previous tools that allow interviewers to simultaneously conduct a 24-hour recall and enter the data provided by respondents have been Windows-based software packages run on a desktop or laptop computer.¹ The Automated Multiple Pass Method (AMPM) and Post Interview Processing System software developed by the USDA, Nutrition Data System Research (NDSR) package by the University of Minnesota and the EPIC-SOFT program developed for use in the European Prospective Inquiry into Cancer and Nutrition are examples of computer-based, interviewer-administered 24-hour recall tools.^{30–32} Related tools have also been developed for conducting 24-hour recalls among upper primary school children and adolescents.³³ Tools have largely focused on North American or European populations. One recent exception is the New Interactive Nutrition Assistant – Diet in India Study of Health (NINA-DISH) program developed for a nationally representative survey of dietary intakes among Indian adults.³⁴ All of the above tools guide interviewers and support direct data entry. They also calculate nutrient intakes from the recorded data, but in order to do so, require the computing power and memory of a personal computer and currently cannot be run on the more portable tablet devices.

A second set of tools for dietary intake data collection are the self-administered, often web-based programs such as the Automated Self-Administered 24 Hour Recall (ASA24) developed by the US National Cancer Institute, Oxford WebQ dietary recall program for use in the UK, and the Computer-Assisted Personal Interview System, which was developed for use in Korea and includes a 24-hour recall.^{35–37} Like the computer-based tools, these web-based, self-administered tools have been adapted for use by older children and adolescents.^{38,39} This group of tools offers tremendous advantages to researchers studying diet among populations where

most potential respondents have the computer access and skills needed for independently completing a 24-hour recall. However, this is not the case in many low-income countries.

Several studies have demonstrated the promise of using mobile data collection in low-income countries or remote settings. Reported benefits include: reduced data entry errors;³ improved adherence to protocol, reduced rate of omitted data fields, reduced typographical errors, and faster data checking time;⁴ and high data completeness and faster data checking and processing.⁵ Common observations include cost savings, particularly in large surveys where the cumulative costs of printing and data entry are greater than the one-time cost of purchasing tablets.³⁻⁵ Though we did not do a cost comparison, the total number of survey forms completed using tablets in our study, including the 24-hour recall and most other data collection, far outnumbered the point at which paper forms would exceed tablet-related costs. A second common observation is the need for technical expertise. Our study employed one full time and two part time IT specialists to support forms development, design and use of the data management system, data checks and cleaning. A final common observation is the general acceptability of tablets or PDAs among both study staff and respondents. Our experience matches those described by other authors, that field staff quickly mastered tablet use despite lack of prior experience, and respondents were accepting of the technology.^{3,5}

The tablet-based 24-hour recall tool we developed was designed to fill a need that was not met by existing tools or mobile device-based surveys. Detailed dietary data are needed to address public health problems such as micronutrient inadequacy, potential exposure to toxins, and food insecurity. The use of an interviewer-administered, tablet-based tool meets that need and offers several advantages. Using one set of 30 tablets, we collected data monthly among 1226 children over a study period of seven months, in a remote setting where interviewers traveled extensively by motorcycle to reach participants' homes. We did not experience reliability

problems with general tablet operability or with the ODK survey software, nor did we have instances of data loss due to lack of battery power or tablet malfunction. Issues such as freezing screens were found to be the result of operator error, quickly addressed through re-training. The tablet and minimal supporting equipment fit in small, messenger-style packs for easy transport, and the tablet allowed interviewers to work indoors or outdoors. The tablets were inexpensive (~USD 200) and the ODK survey software is available free of charge. Tablet battery life was sufficient for a full day of field work in most cases. In the event of low battery power, back-up tablets and battery chargers were available from interviewer team leaders. Data collection and transfer did not require an internet connection or cellular reception. Finally, using mobile devices for data collection has been shown to reduce data recording errors through the addition of range checks, required fields, and conditioned ordering of questions, which prevents skip logic errors.⁴⁰ Through the use of required fields and conditioned ordering of questions, our data had low rates of missing or unknown values.

We encountered several challenges in developing a tool for conducting repeated 24-hour recalls in rural Zambia. Limitations in the current version of ODK software shaped our data collection tool, and use of tablets for all types of data collection requires the time and expertise of skilled IT staff. Particularly for the 24-hour recall tool, programming is made challenging by the conditional logic needed to create the correct ordering of food identification questions. Though our tool could be used as a template for future 24-hour recalls in other settings, the ODK program and food identification framework would need to be re-written to accommodate a different local foods list. We were unable to record a list of foods consumed in the day and add detailed information to it, as is done in other 24-hour recall programs. All information on each food needed to be collected together in a series of questions. This method of data collection, however, provided an advantage; breaking the traditional table into a series of questions

enabled the addition of prompts to support interview quality and collection of all relevant details about each food. Future updates to our tool would be to integrate an initial quick list stage into the interview protocol using new features in ODK. The quick listing of all foods respondents remember consuming during the previous day is an important feature of other 24-hour recall programs using the multi-pass method developed as part of AMPM.⁴¹ Another desired addition to this tool would be the collection of household recipes. A final key challenge, identifying food composition data for local foods, is common to dietary assessments in diverse settings. We combined available data from many different sources to arrive at estimates of nutrient contents, food weights and nutrient retention factors, yet for some traditional and local foods, complete data were simply unavailable.

Conclusion

Tablet-based programming technology has advanced to the point that conducting 24-hour recall interviews in remote settings using tablets is not only feasible, but will also make dietary data easier and more affordable to collect. Critical next steps include a validation study of the tablet-based tool, developing recall tools that are readily adaptable to different contexts, improving the availability and quality of food composition data, and linking dietary data collected on tablets to analysis software. These advances will enable efficient collection and analysis of high-quality dietary intakes data for research as well as program or intervention assessment.

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Tables and Figures

Table A1.1. Characteristics of children and households in Mkushi, Zambia (n=938*)

| | n | Percent |
|------------------------------------|-----|---------|
| Child Characteristics | | |
| Male sex | 479 | 51.1 |
| Age (y) | | |
| <5 years | 332 | 35.4 |
| 5-7 years | 436 | 46.5 |
| >7 years | 170 | 18.1 |
| Stunted [†] | 235 | 26.3 |
| Underweight [†] | 122 | 13.6 |
| Household Characteristics | | |
| Literate head of household | 586 | 83.0 |
| <u>Household head's occupation</u> | | |
| Self-employed / running a business | 212 | 30.0 |
| Farming | 123 | 17.4 |
| Salaried employment | 115 | 16.6 |
| Farm labor | 78 | 11.1 |
| Piecework or hourly | 117 | 16.6 |
| Other | 61 | 8.6 |
| <u>Asset ownership</u> | | |
| Mobile phone | 510 | 72.2 |
| Radio | 477 | 67.6 |
| Bicycle | 393 | 55.7 |
| Electricity in the home | 34 | 4.8 |

* Number of children, living in 716 households. Data missing from a maximum of n=44 children for any one variable.

[†] Height and weight were assessed by trained anthropometrists using portable stadiometers (ShorrBoards, Weigh and Measure, LLC, Olney, Maryland) and flat scales (Seca, Model 874, Hanover, Maryland). Stunting was defined as height-for-age Z-score of less than -2SD and underweight as weight-for-age Z-score of less than -2SD, based on the 2006 WHO growth standards for children 0 – 60 months and 2007 WHO growth reference for children and adolescents 5 - 19 years.^{21,22}

Table A1.2. Common foods consumed by children aged 4-8 years in Mkushi, Zambia derived from tablet-based 24-hour recall tool (n=938*)

| | Children consuming food item | | Times per day consumed | | Reported foods | |
|------------------------|------------------------------|------|------------------------|-----|----------------|------|
| | N | % | Mean | SD | N | % |
| White maize nshima | 922 | 99.4 | 2.2 | 0.5 | 2021 | 29.9 |
| Leafy vegetable relish | 801 | 86.3 | 1.7 | 0.7 | 1374 | 20.3 |
| Small fish relish | 500 | 55.0 | 1.3 | 0.5 | 671 | 9.9 |
| Large fish relish | 246 | 25.1 | 1.4 | 0.6 | 334 | 4.9 |
| White maize porridge | 202 | 21.8 | 1.1 | 0.2 | 213 | 3.1 |
| Tea | 193 | 20.8 | 1.1 | 0.3 | 210 | 3.1 |
| Beans relish | 161 | 17.3 | 1.2 | 0.5 | 199 | 2.9 |
| Root vegetable relish | 143 | 15.4 | 1.1 | 0.3 | 157 | 2.3 |
| Sweet snack | 126 | 13.6 | 1.1 | 0.4 | 143 | 2.1 |
| Plain vegetable | 120 | 12.9 | 1.1 | 0.3 | 136 | 2.0 |
| Chicken | 113 | 12.2 | 1.3 | 0.5 | 150 | 2.2 |
| Bread / bun | 110 | 11.9 | 1.1 | 0.3 | 118 | 1.7 |
| Egg relish | 101 | 10.9 | 1.1 | 0.4 | 115 | 1.7 |
| Fritter / donut | 100 | 10.8 | 1.0 | 0.2 | 103 | 1.5 |

* Dietary recall data missing from n=10 children

Table A1.3. Energy and nutrient intakes of children aged 4-8 years in Mkushi, Zambia derived from tablet-based 24-hour dietary recall tool (n=938*)

| | Median | 25th Percentile | 75th Percentile | Estimated average requirement⁽²⁴⁻²⁸⁾ |
|--|---------------|------------------------|------------------------|--|
| Total energy (kJ (kcal)) | 6146 (1469) | 4703 (1124) | 7653 (1829) | -- |
| Total protein (g) | 47 | 32 | 61 | -- |
| Total fats (g) | 44 | 29 | 64 | -- |
| Total carbohydrates (g) | 222 | 173 | 278 | 100 |
| Total calcium (mg) | 263 | 157 | 400 | 800 |
| Total iron (mg) | 8.8 | 6.6 | 12.1 | 4.1 |
| Total zinc (mg) | 5.9 | 4.4 | 7.6 | 4.0 |
| Total vitamin C (mg) | 42 | 26 | 66 | 22 |
| Total thiamin (mg) | 0.7 | 0.5 | 1.0 | 0.5 |
| Total riboflavin (mg) | 0.6 | 0.4 | 0.9 | 0.5 |
| Total niacin (mg) | 11 | 7 | 17 | 6 |
| Total vitamin B6 (mg) | 1.2 | 0.8 | 1.7 | 0.5 |
| Total folate (µg) | 117 | 75 | 208 | 160 |
| Total vitamin B12 (µg) | 0.7 | 0.3 | 1.4 | 1.0 |
| Total vitamin A (µg RAE [†]) | 527 | 308 | 797 | 275 |

* Dietary recall data missing from n=10 children

† RAE, retinol activity equivalents

Figure A1.1. Illustration of the branching food identification framework used to program a pre-set list of local foods into a series of food identification questions in the tablet-based 24-hour recall tool

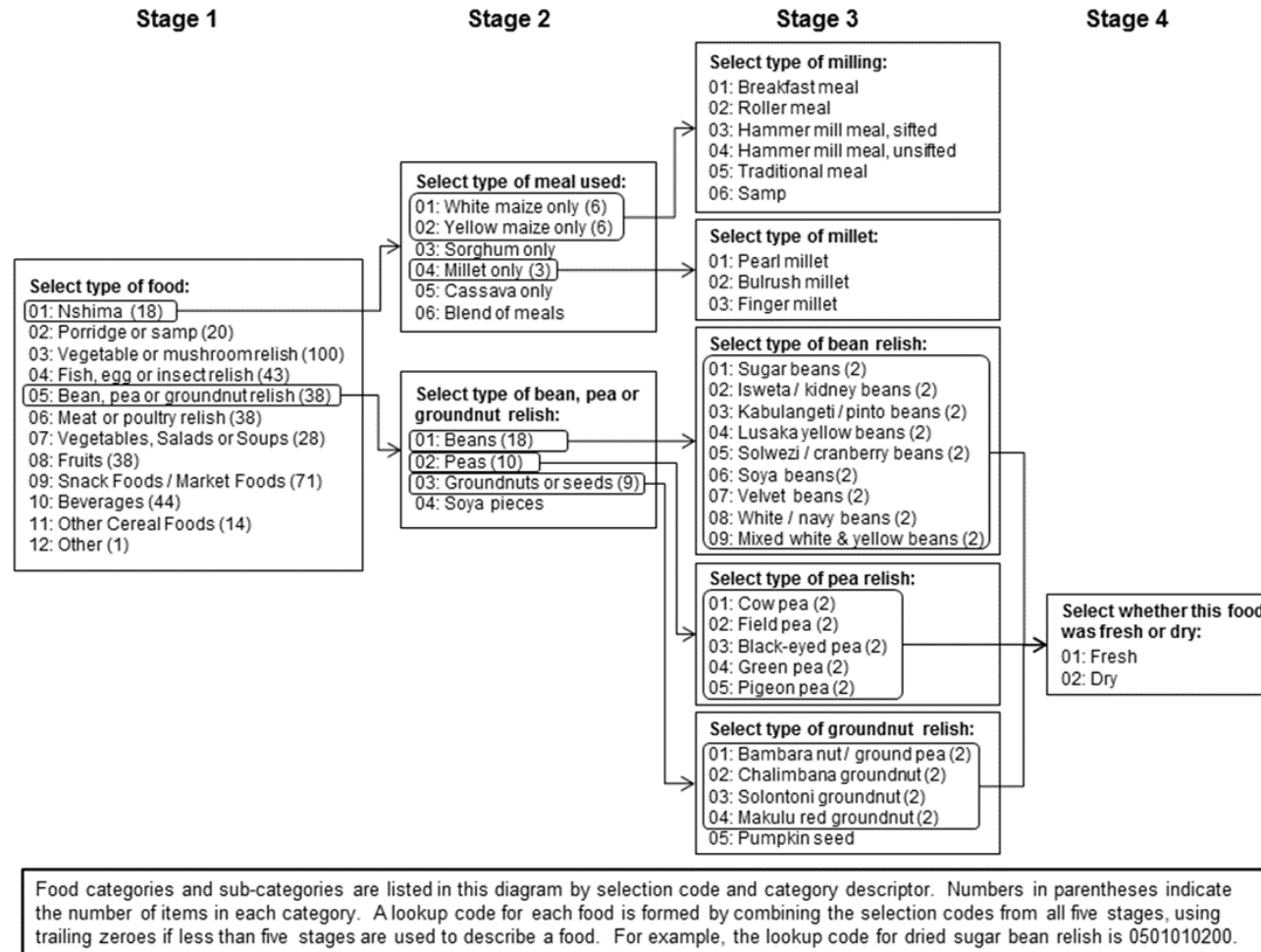


Figure A1.2. Screen shots from the tablet-based 24-hour recall tool showing examples of the series of food identification questions (a), questions on added ingredients (b) and portion size estimation (c)

(a) Food identification

| | | |
|--|--|--|
| <p>SELECT TYPE OF FOOD:</p> <input type="radio"/> NSHIMA <input type="radio"/> PORRIDGE OR SAMP <input type="radio"/> RELISH: VEGETABLE OR MUSHROOM <input type="radio"/> RELISH: FISH, EGG OR INSECT <input type="radio"/> RELISH: BEANS, PEAS OR GROUNDNUTS <input type="radio"/> RELISH: MEAT OR POULTRY <input type="radio"/> VEGETABLES, SALADS OR SOUPS <input type="radio"/> FRUITS <input type="radio"/> SNACK FOODS / MARKET FOODS <input type="radio"/> BEVERAGES <input type="radio"/> OTHER CEREAL FOODS <input type="radio"/> OTHER <input type="radio"/> INGREDIENTS | <p>SELECT TYPE OF VEGETABLE OR MUSHROOM RELISH:</p> <input type="radio"/> LEAFY VEGETABLE <input type="radio"/> NON-LEAFY VEGETABLE <input type="radio"/> ROOT VEGETABLE <input type="radio"/> MUSHROOM <input type="radio"/> CHIKANDA <input type="radio"/> OTHER VEGETABLE RELISH <input type="radio"/> UNKNOWN VEGETABLE RELISH | <p>SELECT TYPE OF LEAFY VEGETABLE RELISH:</p> <input type="radio"/> AMARANTH LEAVES (BONDWE) <input type="radio"/> BEAN LEAVES (CHIMPAPILA) <input type="radio"/> CABBAGE, GREEN <input type="radio"/> CASSAVA LEAVES (KATAPA) <input type="radio"/> CHINESE CABBAGE <input type="radio"/> COWPEA LEAVES (KACHESHA) <input type="radio"/> OKRA LEAVES <input type="radio"/> PUMPKIN LEAVES (CHIBWABWA) <input type="radio"/> RAPE / MUPILU <input type="radio"/> SWEET POTATO LEAVES <input type="radio"/> TRADITIONAL / WILD LEAFY VEGETABLES <input type="radio"/> OTHER LEAFY VEGETABLE <input type="radio"/> UNKNOWN LEAFY VEGETABLE |
|--|--|--|

(b) Added ingredients

| SELECT ALL INGREDIENTS ADDED TO THIS DISH: | |
|--|-----------------------|
| | YES |
| TOMATO | <input type="radio"/> |
| ONION | <input type="radio"/> |
| OIL | <input type="radio"/> |
| GROUNDNUT PASTE OR MEAL | <input type="radio"/> |
| SPICES | <input type="radio"/> |
| SALT | <input type="radio"/> |
| OTHER | <input type="radio"/> |
| DO NOT KNOW | <input type="radio"/> |

(c) Portion estimation

| |
|---|
| <p>Which picture best matches the portion size your child had at this time? Enter 9 for 'Cannot report'</p> <input type="radio"/> A <input type="radio"/> B <input type="radio"/> C <input type="radio"/> D <input type="radio"/> E <input type="radio"/> Cannot report |
| <p>SHOW RESPONDENT PHOTO BOOK PAGE P1.2 (LEAFY VEGETABLE RELISH). ENTER NUMBER OF THE SELECTED PHOTO</p> |

Appendix 2. Estimating Usual Nutrient Intakes and Probability of Intake Inadequacy

This appendix provides detailed descriptions of the methods used to estimate usual nutrient intake distributions, to predict individual usual nutrient intakes and to calculate probability of nutrient intake adequacy. Distributions of usual nutrient intakes and prevalence of intake inadequacy are presented in Chapter 3 ('Aim 1: Usual Nutrient Intake Adequacy Among 4- to 8-year-old Rural Zambian Children'). The usual nutrient intakes of individuals are predicted for use in linear regression models relating dietary diversity scores to usual nutrient intakes or to mean probability of adequacy in Chapter 5 ('Aim 3: Seasonal Variation in Performance of Two Recommended Dietary Diversity Scores among Rural Zambian Children').

Estimation of usual nutrient intake distributions

Background

When assessing nutrient intakes and their adequacy, the measurement of interest is usual intake, that is, the average daily intake over time, rather than intake on a single day or simple average over several days. Intake on a single day is an inaccurate estimate of usual intake due to both day-to-day variation and measurement error.¹ The resulting inflation of variance produces biased estimates of percentiles in the tails of the distribution. Therefore, appropriate statistical models to remove within-person variance are required to describe usual nutrient intakes.¹

Several methods to estimate the usual intake distribution by removing within-person variance have been developed by the National Research Council / Institute of Medicine (NRC/IOM), Iowa State University (ISU), the National Cancer Institute (NCI) and other research groups.¹⁻⁶

Methods developed by ISU and NCI were designed to describe the usual intake of episodically consumed foods, for which many individuals who occasionally consume the food may report no intake during a 24-hour recall. The ISU and NCI approaches address this issue through a two-step modeling process: first, the probability of consumption on a given day is modeled; second, the amount consumed on a consumption day is modeled. For nutrients, which in nearly all cases are consumed daily (i.e. probability of consumption can be assumed to be 1), only the second modeling step is needed.⁴ This stand-alone second step is similar to the general approach used by other methods to estimate usual nutrient intake.

The methods all follow a common basic framework in which nutrient intakes are transformed to approximate a normal distribution, adjusted using a shrinkage factor based on the within- and between-subject components of variance of the transformed data, and back-transformed to describe the estimated usual intake distribution in the original unit of measurement.^{1,3,4} The components of observed nutrient intake by individual i on day j (R_{ij}) are described by the following equation:

$$R_{ij} = \bar{R}_{..} + (\bar{R}_i - \bar{R}_{..}) + e_{ij}$$

where $\bar{R}_{..}$ is the overall mean usual intake, \bar{R}_i is the usual intake of individual i , $(\bar{R}_i - \bar{R}_{..})$ is the difference between usual intake of individual i and the overall mean, which has a mean of zero and variance σ_b^2 , and e_{ij} is the within-individual variation in intake, which has a mean of zero and variance σ_w^2 . Because nutrient intake data are rarely normally distributed, the values for R_{ij} are transformed by a power or logarithmic function to approximate a normal distribution.¹ The within- and between-subject components of variance (σ_w^2 , σ_b^2) in the transformed data are estimated and used to calculate the shrinkage factor, w , which is applied to each transformed observation, R_{ij}^* , in order to achieve a distribution with the mean $\bar{R}_{..}^*$ and variance σ_b^2 .

$$R_{ij}^* = \bar{R}_{..}^* + w(\bar{R}_{i.}^* - \bar{R}_{..}^*)$$

$$\text{where } w = \sqrt{\frac{\sigma_b^2}{\sigma_b^2 + (\sigma_w^2/n)}}$$

and n is the number of observations per individual. This shrinkage estimate produces adjusted values that are close to the individual means when within-person variance is small or there are many replicates, but the adjusted values are closer to the overall mean if the within-person variance is large or replicate number is small.^{1,4} For most methods, rather than a simple inversion of the transformation step, a back-transformation procedure that includes a bias adjustment step is used to return the intake values to original scale.¹ The empirical distribution of the back-transformed and bias-adjusted values forms the estimated usual intake distribution.¹

The specific methods developed around this common framework vary in the choice of transformation and back-transformation procedures, assumptions about the intake measured by 24-hour recall as an estimator of usual intake, ability to include covariates or sub-group analyses, and extension to estimation of individual usual intakes.^{1,3,4} The method developed at NCI is one of the most recent methods -- having been developed to address weaknesses in the ability of prior methods to estimate usual intakes of episodically consumed foods -- and was chosen for estimation of usual nutrient intakes in this study for several reasons. First, it has been demonstrated to perform well in simulation and comparison studies.^{3,4} Souverein et al. found that the NCI method produced unbiased estimates of usual intake distribution percentiles and means from simulated datasets that varied in number of observations, skewness and ratio of within- to between-subject variance, except when the within- to between-subject variance ratio was very high.³ In a subsequent study, Tooze et al. concluded that in datasets with high within-subject variance, the NCI method combined with a back-transformation approach

developed by ISU produces better estimates of the moments of the usual intake distribution.⁴ Both studies found that the NCI method generally produces smaller standard errors around estimated distribution parameters than does the ISU method.^{3,4} Second, the NCI method allows for adjustment by covariates.^{3,4} Whereas the ISU method includes a preliminary data adjustment step to account for factors such as season or interview day, the NCI method is able to include such factors as well as covariates such as age and sex in the model for the transformed intakes. Finally, the NCI method enables estimation of individual usual intakes through an additional SAS macro (for use with SAS statistical analysis software package, SAS Institute, Cary, NC), available along with the core SAS macros for the estimation procedures on the NCI website.⁷ This extension of the usual intake modeling procedure was used in Aim 3 (Chapter 5) analyses and is discussed further below.

Implementation

The procedure for estimating usual intake distributions of individual nutrients was used in Aim 1 (Chapter 3) analyses. It was implemented using two SAS macros provided by NCI, titled MIXTRAN and DISTRIB.⁸ The macros enable either one-part (amount only) or two-part (probability and amount) modeling. In the first macro, MIXTRAN, observed intake values are transformed to approximate a normal distribution and mixed effect models (SAS PROC NLMIXED) are fit, producing parameter estimates for the distribution of intakes and predicted values for each child. The parameter estimates include the overall intercept, regression coefficients for covariates such as age, sex or socio-economic status indicators that are specified by the user as fixed effects, variance of an individual-level random intercept and error variance. The models estimated in MIXTRAN can be optionally extended to estimate a fixed effect and

separate residual variance for observation days that fall on a weekend or other day when intakes may be systematically different from most days, referred to as a 'weekend' effect.

The second macro, DISTRIB, uses the parameter estimates produced by the MIXTRAN modeling procedures to run a Monte Carlo simulation. A simulated draw of 100 random samples per child is taken from a distribution with a mean of the child's predicted value and variance equal to the random intercept variance estimated by MIXTRAN. When a weekend effect is modeled, draws are pulled from distributions for weekend and non-weekend days and weighted according to user specification. The simulated samples are pooled into a single distribution. The percentiles of this distribution are the estimated percentiles of the usual intake distribution. Because these values are still in transformed terms, the final step is a back-transformation procedure to state the percentiles of the usual intake distribution in the original units of nutrient intake.

Within the MIXTRAN macro, models for both amount of food or nutrient intake and probability of food or nutrient intake can be run. When both models are requested by the user, the user may further specify whether the two models should be linked by an assumption of correlation between their random effects. Amount only models are appropriate for estimating usual intake of foods or nutrients when there are few or no instances of zero values for observed daily intakes.⁴ Usual intake distributions of energy, protein, carbohydrates, fat, iron, calcium, zinc, vitamin A, thiamin, riboflavin, niacin, vitamin B6, folate and vitamin C were modeled using this one-part procedure since very few or no observations with zero intakes occurred. The two-part procedure, modeling both amount and probability of intake, is appropriate for foods or nutrients with a high number of zero intake instances.⁹ This was the case for vitamin B12, so its usual intake distribution was modeled using the two-part, probability and amount procedure, which first models the probability of consumption on any given day, then estimates the amount of consumption on a consumption day and multiplies these two estimates to arrive at usual

intake. Models of vitamin B12 intake were fit with and without the assumption of correlated random effects. Because the covariance in random terms was not significant when the correlated modeling procedure was used, model fit diagnostics (AIC) were better for the uncorrelated model, and estimated probabilities of inadequacy using the two modeling approaches were very similar, the uncorrelated, two-part model was used in the final estimation of the usual vitamin B12 intake distribution.

In order to control for differences by age and sex in mean intakes and intake variance, the MIXTRAN modeling step was run separately by age*sex subgroups. Age group was defined as less than five years old or five years and older. This definition of two age groups was chosen for better balance of group sizes and comparability to other studies. Change in intake with increasing age within age groups was not observed, and slightly higher intakes among boys were noted in exploratory data analysis. Differences in variance terms by age and by sex were found in model estimates of variance parameters and visual inspection of observed intake distributions. Modeling by age*sex subgroup was therefore considered sufficient and appropriate to control for differences in mean intakes or intake variance.

Days reported as market days, feast days or fasting days, as well as whether the recall period was a Sunday were considered as possible days on which intakes might systematically differ from other days. No differences in intakes were observed when comparing feast to non-feast or fast to non-fast days, or when comparing Sundays to weekdays. However, nutrient intakes were significantly lower on market days than on non-market days for many nutrients. In order to control for this market day effect, a binary variable indicating whether the recall period was a market day was included in the MIXTRAN macro using the 'weekend' option. Significant differences in error variance between market and non-market days were not observed when estimated by age*sex subgroup and inclusion of a separate error variance term for market days

did not improve model fit criteria (AIC) or substantially change other model parameter estimates, so separate estimates of residual variance on non-market and market days were not included in the final estimation of usual intake distributions. Market day was retained as a fixed effect only.

A separate estimation procedure using bivariate modeling must be used to estimate the distribution of the ratio of two usual nutrient intakes, such as the percent of total energy intake from carbohydrates.¹⁰ NCI provides macros for this procedure.⁸ Two macros, NLMIXED_UNIVARIATE and NLMIXED_BIVARIATE, extend the procedures in the MIXTRAN macro to estimate parameters of the bivariate distribution of two nutrients, and an additional macro, DISTRIB_BIVARIATE, parallels the procedures of the DISTRIB macro but produces percentiles of the usual intake ratio distribution. The NLMIXED_UNIVARIATE macro generates initial parameter estimates for the intake distributions and must be run separately for each nutrient in the ratio, and by any subgroups for which separate estimates of random effect and error variance are needed. The NLMIXED_BIVARIATE macro uses the output from the NLMIXED_UNIVARIATE macro and generates the parameters of the bivariate distribution of both nutrients in the ratio. The DISTRIB_BIVARIATE macro uses a Monte Carlo procedure to generate the distribution of the two nutrients, and the PERCENTILE_SURVEY macro uses the output from the DISTRIB_BIVARIATE macro to generate the moments of the distribution of the usual intake ratio. This set of macros was used to estimate distributions of the usual percent of total energy coming from protein, fat and carbohydrates.

For each nutrient, energy or macronutrient to energy ratio, after running MIXTRAN or NLMIXED_UNIVARIATE and NLMIXED_BIVARIATE macros separately by age*sex subgroup with adjustment for a market day fixed effect, the parameter estimates and predicted values from each subgroup were merged into single datasets for use in the DISTRIB macro. The DISTRIB

macro outputs datasets containing estimates for all percentiles of the usual intake distribution, in original scale of nutrient intake measurement. These percentiles were used to estimate probability of adequacy as will be described below.

Prediction of usual energy and nutrient intakes and usual dietary diversity

As has been discussed above and in Chapter 1, day-to-day variation in food and nutrient intakes is a form of error when the measure of interest is usual intakes. This error biases measures of association between two variables toward null. To address the third aim of the dissertation (Chapter 5), methods for accounting for this day-to-day variation in both nutrient intakes and dietary diversity scores were required. Failure to apply such methods may have introduced inaccuracy to our estimates of association between dietary diversity and usual nutrient intakes or between dietary diversity and overall nutrient intake adequacy. To account for this error in energy and nutrient intakes and in dietary diversity scores while examining seasonal effects, techniques previously described for cross-sectional analyses of dietary data were adapted for use in longitudinal models. This section details the extension of the NCI method for estimation of usual intakes to the prediction of individual usual intakes for use in regression analyses. The combination of this method with modeling approaches recommended for validation of dietary diversity scores against usual nutrient intakes or intake adequacy is then described.

Kipnis et al. demonstrate an extension of the NCI method described above for use when modeling an association between usual food or nutrient intakes and another variable such as nutritional status or a health outcome measure.¹¹ This method uses the one- or two-part procedure for modeling the amount of foods or nutrients consumed daily or the amount and probability of consumption for foods or nutrients consumed episodically. The extension of the method is to use the parameter estimates and predicted values from the one- or two-part

model of intakes to predict the usual intakes of individuals. This is in contrast to the methods described for Aim 1 analyses, in which the distribution of usual intakes is estimated but individual usual intakes are not. When the measurement error model of usual intake is linear, the predictor recommended in the approach described by Kipnis et al. takes the form of the best linear unbiased predictor (BLUP) of usual intake.¹¹

Where Kipnis et al. demonstrated the use of BLUPs of usual intake as independent variables in a model of a health outcome, this analysis uses BLUPs of usual intake as dependent variables in relation to DDS or as the basis for calculating a more complex indicator of overall nutrient adequacy, mean probability of adequacy.¹¹ Joseph and Carriquiry address this simultaneous use of BLUPs of usual intake and of DDS for minimizing bias due to day-to-day variation in cross-sectional models of the association between DDS and usual intakes or mean probability of adequacy (MPA).¹² MPA is an indicator of overall nutrient intake adequacy based on the probability approach, described below. Joseph and Carriquiry demonstrate an error-in-the-equation model that can account for both the day-to-day variation error in estimates of usual intake and DDS and potential correlation in the intake and DDS errors in cross-sectional associations.¹² The application of this approach to this study was limited by our goal of studying seasonal effects, which requires a longitudinal model. Therefore, a further recommendation from Joseph and Carriquiry was followed; when the full error in the equation modeling approach cannot be implemented, the use of BLUPs of both the DDS and usual intake can be used to reduce bias in simple linear regression approaches.¹² The authors recommend that for MPA, the BLUPs of usual nutrient intakes be used to calculate the probability of adequacy for each of the nutrients included, in order to reflect overall probability of adequacy of usual intakes.¹² This has been demonstrated in a recent paper by Arsenault et al., who used MPA based on BLUPs of usual nutrient intakes, though this study used mean DDS, in contrast to Joseph and Carriquiry

and this dissertation research.¹³ The use of BLUPs of usual intakes when examining associations between DDS and overall nutrient adequacy was also used in the Women’s Dietary Diversity Projects from which the FAO-recommended DDS used in Chapter 5 was developed.^{14,15}

Drawing on the work by Joseph and Carriquiry and using the NCI SAS macros as demonstrated by Kipnis et al., BLUPs of usual intakes of energy and 11 micronutrients were calculated, as well as BLUPs of the 7-food group and 10-food group versions of DDS used in Chapter 5. Instead of estimating usual intakes over the full six-month trial period as was done for Aim 1, BLUPs were predicted for each survey round using the data from the round of interest and adjacent rounds. For example, baseline usual intakes or usual DDS were calculated using the observed values from baseline and the first round of monthly monitoring, and monitoring round 1 usual intakes were calculated using the observed intakes from baseline and monitoring rounds 1 and 2. This was implemented using the MIXTRAN macro described above and an additional macro, INDIVINT, which uses the parameter estimates and predicted values to calculate BLUPS.⁸ For the reasons described in the section on estimating usual intake distributions, the amount-only model was used for energy and all nutrients other than vitamin B12, for which the amount and probability model was used. In this case, and as was described by Joseph and Carriquiry, covariates such as fixed or ‘weekend’ effects were not included when running the mixed models in MIXTRAN.¹² Without covariates, the formula for the BLUP of usual intake or usual DDS, \hat{T}_i , reduces to

$$\hat{T}_i = \hat{w}_i \bar{R}_i + (1 - \hat{w}_i)(\bar{R}_{..})$$

$$\text{where } \hat{w}_i = \frac{\hat{\sigma}_u^2}{\hat{\sigma}_u^2 + (\hat{\sigma}_\epsilon^2/n_i)}$$

\bar{R}_i is the within-person mean of observed values

$\bar{R}_{..}$ is the overall mean of observed values

$\hat{\sigma}_u^2$ is the estimated between-person variance

$\hat{\sigma}_\varepsilon^2$ is the estimated within-person variance,

and n_i is the number of replicates for individual i .

Probability of nutrient intake inadequacy

In Chapter 3, the prevalence of intake inadequacy among 4- to 8-year-old rural Zambian children is presented. In Chapter 5, mean probability of adequacy, an indicator of nutrient intake adequacy across eleven micronutrients, is used as a measure of overall nutrient adequacy against which dietary diversity scores are validated. This section describes how, in Chapter 3, the probability approach is used to estimate group prevalence of inadequacy based on the usual nutrient intake distributions describe above. The estimation probability of nutrient adequacy and mean probability of adequacy based on predicted usual intakes (BLUPs), as used in Chapter 5, is also described.

The probability of adequacy (PA) approach is recommended by the IOM for describing nutrient intake adequacy.¹⁶ The probability approach is a method for estimating the percentage of individuals at risk for inadequate nutrient intakes based on the distribution of nutrient requirements and the distribution of usual nutrient intakes.¹⁷ At the individual level, PA is equal to the probability that the intake requirement for an individual falls below their usual intake on the requirement distribution. That is to say, where r_i is the intake requirement for the i^{th} individual and T_i is the usual nutrient intake for the i^{th} individual, the probability of adequacy is equal to $Pr(r_i \leq T_i)$.¹² The group average probability of inadequacy is equal to the prevalence of inadequacy. It can be calculated by plotting the moments of the group's nutrient intake distribution against the risk curve for inadequacy by intake level.¹⁶

This approach can only be used for nutrients for which the Estimated Average Requirement (EAR) and coefficient of variation have been determined.^{16,17} For most nutrients -- those with approximately normal requirement distributions -- the EAR and standard deviation (SD) or coefficient of variation (CV) can be used to describe the distribution and calculate probability of adequacy for a particular intake as the proportion of the population with intake requirements at or below that intake.^{12,18} An important nutrient for which intake requirements cannot be accurately described by an approximately normal distribution is iron.¹⁶⁻¹⁸ Tables describing percentiles of the iron requirement distribution are used to determine the probability of adequacy for ranges of intakes.^{18,19}

Nutrient requirement distributions described by the IOM were used in calculations of probability of adequacy for most nutrients.²⁰⁻²³ For iron and zinc, different requirements were used to account for low bioavailability of these minerals in plant-based diets such as are typical in rural Zambia. The IOM Dietary Reference Intakes for iron were set assuming 18% bioavailability associated with a Western diet.²⁴ We adjusted the percentile values of the IOM iron requirement distribution for 4- to 8-year-old children to reflect an estimated 10% bioavailability of a high phytate, low meat diet, as recommended by the World Health Organization (WHO).²⁵ The rural Zambian diet is characterized by high intakes of phytate-containing cereals, vegetables and legumes and low intakes of meat, poultry and fish (Chapter 3, Appendix 1).^{26,27} Bioavailability of 10% rather than 5% is chosen based on inclusion of fish and occasionally chicken in the diet. This method is the same as that described by previous authors.^{12,13,28,29} Each percentile from the IOM iron requirement distribution is adjusted to shift from the 18% bioavailability assumed in the requirement distribution estimation to 10% bioavailability assumed based on WHO guidance by the following calculation:

$$\text{Adjusted percentile} = \text{IOM percentile} \times \left(\frac{18\%}{10\%}\right) = \text{IOM percentile} \times 1.8$$

To estimate probability of zinc intake adequacy, we used guidelines from the International Zinc Nutrition Consultative Group (IZiNCG).³⁰ IZiNCG recommends classifying diets into one of two types: a mixed or refined vegetarian diet with phytate : zinc molar ratio of 4–18, or an unrefined, cereal-based diet with phytate : zinc molar ratio >18. Based on their review of zinc absorption and requirement studies, iZiNCG estimated an EAR for 4- to 8-year-old children of 3 mg/day for the mixed or refined vegetarian diet or 4 mg/day for the unrefined, cereal-based diet. The recommended dietary allowances (RDAs) are 4 and 5 respectively, set 2 SD above the mean based on an estimated CV of 12.5%.³⁰ We used the EAR of 4 mg/day for the low bioavailability, unrefined, cereal-based diet. Two other recent papers have used the EAR and CV of zinc requirements suggested by IZiNCG when calculating probability of adequacy.^{13,28}

To estimate prevalence of nutrient intake inadequacy (Chapter 3), percentiles of the estimated usual nutrient intake distributions produced by the SAS macros were used. The midpoint between each pair of adjacent percentiles from the intake distribution ($pctile_x$, $pctile_{x+1}$) was converted to a z-score on the requirement distribution by the formula

$$z = \frac{[(pctile_{x+1} - pctile_x)/2] - EAR}{SD}$$

The SAS PROBNORM function was used to return the probability that a random draw from the requirement distribution falls below that z-score. This value is subtracted from 1 to obtain the probability of inadequacy for the midpoint. The probabilities of inadequacy for all 100 percentile midpoints are summed to arrive at the prevalence of inadequacy for each nutrient. This procedure was used to calculate prevalence of inadequacy in the full sample and by age and

sex subgroups (for which estimated intake distributions were output separately from the DISTRIB macro described above).

The probability of iron inadequacy is calculated using the percentiles of the iron distribution requirement provided by the IOM, adjusted for 10% bioavailability as described above.^{24,25} Since 13 percentile values are provided by the IOM to describe the requirement distribution, the probabilities of inadequacy for each 1 percent of the intake distribution (midpoint between adjacent percentiles as described above) is assigned as the average of the two requirement percentiles the intake falls between. For example, an intake of 8.88mg iron is assigned a probability of inadequacy of 35% because it falls between the 60th and 70th percentiles of the requirement distribution.

In analyses described in Chapter 5 (Aim 3), a similar procedure was used to estimate probability of adequacy for each of eleven micronutrients for each participating child. Probability of adequacy was calculated for each nutrient, for each child, in each survey round. These probabilities of inadequacy were calculated using the BLUPs of usual nutrient intakes described above. A child's probability of adequacy for a given nutrient in a given round is calculated as the area under the requirement distribution curve that falls below their usual intake. The probabilities of adequacy for the eleven nutrients were averaged to calculate a mean probability of adequacy for each child in each round.

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- Assisted with research design and protocol development, including initial site visits
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- Developed tablet-based 24 hour dietary recall tool
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- Coordinated and supervised mapping and census of study area
- Developed protocol and survey tools for collecting household food security and food acquisition data and market food prices and availability

- Jan–May 2015 Fellow, Gordis Teaching Fellowship, Johns Hopkins University
Public Health Studies Program, Krieger School of Arts & Sciences
Course Title: Global Food and Nutrition Security
- Designed ‘Global Food and Nutrition Security’, a seminar-style course on food security from a public health nutrition perspective
 - Responsible for all aspects of course design and implementation
- Oct–Dec 2011 Teaching Assistant, The Johns Hopkins Bloomberg School of Public Health
Sept 2013– Measurement Methods in Humanitarian Emergencies (Oct–Dec 2011)
Oct 2015 Food and Nutrition Policy (Sept–Oct 2013; Sept–Oct 2014; Sept–Oct 2015)
Nutrition Epidemiology (Jan–Mar 2014; July 2014; Jan–Mar 2015; June 2015)
Designing Healthy Diets (Mar–May 2014; Mar–May 2015)
- Assisted with course administration
 - Provided guidance and feedback to students via office hours and online
 - Created and graded quizzes and assignments
 - Guest lectured on implications of measurement error in nutrition data and calculating nutrient contents of mixed foods
- 2010–2011 Research Assistant, The Johns Hopkins Bloomberg School of Public Health
Center for Refugee and Disaster Response, Baltimore, MD
- Identification and cataloging of grants and foundation funding opportunities
 - Developed rapid community assessment tool for identification of food insecure communities in the Democratic Republic of Congo
 - Member of investigative team planning operations research on targeted food aid program effectiveness in South Sudan
 - Made planning visits to offices and field sites in Juba and Kuajok, South Sudan (June 2011)
 - Developed household survey instruments and data entry system for operations research in South Sudan
- 2007–2010 Graduate Student Researcher, University of California at Davis,
Davis, California and Bangladesh
Project title: Rice and Zinc dietary intakes among small children and their caregivers in rural Bangladesh
- Designed surveys on household food production and socio-economic status
 - Provided on-site supervision of the establishment and operation of two field research offices
 - Procured field supplies and equipment in accordance with study protocol, research institution procedures, and project budget
 - Trained field staff and supervisors on implementation of study protocol, including anthropometry, food weighing, dietary data recording and interviewing
 - Analyzed data and reported results in Master’s thesis, “Effect of Household Animal Production on Household Meat Consumption in Rural Bangladesh”

- Jan–Oct 2004 Volunteer, Peace Corps, Niger, Natural Resources Management program
- Trained in Zarma language and culture, natural resources management strategies and community needs assessment techniques
 - Initiated women’s small income generation projects
 - Engaged in cultural exchange and project planning with Nigerien professional counterparts and community members
 - Organized and conducted tree plantings with local youth and government-run tree nursery

PROFESSIONAL ACTIVITIES

- 2010–2016 American Society for Nutrition, Member
- 2008–2009 Association for International Agricultural and Rural Development, Member and Future Leaders Forum participant

TEACHING TRAINING

- 2014–2015 Preparing Future Faculty Teaching Academy
- Completed a teaching certification program through Johns Hopkins University, including classes, seminars and hands-on experience
- 2014 An Introduction to Evidence-Based Undergraduate STEM Teaching
- Completed the seven-week course offered by Vanderbilt University and the Center for Integration of Research, Teaching and Learning (CIRTL) Network via the Coursera online learning platform
- 2014 Teaching Assistanceships 1: Essential Elements
Teaching Assistanceships 2: Interactive Methods
- Completed the two-part teaching assistant training program offered by the Johns Hopkins Bloomberg School of Public Health Center for Teaching and Learning
- 2014 Exploring New Technologies for Active Learning: Maximizing Student Engagement
- Completed course offered by the Center for Integration of Research, Teaching and Learning (CIRTL) Network on flipped classrooms and online courses
- 2014 University Teaching 101
- Completed course offered by the Johns Hopkins University on the Coursera online course platform

HONORS AND AWARDS

- 2015 Johns Hopkins Bloomberg School of Public Health, Program in Human Nutrition
Harry D. Kruse Publication Award
- 2015 Johns Hopkins Bloomberg School of Public Health, Program in Human Nutrition
Elsa Orent Keiles Fellowship
- 2011 Johns Hopkins Bloomberg School of Public Health, Program in Human Nutrition
Richard and Barbara Hall Fund Award
- 2010 Johns Hopkins Bloomberg School of Public Health,
Bacon Field Chow Memorial Fellowship
- 2008 Association for International Agriculture and Rural Development
Scholarship recipient to the Future Leaders Forum and annual meeting
- 2006 University of California, Davis
Jesse D. Carr Fellowship
- 1999–2002 University of Iowa Undergraduate Dean’s List, 5 semesters

PUBLICATIONS

*Published as B. L. Lewis prior to September 2015

Na M, Gross A, Wu L, **Caswell B**, Talegawkar S, Palmer A. Internal Validity of the Food Access Survey Tool in assessing household food insecurity in rural Zambia. *Food Secur* 2016; 8(3):679-688.

Palmer AC, Siamusantu W, Chileshe J, Schulze KJ, Barffour M, Molobeka N, Kalungwana N, Arguello MA, **Caswell BL**, Klemm RDW, West KP. Provitamin A biofortified maize raises serum beta-carotene, but not retinol, in marginally nourished children: a cluster-randomized trial in rural Zambia. *Am J Clin Nutr.* 2016; 104(1):181-190.

Caswell BL, Talegawkar SA, Dyer B, Siamusantu W, Klemm RDW, Palmer AC. Assessing Child Nutrient Intakes Using a Tablet-based 24-hour Recall Tool in Rural Zambia. *Food Nutr Bull.* 2015; 36(4):467-480.

Arsenault JE, Yakes EA, Islam MM, Hossain MB, Ahmed T, Hotz C, **Lewis B**, Rahman AS, Jamil KM, Brown KH. Very Low Adequacy of Micronutrient Intakes by Young Children and Women in Rural Bangladesh Is Primarily Explained by Low Food Intake and Limited Diversity. *J Nutr.* 2013;143:197-203.

Lewis BL. Effect of household animal production on household meat consumption in rural Bangladesh. Diss. University of California, Davis, 2010. Dissertations & Theses: Full Text, ProQuest. Web. 24 Feb. 2011.

Yakes EA, Arsenault JE, Islam MM, Hossain MB, Ahmed T, German JB, Gillies LA, Rahman AS, Drake C, Jamil KM, **Lewis BL**, Brown KH. Intakes and breast-milk concentrations of essential fatty acids are low among Bangladeshi women with 24-48-month-old children. *Br J Nutr* 2011; 16: 1-11.

Arsenault JE, Yakes EA, Hossain MB, Islam MM, Ahmed T, Hotz C, **Lewis B**, Rahman AS, Jamil KM, Brown KH. The current high prevalence of dietary zinc inadequacy among children and women in rural Bangladesh could be substantially ameliorated by zinc biofortification of rice. *J Nutr* 2010; 140: 1683-1690.