Micronutrient adequacy and dietary diversity exert positive and distinct effects on linear growth in urban Zambian infants[1-3]

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[3]**Abbreviations used:** CIGNIS, Chilenje Infant Growth, Nutrition, and Infection Study; EFSA, European Food Safety Authority; IYCF, infant and young child feeding; LAZ, length-for-age Z-score; MAR, mean adequacy ration; MMDA, mean micronutrient density

adequacy; VIF, variance inflation factor; WHO, World Health Organization; WLZ, weight-for-length Z-score.

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ABSTRACT

Background: In the monitoring of infant and young child feeding, dietary diversity is used as an indicator of micronutrient adequacy; however, their relation may have weakened with the rising use of fortified complementary foods.

Objective: To assess the relation between dietary diversity and micronutrient adequacy in an urban infant population with a high consumption of fortified foods, and to investigate whether dietary diversity and micronutrient adequacy were independently associated with subsequent growth.

Methods: We used longitudinal data on 811 infants in the Chilenje Infant Growth, Nutrition, Infection Study conducted in Lusaka, Zambia. The relation between mean micronutrient adequacies and dietary diversity scores derived from 24-h diet recalls at 6 mo of age was investigated using Spearman rank correlation. Multiple linear regression was used to assess the association between micronutrient adequacy, dietary diversity and subsequent growth to 18 mo of age.

Results: Overall mean micronutrient density adequacy (MMDA) and MMDA of "problem micronutrients," defined as those micronutrients with mean density adequacies less than half of estimated needs (calcium, iron, zinc), were correlated with dietary diversity scores (ρ = 0.36 and 0.30, respectively, both $P < 0.0001$). Consumption of "sentinel foods" (iron-rich, fortified, animal-source, dairy) showed better correlation with MMDA than did dietary diversity (ρ = 0.58 to 0.69, all $P < 0.0001$). In fully adjusted analyses, MMDA Ca Fe Zn and dietary diversity, but not overall MMDA, were associated with linear growth to 18 mo (both $P \leq 0.028$).

Conclusions: Micronutrient adequacy among infants consuming fortified foods may be more accurately assessed using locally specific sentinel food indicators rather than dietary diversity scores. Nonetheless, dietary diversity has a positive effect on subsequent linear growth separate to that of micronutrient adequacy, warranting its continued monitoring and further investigation into the mechanisms underlying this finding. This trial was registered at www.controlled-trials.com as ISRCTN37460449.

Key words: complementary feeding, Zambia, micronutrient adequacy, dietary diversity, infant growth, fortification

INTRODUCTION

The first 1000 days of life are recognised as being critical for growth, with faltering in this period having proximal effects on child morbidity and mortality and enduring effects on attained height and work productivity in adulthood (1-4). Within this 1000-day window, the transition period from a milk-only diet to a diet that includes complementary foods presents the greatest challenge in terms of supplying adequate nutrition to support growth (5). This is attributable to the limited gastric capacity and rapid development of infants during this period, and is reflected in the micronutrient density (micronutrients per 100 kcal of food) needs of infants aged 6-8 mo, which are the greatest of any age group (5, 6).

Previously we examined the longitudinal relation between WHO infant and young child feeding (IYCF) indicators (7) and subsequent growth in a large group of Zambian infants (8). As hypothesized, we found that dietary diversity–which is used as an indicator of micronutrient adequacy–at 6 mo of age was positively associated with subsequent linear growth (length-for-age Z-score, LAZ) and weight gain (weight-for-length Z-score, WLZ) to 18 mo. Findings such as these are important for facilitating the improvement of infant feeding practices that promote optimal growth.

Although accepted as a proxy measure of micronutrient adequacy, the relation between dietary diversity and micronutrient adequacy has been examined in few populations (9). This relation may be particularly poor in the context of high rates of fortified food consumption, such as in the current urban Zambian population, where the HIV-positive status of one-fifth of mothers led many to utilise fortified breastmilk substitutes, a practice recommended by the WHO at the time (10). Therefore, we sought to examine how well dietary diversity scores correlated with the adequacy of micronutrient intakes according to breastfeeding status and fortified food consumption. In addition, we aimed to extend our previous findings and determine whether the adequacy of micronutrient intakes, like dietary diversity, was also related to subsequent growth. Because dietary diversity may affect aspects of health other than micronutrient adequacy, such as the gut microbiota, we also sought to investigate whether the relation between dietary diversity and growth was separate to that of micronutrient adequacy, while controlling for socio-demographic factors.

METHODS

Study design and population

The Chilenje Infant Growth, Nutrition and Infection Study (CIGNIS) was a randomized controlled fortification trial conducted in the middle-income area of Chilenje in Lusaka, the capital city of Zambia, the details and primary outcomes of which have been published previously (11). Lusaka had the highest prevalence of HIV amongst reproductive-aged women in HIV-endemic Zambia in 2007 at 22% (12). Between October 2005 and July 2009, all women attending the local government health clinic for infant vaccinations or growth monitoring were informed of the study. Infants were eligible for inclusion provided they were aged 6 mo ± 2 wks, free from severe disease, and their parents or guardians gave written informed consent. Infants were randomly assigned to receive one of two micronutrient-fortified porridges for a year: one was richly fortified at levels designed to meet the WHO estimated micronutrient needs of infants aged 9-11 mo with low breastmilk intake (13), and the other was fortified at proposed national maize flour fortification levels. The analyses presented in the current paper use baseline dietary intake data collected at 6 mo of age, prior to the provision of the micronutrient-fortified porridges. In Zambia, as in many countries, complementary food–defined as all non-breastmilk foods and drinks–is commonly introduced between 4 to 6 mo of age (12), and all infants in this study had been introduced to complementary foods prior to baseline.

Dietary intakes

Single, 24-h diet recall interviews were conducted by trained researchers with the caregiver of each infant at baseline, and when children were 12 and 18 mo of age. Baseline diet recalls, conducted prior to treatment initiation, were used in the current analyses. Although a second 24-h diet recall was repeated in a subset of infants, the treatment had already begun at this time and thus these recalls were not used in the current analyses. Interviews were conducted in the home, and portion sizes were determined by calibrating household utensils against graduated measuring cylinders, cups, or spoons. Recipe data were collected for composite dishes (14). A food composition database was created for the study using data primarily sourced from the South African Food Composition Tables (15) and ProPAN 2.0 software (16), with the iron, zinc and calcium content of commercial plant-based complementary foods consumed analyzed directly at the Department of Human Nutrition, University of Otago, New Zealand (17). Supplementary data were obtained where necessary from the West African Food Composition Table (18) and the USDA nutrient database (19). Following the calculation of nutrient intakes for each child, those with energy intakes from complementary food >3 SD above the mean (n = 11) were excluded from analyses.

Estimated nutrient intakes from breastmilk were derived using mean breastmilk volumes consumed by 6-8 mo infants in developing countries (20), and mature breastmilk composition data from WHO 1998 (6) and 2002 (20) publications, and the 2014 European Food Safety Authority (EFSA) external scientific report on breastmilk composition (21). Because the zinc concentrations in breastmilk decline markedly over time, data on zinc concentrations in breastmilk at 6 mo lactation were obtained from Brown et al. 2009 (22).

Recommended daily nutrient intakes for infants aged 6-12 mo were obtained from the 2013 EFSA scientific opinion document on infant and young child dietary intake requirements, which summarizes current international recommendations (23). Critical nutrient densities were calculated as the nutrient densities per 100 kcal of complementary foods required to meet the recommended nutrient intakes (6, 24). Critical nutrient densities were originally devised using estimated energy requirements as the denominator (6); however, due to a decrease in estimated energy requirements, critical densities calculated in this way have increased. Therefore, we chose to use median energy intakes as the denominator to produce more attainable critical nutrient densities for our population based on their actual energy intakes. For non-breastfed infants, the EFSA recommended intake values were used directly to calculate the critical nutrient densities, with the median energy intake of the non-breastfed group employed as the denominator. For breastfed infants, estimated nutrient needs from complementary foods were calculated by subtracting the estimated nutrient intakes from breastmilk from the total recommended nutrient intakes (24). These values were then divided by the median complementary food energy intake of the breastfed group to generate critical nutrient densities per 100 kcal of complementary food.

Micronutrient density adequacies for individuals were calculated as percentages of the critical intake densities, by dividing their actual intake densities by the corresponding critical intake densities (9). These were then capped at 100%, and averaged to create an overall mean micronutrient density adequacy (overall MMDA). “Problem micronutrients” were defined as those micronutrients for which the capped density adequacy averaged across all infants fell below 50%, indicating a large discrepancy between the estimated requirement and the actual intake of the study population. Micronutrient density adequacies of these problem nutrients were then averaged for individuals. Mean adequacy ratios (MAR) were also generated for comparison with MMDA, as these are reflective of the adequacy of micronutrient intakes rather than the adequacy of micronutrient densities (25). Nutrient adequacy ratios for individuals were calculated as percentages, by dividing their actual nutrient intakes from complementary foods by the recommended intakes, without taking energy intakes into account. These were again capped at 100%, and averaged to create an overall MAR for individuals.

Dietary diversity scores were generated by summing the number of WHO-defined food groups consumed over the 24-h recall period: 1) grains, roots and tubers; 2) legumes and nuts; 3) dairy products (milk, yoghurt, cheese); 4) eggs; 5) flesh foods (meat, fish, poultry and liver/organ meats); 6) vitamin A-rich fruits and vegetables; and 7) other fruits and vegetables (7). One point was awarded for each of the food groups consumed, generating a dietary diversity score for each individual with a range from 0 to 7. In calculating the dietary diversity score, all food groups within a mixed dish were counted separately, condiments and clear broths were not included, and no minimum quantity of consumption was defined (7).

Anthropometry

Length and weight measurements were taken at baseline and every 3 mo thereafter, with infants nude or wearing a diaper. Measurements were performed by trained anthropometrists at the study clinic, using standardized techniques and calibrated equipment (all anthropometric equipment was from Chasmor Ltd, London, UK). A length board (to 1 mm) and digital balance (to 10 g) were used for infant length and weight, respectively. Maternal height at recruitment was measured (to 1 mm) using a wall-mounted microtoise tape. All measurements were performed in triplicate and the median was used in analyses, with inter- and intra-examiner technical error of the measurements indicating good precision (26). Z-scores for LAZ and WLZ were generated using 2011 WHO growth reference data macros for Stata (27).

Socio-demographics and morbidity

Maternal education was categorized as primary school or less, secondary school, or college/university. Using principle component analysis, an asset-based index of household wealth was generated using the following variables: home ownership; floor type; connection to water, electricity and telephone; sanitation facilities; transport type; ownership of electrical appliances; number of meals per day; and ownership of animals and a vegetable garden. The index was divided into quintiles for use as a covariate in analyses.

Maternal HIV status was established using HIV antibody test results from the government antenatal health service. Infants were defined as HIV exposed if their mothers were HIV-positive. Basic care and prescription of antibiotics or antimalarials were available for infants at all clinic visits, including unscheduled visits, while referrals for other treatments were made to Chilenje main clinic or to the University Teaching Hospital. Data on hospital admissions prior to 6 mo of age were not collected, however reports of diarrhea (at least 3 loose stools or one bulky, watery stool in a 24-h period) in the last 3 mo were recorded at baseline. Infant hemoglobin concentrations (g/L) were measured in fingerprick blood samples (Hemocue, Dronfield, UK) at baseline.

Statistical analyses

Wilcoxon rank sum tests were used to determine whether mean micronutrient adequacy differed by breastfeeding status or consumption of fortified foods. Spearman rank correlation was used to examine the relationship between micronutrient adequacy and dietary diversity scores (all treated as continuous variables). The linearity of these relations was then tested using linear regression, with a significant quadratic dietary diversity term indicating deviation from linearity. Spearman rank correlation was also used to investigate the relationship between micronutrient adequacy and consumption of locally specific, nutrient-dense “sentinel foods" (yes/no): animal-source foods (flesh foods, eggs, dairy products), fortified foods, flesh foods, iron-rich foods, dairy foods and vitamin A-rich fruits and vegetables. These associations were tested overall and by breastfeeding status.

Multiple linear regression was used to determine the association between LAZ and WLZ at 18 mo and the explanatory variables overall MMDA, MMDA of problem nutrients, and MAR (all continuous variables). All analyses controlled for baseline LAZ or WLZ, and models adjusted for the following *a priori* confounders were produced: baseline hemoglobin concentration (continuous), birth weight (continuous), maternal height (continuous), sex, HIV exposure (yes/no), diarrhea in the last 3 mo (yes/no), treatment group, household wealth (5 categories) and maternal education (3 categories). Models further adjusted for fortified food intake (yes/no), baseline breastfeeding status (yes/no) and energy intake (continuous) were generated. Final, fully adjusted models additionally controlled for dietary diversity score (5 categories). Multicollinearity of all variables was assessed using variance inflation factors (VIF) (28) and model assumptions were checked (29). All analyses were conducted using Stata 11.2 (Stata Corporation 2010, College Station, Texas, United States), and a two-sided 0.05 level of significance was used in all cases.

Ethics

Ethical approval for the CIGNIS protocol was granted by the University of Zambia and the London School of Hygiene and Tropical Medicine and all mothers gave written informed consent. According to the standard protocol of care in Zambia, all infants received vitamin A supplements at their 6, 12, and 18 mo clinic visits under the national supplementation program.

RESULTS

Of the 1835 infants screened, 1316 were deemed eligible to participate in CIGNIS, and of these a total of 811 infants were enrolled (62% of all eligible). Reasons for refusal included: agreed but did not return (48%), family objected/family consultation required (39%), no interest/no reason (10%), and time commitment (3%). The prevalence of stunting and wasting among infants at baseline was 12% and 3%, respectively. Two-thirds (67%) of mothers were educated to high school level or above, with 29% having attained college or university qualifications (**Table 1**). Nine percent (9%) of all mothers were unaware of or did not disclose their HIV status and 22% were HIV-positive. At the conclusion of the trial, loss to follow-up was 22%; reasons for non-completion (moved away (32%), family objected (13%), child died (7%), lost interest (5%), would not disclose/other (43%)) did not differ by maternal education or household wealth (11). Among the 12 children who died during the study, eight were born to HIV-infected mothers, and 17 children tested HIV-positive at 18 mo.

Sixty-one percent (61%) of all infants consumed fortified foods, with lower rates among breastfed infants (55%) and HIV-unexposed infants (57%), and higher rates among of non-breastfed infants (90%) and HIV-exposed infants (72%). Among breastfed infants, the majority of fortified food consumed was infant cereal (70%), while for non-breastfed infants, infant cereal comprised 18% of all fortified food consumed. The remainder of fortified food consumed was infant formula, comprising 30% of all fortified foods consumed by breastfed infants and 82% of all fortified foods consumed by non-breastfed infants.

Micronutrient adequacy

Breastfed infants (*n* = 650) did not meet their estimated needs from complementary foods for most nutrients assessed, including folate, niacin, riboflavin, vitamin B6, thiamin, vitamin A, calcium, iron, zinc, phosphorus and magnesium, whereas non-breastfed infants (*n* = 137) did not achieve recommended intakes for folate, iron and zinc (**Table 2**). The intake of energy was sufficient in both groups. Mean nutrient density adequacies across all infants were less than 50% for calcium, iron and zinc. These three problem micronutrient density adequacies were averaged for individuals to create MMDA Ca Fe Zn.

Mean micronutrient adequacies, including overall MMDA, MMDA Ca Fe Zn, and MAR, differed according to breastfeeding status and consumption of fortified foods (all $P < 0.0001$ for Wilcoxon rank-sum tests) (**Table 3**). Non-breastfed infants consuming fortified foods achieved the highest mean micronutrient adequacies (89% to 93%), while breastfed infants consuming no fortified foods had the lowest mean micronutrient adequacies (19% to 49%). Infants consuming just one food group were most likely to be consuming the food group grains, roots or tubers, and had mean micronutrient adequacies of less than 50% across all measures of adequacy (data not shown). Mean micronutrient adequacies were generally higher as dietary diversity increased, however the relationship deviated from linearity in linear regression models, with statistically significant quadratic dietary diversity terms (data not shown).

Correlation between micronutrient adequacy, dietary diversity and sentinel foods

Overall, MAR displayed the best association with dietary diversity in Spearman rank correlation analyses, with positive and statistically significant correlations for both breastfed and non-breastfed infants alike, including those consuming fortified foods ($\rho = 0.25$ to 0.70, all $P < 0.05$) (Table 3). MMDA Ca Fe Zn was statistically significantly negatively correlated with dietary diversity among breastfed infants consuming fortified foods ($\rho = -0.25$, $P < 0.05$), likely because consumption of a single fortified food would provide a high quantity of these micronutrients. Indeed, in sentinel food analyses, correlation coefficients between the consumption of fortified foods and MMDA were high and statistically significant for breastfed infants (both $\rho = 0.71$, $P < 0.0001$) (**Table 4**). Overall correlation coefficients between mean micronutrient adequacies and consumption of locally specific sentinel foods (30) (animal-source foods, fortified foods, dairy foods, and iron-rich foods) ranged from ρ = 0.41 to 0.69 (all $P < 0.0001$). Few infants consumed flesh foods or vitamin A-rich fruits and vegetables, and these sentinel foods were not well correlated with micronutrient adequacies.

Micronutrient adequacy and dietary diversity in relation to subsequent growth

In multiple linear regression models adjusted for baseline LAZ or WLZ and other *a priori* confounders, all measures of mean micronutrient adequacy at 6 mo were positively associated with LAZ and WLZ at 18 mo of age (all $P \leq 0.02$) (**Table 5**). Introducing other dietary factors (fortified food consumption, breastfeeding status, energy intake) into the model rendered the effect of micronutrient adequacies at 6 mo on WLZ at 18 mo non-statistically significant (all $P \geq 0.11$), likely due to the inclusion of energy intake. In final models additionally adjusted for dietary diversity, the association between MMDA Ca Fe Zn at 6 mo and LAZ at 18 mo remained statistically significant ($P = 0.028$), while all other associations between micronutrient adequacy and LAZ became non-significant (both $P \geq 0.19$) (**Table 6**). In all three of these fully adjusted models, dietary diversity at 6 mo was positively associated with LAZ at 18 mo (all $P \leq 0.014$ for linear trend). There was no indication of problematic multicollinearity between dietary diversity, micronutrient adequacy, energy intake, socio-economic position or other covariates (mean VIF ≤ 2.4) (28).

DISCUSSION

In this urban setting, more than half of all infants studied consumed fortified complementary foods, and among these infants, dietary diversity scores were not well correlated with micronutrient adequacy. However, among infants not receiving fortified foods, correlations between overall micronutrient adequacies and dietary diversity were comparable to those reported elsewhere (9, 30-32). Given that the use of fortified complementary foods is likely to become increasingly widespread, the utility of dietary diversity as an indicator of micronutrient adequacy during infancy may become limited. Including locally specific sentinel foods indicators may thus be required to accurately assess the adequacy of micronutrient intakes in modern, urban settings. Mean micronutrient density adequacies of the three problem nutrients calcium, iron and zinc remained significantly associated with subsequent linear growth in models including dietary diversity, while the effect of overall micronutrient density adequacy on growth was rendered non-significant. Although it was not possible to elucidate the precise mechanism by which dietary diversity affects growth in this study, it appears to have actions separate to those of socio-demographic position and micronutrient adequacy.

Up to three decades have passed since the collection of the dietary data used in the pioneering research into the utility of dietary diversity as a simple indicator of micronutrient adequacy among infants in developing countries (9). Since the time these dietary data were collected, international guidelines on the fortification of complementary foods have been published (33, 34), and the availability and use of these foods has increased with the rise in urbanization and the movement of women into paid employment (35-38). With our large dataset from a middle-income, urban location where >60% of infants received fortified foods, we were in a unique position to re-examine the relationship between dietary diversity and micronutrient adequacy in this modern context. While the positive correlation between greater dietary diversity and higher micronutrient adequacy held overall, for breastfed infants consuming fortified foods, a statistically significant inverse correlation between dietary diversity and MMDA of problem micronutrients was observed. This finding highlights the need to assess additional measures of micronutrient intake adequacy alongside dietary diversity, such as the consumption of locally specific sentinel food groups (9). Fortunately, the consumption of iron-rich foods is already used to monitor dietary quality, being included in the WHO set of IYCF indicators (7). Difficulties may arise in the use of fortified products as sentinel food indicators due to varying levels of fortificants. While details on manufacturers' nutrition labels may be limited or inaccurate, our findings benefited from the use of laboratory-analyzed values for calcium, iron and zinc of commercial plant-based complementary foods. In a review of processed complementary foods, most of which were fortified, few contained WHO-recommended levels of calcium, iron and zinc (17), which may undermine their utility as sentinel food indicators in settings where such fortified complementary foods are consumed.

Calcium, iron and zinc have long been recognised as problem micronutrients for complementary-fed infants in resource-constrained countries, with target nutrient densities difficult to achieve due to the low levels and poor bioavailability of these minerals in traditional plant-based complementary foods used in these settings (6). Thus, it is not surprising that the supply of these micronutrients was the most limited in the current population, and that their MMDA at 6 mo was related to subsequent linear growth in fully adjusted models. Evidence from meta-analyses of randomized controlled trials supports our finding, with both zinc (39) and iron supplementation (40) improving linear growth in childhood. Calcium intakes have also recently been associated with improved linear growth from infancy to adulthood in a large cohort study in the Philippines (41).

Although we did not find an association between overall micronutrient adequacy and linear growth when controlling for dietary diversity, we cannot completely discount that an association exists. Being derived from single 24-h diet recalls, our estimates of dietary intakes in this population are subject to random error as a consequence of within-subject variation. While this may have attenuated the association between micronutrient adequacy and subsequent growth, dietary diversity scores are less affected by this type of random error (42). The refusal rate was greater than expected, and although birth weights of CIGNIS study participants were virtually identical to those of the total population of infants born in the Chilenje clinic during the study period (11), selection bias may have also affected estimates of associations between micronutrient adequacy, dietary diversity and subsequent growth (43).

While residual confounding may account for the relation between dietary diversity and linear growth noted here, it is important to consider other potential avenues by which dietary diversity may be operating. Namely, it is known that a more diverse diet is linked to a greater diversity in the human gut microbiota (44, 45), and current evidence suggests that macronutrients as opposed to micronutrients are largely responsible for this relationship (45-47). Infants' gut microbiota diversifies immediately following the introduction of complementary foods, mirroring the diversification in the food substrates provided (48-53), and continues to mature until around 2 to 3 years of age when it becomes adult-like (54). Reductions in gut microbial diversity were associated with the severity of growth faltering in two infant cohorts from Malawi and Bangladesh, while the increased relative abundance of a single bacterial genus was associated longitudinally with impaired linear growth (55, 56). We could not investigate here whether the effect of dietary diversity on growth is mediated by the gut microbiota, thus the exploration of the inter-relationships among infants' diet, the gut microbiome and growth in several ongoing trials in Malawi (57) and Zimbabwe (44) is timely. The beneficial effect of increased dietary diversity may also have implications in more advantaged settings. Dietary diversity among breastfed US infants aged 6 to 12 mo was recently reported to be lower than that in Mexico and China (58, 59), and in an earlier study of infants in Burkina Faso and Italy it was reported that the lower gut microbial diversity among the European children was attributable to a comparatively high-fat, high-sugar, high-protein diet low in plant polysaccharides (50).

Other potential benefits of increased dietary diversity beyond the increased intake of micronutrients include exposure to foods of different textures and flavors, enabling infants to develop healthy food preferences (60), and the intake of bioactive constituents that may not be present in fortified foods (61). The WHO IYCF indicators include “minimum dietary diversity”, with infants achieving this indicator receiving ≥ 4 food groups/d (7). Minimum dietary diversity is intended to indicate not only micronutrient adequacy, but also a high likelihood of consuming at least one animal-source food and at least one fruit or vegetable in addition to a staple food (7).

In sum, our investigation into the relation between dietary diversity and the adequacy of micronutrient intakes updates existing knowledge, providing new estimates of their association in the framework of changing infant complementary diets. While dietary diversity was poorly correlated with micronutrient adequacy among fortified food consumers, we observed that locally specific sentinel food group indicators, such as the already established WHO IYCF indicator “iron-rich foods,” provided a practicable additional measure for estimating micronutrient adequacy among these urban Zambian infants. The separate effects of the micronutrient adequacy of calcium, iron, and zinc and dietary diversity on subsequent infant linear growth demonstrated here underscores the importance of monitoring and improving dietary intakes at this critical period of development, and warrants further investigation into the mechanisms underlying the effect of dietary diversity on growth.

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SF and RSG designed the original CIGNIS trial; AM, MC, JS and LK conducted the research; SRM, SF, LAH and RSG designed the current study and wrote the paper. In addition, SRM analyzed the data and had primary responsibility for final content of the manuscript. All authors reviewed and approved the final manuscript.

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Table 1. Characteristics of the study population at baseline[1]

Characteristics	Value
Infant characteristics	
Total *n*	811
Age, *d*	184 ± 9
Female, *%*	53
Anthropometrics	
Birth weight, *kg*	3.05 ± 0.49
Length, *cm*	64.9 ± 2.48
LAZ	-0.81 ± 1.03
Weight, *kg*	7.28 ± 1.08
WLZ	0.15 ± 1.15
Currently breastfed, *%*	82
Diarrhea in last 3 mo, *%*	20
Maternal characteristics	
Height, *cm*	159.7 ± 6.00
Antenatal HIV status, *%*	
HIV-negative	70
HIV-positive	22
HIV status unknown	9
Education, *%*	
Primary school or less	33
Secondary school	38
College/university	29

[1] Values are means ± SD unless otherwise indicated. Percentages may not total to 100 due to rounding. LAZ, length-for-age Z-score; WLZ, weight-for-length Z-score.

Table 2. Micronutrient intakes and densities among urban Zambian infants at 6 mo of age[1]

Nutrient	Estimated daily nutrient intakes from BM[2]	Recommended daily nutrient intakes from CF[3]		Observed median nutrient intakes from CF (median, IQR)		Critical nutrient densities in CF[4]		Observed median nutrient densities in CF per 100 kcal (median, IQR)	
		BF	Non-BF	BF	Non-BF	BF	Non-BF	BF	Non-BF
n				650	137			650	137
Energy, *kcal*	409	164	573	219 (132-329)	650 (530-777)				
Folate, *µg*	30	50	80	23 (10-39)	61 (44-84)	22.9	12.3	9.6 (6.0-14.6)	9.1 (7.8-11.4)
Niacin, *mg*	0.9	4.1	5	2.5 (1.2-4.3)	5.7 (4.3-8.1)	1.9	0.8	1.0 (0.8-1.8)	0.9 (0.7-1.2)
Riboflavin, *mg*	0.21	0.19	0.4	0.18 (0.10-0.33)	0.8 (0.6-1.0)	0.09	0.06	0.08 (0.07-0.11)	0.12 (0.11-0.13)
Vitamin B6, *mg*	0.08	0.32	0.4	0.2 (0.1-0.3)	0.5 (0.4-0.6)	0.15	0.06	0.08 (0.06-0.09)	0.08 (0.07-0.08)
Thiamin, *mg*	0.04	0.26	0.3	0.13 (0.06-0.25)	0.4 (0.3-0.6)	0.12	0.05	0.06 (0.03-0.12)	0.06 (0.05-0.09)
Vitamin B12, *µg*	0.6	0	0.5	0.2 (0-0.5)	1 (0.6-1.3)	0	0.08	0.10 (0.0-0.19)	0.14 (0.11-0.18)
Vitamin C, *mg*	24	0	20	13 (2-29)	45 (30-59)	0	3.1	7.1 (1.2-11.6)	7.2 (5.1-8.6)
Vitamin A, *µg RE*	204	146	350	133 (69-236)	499 (368-644)	67	54	63.7 (45.9-81.4)	80.0 (69.4-86.8)
Calcium, *mg*	143	257	400	59 (18-134)	471 (307-675)	117	61.2	31.9 (8.7-66.3)	75.9 (52.2-114.5)

Iron, *mg*	0.18	7.82	8	1.37 (0.68-2.47)	6.63 (4.07-9.85)	3.6	1.2	0.6 (0.5-0.9)	1.05 (0.76-1.33)
Zinc, *mg*	0.47	3.53	4	0.98 (0.47-1.71)	3.93 (3.12-4.90)	1.6	0.6	0.44 (0.3-0.6)	0.61 (0.5-0.7)
Phosphorus, *mg*	80.5	219.5	300	110 (55-177)	409 (304-597)	100	45.9	52 (35-73)	66 (49-103)
Magnesium, *mg*	21.4	58.6	80	37.6 (17.3-61.5)	90 (62-122)	26.8	12.2	16.6 (10.8-26.4)	14.6 (10.9-18.2)

[1] BM, breastmilk; BF, breastfed; CF, complementary food (defined as all non-breastmilk foods); IQR, interquartile range; Non-BF, non-breastfed

[2] Mean BM volume consumed sourced from the WHO (20); nutrient composition sourced from the WHO (6, 20) and EFSA (21), with the exception of zinc, which was sourced from Brown et al. (22).

[3] Sourced from EFSA (23); energy requirements for infants 6-7 mo; calculated as estimated nutrient needs for BF infants by subtracting intake from BM from recommended intakes.

[4] Critical nutrient densities were calculated as the nutrient densities per 100 kcal of CF required to meet the recommended nutrient intakes (23), based on observed median energy intakes.

Table 3. Mean micronutrient adequacies and their correlation with dietary diversity scores among urban Zambian infants at 6 mo of age[1]

	n	MMDA Ca Fe Zn	Overall MMDA	MAR
Mean micronutrient adequacies, *%*, ± *SD*				
Overall	787	39 ± 26	64 ± 17	62 ± 24
Breastfed	650	29 ± 14*	58 ± 11*	56 ± 22*
No fortified foods consumed	293	19 ± 10*	49 ± 10*	48 ± 22*
Fortified foods consumed	357	37 ± 11*	65 ± 7*	63 ± 21*
Non-breastfed	137	86 ± 16*	92 ± 6*	88 ± 14*
No fortified foods consumed	14	61 ± 15*	81 ± 9*	71 ± 23*
Fortified foods consumed	123	89 ± 13*	93 ± 5*	90 ± 11*
Correlation coefficients between DDS and mean micronutrient adequacies				
Overall	787	0.30***	0.36***	0.46***
Breastfed	650	0.27***	0.34***	0.46***
No fortified foods consumed	293	0.46***	0.54***	0.54***
Fortified foods consumed	357	-0.25**	-0.08	0.25***
Non-breastfed	137	-0.07	0.07	0.25**
No fortified foods consumed	14	0.19	0.55**	0.70**
Fortified foods consumed	123	-0.10	0.07	0.25**

[1] DDS, dietary diversity scores; MMDA Ca Fe Zn, mean micronutrient density adequacy of Ca, Fe, Zn; Overall MMDA, mean micronutrient density adequacy of all measured micronutrients; MAR, mean adequacy ratio of all measured micronutrients.

*$P < 0.0001$ for Wilcoxon rank sum test with corresponding group (breastfed vs. non-breastfed; breastfed no fortified foods vs. breastfed fortified foods; non-breastfed no fortified foods vs. non-breastfed fortified foods).

**$P < 0.05$ for Spearman rank correlation test.

***$P < 0.0001$ for Spearman rank correlation test.

Table 4. Correlation between mean micronutrient adequacies and consumption of sentinel foods among urban Zambian infants at 6 mo of age[1]

		Correlation coefficients between micronutrient adequacy and sentinel foods		
	n consuming (%)	MMDA Ca Fe Zn	Overall MMDA	MAR
Overall, *n* = 787				
Animal-source foods	555 (71)	0.58**	0.58**	0.46**
Fortified foods	480 (61)	0.68**	0.69**	0.41**
Dairy foods	508 (65)	0.63**	0.63**	0.47**
Iron-rich foods	483 (61)	0.68**	0.68**	0.41**
Flesh foods	13 (2)	-0.02	-0.003	0.05
Vitamin A-rich F&V	27 (3)	-0.03	0.02	-0.003
Breastfed, *n* = 650				
Animal-source foods	421 (65)	0.56**	0.55**	0.39**
Fortified foods	357 (55)	0.71**	0.71**	0.32**
Dairy foods	374 (58)	0.58**	0.59**	0.37**
Iron-rich foods	360 (55)	0.70**	0.70**	0.32**
Flesh foods	12 (2)	-0.003	0.02	0.08*
Vitamin A-rich F&V	27 (4)	0.04	0.10*	0.06
Non-breastfed, *n* = 137				
Animal-source foods	134 (98)	0.25*	0.25*	0.25*
Fortified foods	123 (90)	0.46**	0.47**	0.35**
Dairy foods	134 (98)	0.25*	0.25*	0.25*
Iron-rich foods	123 (90)	0.46**	0.47**	0.35**
Flesh foods	1 (<1)	0.03	0.05	0.02

Vitamin A-rich F&V	0 (0)	-	-	-

[1] MMDA Ca Fe Zn, mean micronutrient density adequacy of Ca, Fe, Zn; F&V, fruit and vegetables; Overall MMDA, mean micronutrient density adequacy of all measured micronutrients; MAR, mean adequacy ratio of all measured micronutrients.

*$P < 0.05$ for Spearman rank correlation test.

**$P < 0.0001$ for Spearman rank correlation test.

Table 5. Mean micronutrient adequacies among urban Zambian infants at 6 mo of age in relation to LAZ and WLZ at 18 mo[1]

	LAZ 18 mo		WLZ 18 mo	
	β (95% CI)	*P*	β (95% CI)	*P*
Model 1 (*n* = 618)[2]				
MMDA Ca Fe Zn	0.39 (0.17, 0.61)	0.001	0.49 (0.25, 0.73)	<0.001
Overall MMDA	0.70 (0.36, 1.04)	<0.001	0.68 (0.30, 1.05)	<0.001
MAR	0.42 (0.18, 0.66)	0.001	0.68 (0.42, 0.94)	<0.001
Model 2 (*n* = 551)[3]				
MMDA Ca Fe Zn	0.52 (0.24, 0.80)	<0.001	0.61 (0.28, 0.93)	<0.001
Overall MMDA	0.81 (0.38, 1.24)	<0.001	0.78 (0.29 1.28)	0.002
MAR	0.31 (0.05, 0.56)	0.019	0.68 (0.39, 0.97)	<0.001
Model 3 (n = 551)[4]				
MMDA Ca Fe Zn	0.61 (0.10, 1.13)	0.018	0.48 (-0.10, 1.05)	0.11
Overall MMDA	0.88 (0.14, 1.62)	0.02	0.23 (-0.61, 1.07)	0.59
MAR	0.54 (0.03, 1.06)	0.038	0.44 (-0.15, 1.04)	0.14

[1] LAZ, length-for-age Z-score; MMDA Ca Fe Zn, mean micronutrient density adequacy of Ca, Fe, Zn; Overall MMDA, mean micronutrient density adequacy of all measured micronutrients; MAR, mean adequacy ratio of all measured micronutrients; WLZ, weight-for-length Z-score.

[2] Adjusted for LAZ or WLZ at baseline.

[3] Adjusted for baseline LAZ/WLZ and hemoglobin, sex, birth weight, treatment group, HIV exposure, diarrhea in last 3 mo, maternal height and education, and household wealth.

[4] Adjusted for covariates in Model 2 plus energy intake, baseline breastfeeding status, and consumption of fortified foods.

Table 6. Mean micronutrient adequacies and dietary diversity among urban Zambian infants at 6 mo of age in relation to LAZ and WLZ at 18 mo[1]

	LAZ 18 mo		WLZ 18 mo	
	β (95% CI)	*P*	β (95% CI)	*P*
MMDA Ca Fe Zn model (*n* = 551)[2]				
MMDA Ca Fe Zn	0.58 (0.06, 1.10)	0.028	0.44 (-0.15, 1.03)	0.14
Dietary diversity		0.002[3]		0.18[3]
DDS 1	referent		referent	
DDS 2	0.16 (-0.06, 0.38)		0.10 (-0.16, 0.35)	
DDS 3	0.24 (0.01, 0.48)		0.13 (-0.14, 0.39)	
DDS 4	0.35 (0.08, 0.63)		0.15 (-0.17, 0.47)	
DDS ≥5	0.49 (0.05, 0.94)		0.37 (-0.14, 0.88)	
Overall MMDA model (*n* = 551)[2]				
Overall MMDA	0.55 (-0.27, 1.37)	0.19	-0.03 (-0.97, 0.91)	0.95
Dietary diversity		0.014[3]		0.18[3]
DDS 1	referent		referent	
DDS 2	0.14 (-0.10, 0.38)		0.14 (-0.14, 0.41)	
DDS 3	0.20 (-0.05, 0.46)		0.15 (-0.14, 0.45)	
DDS 4	0.31 (0.004, 0.61)		0.18 (-0.12, 0.52)	
DDS ≥5	0.45 (-0.02, 0.91)		0.41 (-0.12, 0.94)	
MAR model (*n* = 551)[2]				
MAR	0.35 (-0.18, 0.89)	0.2	0.36 (-0.27, 0.98)	0.26
Dietary diversity		0.008[3]		0.29[3]
DDS 1	referent		referent	
DDS 2	0.16 (-0.07, 0.39)		0.09 (-0.17, 0.35)	
DDS 3	0.23 (-0.02, 0.47)		0.10 (-0.18, 0.38)	
DDS 4	0.33 (0.04, 0.62)		0.11 (-0.22, 0.45)	

DDS ≥5	0.48 (0.03, 0.94)	0.36 (-0.16, 0.87)

[1] DDS, dietary diversity score; LAZ, length-for-age Z-score; MMDA Ca Fe Zn, mean micronutrient density adequacy of Ca, Fe, Zn; Overall MMDA, mean micronutrient density adequacy of all measured micronutrients; MAR, mean adequacy ratio of all measured micronutrients; WLZ, weight-for-length Z-score.

[2] Adjusted for baseline LAZ or WLZ and hemoglobin, sex, birth weight, treatment group, HIV exposure, diarrhea in last 3 mo, maternal height and education, household wealth, energy intake, baseline breastfeeding status, consumption of fortified foods, mean micronutrient adequacy, and DDS.

[3] *P* value for linear trend.