

1                    Micronutrient intake and prevalence of micronutrient inadequacy among  
2                    women (15-49 y) and children (6-59 mo) in South Kivu and  
3                    Kongo Central, Democratic Republic of the Congo (DRC)  
4

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## 34 **Abstract**

35 Iron biofortified beans and carotenoid enriched cassava are proposed as a solution to  
36 combat iron and vitamin A deficiencies, respectively, in the Democratic Republic of Congo  
37 (DRC). To inform the need for biofortified foods we conducted a survey in 2014, in two  
38 provinces of the DRC, South Kivu and Kongo Central. Unexpectedly, women of  
39 reproductive age (WRA; 15-49 y) and their children (6-59 m) had a low prevalence of  
40 biochemical iron and vitamin A deficiency, based on ferritin and retinol binding protein,  
41 respectively. However, to better understand the lack of biochemical deficiency of these  
42 nutrients we examined the prevalence of inadequate intake for these and other select  
43 nutrients. Dietary intake was assessed using 24-hour recalls (with repeats) among 744  
44 mother-child dyads. In WRA the prevalence of inadequate iron intakes was 32% (10%  
45 bioavailability). The prevalence of inadequate vitamin A intakes was low in South Kivu  
46 (18%) and negligible in Kongo Central (1%). The prevalence of inadequate iron intake  
47 was 74% in infants (6-11 m) and ~22% in the older children (12-59 months). There was  
48 a high prevalence of inadequate zinc intake in women and children (i.e. 83% WRA)  
49 consistent with our findings of a high prevalence of low serum zinc in the same sample.  
50 Dietary data here corroborate the low prevalence of biochemical vitamin A deficiency but  
51 not iron. However, any change to the supply of red palm oil would dramatically reduce  
52 population vitamin A intakes, thus a carotenoid enriched cassava program may be  
53 beneficial as a safety net measure. Additionally, iron biofortified beans may be warranted  
54 given the high rates of dietary inadequacy and uncertainty around the validity of iron

55 biomarkers. Crops biofortified with zinc also appear warranted. We caution that our  
56 findings cannot be extrapolated to the entire Congo where diverse agro-ecological  
57 landscape exist or when political and environmental shocks occur which challenge food  
58 production.

## 60 Introduction

61           Micronutrient deficiencies leading to anemia are thought to be common in the  
62 Democratic Republic of the Congo (DRC), especially among children and women of  
63 reproductive age. According to the most recent population survey (2013–2014), the  
64 overall prevalence of anemia was ~35% among women of reproductive age (WRA) (15–  
65 49 years) and ~60% in children (6-59 years).(1) Anemia in the DRC has been attributed  
66 to micronutrient deficiencies, mainly iron, but also zinc, folate, vitamins A and B<sub>12</sub>.  
67 Unfortunately, very little is known about the micronutrient status of the Congolese.  
68 HarvestPlus has plans to scale up their program on iron biofortified beans and carotenoid  
69 biofortified cassava in the DRC to help control anemia as well as reduce blindness and  
70 decrease mortality from infectious disease caused by vitamin A deficiency.

71           To inform the need for the intervention we conducted a micronutrient survey of 744  
72 mother-child pairs in two provinces in the DRC, South Kivu and Kongo Central. Despite  
73 relatively high rates of anemia we found very low rates of biochemical iron (low ferritin) or  
74 vitamin A deficiency (low retinol binding protein). Iron deficiency anemia (low ferritin with  
75 anemia) was present in less than 1% of WRA and affected about 4% of children overall,  
76 rising to 9% in children 6-23 months in South Kivu. We found very little evidence of  
77 biochemical vitamin B<sub>12</sub> or folate deficiency but found low serum zinc in 50% of WRA and  
78 around 25% of young children.

79           We are aware that there are other causes of anemia such as malaria, helminths  
80 infection, and the presence of genetic blood disorders. Nevertheless, we were perplexed  
81 by the general lack of micronutrient deficiencies in this population and sought  
82 corroborating evidence from dietary intake information that was obtained from 24 hour

83 recalls in the study group. Here we aim to estimate the prevalence of inadequacy for  
84 select nutrients among WRA (15-49 y) and children (6-59 m), to identify major dietary  
85 sources of nutrients, and to quantify the intakes of potential vehicles (i.e. bean) to assess  
86 the potential impact of biofortification.

## 87 **Materials and Methods**

### 88 **Study design**

89           A cross-sectional survey using the multiple pass 24-hour dietary recall (24HDR)  
90 interview method was conducted from August to October 2014 to assess usual dietary  
91 intake and estimate the prevalence of inadequacy for select nutrients in two contrasting  
92 provinces in the DRC, South Kivu and Kongo Central. This was part of a larger nutritional  
93 assessment survey in the two provinces, the biochemical results of which have been  
94 reported on in Harvey-Leeson et al.(2) These two provinces were selected as they were  
95 politically stable at the time of data collection and captured the country's diverse  
96 geographical landscape. The densely populated eastern province of South Kivu was  
97 selected for its high altitude, where most of the population lives approximately 1000 m  
98 above sea level. In contrast, Kongo Central, the westernmost province, traversed by the  
99 Congo River, was selected as it was located in a lowland area. Ethical approval was  
100 obtained from the Clinical Research Ethics Board at the University of British Columbia  
101 (H14-01279), the Université de Kinshasa (ESP/CE/033/14), and the Université  
102 Catholique de Bukavu (UCB/CIENC/25/2014).

### 103 **Participants**

104           The sampling method used for this study has been described in detail  
105 elsewhere.(2) Briefly, participants were selected using a three-stage probability  
106 proportionate to size (PPS) sampling method. During the first stage, only those health  
107 zones in closest proximity to biological sample processing centres in Bukavu and  
108 Kinshasa were included in the sampling frame to maintain the integrity of the blood

109 samples collected concurrently with the 24 HDRs. In South Kivu, 10 health zones within  
110 a 60-km radius of Bukavu were selected, and in Kongo Central all six health zones within  
111 the district of Lukaya (nearest to Kinshasa) were selected. Villages were then randomly  
112 selected proportionate to population size (South Kivu: n=40 villages; Kongo Central: n=25  
113 villages). Finally, households were randomly selected using the random walk method;  
114 three enumerators walked in opposite directions from one another at 120° starting from  
115 the geographical centre of each village. Every tenth household that met inclusion criteria  
116 was then selected and enrolled into the study until 12 households were selected from  
117 each village. If the household did not meet criteria or refused to participate, the next  
118 available household in sequence that met eligibility criteria was chosen.

119         Households were included in the cross-sectional study if participating mothers met  
120 the following criteria: 1) mothers were the female heads of the selected household; 2)  
121 were non-pregnant and between 15-49 years of age; 3) had no serious health conditions  
122 or chronic diseases; 4) had a child 6-59 months of age living in the home; and 5) agreed  
123 to provide consent to draw blood samples for themselves and their child.

124         The sample sizes in this study were calculated to measure the expected point  
125 estimates of the prevalence of anemia among children 6-59 months of age in the two  
126 study provinces. According to the most recent national demographic and health survey,  
127 the prevalence of anemia among this age group was 60% in South Kivu and 71% in  
128 Kongo Central. (1) With a margin of error of  $\pm 7.5\%$ , and a 95% confidence interval with  
129 alpha 0.05, we estimated a minimum sample size of 328 and 287 mother-child pairs in  
130 South Kivu and Kongo Central respectively would be needed. The number of villages  
131 (clusters) and units of observation were determined in keeping with micronutrient survey

132 sampling guidelines and adjusted proportionate to population size.(3) In total, n=40 and  
133 n=25 clusters with 12 households per cluster, in South Kivu and Kongo Central,  
134 respectively were included. However, due to unexpected security issues in one health  
135 zone, the final sample size in South Kivu was reduced to n=444 mother-child pairs. In  
136 Kongo Central, the final sample size was n=300 mother-child pairs.

## 137 **Data collection**

138 After eligibility was confirmed, women heads of selected households completed a  
139 baseline questionnaire designed to elicit information on household demographics, as well  
140 as current knowledge and practices regarding health, nutrition, and water, sanitation and  
141 hygiene (WASH) behaviours. Following this questionnaire, anthropometric  
142 measurements (height/length, weight and mid upper arm circumference [(MUAC) children  
143 only]) were taken, along with non-fasting blood samples for each mother-child pair.  
144 Finally, a multi-pass 24 HDR interview was completed with all participants, the results of  
145 which are presented in this paper.

## 146 **Dietary Assessment**

147 The 24 HDR is a validated and widely used data collection instrument that helps  
148 gather detailed information on all foods and beverages consumed by a respondent in the  
149 preceding 24 hours.(4, 5) This method of dietary assessment is particularly ideal for use  
150 in low resource settings as it is inexpensive, uses little equipment, and takes on average  
151 less than one hour to complete. Despite its ease of use however, there are limitations  
152 including: over/under estimation of food and beverages consumed; poor estimation of  
153 portion sizes; and failure to capture day-to-day variations in food intake. Gibson and  
154 Ferguson addressed these limitations and subsequently developed the multi-pass 24

155 HDR.(6) For our study, an adapted version of the multi-pass 24 HDR method, tailored for  
156 use in the DRC context, was used.

## 157 **Food composition table development**

158 To develop a locally appropriate food composition table and means of determining  
159 portion size, focus group discussions were held with women in communities neighbouring  
160 the study villages. From these discussions, information on common and unique  
161 household recipes was systematically collected. This information was used to generate  
162 conversion factors (factors to allow reported quantities of food to be converted to grams,  
163 including volume equivalents, household measures, clay models, and linear dimensions)  
164 and a nutrient composition table of local foods. A nutrient composition table from a  
165 HarvestPlus study in Uganda (7) was used as the starting point and then completed with  
166 the USDA Nutrient Database 22 (8). Because red palm oil is an important source of  
167 provitamin A carotenoids (pVACS) in the DRC, we conducted a post hoc sampling of red  
168 palm oil from street side vendors in nine distinct markets located in South Kivu, Kongo  
169 Central and three additional provinces in the DRC and analysed the samples for  
170 carotenoids, which had a mean of 400 µg/g pVAC (range: 273 – 600 µg/g pVAC). The  
171 average of the results of was then applied to the food composition table, assuming a  
172 bioconversion factor of 7 to 1 micrograms of beta carotene equivalents to retinol and a  
173 retention of 70% from cooking.(9) Nutrient intakes from breastmilk were also estimated  
174 for partially breastfed children based on the World Health Organization (WHO) average  
175 daily intake scenario based on age category.(10) Finally, we created new variables for  
176 cassava and beans to express the reported intakes as dry weight beans and fresh weight  
177 peeled cassava, by applying conversion factors to the most commonly reported foods.

178 Gram weight of cooked beans was divided by 2.54 to derive dry weight bean  
179 consumption, and uncooked cassava flour used for *fufu* was divided by 0.31 and  
180 uncooked fermented dough used for *chikwangwe* by 0.23 to derive gram weight of peeled  
181 cassava roots. Nearly all the maize consumed in the 24HDR was reported as maize flour,  
182 thus no conversion factors were applied.

### 183 **Structured interviews**

184 A pre-interview visit from trained enumerators was conducted two days before  
185 each 24 HDR. Respondents were presented with a picture chart of locally appropriate  
186 foods and beverages and asked to tick all items consumed by themselves and their child  
187 for the following day. In addition, respondents were asked to save all packaging from  
188 commercial foods and beverages and use standardized plates, cups, bowls and utensils  
189 to help quantify their consumption throughout the following day. On the interview day,  
190 respondents were asked to produce the picture chart and all food packages from the  
191 previous day. The interview was then conducted in four separate sections and information  
192 on the quantity, type, and method of preparation for each food item was recorded.  
193 Probing techniques were used to obtain detailed descriptions of foods, and visual aids  
194 (playdough and rice) were used to assist with estimation of portion sizes. Repeat recalls  
195 were completed on non-consecutive days with a subset of the sample, ~20% of  
196 participants, to account for day-to-day variation in intake. All interviews were conducted  
197 with the mother; if another caregiver fed the child, they were interviewed for the child's  
198 recall. Mothers were asked about their own food and beverage consumption first, followed  
199 by their child. All data collection took place between August and October 2014.

## 200 **Data management and analysis**

201 Trained enumerators using established guidelines recorded all interview data on  
202 hard-copy questionnaires.(6) Field supervisors reviewed each questionnaire for accuracy  
203 and completion before data entry into CS Dietary software.(11) Using this software,  
204 macronutrient (energy) and micronutrient (iron, zinc, vitamin C, thiamine, riboflavin,  
205 niacin, vitamin B6, folate, vitamin B<sub>12</sub>, and calcium) intakes were quantified. Within-person  
206 variability in intake was adjusted for by implementing the ISU method (12) with the Intake  
207 Modelling, Assessment and Planning Program (IMAPP) software.(13) For foods and  
208 nutrients that were not consumed nearly every day, we used the PC Software for Intake  
209 Distribution Estimation program which estimates the joint distribution of usual intake and  
210 probability of consumption.(14) The estimated average requirement (EAR) cut point  
211 method developed by the Institute of Medicine (IOM) was used to estimate prevalence of  
212 inadequate intake for all micronutrients except iron, for which the probability density  
213 approach was used due to the skewed distribution of requirements in women and  
214 children.(15) The probability of inadequacy was calculated by comparing the sample's  
215 iron distribution to the iron requirement distribution percentiles at 7.5% and 10%  
216 bioavailability.(6) For infants, we adopted the iron intake variation components from  
217 among the 1 to 3 year olds to estimate prevalence of inadequacy since the sample size  
218 and number of repeated recalls for infants was too small to estimate these parameters.  
219 The Institute of Medicine EAR values were used for all nutrients, except for zinc.(15) For  
220 zinc, we used the zinc physiological requirements from the European Food Safety  
221 Authority (16) and applied bioavailability estimates for an unrefined cereal diet from  
222 IZINCG to calculate an EAR appropriate for this setting. These were determined to be the

223 most up-to-date evidence on zinc requirements, given the marked differences in zinc  
224 physiological requirements that are reported by various expert groups (17, 18). Data  
225 analysis was conducted using Stata version 15.(19)

## 226 **Results**

227           The response rates for the dietary surveys were 87% in South Kivu and 97% in  
228 Kongo Central. The participation rate in South Kivu was lower due to security concerns  
229 as one of the villages was the site of a rebel conflict and was therefore withdrawn from  
230 data collection. Households were slightly larger in South Kivu with  $6.6 \pm 2.6$  members  
231 and had a greater proportion of women, 48%, reporting no formal schooling. Kongo  
232 Central, on the other hand, had smaller household sizes with  $5.7 \pm 2.3$  members and had  
233 a higher proportion of women, 43%, reporting completion of secondary school (Table1).  
234 Access to health services were similar between provinces. Approximately 90% of all  
235 children received vitamin A capsules, and roughly 80% were treated with de-worming  
236 tablets in the past six months. Iron supplementation was less common with less than  
237 10% of children receiving tablets or syrup in the preceding six months.

238 **Table 1.** Household characteristics of the study populations South Kivu and Kongo  
 239 Central, Democratic Republic of the Congo<sup>a</sup>

	South Kivu N=444	Kongo Central N=300
Household size, mean ± SD	6.6 ± 2.6	5.7 ± 2.3
Mother's age, y, mean ± SD	29.2 ± 0.4	29.9 ± 0.4
Lactating Mothers	386 (87)	219 (73)
Mother's education level		
No schooling	214 (48)	29 (10)
Primary school	139 (31)	141 (47)
Secondary school	87 (20)	128 (43)
Post-Secondary	4 (1)	1 (0)
Child currently breastfeeding	322 (73)	200 (67)
Child received vitamin A capsule in past 6 mo	397 (89)	279 (93)
Child received de-worming in past 6 mo	368 (83)	232 (77)
Child received oral iron in past 6 mo	36 (8)	30 (10)

240 <sup>a</sup>Values are n (%) unless otherwise stated

### 241 **Mean nutrient intake and prevalence of inadequacy**

242       The mean daily nutrient intakes and prevalence of inadequacy among WRA in  
 243 South Kivu and Kongo Central are summarized in Table 2, and in Tables 3-5 for their  
 244 children 6-59 months of age. Mean ± SE vitamin A intakes were lower among WRA in  
 245 South Kivu than Kongo Central (757 ± 14 µg vs. 1109 ± 19 µg RAE/d). The prevalence  
 246 of Vitamin A inadequacy among WRA was moderate in South Kivu (18%) and absent in  
 247 Kongo Central (1%). Iron intake was similar among women in South Kivu and Kongo  
 248 Central at 18.8 ± 0.3 mg/d and 20.3 ± 0.5 mg/d, respectively. Assuming a mixed  
 249 vegetarian diet and moderate bioavailability of 10%, the prevalence of inadequacy for iron  
 250 was similar in both provinces at 32%. The prevalence of inadequate zinc intakes was  
 251 high in both provinces at 86% in South Kivu and 79% Kongo Central. Among WRA, the  
 252 prevalence of inadequacy was high for thiamine (50%), riboflavin (47%), and calcium  
 253 (75%), but moderate for folate (22%), niacin (29%) and vitamin B<sub>12</sub> (24%)

**Table 2:** Daily energy and micronutrient intakes of the mothers and prevalence of inadequate micronutrient intakes.

	South Kivu (n=441)			Kongo Central (n=290)		All (n=731)	
	EAR	Intake <sup>a</sup>	Prevalence of Inadequacy <sup>b</sup>	Intake	Prevalence of Inadequacy <sup>b</sup>	Intake <sup>a</sup>	Prevalence of Inadequacy <sup>b</sup>
Energy, kcal	-	2372 ± 40		2338 ± 48		2359 ± 31	
Vitamin A, µg RAE <sup>c</sup>	500	757 ± 14	18 ± 8	1109 ± 19	1 ± 5	898 ± 13	10 ± 7
Iron, mg	8.1	18.8 ± 0.3		20.3 ± 0.5		19.4 ± 0.3	
10% bioavailability			33		29		32
7.5% bioavailability			58		52		56
Zinc, mg	11.6	8.3 ± 0.2	86 ± 5	9.0 ± 0.3	79 ± 4	8.6 ± 0.1	83 ± 3
Vitamin C, mg	60	83 ± 1	22 ± 9	90 ± 1	8 ± 24	86 ± 1	15 ± 12
Thiamine, mg	0.9	0.95 ± 0.02	53 ± 3	1.0 ± 0.02	43 ± 3	0.98 ± 0.02	50 ± 2
Riboflavin, mg	0.9	0.98 ± 0.02	47 ± 3	1.0 ± 0.03	46 ± 4	1.0 ± 0.01	47 ± 2
Niacin, mg	11	14.4 ± 0.2	26 ± 5	13.7 ± 0.3	35 ± 4	14 ± 0.2	29 ± 3
Vitamin B6, mg	1.1	2.1 ± 0.03	4 ± 4	1.6 ± 0.04	20 ± 5	1.9 ± 0.03	9 ± 3
Folate, µg	320	522 ± 13	21 ± 9	508 ± 15	24 ± 5	517 ± 10	22 ± 5
Vitamin B <sub>12</sub> , µg	2	14.5 ± 12	<1	2.0 ± 0.2 <sup>d</sup>	NE	9.5 ± 3.9	18
Calcium, mg	800	581 ± 11	85 ± 6	789 ± 20	59 ± 5	665 ± 11	75 ± 4

254 <sup>a</sup>Intake data are presented as mean ± SEM and represent the usual nutrient intake for women in South Kivu and in Kongo  
255 Central.

256 <sup>b</sup>Prevalence of inadequacy was estimated as the percent of the usual intake distribution below the estimated average  
257 requirement (EAR) for each micronutrient (IOM, 2000) except for iron. For iron, the full probability approach at 7.5% and  
258 10% bioavailability was used (Gibson and Ferguson, 2008). Estimated average requirement for zinc was calculated using  
259 physiologic requirements reported by EFSA (EFSA, 2014) and bioavailability assumptions for unrefined cereal diets  
260 (IZINCG, 2004). The Institute of Medicine's estimated average requirements for all other nutrients were used. Standard  
261 errors (SE) are estimated and reported by IMAPP for the cut-point approach and are reported here.

262 <sup>c</sup>As retinol activity equivalents (RAE). 1 RAE=1µg retinol, 12 µg β-carotene, 24 µg α-carotene, or 24 µg β-cryptoxanthin.  
263 The RAE for dietary provitamin A carotenoids is two-fold greater than retinol equivalents (RE), whereas the RAE for  
264 preformed vitamin A is the same as RE.  
265 <sup>d</sup>Usual intake could not be estimated thus mean ± SEM of a single day's intake for each woman are presented.

266 Among children, age-specific EARs, IZINCG values, or iron distribution percentiles were  
267 applied. The prevalence of inadequacy for infants 6-11 months was calculated for iron  
268 and zinc only because other nutrients lack an EAR (Table 3). The prevalence of zinc  
269 inadequacy was high among infants; ~ 90% of all infants had inadequate intakes, with  
270 higher rates in in South Kivu (91%) than Kongo Central (67%). Similarly, 82% of infants  
271 had inadequate iron (10% bioavailability) intakes in South Kivu compared with 64% in  
272 Kongo Central.

**Table 3:** Mean daily energy and micronutrient intakes of infants 6-11 mo. and prevalence of inadequate micronutrient intakes

	EAR	South Kivu (n=49; R=10)		Kongo Central (n=38; R=6)		All (n=87; R=16)	
		Intake <sup>a</sup>	Prevalence of Inadequacy <sup>b</sup>	Intake <sup>a</sup>	Prevalence of Inadequacy <sup>b</sup>	Intake <sup>a</sup>	Prevalence of Inadequacy <sup>b</sup>
Energy, kcal		942 ± 35		1040 ± 40		980 ± 26	
Vitamin A, µg RAE <sup>c</sup>	--	349 ± 16	--	641 ± 43	--	451 ± 18	--
Iron, mg	6.9	4.3 ± 0.3		6.4 ± 0.6		5.2 ± 0.3	
10% bioavailability			82		64		74
7.5% bioavailability			92		76		86
Zinc, mg	3.5	2.3 ± 0.1	91 ± 18	3.1 ± 0.2	67 ± 15	2.6 ± 0.1	88 ± 17
Vitamin C, mg		45 ± 1	--	48 ± 2	--	46 ± 1	--
Thiamine, mg		0.33 ± 0.02	--	0.42 ± 0.02	--	0.37 ± 0.01	--
Riboflavin, mg		0.47 ± 0.02	--	0.51 ± 0.02	--	0.49 ± 0.01	--
Niacin, mg		5.1 ± 0.3	--	5.1 ± 0.3	--	5.1 ± 0.2	--
Vitamin B6, mg		0.64 ± 0.04	--	0.57 ± 0.04	--	0.59 ± 0.03	--
Folate, µg		134 ± 4	--	152 ± 8	--	152 ± 4	--
Vitamin B <sub>12</sub> , µg <sup>2</sup>		5.6 ± 1.2	--	1.2 ± 0.1	--	3.7 ± 0.7	--
Calcium, mg		309 ± 6	--	449 ± 14	--	363 ± 7	--

273 <sup>a</sup>Intake data are presented as mean ± SEM and represent the usual nutrient intake for infants in South Kivu and Kongo  
274 Central; the SEM refers to inter-individual variation. <sup>b</sup>Prevalence of inadequacy was estimated as the percent of the usual  
275 intake distribution below the estimated average requirement (EAR) for each micronutrient (IOM, 2000) except iron and zinc.  
276 For iron, the full probability approach at 7.5% and 10% bioavailability was used (Gibson and Ferguson, 2008), and for zinc,  
277 IZINCG physiologic requirements were used (EFSA, 2014). Standard errors (SE) are estimated and reported by IMAPP for  
278 the cut-point approach and are reported here.

279 <sup>c</sup>As retinol activity equivalents (RAE). 1 RAE=1µg retinol, 12 µg β-carotene, 24 µg α-carotene, or 24 µg β-cryptoxanthin.  
280 The RAE for dietary provitamin A carotenoids is two-fold greater than retinol equivalents (RE), whereas the RAE for  
281 preformed vitamin A is the same as RE

282           Results for children 1-3 y and 4-6 y of age are summarized in Tables 4 and 5,  
283 respectively. Similar patterns observed with maternal vitamin A, iron, and zinc intakes  
284 were found among children. Among both age groups, mean vitamin A intakes were lower  
285 in South Kivu than in Kongo Central, but the prevalence of inadequate vitamin A intakes  
286 was less than 10% in both provinces and age groups. The probability of inadequate iron  
287 intakes (10% bioavailability) was much lower among children than their mothers at only  
288 20-25% . In contrast, zinc inadequacy was equally as high, although slightly higher  
289 among children 4-6 y compared to 1-3 y at 69% and 57%, respectively. Calcium  
290 inadequacy was quite pronounced among all children, 76% among 1-3 years of age and  
291 95% among the 4-6 year old age group. For other nutrients the prevalence of inadequacy  
292 was similar to their mothers.

**Table 4:** Mean daily energy and micronutrient intakes of children 1-3 y and prevalence of inadequate micronutrient intakes

	EAR	South Kivu (n=334; R=72)		Kongo Central (n=225; R=48)		All (n=559; R =120)	
		Intake <sup>a</sup>	Prevalence of Inadequacy <sup>b</sup>	Intake <sup>a</sup>	Prevalence of Inadequacy <sup>b</sup>	Intake <sup>a</sup>	Prevalence of Inadequacy <sup>b</sup>
Energy, kcal		1343 ± 29		1190 ± 13		1296 ± 19	
Vitamin A, µg RAE <sup>c</sup>	210	497 ± 12	6 ± 5	656 ± 18	1 ± 3	564 ± 11	4 ± 3
Iron, mg	3.0	10.0 ± 0.3		10.0 ± 0.3		9.9 ± 0.2	
10% bioavailability			23		21		21
7.5% bioavailability			37		35		36
Zinc, mg	4.7	4.8 ± 0.1	56 ± 3	4.7 ± 0.1	58 ± 4	4.7 ± 0.1	57 ± 3
Vitamin C, mg	13	57 ± 1	<1 ± <1	52 ± 1	<1 ± <1	55 ± 0.9	<1 ± <1
Thiamine, mg	0.4	0.56 ± 0.01	31 ± 4	0.59 ± 0.01	15 ± 8	0.57 ± 0.01	25 ± 4
Riboflavin, mg	0.4	0.61 ± 0.01	18 ± 6	0.66 ± 0.02	14 ± 6	0.63 ± 0.01	16 ± 4
Niacin, mg	5	8.3 ± 0.2	16 ± 5	7.1 ± 0.2	21 ± 5	7.8 ± 0.1	17 ± 4
Vitamin B6, mg	0.4	1.2 ± 0.03	1 ± 2	0.81 ± 0.02	5 ± 4	1.0 ± 0.2	3 ± 2
Folate, µg	120	315 ± 10	6 ± 5	288 ± 6	<1 ± 3	302 ± 6	3 ± 4
Vitamin B <sub>12</sub> , µg	0.7	7.8 ± 3.1	6	1.1 ± 0.03	0.8	5.2 ± 0.5	7
Calcium, mg	500	363 ± 8	84 ± 6	466 ± 13	65 ± 7	405 ± 8	76 ± 5

293 <sup>a</sup>Intake data are presented as mean ± SEM and represent the usual nutrient intake of children 1 – 3 years old; the SEM  
294 refers to inter-individual variation.

295 <sup>b</sup>Prevalence of inadequacy was estimated as the percent of the usual intake distribution below the estimated average  
296 requirement (EAR) for each micronutrient (IOM, 2000) except for iron. For iron, the full probability approach at 7.5% and  
297 10% bioavailability was used (Gibson and Ferguson, 2008). Estimated average requirement for zinc was calculated using  
298 physiologic requirements reported by EFSA (EFSA, 2014) and bioavailability assumptions for unrefined cereal diets  
299 (IZINCG, 2004). The Institute of Medicine's estimated average requirements for all other nutrients were used. Standard  
300 errors (SE) are estimated and reported by IMAPP for the cut-point approach and are reported here.

301 <sup>c</sup>As retinol activity equivalents (RAE). 1 RAE=1µg retinol, 12 µg β-carotene, 24 µg α-carotene, or 24 µg β-cryptoxanthin.  
302 The RAE for dietary provitamin A carotenoids is two-fold greater than retinol equivalents (RE), whereas the RAE for  
303 preformed vitamin A is the same as RE.

**Table 5:** Mean daily energy and micronutrient intakes of children 4-6 y and prevalence of adequate micronutrient intakes.

	EAR	South Kivu (n=56; R=12)		Kongo Central (n=29; R=4)		All (n=85; R=16)	
		Intake <sup>a</sup>	Prevalence of Inadequacy <sup>b</sup>	Intake <sup>a</sup>	Prevalence of Inadequacy <sup>b</sup>	Intake <sup>a</sup>	Prevalence of Inadequacy <sup>b</sup>
Energy, kcal		1376 ± 47		1463 ± 88		1402 ± 30	
Vitamin A, µg RAE <sup>c</sup>	275	503 ± 25	10 ± 13	814 ± 89	7 ± 8	588 ± 28	9 ± 9
Iron, mg	4.1	11.4 ± 2.0		13.2 ± 1.5		11.8 ± 0.4	
10% bioavailability			24		24		22
7.5% bioavailability			44		41		39
Zinc, mg	6	5.2 ± 0.2	71 ± 15	5.8 ± 0.6	66 ± 9	5.4 ± 0.2	69 ± 15
Vitamin C, mg	22	53 ± 4	12 ± 6	79 ± 5	0	60 ± 4	6 ± 5
Thiamine, mg	0.5	0.60 ± 0.02	19 ± 63	0.70 ± 0.06	29 ± 14	0.63 ± 0.02	20 ± 35
Riboflavin, mg	0.5	0.68 ± 0.04	33 ± 7	0.64 ± 0.04	31 ± 10	0.66 ± 0.03	31 ± 7
Niacin, mg	6	8.2 ± 0.4	28 ± 9	8.9 ± 0.8	23 ± 15	8.4 ± 0.3	22 ± 11
Vitamin B6, mg	0.5	1.2 ± 0.05	2 ± 4	1.0 ± 0.1	2 ± 5	1.2 ± 0.04	1 ± 3
Folate, µg	160	375 ± 29	8 ± 26	398 ± 43	4 ± 12	378 ± 22	6 ± 18
Vitamin B <sub>12</sub> , µg	1	6.0 ± 1.8	16	1.1 ± 0.2 <sup>d</sup>	NE	5.5 ± 2.4	24
Calcium, mg	800	411 ± 27	96 ± 4	525 ± 57	85 ± 11	449 ± 22	95 ± 6

304 <sup>a</sup>Intake data are presented as mean ± SEM and represent the mean of a single day's intake for each woman; the SEM  
305 refers to inter-individual variation.

306 <sup>b</sup>Prevalence of inadequacy was estimated as the percent of the usual intake distribution below the estimated average  
307 requirement (EAR) for each micronutrient (IOM, 2000) except for iron. For iron, the full probability approach at 7.5% and  
308 10% bioavailability was used (Gibson and Ferguson, 2008). Estimated average requirement for zinc was calculated using  
309 physiologic requirements reported by EFSA (EFSA, 2014) and bioavailability assumptions for unrefined cereal diets  
310 (IZINCG, 2004). The Institute of Medicine's estimated average requirements for all other nutrients were used. Standard  
311 errors (SE) are estimated and reported by IMAPP for the cut-point approach and are reported here.

312 <sup>c</sup>As retinol activity equivalents (RAE). 1 RAE=1µg retinol, 12 µg β-carotene, 24 µg α-carotene, or 24 µg β-cryptoxanthin.  
313 The RAE for dietary provitamin A carotenoids is two-fold greater than retinol equivalents (RE), whereas the RAE for  
314 preformed vitamin A is the same as RE.

315 <sup>d</sup>Usual intakes could not be computed, thus arithmetic mean ± SEM are reported.

316 **Food sources of nutrients**

317 The major food sources contributing to vitamin A, iron and zinc intakes among  
 318 women are presented in **Table 6**. In South Kivu, the three largest contributors to both  
 319 iron and zinc intake among women were cassava flour, beans, and fish. Together these  
 320 three sources accounted for two thirds of all iron intake and over half of all zinc intake.  
 321 Women in this province also consumed more red meat than women in Kongo Central;  
 322 beef consumption accounted for 6% of zinc intake. Among women in Kongo Central,  
 323 plant-based sources, specifically, cassava flour, amaranth/bean/cassava leaves, and  
 324 beans provided most of the iron and zinc. As for vitamin A, similar trends were observed  
 325 in both provinces; red palm oil provided ~70% of the vitamin A among women.

326 **Table 6:** Top contributing food sources to iron, zinc, and vitamin A intake in mothers

Food source	South Kivu			Kongo Central		
	Iron (%)	Zinc (%)	Vitamin A (%)	Iron (%)	Zinc (%)	Vitamin A (%)
Cassava flour	31	27	--	36	29	--
Beans	20	20	--	15	15	--
Amaranth/bean/cassava leaves	11	9	16	19	14	19
Sesame	--	--	--	11	12	--
Fish	11	10	--	--	6	--
Beef	--	6	--	--	--	--
Antelope	--	--	--	--	--	--
White sweet potato	--	6	--	--	--	--
Red palm oil	--	--	70	--	--	72
Other sources (<5%)	24	22	15	19	24	9

328 For children in both provinces, the top three food sources for iron and zinc were  
 329 similar to their mothers (Table 7). In both provinces, cassava flour contributed to  
 330 approximately 30% and 20% of dietary iron and zinc, respectively. Like their mothers,  
 331 animal source food consumption was higher among children in South Kivu, where fish  
 332 and beef contributed to ~20% of dietary zinc. Red palm oil also accounted for 70% of  
 333 total vitamin A intake among children in both provinces

334 **Table 7:** Top contributing food sources to iron, zinc, and vitamin A intake in mothers

Food source	South Kivu			Kongo Central		
	Iron (%)	Zinc (%)	Vitamin A (%)	Iron (%)	Zinc (%)	Vitamin A (%)
Cassava flour	27	21	--	28	23	--
Beans	21	20	--	18	18	--
Amaranth/bean/cassava leaves	13	11	16	20	14	18
Sesame	--	--	--	13	13	--
Fish	12	11	--	--	8	--
Beef	--	8	--	--	--	--
Antelope	--	--	--	--	--	6
White sweet potato	--	5	--	--	--	--
Red palm oil	--	--	67	--	--	70
Other sources (<5%)	27	26	17	21	25	6

335

336 Staple food intakes for mothers and children are reported in **Table 8**. Beans,  
 337 expressed as dry weight, were consumed regularly in both regions, although not every  
 338 day. On days when women reported consuming beans, ~240 g/d were consumed in

339 South Kivu and 180 g/d in Kongo Central. On average, mean bean intake among women  
 340 was  $93 \pm 7$  g/d in South Kivu and  $59 \pm 11$  g/d in Kongo Central. Children also consumed  
 341 a sizeable amount of beans on days of consumption ( $\sim 130$  g/d in South Kivu and 100 g/d  
 342 in Kongo Central); however, usual intake on all days averaged out to  $47 \pm 2$  g/d in South  
 343 Kivu and  $37 \pm 4$  g/d in Kongo Central. Cassava intake, expressed as fresh weight peeled  
 344 cubes, was consumed in large quantities at  $\sim 1260$  g/d among women in South Kivu and  
 345  $\sim 1480$  g/d in Kongo Central. Cassava consumption among children was also high at  $\sim$   
 346 450 g/d and 640 g/d in Kongo Central. Maize was consumed infrequently by half the  
 347 population in South Kivu and a third of the population in Kongo Central at the time of this  
 348 study, with an estimated mean intake of  $20 \pm 5$  g/d among women in South Kivu and  $4 \pm$   
 349 1 g/d intake in Kongo Central. Usual mean intakes of maize among children were similarly  
 350 very low.

**Table 8:** Mean usual intakes of selected staples among mothers and children.

	South Kivu			Kongo Central		
	% Consumers	Usual intake on consumption days, g/d $\pm$ SEM	Usual Intake on all days for entire population, g/d $\pm$ SEM <sup>d</sup>	% Consumers	Usual intake on consumption days, g/d $\pm$ SEM <sup>d</sup>	Usual Intake on all days for entire population, g/d $\pm$ SEM <sup>d</sup>
<b>Women 15 - 49 yr</b>						
<sup>a</sup> Bean	100%	$243 \pm 14$	$93 \pm 7$	86%	$180 \pm 17$	$59 \pm 11$
<sup>b</sup> Cassava	100%	$1262 \pm 50$	$1178 \pm 47$	100%	$1486 \pm 72$	$1471 \pm 74$
<sup>c</sup> Maize	64%	$83 \pm 10$	$20 \pm 5$	30%	$61 \pm 16^1$	$4 \pm 1^1$
<b>Children 0.5 - 5 yr</b>						
<sup>a</sup> Bean	100%	$129 \pm 3$	$47 \pm 2$	100%	$97 \pm 10$	$37 \pm 4$
<sup>b</sup> Cassava	100%	$500 \pm 33$	$456 \pm 30$	100%	$640 \pm 33$	$604 \pm 33$
<sup>c</sup> Maize	53%	$28 \pm 2$	$7 \pm 2$	31%	$32 \pm 5^e$	$2 \pm 0.6^e$

351 <sup>a</sup>Bean intakes expressed as dry weight

352 <sup>b</sup>Cassava intakes expressed as fresh peeled cassava.

353 <sup>c</sup>Maize intakes expressed as maize flour.

354 <sup>d</sup>Usual mean intakes were computed, applying a probability of consumption for foods not  
 355 consumed every day.

356 <sup>e</sup>Usual intake could not be computed thus arithmetic mean  $\pm$  SEM is reported.

## 357 **Discussion**

358           In this paper, we present the results on dietary intakes and prevalence of nutrient  
359 inadequacy from our 24 HDR survey to better understand the lack of biochemical  
360 deficiency among women and children in South Kivu and Kongo Central in the DRC. To  
361 our knowledge, this is the only assessment of dietary intake and micronutrient status  
362 among women of childbearing age and their children 6-59 months in these two provinces.  
363 When comparing nutrient intakes at the regional level, respondents in Kongo Central had  
364 higher mean intakes for most nutrients and consequently lower prevalence of inadequacy.  
365 Kongo Central is located in proximity to Kinshasa, the nation's capital, has more  
366 commercial activity than South Kivu and is a large supplier of cassava. South Kivu, on  
367 the other hand, borders Rwanda and Burundi, thus we expected similar dietary patterns  
368 to these bordering countries characterized by more bean and maize consumption. Given  
369 that South Kivu is situated within Lake Kivu and Lake Tanganyika, we also expected  
370 respondents in this region to have significantly higher fish consumption than those in  
371 Kongo Central. Food level data revealed greater consumption of animal source foods,  
372 specifically fish and beef, among women and children in South Kivu compared to Kongo  
373 Central, however the difference was as not as marked (Tables 6 and 7). One of the  
374 reasons for this is due to renewed rebel conflict and political instability in North Kivu that  
375 had disrupted the province's agricultural production.(20) Because 68% of households in  
376 South Kivu depend on market access to North Kivu to meet food needs, particularly staple  
377 crops, political instability contributed to increased food and nutrition insecurity in the  
378 region. According to a report by the United States Agency for International Development  
379 Office of Food for Peace, the proportion of the population in South Kivu categorized as

380 food insecure at the time of our survey was highest in the country at 64%, of which 14%  
381 were classified as severely food insecure.(21) Despite this, our results remarkably show  
382 nutrient adequacy for most nutrients.

383 Nutrient intake patterns and prevalence of inadequacy among women and children  
384 in the respective provinces were nearly identical. Calcium and zinc intakes in both regions  
385 were low and presented as the greatest micronutrient vulnerabilities in these two regions.  
386 The prevalence of inadequate calcium intakes ranged from 60 to 95%, depending on the  
387 region and age group. Similarly, prevalence of inadequate zinc intakes was high, ranging  
388 from 55 to 90%. It is important to note that there is still considerable debate on the  
389 physiological requirements for zinc (17), which has important ramifications on the  
390 estimates of prevalence of inadequacy. However, the general conclusions are consistent  
391 with those from the biochemical results that show a moderate to high prevalence of zinc  
392 deficiency, in which more than half of women and a quarter of children had low serum  
393 zinc concentrations.(2)

394 Our dietary assessment of vitamin B<sub>12</sub>, vitamin A, and folate intakes supports the  
395 results from the biochemical survey that show little to no evidence of deficiency in these  
396 nutrients. Consumption of animal source foods such as red meat and fish likely explains  
397 B<sub>12</sub> sufficiency, and food level data revealed vitamin A sufficiency was primarily due to  
398 the ubiquitous presence of red palm oil in these regions. Folate intake on the other hand  
399 appeared to be moderately inadequate among women at ~20%, and mild among children  
400 at ~5% among children. Although prevalence of inadequacy is slightly higher, the results  
401 are reasonably consistent with biochemical findings suggesting this is not a problem  
402 nutrient.

403 Our results also show that approximately 30% of women and 20 to 25% of children  
404 1y and older in our sample had inadequate iron intakes, based on a 10% bioavailability  
405 assumption. According to our findings from biochemical measures of haemoglobin,  
406 ferritin, and soluble transferrin receptor, however, we found little evidence of iron  
407 deficiency anemia among women regardless of the biomarker used (2). In fact, less than  
408 3% of anemia observed was due to iron deficiency. Among children, iron deficiency  
409 anemia was higher but remained below 20%. The discrepancy in dietary assessment  
410 and biochemical results has been reported in other surveys. Similar results were found  
411 in a study conducted by Verbowski et al where dietary intake data contradicted  
412 biochemical indicators.(22) In this study, iron deficiency anemia was only attributable to  
413 1.5% of women in their sample, while prevalence of inadequacy was estimated to be  
414 50%. As was highlighted by Verbowski et al, the reason for the discrepancy may be due  
415 to the use of nutrient reference values that are not suitable to the Congolese population  
416 where starchy roots and tubers contribute roughly 40% of dietary energy intake.

417 As expected, cassava is a major staple in the daily diets of the rural Congolese in  
418 South Kivu and Kongo Central. Our study showed that women consumed more than  
419 1200 g/d and children more than 500 g/d of cassava thus holding great potential for a  
420 cassava biofortification program. Currently, the biofortified cassava program  
421 conventionally breeds for pVACS with breeding targets set for 15 µg/g. A recent retention  
422 study showed great losses of pVACs during processing and cooking of two primary forms  
423 of cassava consumed, *fufu* and *chikwangwe*, with a mere ~ 2% and ~ 10% of pVACS  
424 remaining, respectively.(23) These large losses in pVACs are in part a result of the large  
425 losses of solid mass due to the processing, with primary cassava products *fufu* and

426 *chikwangwe* containing about one-quarter to one-third of solid mass of the cassava cubes  
427 after processing. Despite these losses, our study indicates that a biofortified cassava  
428 program can still have important contributions to vitamin A intakes in the population given  
429 the substantial amount of cassava consumed on a daily basis. If only 5% of pVACs are  
430 retained, children consuming 500 g/d and women 1000 g/d will still receive an additional  
431 30 to 40% of the average vitamin A requirement from a biofortification cassava  
432 intervention. While our results show no evidence of vitamin A inadequacy in the diets of  
433 women and children, more than two-thirds of the vitamin A in the diet came from red palm  
434 oil. Any disruption to this food source, such as vegetable oil refining, supply chain breaks,  
435 or price increases, could cause a dramatic reduction in population vitamin A intakes.  
436 Thus, additional dietary sources of vitamin A including carotenoid enriched cassava would  
437 be an important safety net.

438 Beans were also an important part of the diet in both regions, although consumed  
439 more often and in greater amounts in South Kivu. Usual intakes of 93 g/d among women  
440 and 47 g/d among children would provide ~ 20 to 25% of the iron requirement for women  
441 and young children, respectively. Our results on dietary inadequacy show that the age  
442 group in greatest need of an iron intervention are infants <1y, but they are also the least  
443 likely to benefit from an iron bean biofortification program due to the small amount of food  
444 infants consume and their higher iron requirements. Instead, beans may be a more  
445 important vehicle for increasing the zinc density in the diets of the women and children in  
446 the DRC, which we show is one of the nutrients most lacking in the diet. Indeed, an added  
447 benefit to conventionally breeding for iron is that zinc concentrations also increase.(24)  
448 Achievements have been made in breeding for low phytic acid content in beans,

449 increasing the potential for zinc absorption.(25, 26) However, zinc absorption studies of  
450 low phytic acid beans are required to prove this concept.

451 In contrast, maize was consumed infrequently and in small quantities during the  
452 time of this survey. This could be, in part, a seasonal effect as the harvest season of  
453 maize in the eastern provinces was in January and February.(20) Production patterns  
454 may also explain the low maize consumption, particularly in Kongo Central where maize  
455 production is nominal in comparison to cassava, rice and beans.(20) Maize, however, is  
456 one of three staple crops that are produced above subsistence levels, and active  
457 production chains exist, many of which cluster in the southern territories of the DRC.(27)  
458 Thus, it is also quite possible we did not survey populations in the DRC in which maize  
459 constitutes a large share of the diet.

460 There are a number of strengths in our study, namely that the results from our  
461 dietary assessment survey mimic our findings from the biochemical survey. Thus, we  
462 can draw conclusions with confidence that zinc deficiency prevails as one of the most  
463 important micronutrient deficiencies affecting women and children in South Kivu and  
464 Kongo Central. Furthermore, there does not appear to be under-reporting of dietary  
465 intakes, which frequently occurs with self-reported dietary surveys, as energy intakes  
466 were reasonable. Finally, we developed a locally specific food composition table that  
467 accurately reflects the Congolese diet giving us further confidence in our results.

468 There are also important considerations and limitations to keep in mind when  
469 interpreting these results. The first is that the timing of the dietary assessment occurred  
470 during a period of very high political instability. Thus, the generalizability should be  
471 interpreted with some amount of caution. In addition, we did not measure breastmilk

472 intakes among children, which constitutes an important supply of energy and  
473 micronutrients in their diets. To account for this limitation, we applied WHO estimates of  
474 average breastmilk intakes among children in low and middle income countries, however  
475 we have little knowledge if these averages reasonably represent breastmilk intake among  
476 children in the DRC region or if they are an over or underestimate. Finally, estimation of  
477 probability of consumption and quantity of staple crops requires a minimum two days,  
478 thus the data used to conduct these analyses was limited. However, mean usual intakes  
479 of all crops reported are in agreement with the arithmetic mean from the full sample, thus  
480 giving confidence to our results.

## 481 **Conclusions**

482 Dietary data here corroborate the low prevalence of biochemical vitamin A  
483 deficiency but not iron. We found respondents in both provinces to be replete with Vitamin  
484 A, and this was largely due to a single dietary source, red palm oil. Carotenoid enriched  
485 cassava would be a prudent safety net measure to ensure a sustainable source of vitamin  
486 A in these provinces of the DRC, in addition to red palm oil and routine supplementation  
487 programs. Iron biofortified beans may be warranted given the high rates of dietary  
488 inadequacy and uncertainty around the validity of iron biomarkers. There was, however,  
489 a high burden of zinc inadequacy ranging from and 55-90%. The high zinc burden was  
490 confirmed by biochemical data where more than 50% of women and 25% of children had  
491 low serum zinc concentrations. Crops biofortified with zinc therefore appear warranted.  
492 We caution that our findings cannot be extrapolated to the entire Congo where diverse  
493 agro-ecological landscape exist or when political and environmental challenges and  
494 shocks occur which challenge food production.

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499 **Author Contributions**

500 P.T., Er.B., Es.B., J.F., MM and T.G. designed the research; T.G., M.H. and J.F. drafted  
501 the research protocol and Er.B. contributed to the revision of the protocol to the final  
502 version. M.D.H, J.F., P.L.T., P.A. K.A.S and Es.B. conducted the research and  
503 managed the data. K.D.M., J.F. and K.C.W. drafted the data collection tools. T.G. and  
504 M.M provided oversight and input into all aspects of the study. C.D.K. and M.D.A  
505 conducted the statistical analysis. N.A.M., M.D.A. and T.G. drafted the manuscript. All  
506 authors contributed to the review and editing of the manuscript to the final version. T.G.  
507 had primary responsibility for the final content. All authors read and approved the final  
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