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Micronutrient adequacy and dietary diversity exert positive and distinct effects on linear growth in urban Zambian infants¹⁻³

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³Abbreviations 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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1 **ABSTRACT**

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

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

9 **Methods:** We used longitudinal data on 811 infants in the Chilenje Infant Growth, Nutrition,
10 Infection Study conducted in Lusaka, Zambia. The relation between mean micronutrient
11 adequacies and dietary diversity scores derived from 24-h diet recalls at 6 mo of age was
12 investigated using Spearman rank correlation. Multiple linear regression was used to assess
13 the association between micronutrient adequacy, dietary diversity and subsequent growth to
14 18 mo of age.

15 **Results:** Overall mean micronutrient density adequacy (MMDA) and MMDA of “problem
16 micronutrients,” defined as those micronutrients with mean density adequacies less than half
17 of estimated needs (calcium, iron, zinc), were correlated with dietary diversity scores ($\rho =$
18 0.36 and 0.30, respectively, both $P < 0.0001$). Consumption of “sentinel foods” (iron-rich,
19 fortified, animal-source, dairy) showed better correlation with MMDA than did dietary
20 diversity ($\rho = 0.58$ to 0.69 , all $P < 0.0001$). In fully adjusted analyses, MMDA Ca Fe Zn and
21 dietary diversity, but not overall MMDA, were associated with linear growth to 18 mo (both
22 $P \leq 0.028$).

23 **Conclusions:** Micronutrient adequacy among infants consuming fortified foods may be more
24 accurately assessed using locally specific sentinel food indicators rather than dietary diversity
25 scores. Nonetheless, dietary diversity has a positive effect on subsequent linear growth

26 separate to that of micronutrient adequacy, warranting its continued monitoring and further
27 investigation into the mechanisms underlying this finding. This trial was registered at
28 www.controlled-trials.com as ISRCTN37460449.

29

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

32 INTRODUCTION

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

41 Previously we examined the longitudinal relation between WHO infant and young
42 child feeding (IYCF) indicators (7) and subsequent growth in a large group of Zambian
43 infants (8). As hypothesized, we found that dietary diversity—which is used as an indicator of
44 micronutrient adequacy—at 6 mo of age was positively associated with subsequent linear
45 growth (length-for-age Z-score, LAZ) and weight gain (weight-for-length Z-score, WLZ) to
46 18 mo. Findings such as these are important for facilitating the improvement of infant feeding
47 practices that promote optimal growth.

48 Although accepted as a proxy measure of micronutrient adequacy, the relation
49 between dietary diversity and micronutrient adequacy has been examined in few populations
50 (9). This relation may be particularly poor in the context of high rates of fortified food
51 consumption, such as in the current urban Zambian population, where the HIV-positive status
52 of one-fifth of mothers led many to utilise fortified breastmilk substitutes, a practice
53 recommended by the WHO at the time (10). Therefore, we sought to examine how well
54 dietary diversity scores correlated with the adequacy of micronutrient intakes according to
55 breastfeeding status and fortified food consumption. In addition, we aimed to extend our
56 previous findings and determine whether the adequacy of micronutrient intakes, like dietary

57 diversity, was also related to subsequent growth. Because dietary diversity may affect aspects
58 of health other than micronutrient adequacy, such as the gut microbiota, we also sought to
59 investigate whether the relation between dietary diversity and growth was separate to that of
60 micronutrient adequacy, while controlling for socio-demographic factors.

61

62 **METHODS**

63

64 **Study design and population**

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

82

83 Dietary intakes

84 Single, 24-h diet recall interviews were conducted by trained researchers with the caregiver of
85 each infant at baseline, and when children were 12 and 18 mo of age. Baseline diet recalls,
86 conducted prior to treatment initiation, were used in the current analyses. Although a second
87 24-h diet recall was repeated in a subset of infants, the treatment had already begun at this
88 time and thus these recalls were not used in the current analyses. Interviews were conducted
89 in the home, and portion sizes were determined by calibrating household utensils against
90 graduated measuring cylinders, cups, or spoons. Recipe data were collected for composite
91 dishes (14). A food composition database was created for the study using data primarily
92 sourced from the South African Food Composition Tables (15) and ProPAN 2.0 software
93 (16), with the iron, zinc and calcium content of commercial plant-based complementary foods
94 consumed analyzed directly at the Department of Human Nutrition, University of Otago, New
95 Zealand (17). Supplementary data were obtained where necessary from the West African
96 Food Composition Table (18) and the USDA nutrient database (19). Following the calculation
97 of nutrient intakes for each child, those with energy intakes from complementary food >3 SD
98 above the mean ($n = 11$) were excluded from analyses.

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

105 Recommended daily nutrient intakes for infants aged 6-12 mo were obtained from the
106 2013 EFSA scientific opinion document on infant and young child dietary intake

107 requirements, which summarizes current international recommendations (23). Critical nutrient
108 densities were calculated as the nutrient densities per 100 kcal of complementary foods
109 required to meet the recommended nutrient intakes (6, 24). Critical nutrient densities were
110 originally devised using estimated energy requirements as the denominator (6); however, due
111 to a decrease in estimated energy requirements, critical densities calculated in this way have
112 increased. Therefore, we chose to use median energy intakes as the denominator to produce
113 more attainable critical nutrient densities for our population based on their actual energy
114 intakes. For non-breastfed infants, the EFSA recommended intake values were used directly
115 to calculate the critical nutrient densities, with the median energy intake of the non-breastfed
116 group employed as the denominator. For breastfed infants, estimated nutrient needs from
117 complementary foods were calculated by subtracting the estimated nutrient intakes from
118 breastmilk from the total recommended nutrient intakes (24). These values were then divided
119 by the median complementary food energy intake of the breastfed group to generate critical
120 nutrient densities per 100 kcal of complementary food.

121 Micronutrient density adequacies for individuals were calculated as percentages of the
122 critical intake densities, by dividing their actual intake densities by the corresponding critical
123 intake densities (9). These were then capped at 100%, and averaged to create an overall mean
124 micronutrient density adequacy (overall MMDA). “Problem micronutrients” were defined as
125 those micronutrients for which the capped density adequacy averaged across all infants fell
126 below 50%, indicating a large discrepancy between the estimated requirement and the actual
127 intake of the study population. Micronutrient density adequacies of these problem nutrients
128 were then averaged for individuals. Mean adequacy ratios (MAR) were also generated for
129 comparison with MMDA, as these are reflective of the adequacy of micronutrient intakes
130 rather than the adequacy of micronutrient densities (25). Nutrient adequacy ratios for
131 individuals were calculated as percentages, by dividing their actual nutrient intakes from

132 complementary foods by the recommended intakes, without taking energy intakes into
133 account. These were again capped at 100%, and averaged to create an overall MAR for
134 individuals.

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

143

144 **Anthropometry**

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

155

156

157 **Socio-demographics and morbidity**

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

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

173

174 **Statistical analyses**

175 Wilcoxon rank sum tests were used to determine whether mean micronutrient adequacy
176 differed by breastfeeding status or consumption of fortified foods. Spearman rank correlation
177 was used to examine the relationship between micronutrient adequacy and dietary diversity
178 scores (all treated as continuous variables). The linearity of these relations was then tested
179 using linear regression, with a significant quadratic dietary diversity term indicating deviation
180 from linearity. Spearman rank correlation was also used to investigate the relationship
181 between micronutrient adequacy and consumption of locally specific, nutrient-dense “sentinel

182 foods” (yes/no): animal-source foods (flesh foods, eggs, dairy products), fortified foods, flesh
183 foods, iron-rich foods, dairy foods and vitamin A-rich fruits and vegetables. These
184 associations were tested overall and by breastfeeding status.

185 Multiple linear regression was used to determine the association between LAZ and
186 WLZ at 18 mo and the explanatory variables overall MMDA, MMDA of problem nutrients,
187 and MAR (all continuous variables). All analyses controlled for baseline LAZ or WLZ, and
188 models adjusted for the following *a priori* confounders were produced: baseline hemoglobin
189 concentration (continuous), birth weight (continuous), maternal height (continuous), sex, HIV
190 exposure (yes/no), diarrhea in the last 3 mo (yes/no), treatment group, household wealth (5
191 categories) and maternal education (3 categories). Models further adjusted for fortified food
192 intake (yes/no), baseline breastfeeding status (yes/no) and energy intake (continuous) were
193 generated. Final, fully adjusted models additionally controlled for dietary diversity score (5
194 categories). Multicollinearity of all variables was assessed using variance inflation factors
195 (VIF) (28) and model assumptions were checked (29). All analyses were conducted using
196 Stata 11.2 (Stata Corporation 2010, College Station, Texas, United States), and a two-sided
197 0.05 level of significance was used in all cases.

198

199 **Ethics**

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

205

206 **RESULTS**

207 Of the 1835 infants screened, 1316 were deemed eligible to participate in CIGNIS, and of
208 these a total of 811 infants were enrolled (62% of all eligible). Reasons for refusal included:
209 agreed but did not return (48%), family objected/family consultation required (39%), no
210 interest/no reason (10%), and time commitment (3%). The prevalence of stunting and wasting
211 among infants at baseline was 12% and 3%, respectively. Two-thirds (67%) of mothers were
212 educated to high school level or above, with 29% having attained college or university
213 qualifications (**Table 1**). Nine percent (9%) of all mothers were unaware of or did not disclose
214 their HIV status and 22% were HIV-positive. At the conclusion of the trial, loss to follow-up
215 was 22%; reasons for non-completion (moved away (32%), family objected (13%), child died
216 (7%), lost interest (5%), would not disclose/other (43%)) did not differ by maternal education
217 or household wealth (11). Among the 12 children who died during the study, eight were born
218 to HIV-infected mothers, and 17 children tested HIV-positive at 18 mo.

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

226

227 **Micronutrient adequacy**

228 Breastfed infants ($n = 650$) did not meet their estimated needs from complementary foods for
229 most nutrients assessed, including folate, niacin, riboflavin, vitamin B6, thiamin, vitamin A,
230 calcium, iron, zinc, phosphorus and magnesium, whereas non-breastfed infants ($n = 137$) did
231 not achieve recommended intakes for folate, iron and zinc (**Table 2**). The intake of energy

232 was sufficient in both groups. Mean nutrient density adequacies across all infants were less
233 than 50% for calcium, iron and zinc. These three problem micronutrient density adequacies
234 were averaged for individuals to create MMDA Ca Fe Zn.

235 Mean micronutrient adequacies, including overall MMDA, MMDA Ca Fe Zn, and
236 MAR, differed according to breastfeeding status and consumption of fortified foods (all $P <$
237 0.0001 for Wilcoxon rank-sum tests) (**Table 3**). Non-breastfed infants consuming fortified
238 foods achieved the highest mean micronutrient adequacies (89% to 93%), while breastfed
239 infants consuming no fortified foods had the lowest mean micronutrient adequacies (19% to
240 49%). Infants consuming just one food group were most likely to be consuming the food
241 group grains, roots or tubers, and had mean micronutrient adequacies of less than 50% across
242 all measures of adequacy (data not shown). Mean micronutrient adequacies were generally
243 higher as dietary diversity increased, however the relationship deviated from linearity in
244 linear regression models, with statistically significant quadratic dietary diversity terms (data
245 not shown).

246

247 **Correlation between micronutrient adequacy, dietary diversity and sentinel foods**

248 Overall, MAR displayed the best association with dietary diversity in Spearman rank
249 correlation analyses, with positive and statistically significant correlations for both breastfed
250 and non-breastfed infants alike, including those consuming fortified foods ($\rho = 0.25$ to 0.70 ,
251 all $P < 0.05$) (Table 3). MMDA Ca Fe Zn was statistically significantly negatively correlated
252 with dietary diversity among breastfed infants consuming fortified foods ($\rho = -0.25$, $P <$
253 0.05), likely because consumption of a single fortified food would provide a high quantity of
254 these micronutrients. Indeed, in sentinel food analyses, correlation coefficients between the
255 consumption of fortified foods and MMDA were high and statistically significant for
256 breastfed infants (both $\rho = 0.71$, $P < 0.0001$) (**Table 4**). Overall correlation coefficients

257 between mean micronutrient adequacies and consumption of locally specific sentinel foods
258 (30) (animal-source foods, fortified foods, dairy foods, and iron-rich foods) ranged from $\rho =$
259 0.41 to 0.69 (all $P < 0.0001$). Few infants consumed flesh foods or vitamin A-rich fruits and
260 vegetables, and these sentinel foods were not well correlated with micronutrient adequacies.
261

262 **Micronutrient adequacy and dietary diversity in relation to subsequent growth**

263 In multiple linear regression models adjusted for baseline LAZ or WLZ and other *a priori*
264 confounders, all measures of mean micronutrient adequacy at 6 mo were positively associated
265 with LAZ and WLZ at 18 mo of age (all $P \leq 0.02$) (**Table 5**). Introducing other dietary factors
266 (fortified food consumption, breastfeeding status, energy intake) into the model rendered the
267 effect of micronutrient adequacies at 6 mo on WLZ at 18 mo non-statistically significant (all
268 $P \geq 0.11$), likely due to the inclusion of energy intake. In final models additionally adjusted
269 for dietary diversity, the association between MMDA Ca Fe Zn at 6 mo and LAZ at 18 mo
270 remained statistically significant ($P = 0.028$), while all other associations between
271 micronutrient adequacy and LAZ became non-significant (both $P \geq 0.19$) (**Table 6**). In all
272 three of these fully adjusted models, dietary diversity at 6 mo was positively associated with
273 LAZ at 18 mo (all $P \leq 0.014$ for linear trend). There was no indication of problematic
274 multicollinearity between dietary diversity, micronutrient adequacy, energy intake, socio-
275 economic position or other covariates (mean VIF ≤ 2.4) (28).
276
277

278 DISCUSSION

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

294 Up to three decades have passed since the collection of the dietary data used in the
295 pioneering research into the utility of dietary diversity as a simple indicator of micronutrient
296 adequacy among infants in developing countries (9). Since the time these dietary data were
297 collected, international guidelines on the fortification of complementary foods have been
298 published (33, 34), and the availability and use of these foods has increased with the rise in
299 urbanization and the movement of women into paid employment (35-38). With our large
300 dataset from a middle-income, urban location where >60% of infants received fortified foods,
301 we were in a unique position to re-examine the relationship between dietary diversity and
302 micronutrient adequacy in this modern context. While the positive correlation between greater

303 dietary diversity and higher micronutrient adequacy held overall, for breastfed infants
304 consuming fortified foods, a statistically significant inverse correlation between dietary
305 diversity and MMDA of problem micronutrients was observed. This finding highlights the
306 need to assess additional measures of micronutrient intake adequacy alongside dietary
307 diversity, such as the consumption of locally specific sentinel food groups (9). Fortunately,
308 the consumption of iron-rich foods is already used to monitor dietary quality, being included
309 in the WHO set of IYCF indicators (7). Difficulties may arise in the use of fortified products
310 as sentinel food indicators due to varying levels of fortificants. While details on
311 manufacturers' nutrition labels may be limited or inaccurate, our findings benefited from the
312 use of laboratory-analyzed values for calcium, iron and zinc of commercial plant-based
313 complementary foods. In a review of processed complementary foods, most of which were
314 fortified, few contained WHO-recommended levels of calcium, iron and zinc (17), which may
315 undermine their utility as sentinel food indicators in settings where such fortified
316 complementary foods are consumed.

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

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

337 While residual confounding may account for the relation between dietary diversity and
338 linear growth noted here, it is important to consider other potential avenues by which dietary
339 diversity may be operating. Namely, it is known that a more diverse diet is linked to a greater
340 diversity in the human gut microbiota (44, 45), and current evidence suggests that
341 macronutrients as opposed to micronutrients are largely responsible for this relationship (45-
342 47). Infants' gut microbiota diversifies immediately following the introduction of
343 complementary foods, mirroring the diversification in the food substrates provided (48-53),
344 and continues to mature until around 2 to 3 years of age when it becomes adult-like (54).
345 Reductions in gut microbial diversity were associated with the severity of growth faltering in
346 two infant cohorts from Malawi and Bangladesh, while the increased relative abundance of a
347 single bacterial genus was associated longitudinally with impaired linear growth (55, 56). We
348 could not investigate here whether the effect of dietary diversity on growth is mediated by the
349 gut microbiota, thus the exploration of the inter-relationships among infants' diet, the gut
350 microbiome and growth in several ongoing trials in Malawi (57) and Zimbabwe (44) is
351 timely. The beneficial effect of increased dietary diversity may also have implications in more

352 advantaged settings. Dietary diversity among breastfed US infants aged 6 to 12 mo was
353 recently reported to be lower than that in Mexico and China (58, 59), and in an earlier study
354 of infants in Burkina Faso and Italy it was reported that the lower gut microbial diversity
355 among the European children was attributable to a comparatively high-fat, high-sugar, high-
356 protein diet low in plant polysaccharides (50).

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

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

376

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380 analyzed the data and had primary responsibility for final content of the manuscript. All

381 authors reviewed and approved the final manuscript.

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Table 1. Characteristics of the study population at baseline¹

Characteristics	Value
Infant characteristics	
Total <i>n</i>	811
Age, <i>d</i>	184 ± 9
Female, %	53
Anthropometrics	
Birth weight, <i>kg</i>	3.05 ± 0.49
Length, <i>cm</i>	64.9 ± 2.48
LAZ	-0.81 ± 1.03
Weight, <i>kg</i>	7.28 ± 1.08
WLZ	0.15 ± 1.15
Currently breastfed, %	82
Diarrhea in last 3 mo, %	20
Maternal characteristics	
Height, <i>cm</i>	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 age¹

Nutrient	Estimated	Recommended		Observed median nutrient intakes		Critical nutrient		Observed median nutrient densities in	
	daily nutrient intakes from BM ²	daily nutrient intakes from CF ³		from CF (median, IQR)		densities in CF ⁴		CF per 100 kcal (median, IQR)	
		BF	Non-BF	BF	Non-BF	BF	Non-BF	BF	Non-BF
<i>n</i>				650	137			650	137
Energy, <i>kcal</i>	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, <i>mg</i>	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, <i>mg</i>	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, <i>mg</i>	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, <i>mg</i>	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, <i>mg</i>	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, $\mu\text{g RE}$	204	146	350	133 (69-236)	499 (368-644)	67	54	63.7 (45.9-81.4)	80.0 (69.4-86.8)
Calcium, <i>mg</i>	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, <i>mg</i>	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, <i>mg</i>	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, <i>mg</i>	80.5	219.5	300	110 (55-177)	409 (304-597)	100	45.9	52 (35-73)	66 (49-103)
Magnesium, <i>mg</i>	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 age¹

	<i>n</i>	MMDA			Overall MMDA	MAR
		Ca	Fe	Zn		
Mean micronutrient adequacies, %, ± <i>SD</i>						
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 age¹

	<i>n</i> consuming (%)	Correlation coefficients between micronutrient adequacy and sentinel foods		
		MMDA Ca Fe Zn	Overall MMDA	MAR
Overall, <i>n</i> = 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, <i>n</i> = 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, <i>n</i> = 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)	-	-	-
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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 mo¹

	LAZ 18 mo		WLZ 18 mo	
	β (95% CI)	<i>P</i>	β (95% CI)	<i>P</i>
Model 1 (<i>n</i> = 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 (<i>n</i> = 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 (<i>n</i> = 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 mo¹

	LAZ 18 mo		WLZ 18 mo	
	β (95% CI)	<i>P</i>	β (95% CI)	<i>P</i>
MMDA Ca Fe Zn model (<i>n</i> = 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 \geq 5	0.49 (0.05, 0.94)		0.37 (-0.14, 0.88)	
Overall MMDA model (<i>n</i> = 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 \geq 5	0.45 (-0.02, 0.91)		0.41 (-0.12, 0.94)	
MAR model (<i>n</i> = 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.