

**Comparison of an interactive 24-hour recall and weighed food record for measuring energy and nutrient intakes from complementary foods among 9-10 month-old Malawian infants consuming Lipid-Based Nutrient Supplements**

Journal:	<i>British Journal of Nutrition</i>
Manuscript ID	BJN-RA-17-0873.R2
Manuscript Type:	Research Article
Date Submitted by the Author:	n/a
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Keywords:	LNS, weighed record, 24-hr recall, dietary assessment, infants
Subject Category:	Dietary Surveys and Nutritional Epidemiology

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## Comparison of an interactive 24-hour recall and weighed food record for measuring energy and nutrient intakes from complementary foods among 9-10-month-old Malawian infants consuming Lipid-Based Nutrient Supplements

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Running title: Dietary assessment errors of common methods

### Keywords

LNS, weighed record, 24-hr recall, dietary assessment, infants

**Abstract word count: 250, Manuscript body word count: 5 411**

**Number of figures: 1**

**Number of tables: 4**

**Supplementary tables: 2, Supplementary figures: 3**

**Study Funding:** This manuscript is based on research funded by a grant issued to the University of California, Davis from the Bill & Melinda Gates Foundation. The findings and conclusions contained within are those of the authors and do not necessarily reflect positions or policies of the Bill & Melinda Gates Foundation.

All authors declare no conflicts of interest

**1 Abstract**

2 Fortifying complementary foods with lipid-based nutrient supplements (LNS) may improve energy and  
3 nutrient intakes of infants at risk for undernutrition. We aimed to determine the relative validity of an  
4 interactive 24-hour dietary recall (i-24-HR) for assessing the impact of an LNS intervention on dietary  
5 intakes of energy and nutrients among rural Malawian 9-10-month-old infants (n=132) participating in  
6 the iLiNS dose trial. Dietary data were collected for the same day via i-24-HRs and weighed food  
7 records. Inter-method agreements were estimated overall and by intervention group, using Bland-  
8 Altman plots and paired t-tests; measurement error models (differential error); and percentage of food  
9 omissions and intrusions were estimated. Overall, inter-method differences in mean intakes of energy  
10 and most nutrients were not significant. When stratified by group, recalled energy intakes were under-  
11 estimated (-88kcal p=0.01) in the control but not in the intervention group (-10kcal; p=0.6). This  
12 differential reporting error was related to an over-estimation of recalled LNS (8.1g vs 4.5g; p<0.001) in  
13 the intervention group, compensating for an under-estimation of energy and nutrient intakes from  
14 complementary foods. Sources of measurement error in the i-24-HR were under-estimations of starchy  
15 staples, meat/fish/eggs and legumes/nuts/seeds (overall percent agreement between 38-89%; p<0.028);  
16 and over-estimations of added sugar, soups/broths and LNS (overall percent agreement between 138-  
17 149%; p<0.001). Common (>30% eating occasions) omissions were milk/fish/egg, starchy  
18 roots/vegetables, and sweetened snacks. Common intrusions were milk/yogurt. Starchy staples and  
19 LNS were recalled when consumed (>85%) (i.e. matched). These results emphasise the importance of  
20 considering differential error when interpreting dietary results in LNS trials.

## 21 **Introduction**

22 Undernutrition is common among young children living in low income countries (1). Both the short-  
23 and long-term adverse effects of under-nutrition impact health and future livelihoods. This underscores  
24 the need for comprehensive intervention packages, including effective dietary strategies. One such  
25 intervention is the use of lipid-based nutrient supplements (LNS) as home fortification of infant foods  
26 (2). Studies of the effectiveness of LNS for reducing undernutrition have shown mixed results (3-5). In  
27 cases where there was no association between LNS intake and growth outcomes (3), low adherence to  
28 the intervention (LNS consumption) and/or the displacement of other foods in the diet might partially  
29 account for the lack of a physiological effect. Thus, to correctly interpret LNS intervention trial results,  
30 accurate measurement of the LNS exposure and its influence on overall dietary intakes is fundamental.  
31 The assessment of infant dietary intakes is complicated for several reasons: 1) infants eat very small  
32 quantities of food; 2) measuring intake includes measuring not only the amount served, but also  
33 amounts left over, spit-up, spilled or dropped; 3) infants are often cared for and fed by multiple people;  
34 and 4) infants are unable to report their own intakes (6). The weighed food record is considered the  
35 “gold standard” dietary assessment method for quantitative estimates of an individual’s dietary intake,  
36 including for young children, because foods are weighed and recorded as they are consumed (7).  
37 However, for large surveys, the 24-hour recall is more practical because it is relatively rapid to  
38 conduct, has a low respondent burden and is less disruptive for low-literacy communities where, for the  
39 weighed food record, research assistants must weigh and record all foods consumed by participants.  
40 The disadvantages of 24-hour recalls are that they are prone to errors of memory, recall bias, errors in  
41 portion size reporting and potentially a social-desirability bias (8). The interactive multiple pass 24-  
42 hour recall (i-24-HR) was developed specifically for areas with low literacy rates, and includes a  
43 pictorial chart to prospectively record dietary intakes and reduce errors of memory (9).  
44 Previous studies, in Malawi, Ghana, Sweden and the United States, have assessed the validity of the  
45 24-hour dietary recall method relative to weighed food records (WFR) for estimating the energy and  
46 nutrient intakes of young children (10-13). They show recalled compared to weighed energy intakes are  
47 generally over-estimated (10, 12, 14), which for rural Malawian 15-m olds was by 13% (10). This  
48 pattern of over-estimation of energy intakes might be more pronounced for toddlers than infants, if  
49 accurate reporting becomes more difficult as the diet becomes more complex (12, 15). To our  
50 knowledge no study has validated the 24-hour recall for African infants under 12-months of age.  
51 There is also evidence that certain foods are more accurately reported than others (16, 17). Such  
52 differences become important when assessing dietary exposures in a LNS intervention trial because  
53 LNS, which is an energy and nutrient dense food, is not present in the diet of the control group.

54 Systematic under- or over-estimation of LNS intakes would bias between-group comparisons by either  
55 exaggerating or attenuating the observed effect of LNS on infant dietary intakes, of energy and  
56 nutrients. An accurate assessment of dietary exposure is essential in dietary intervention trials to  
57 properly understand the association between dietary exposure and outcome (18-20). To our knowledge,  
58 the i-24-HR has not been validated for use among infants who are participating in an LNS intervention  
59 trial.

60 This study, therefore, aimed to assess the relative validity of the i-24-HR used in an LNS intervention  
61 trial, the iLiNS study (3). The iLiNS study aimed to evaluate the efficacy of three doses of LNS for the  
62 prevention of stunting among infants supplemented from 6 to 18 months of age. In this trial, inter-  
63 group differences in dietary intakes of energy and nutrients were assessed when the infants were 9-10  
64 months of age (21). The specific objectives of the current study were to 1) assess the relative validity of  
65 the i-24-HR method for estimating dietary intakes of energy, protein, fat, iron, zinc, calcium and  
66 vitamin A from complementary foods using a 1-day WFR as the reference method; 2) assess whether  
67 there is a differential bias in i-24-HR measures of energy intake between the control group and  
68 intervention groups, and 3) describe potential sources of measurement error in the i-24-HR, including  
69 errors in the types or amounts of LNS and complementary foods reported.

## 70 **Methods**

### 71 **Design and Study Population**

72 A cross-sectional validation study was nested within a dietary assessment sub-study of infants  
73 participating in a 12-month LNS randomised control trial (iLiNS-DOSE trial) conducted in Mangochi  
74 district, Malawi from November 2009 and July 2012. Data collection for the dietary assessment sub-  
75 study took place between March 2010 and October 2011 when the infants were 9-10 m of age. Data  
76 collection for the dietary validation study took place between October 2010 and October 2011. The  
77 main trial was designed to assess the impact of three different doses of LNS (10g, 20g and 40g) on  
78 linear growth; which was delivered bi-weekly to households in the intervention groups. The objectives  
79 and methods of the iLiNS-DOSE trial (n=1980) and the dietary assessment sub-study (n=688) are  
80 described in more detail in Maleta, et.al. (3) and Hemsworth, et.al. (21), respectively. In the dietary  
81 assessment sub-study, two i-24-HRs were done exactly 7-days apart when the infants were between 9  
82 and 10 months of age. One i-24-HR was done during the week LNS was delivered, and the other in the  
83 subsequent week. In the validation study the WFRs which were done one-day prior to a corresponding  
84 i-24-HR, were done just after the LNS delivery day to maximize capturing the presence of LNS in the

85 child's diet. The other i-24-HR was collected either 7-days before or 7-days after the i-24-HR that  
86 corresponded with the WFR day.

### 87 **Sampling**

88 A random sample of 228 infant-mother dyads was obtained for the validation study (56 in each of the  
89 control, 10g, 20g, and 40g LNS groups). The sample size for the validation study was calculated to  
90 allow detection of a difference of 55kcal (one 10g dose of LNS) between each of the four intervention  
91 groups with power of 80% and  $\alpha=0.05$ , assuming a standard deviation of the difference between the  
92 methods (WFR minus i-24-HR) of 138 kcal (derived from a pilot study), and a 10% attrition rate (e.g.  
93 missed i-24-HR following the WFR).

94 The original inclusion criterion was participation in the dietary assessment sub-study of the iLiNS-  
95 DOSE trial. The validation study, however, began seven months after the trial began, which meant that  
96 one third of participants had already completed the dietary sub-study and were no longer eligible for  
97 the validation study. As a result, to meet our target sample size of 228 age-eligible infants, we selected  
98 additional infants (n=78) at random from the basic sub-study group (i.e., not randomised to any  
99 additional sub-study at baseline to minimise respondent burden) to reach the intended sample size. It  
100 introduced an imbalance in the number of infants from the control and 10g LNS groups versus the 20g  
101 and 40g LNS groups. As such, more infants were in the 20g LNS and 40g LNS groups than the other  
102 two groups in this validation study.

### 103 **Ethical Approval**

104 Ethical approval for this sub-study was granted by the London School of Hygiene and Tropical  
105 Medicine Research Ethics Board as well as by the College of Medicine Research Ethics Board in  
106 Malawi. Informed written consent was obtained from all participating caregivers in this study. The trial  
107 was registered at [clinicaltrials.gov](https://clinicaltrials.gov) with the identifier: NCT00945698

### 108 **Dietary Assessment**

#### 109 *Interactive 24-hour Recall (i-24-HR)*

110 Dietary data were collected using a 4-pass i-24-HR, developed for use in a rural African context (9).  
111 The method was modified specifically for a similar population and included pictorial charts (intended  
112 to reduce intrusions and omissions), bowls/cups/plates, and measured portion sizes using real food  
113 replicas and salted models. In the dietary assessment sub-study, caregivers were given the pictorial  
114 food chart and a plastic cup and bowl 2-days before the i-24-HR was done. On the day before the i-24-  
115 HR, caregivers were asked to prospectively record on the pictorial chart all foods, beverages, and LNS

116 (if appropriate) when given to the child to minimise memory errors; and to feed their child from the cup  
117 and bowl provided to minimise portion size estimation errors. In the first pass, during the i-24-HR  
118 interview, from memory, the caregiver was asked to serially recall all foods, supplements and  
119 beverages that their child had consumed in the previous 24 hours. In the second pass, information  
120 about the time, place, and description of the food or beverage was collected. In the third pass, portion  
121 sizes were estimated by the caregivers showing the amount served and the amount left-over using real  
122 food replicas (with or without excess salt to preserve them) and unit descriptions (e.g. package of  
123 biscuits). The amounts were weighed by the interviewers using digital kitchen scales (Home Elegance,  
124 accurate to  $\pm 1$  g), and recorded. The amount consumed was calculated as the amount served minus the  
125 amount left-over. LNS portion sizes were measured using a pot of LNS, which was weighed before and  
126 after the caregiver had removed the amount of LNS used at each eating occasion. Left-overs were  
127 subtracted from the amount of LNS served. If LNS was mixed with other foods, the amount left over  
128 was calculated by multiplying the amount served by the proportion of the mixed dish that was  
129 consumed, assuming uniform mixing. The consumption of LNS was not specifically probed to prevent  
130 errors of intrusion (i.e. items listed but not actually consumed). To reduce potential differences in  
131 recording, interviewers were given extensive training and used standardised operating procedures,  
132 including a portion size estimation manual, detailing the specific methods for portion size estimations  
133 and probing. At the end of the third pass, interviewers asked for the pictorial chart. Any discrepancies  
134 between the pictorial chart and the food list of the i-24-HR were discussed. In the final pass, the data  
135 collector summarised and confirmed the food and drinks recorded in the i-24-HR.

### 136 ***Weighed Food Record (WFR)***

137 All foods and beverages consumed by the child from 6 a.m. until the final meal of the day were  
138 weighed and recorded by a data collector, using digital kitchen scales (Home Elegance, accurate to  $\pm$   
139 1g). Left-over foods were weighed either individually, if they could be separated on the plate, or as a  
140 mixture, assuming uniform mixing. Recipe data were collected by weighing all raw ingredients and the  
141 final cooked dish. The WFR data collector was not involved in the collection of the i-24-HR data.

### 142 **Questionnaires**

143 Socio-demographic background characteristics of the infants were collected within two weeks of  
144 baseline enrolment in the iLiNS study, when the infants were 6 months old, using an interviewer-  
145 administered questionnaire.

## 146 **Data processing**

147 Conversion factors were developed for the i-24-HR, and used to estimate the grams of food consumed.  
148 Average recipes were calculated for cooked dishes using the individual recipes collected from each  
149 household. These data were used to calculate intakes of ingredients from cooked dishes in the i-24-  
150 HRs. Intakes of energy and nutrients from the WFR and i-24-HRs were estimated, using a food  
151 composition table developed for this study (21).

152 The time each item was consumed was also recorded, and it was used to match the corresponding  
153 eating occasions for inter-method portion size comparisons. Meals and snacks consumed after 19:00  
154 were removed from both the WFR and i-24-HR (i.e. a 12-hour WFR and recall were created) because  
155 there were occasions during the collection of the WFR when the final meal was consumed after the  
156 data collector had left the household.

## 157 **Statistical Analysis**

158 All data analyses were performed using Stata version 12 (StataCorp LLC, College Station, Texas). The  
159 three LNS intervention groups were collapsed to form one large group, for all analyses, because there  
160 were no significant inter-group differences in energy and nutrient intakes from complementary foods  
161 (including LNS), and the group sample sizes were small (21). In all analyses, except the analyses for an  
162 instrument effect (see below), data from only one of the two i-24-HR were used, which was the i-24-  
163 HR collected for the same day as the WFR. Energy and nutrient intake distributions from the WFR and  
164 i-24-HRs were mathematically transformed, when necessary, for the analyses.

165

## 166 ***Sociodemographic variables***

167 A composite variable for socioeconomic status was calculated using principal component analysis  
168 (PCA), and the PCA scores were divided into quintiles using the first principal component. The  
169 following variables were used as part of the composite variable: maternal occupation, household  
170 crowding, source of electricity, source of water, sanitary facilities, material of roofing, and material of  
171 house walls.

172 Chi-squared tests, for categorical socio-demographic variables, and two-sample t-tests, for non-  
173 categorical socio-demographic variables, were used to check for variables associated with  
174 “missingness” of WFRs and for differences between intervention groups (control vs. LNS) in the  
175 validation study.



176 *Assessment of agreement between dietary assessment methods*

177 Paired t-tests were used to compare mean intakes of energy and nutrients from the corresponding i-24-  
 178 HR and WFR. Absolute differences (“error”) in amounts of energy and nutrients between the two  
 179 methods were calculated as follows: i-24-HR – WFR. A two-sample t-test with equal variances was  
 180 used to compare the absolute differences between the control and intervention groups. Bland-Altman  
 181 plots were used to estimate, for energy intakes, the level of agreement between the two methods and  
 182 the 95% limits of agreement.

183 *Assessment of differential error*

184 Measurement error modelling was used to investigate whether error in the i-24-HR differed by  
 185 treatment group. We let  $S_1$  denote the i-24-HR measurement (square-root transformed) made at the  
 186 same time as the WFR, and  $W_1$  denote the WFR measurement itself (square-root transformed). The  
 187 second independent i-24-HR measurement (square-root transformed) was denoted  $S_2$ . The true, but  
 188 unobserved, intakes at time points 1 and 2 were denoted  $Y_1$  and  $Y_2$  respectively. At time point  $j$  ( $j =$   
 189 1,2) the relationships between the observed measurements of dietary intake and the unobserved  
 190 underlying true intake were assumed to be of the following forms, where we allowed separate model  
 191 parameters for individuals in the control (C) and combined intervention (T) groups,

192 **Equation 1**

193

$$\text{Combined intervention group: } S_j = \gamma_{0T} + \gamma_{1T}Y_j + \epsilon_{Tj}$$

$$\text{Control group: } S_j = \gamma_{0C} + \gamma_{1C}Y_j + \epsilon_{Cj}$$

194

$$\text{Combined intervention group: } W_1 = Y_j + \delta_{Tj}$$

$$\text{Control group: } W_1 = Y_j + \delta_{Cj}$$

195 The  $\epsilon$  and  $\delta$  terms are random errors with mean zero and constant variance. The WFR is assumed to  
196 provide an unbiased estimate of true intake in both the control and intervention groups. The intercept  
197 parameters  $\gamma_{0T}$  and  $\gamma_{0C}$ , and slope parameters  $\gamma_{1T}$  and  $\gamma_{1C}$ , represent systematic error in the i-24-HR  
198 measurement. We assessed evidence for differential error based on estimates of the differences  $\gamma_{1T} -$   
199  $\gamma_{1C}$  and  $\gamma_{0T} - \gamma_{0C}$  and corresponding bootstrap confidence intervals. The parameters of the  
200 measurement error model in Equation 1 were estimated via a method of moments approach.

### 201 ***Sources of disagreement between the i-24-HR and WFR***

202 To identify possible sources of disagreement between the two dietary assessment methods, we  
203 categorised each food and drink item (for composite dishes, we matched the individual ingredients) as  
204 an omission (present on WFR, absent on i-24-HR), an intrusion (absent on WFR, present on i-24-HR)  
205 or a match (present on both methods at matching meal/snack times). We calculated the frequency of  
206 each category across food groups (i.e., phala; nsima and rice; added sugar; sweetened snacks; savoury  
207 snacks; meat, fish and egg; legumes, nuts, and seeds; fruit; starchy roots and vegetables; milk and  
208 yogurt; non-dairy beverages; soup/broth from relish; and LNS), a method previously described by  
209 Smith, et.al. (22). We compared the median percentage agreement for each food group, (i.e.  $100 \times$   
210 reported amount (i-24-HR) / reference amount (WFR)), for the intervention and control groups, using  
211 Mann-Whitney rank sum test when the sample was at least five consumers. In the case where one food  
212 within a food group of these is an intrusion, this resulted in a reference amount of zero (at the  
213 individual food level only), and in the case where there is an omission, this resulted in a reported  
214 amount of zero. We also compared the overall inter-method differences, in the grams of food consumed  
215 in each food group, using the Wilcoxon signed-rank test.

### 216 ***Instrument Effect***

217 We tested for an “instrument effect”, because the presence of a data collector on the day of the WFR  
218 might have influenced the caregivers’ ability to recall dietary intakes during its corresponding i-24-HR.  
219 This “instrument effect” was assessed using the Wilcoxon signed-rank test, by comparing the median  
220 intakes of energy and nutrients estimated using the i-24-HR corresponding to the WFR day and the i-  
221 24-HR collected on a day independent of the WFR (i.e., collected one week before or after the WFR).  
222 For this analysis, n=71 matched records were available.

## 223 Results

### 224 Participants

225 A total of 228 infants were selected to participate in the validation study. However, 78 were lost to  
226 follow-up and 18 did not have a matching WFR and i-24-HR. The final sample size analysed was 132  
227 matching i-24HRs and WFRs (**Figure 1**). There were no significant differences in socio-demographic  
228 characteristics comparing those with missing data and those who completed the WFR (data not shown).  
229 Likewise, there were no differences in baseline characteristics between the intervention and control  
230 group (**Table 1**).

### 231 Agreement between dietary assessment methods

232 The reported energy intakes were lower in the i-24-HR compared to the WFR, although the difference  
233 was not statistically significant ( $p=0.09$ ) (**Table 2**). Reported protein intake was significantly  
234 underestimated and calcium intake was significantly over-estimated by the i-24-HR compared to the  
235 WFR ( $p<0.001$ ). There were no significant between-method differences in intakes of fat, iron, zinc or  
236 vitamin A. The Bland-Altman plot showed a systematic bias for under-reporting recalled energy  
237 intakes compared to the WFR and poor agreement at the individual level, with 95% limits of agreement  
238 of -366 kcal to 316 kcal (Online supplement **Figure 1**).

239 When stratified by intervention group, however, there was a significant under-estimation of recalled  
240 energy intakes in the control group ( $p=0.010$ ) but not in the intervention group ( $p=0.60$ ) (**Table 2**).  
241 Recalled intakes of protein, fat, iron and zinc were also significantly underestimated in the control  
242 group. In the intervention group, recalled intakes of protein were significantly under-estimated,  
243 whereas recalled intakes of calcium and zinc were significantly overestimated (Table 2). Further, after  
244 comparing the absolute differences (“error”) calculated between the WFR and i-24-HR in the control  
245 and intervention groups, we found significant differences ( $p\leq 0.05$ ) for energy (kcal) and iron, and all  
246 other nutrients were considered non-significant ( $p>0.05$ ). The Bland-Altman plot by intervention  
247 group (Online supplement **Figures 2a and 2b**) showed poor 95% limits of agreement (LOA) for  
248 energy at an individual level, for both the intervention (95% LOA -358, 337 kcal) and control (95%  
249 LOA -375 to 207 kcal) groups; and a mean systematic under-estimation of energy intakes in the control  
250 group only (-84 kcal).

251

252 By fitting the measurement error models in equation 1, we found that  $\hat{\gamma}_{1C} = -2.4$  (95% CI (-24.9,  
253 29.7)) and  $\hat{\gamma}_{1T} = 2.6$  (95% CI (-20.0, 20.2)),  $\hat{\gamma}_{0C} = 63.2$  (95% CI (58.8, 67.3)) and  $\hat{\gamma}_{0T} = -32.5$  (95%  
254 CI (-34.5,-30.6)). The confidence intervals were obtained from the 2.5 and 97.5 percentiles of 1000

255 bootstrap estimates, using bootstrap samples stratified by intervention group. The expected i-24-HR  
256 measure of energy intake ( $S$ ) given the true intake ( $Y$ ) is therefore  $E(S|Y) = -32.5 + 2.6Y$  in the  
257 combined intervention group, and  $E(S|Y) = 63.2 - 2.4Y$  in the control group. The estimates of the  
258 slope are in opposite directions in the intervention and control groups because the correlation between  
259 the independent i-24 and the WFR is positive in the intervention group, but negative in the control  
260 group; however the CIs are very wide and the 95% bootstrap CI for the difference  $\gamma_{1T} - \gamma_{1C}$  was (-  
261 46.6, 56.5). However, there was strong evidence for a difference in the intercepts; the 95% bootstrap CI  
262 for the difference  $\gamma_{0T} - \gamma_{0C}$  was (-100.1, -90.7) The model-based approach, therefore, suggests that the  
263 relationship between the i-24-HR measure of energy intake and the true intake may be different in the  
264 intervention and groups, i.e. potential differential error.

### 265 Sources of disagreement between the i-24-HR and WFR

#### 266 *LNS intakes*

267 In the intervention group, there was a significant between-method difference in estimated LNS intakes.  
268 The median intake was significantly higher for the recalled (i-24-HR) than reference (WFR) amount  
269 (i.e., 8.1g (4.5, 11.8) vs 4.5g (2.0, 9.0);  $p < 0.001$ ) (**Online Supplement Table 1**). The median (IQR)  
270 percentage agreement (matched LNS portions) indicates recalled LNS consumption was over-estimated  
271 by over 50% compared to the WFR (**Table 3**). Close to 90% of the eating occasions matched on both  
272 the WFR and i-24-HR; and rates of intrusions and omissions were similar and low (**Table 4**).

#### 273 *Complementary food intakes*

274 At the pooled group level, phala, legumes, nuts and seeds, and meat, fish and eggs were significantly  
275 under-estimated; whereas, soups/broths from relish and added sugar were significantly over-estimated  
276 in the i-24-HR compared to the WFR (**Online Supplement Table 1**). There were no significant  
277 differences between intervention- and control groups in reporting accuracy (i.e., percentage agreement  
278 for food groups), except for soups/broths from relish, where the control group showed a higher over-  
279 reporting rate than the intervention group. These comparisons, for four of the 12 food groups, were  
280 limited by the small sample size of the control group (Table 3).

281 In both the intervention and control groups, a comparison of food group matches, intrusions and  
282 omissions showed the highest reporting agreement for staples, where over 88% of the phala and nsima  
283 eating occasions matched between the two methods (Table 4). Episodically consumed foods such as  
284 meat, fish and eggs (which were frequently misreported as soup/broth from relish), starchy roots and  
285 vegetables, and sweetened snacks had poor reporting matching, with a higher tendency for respondents  
286 to omit (i.e. forget) as opposed to intrude (i.e. add in error).

### 287 **The “instrument-effect”**

288 There was no evidence of an “instrument effect”. There were no significant differences in estimated  
289 intakes of energy or nutrients comparing the independent i-24-HR (performed either one week before  
290 or after the WFR) and the corresponding i-24-HR (i.e., for the same day as the WFR). The absolute  
291 differences ranged from zero RAE/d to 34 kcal/d (**Online supplement Table 2**).

### 292 **Discussion**

293 In the context of a LNS supplementation trial, we found there was no significant difference comparing  
294 energy intakes measured using the i-24-HR to the WFR when all groups were pooled. This comparison  
295 was not biased towards agreement by the weighing process, because the independent and  
296 corresponding i-24-HRs provided similar estimates of energy and nutrients intakes. However, this  
297 pooled comparison masked a difference between the intervention and control group. When stratified by  
298 intervention group, the i-24-HR systematically under-estimated dietary energy intakes compared with  
299 the WFR in the control group but not in the intervention group. The significant difference in the “error”  
300 or absolute difference between the methods in control and intervention groups suggest a differential for  
301 recalled energy intakes. This differential error, for estimating median energy intakes, primarily is the  
302 result of an over-estimation of the energy-dense supplement (LNS), which was only consumed by the  
303 intervention group. It compensated for the under-estimation of energy intakes from complementary  
304 foods because most caregivers were able to report whether their infant had consumed it. In contrast,  
305 when using dietary data collected via i-24-HRs to examine associations, the 95% LOA indicate poor  
306 agreement at the individual level, in both groups, which will attenuate associations. These results  
307 highlight, when aiming to estimate inter-group differences in median intakes of energy and nutrients in  
308 an intervention trial, the importance of examining whether systematic measurement error when  
309 quantifying intervention food consumption, contributes to a differential bias. In studies aiming to  
310 examine associations between dietary intakes and functional outcomes (e.g., growth), the i-24-HR is  
311 inferior to more accurate methods of dietary assessment. In our study considerable effort was made to  
312 accurately estimate LNS consumption. The caregivers were asked to spoon out the amount of LNS  
313 served to the infant and estimate the amount left-over, which were both weighed and recorded.

314 There were few differences, comparing the intervention and control group, for between-method  
315 agreement in the estimation of complementary foods intakes. In the pooled group analyses, the main  
316 sources of between-method disagreement were under-estimated recalled portion sizes of dietary staples  
317 (phala, rice and nsima by between 11 and 14%), meat, fish and eggs and legumes, nuts and seeds.  
318 Energy-dense foods, such as added sugar, were overestimated by over 40% compared with the WFR;

319 but it did not compensate for the under-estimation of energy from staples (phala, nsima and rice). This  
320 result is not surprising because dietary staples provide a high percentage of daily energy intakes for  
321 rural infants in Malawi.

322 Underestimation of certain food groups is not unique and has been reported among women in Malawi  
323 (9) as well as preschool aged children in Ghana (11). However, the underestimation in energy intakes  
324 relative to the WFR, in the control group of our study, is in contrast to results from a study of 10-13  
325 month old Senegalese infants (n=45), which showed the 24-hour recall was a relatively good measure  
326 of intake compared to WFR (23, 24); and a study of 15-month old rural Malawian infants (n=169),  
327 which showed a systematic over-estimation in energy and nutrient intakes (10). The sources of  
328 measurement error, in the previous Malawian study, are unknown. These inter-study differences could  
329 be a function of inter-method or age group differences. In our study, we probed for left-overs and  
330 adjusted the portion sizes in the i-24-HR based on recalled left-overs. This adjustment was not reported  
331 in the other studies. It has been suggested that as a diet becomes more complex (as the infant ages), the  
332 reporting accuracy changes (12) and perhaps the direction of the error also changes.

333 The results of this validation study suggest that a differential error might be present when an i-24-HR is  
334 used to measure group mean dietary intakes, which is related to a systematic over-estimation of the  
335 exposure (LNS). Linear calibration techniques could be used to correct the systematic under-estimation  
336 of energy intakes from non-LNS foods. Previous studies have developed correction factors using the  
337 WFR as the reference standard to adjust i-24-HR energy intakes for a systematic overestimation of  
338 energy intakes compared to the WFR. This technique is not recommended for the current study because  
339 the reference method is subject to the same errors as the test method (19, 25), e.g. both the WFR and i-  
340 24-HR are subject to mis-estimation of items that were spilled or spit up. The linear calibration  
341 equations would only have been appropriate if we had used a biomarker, such as the stable isotope  
342 technique to measure total energy expenditure, which is an unbiased and independent measure of long-  
343 term energy intake (6, 20).

#### 344 **Study Limitations and Advantages**

345 The main study limitations were the relatively low sample size and high rate of attrition. The study was  
346 underpowered to detect differential error in the i-24-HR between control vs. intervention groups. The  
347 high rate of attrition occurred because of the logistical demands of this validation study in a large  
348 catchment area (i.e. transportation, communication with households, etc.). No observed background  
349 characteristics were associated with missing the visit.

350 Another limitation was the reference method used. The WFR is the most common reference standard  
351 for comparison with a 24-hour dietary recall because it is less resource-intensive than collection of  
352 biomarkers, and it provides useful robust information about portion size estimation, intrusions and  
353 omissions. However, it does not meet the strict criteria for a valid reference method (26). To validate  
354 the i-24-HR (repeated to provide an estimate of usual intakes), for estimating energy intakes alone, the  
355 doubly labelled water method is the preferred reference method (25, 27). Further, the modelling  
356 approach we used to assess evidence for differential error (equation 1), relies on an assumption that the  
357 WFR provides an unbiased measure of intake, as well as additional assumptions about the form of the  
358 systematic errors.

359 This study also had many advantages. It was carried out several months after the start of the  
360 intervention, which meant that the children were habituated to the intervention food. It was also  
361 conducted over a long period of time which allowed for seasonal variation in dietary patterns and  
362 episodically consumed foods to be captured. This study is also the first study that we are aware of that  
363 has assessed the relative validity of the i-24-HR for estimating the dietary intakes of rural African  
364 infants under 12 months of age who are participating in an LNS intervention trial. Such trials are  
365 important because the process of stunting predominantly occurs before 15 months of age in rural Africa  
366 (28). Detailed and accurate dietary intake information will contribute to an improved understanding of  
367 direct causes of stunting and undernutrition. The study results emphasise the importance of considering  
368 a potential differential bias to avoid the misinterpretation of intervention results.

### 369 **Conclusions**

370 At the pooled group level, the i-24-HR showed relatively good agreement to the WFR. However, there  
371 was an apparent differential bias whereby the mean intakes of energy and some nutrients were under-  
372 estimated compared with the WFR in the control group but not in the intervention group. Considering  
373 the cost and logistical implications of the WFR, the i-24-HR could be used in its place, for estimating  
374 mean intakes, but careful attention should be made during the design stage to the objectives of the  
375 study and whether only measures of absolute intakes or overall between-group differences are required.  
376 Absolute intakes might be under-estimated, if the i-24-HR is used to estimate dietary energy intakes of  
377 9-10-month-old infants who are not consuming an energy dense supplement, such as LNS. Future  
378 interventions evaluating differential dietary exposures (such as LNS) should consider, when comparing  
379 groups, whether a systematic error in intervention food measurement introduced a differential bias.  
380 When designing the study, they should put effort into developing an accurate method of quantifying  
381 intervention food consumption; and where possible, evaluate it in a pilot study before commencing data

382 collection. For researchers aiming to examine associations between dietary intakes and functional  
383 outcomes, such as growth, if resources permit, they should include a dietary assessment validation  
384 study, with a biomarker reference method (or using a gold-standard reference method) to understand  
385 the dietary assessment method's measurement error structure to help avoid misinterpretation of dietary  
386 intakes in relation to final growth outcomes.

### 387 **Acknowledgements**

388 We are grateful for the skilled and dedicated efforts of the data collection team: Mayamiko Banda,  
389 Hamsa Banda, Zikomo Chipatso, Reuben Mbwana, Tony Kansilanga, Mike Njaya, and Yacinta Stima.  
390 We are thankful to Jimmy Ngwaya who carefully prepared the food models which formed the basis of  
391 the data collection tools. A special thank you to Kathryn Dewey and Per Ashorn for their guidance and  
392 leadership in developing the protocol for this study, and expert advice throughout the study  
393 implementation and analysis. We are grateful for the vision, wisdom and professional guidance of the  
394 whole iLiNS study Steering Committee ([http://ilins.org/about-ilins/who-we-are/ilins-steering-](http://ilins.org/about-ilins/who-we-are/ilins-steering-committee)  
395 [committee](http://ilins.org/about-ilins/who-we-are/ilins-steering-committee)).

### 396 **Author contributions**

397 J.H, C.K., K.M., U.A., M.A., & E.L.F designed the research and significantly contributed to the aim  
398 and structure of manuscript; J.H. & C.K. conducted the research; A.M.R. & R.K. provided statistical  
399 guidance and assistance with methods; J.H, R.K. & E.L.F analysed data and performed statistical  
400 analyses; J.H drafted the paper with inputs from R.K. & E.L.F; J.H., R.K. & E.L.F had primary  
401 responsibility for the final content. R.K. & E.L.F have equal contribution to senior authorship. All  
402 authors have read and approved the final manuscript.



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**Table 1 Characteristics of participants at enrolment into the main study (at 6 months of age)**

	<b>Control</b>	<b>Intervention</b>	<b>p-value</b>
Participants (n)	26	106	
Female n (%)	14 (54)	49 (47)	0.50 <sup>a</sup>
<b>Socio-demographic Background Characteristics (n)</b>	<b>24</b>	<b>105</b>	
Maternal age; mean (SD) years	28.8 (7.3)	26.6 (5.9)	0.12 <sup>b</sup>
Maternal Education; mean (SD) years	3.9 (3.4)	4.4 (3.6)	0.52 <sup>b</sup>
Female-headed household n (%)	2 (8.3)	12 (11.9)	0.78 <sup>a</sup>
More than one child under 5 years old in household n (%)	11 (45.8)	44 (41.9)	0.06 <sup>a</sup>
<b>Maternal occupation n (%)</b>			0.64 <sup>a</sup>
Farming/Fishing	17 (77.3)	66 (66.0)	
House wife	3 (16.6)	27 (27.0)	
Indoor / office work	1 (4.6)	3 (3.0)	
Other	1 (4.6)	3 (3.0)	
Unknown	0 (0)	1 (1)	
<b>Information collected during time of visit (n)</b>	<b>26</b>	<b>106</b>	
Season (rainy: October - March) n (%)	12 (46.1)	56 (52.8)	0.80 <sup>a</sup>
Infant Breastfeeding n (%)	25 (100) <sup>c</sup>	104 (98.1)	0.49 <sup>a</sup>

a Chi-square

b Two-sample t-test

c n=25 breastfed, n=1 missing value in this control group

**Table 2: Estimated intakes of energy and selected nutrients (Mean and 95 % Confidence Interval)<sup>a</sup> using the i-24-HR compared to WFR between the hours of 06:00 and 18:00 by intervention group and pooled group**

Nutrient	Control Group (n=26)				Intervention Group- LNS (n=106)					Pooled Group (n=132)			
	WFR	i-24-HR Recall	Abs. Diff <sup>b</sup>	p-value <sup>c</sup>	WFR	i-24-HR Recall	Abs Diff <sup>b</sup>	p-value <sup>c</sup>	p-value <sup>d</sup>	WFR	i-24-HR Recall	Abs Diff <sup>b</sup>	p-value <sup>c</sup>
Energy (kcal/d)	376 (317, 437)	293 (246, 345)	-88	0.010	388 (352, 424)	379 (346, 412)	-10	0.60	0.052	385 (355, 416)	361 (333, 390)	-25	0.09
Protein (g/d)	9.6 (7.7, 11.6)	7.1 (5.8, 8.4)	-2.9	0.009	9.4 (8.4, 10.5)	8.2 (7.3, 9.0)	-1.6	0.007	0.36	9.5 (8.5, 10.4)	8.0 (7.3, 8.6)	-1.8	<0.001
Fat (g/d)	7.3 (5.3, 9.8)	5.3 (4.0, 6.8)	-2.8	0.05	10.0 (8.7, 11.5)	10.4 (9.1, 11.7)	0.1	0.62	0.10	9.6 (8.3, 10.7)	9.2 (8.2, 10.4)	-0.4	0.65
Iron (mg/d)	2.6 (2.1, 3.2)	1.8 (1.4, 2.2)	-0.1	<0.001	3.7 (3.3, 4.2)	4.0 (3.4, 4.5)	0.3	0.25	0.020	3.5 (3.1, 3.9)	3.5 (3.0, 3.9)	0.03	0.68
Zinc (mg/d)	1.6 (1.2, 1.9)	1.1 (0.9, 1.4)	-0.5	<0.001	3.3 (2.8, 3.8)	3.8 (3.1, 4.4)	0.6	0.020	0.07	2.9 (2.5, 3.3)	3.1 (2.6, 3.7)	0.4	0.18
Calcium (mg/d)	38 (25, 54)	53 (33, 77)	21.6	0.20	94 (77, 113)	128 (107, 152)	38.3	<0.001	0.41	81 (68, 96)	111 (93, 130)	35.1	<0.001
Vitamin A (µg RAE/d)	39 (18, 67)	24 (9, 46)	-15	0.19	143 (113, 176)	164 (130, 202)	24.1	0.10	0.23	117 (93, 144)	125 (99, 156)	15.9	0.37

<sup>a</sup> Data back-transformed from square root transformation for presentation

<sup>b</sup> Absolute mean difference - i-24HR Recall – WFR

<sup>c</sup> Matched pairs T-test

<sup>d</sup> Two-group t-test with equal variances between intervention and control group absolute differences

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i-24-HR: interactive 24-hour recall, LNS: Lipid-based nutrient supplement, RAE: retinol activity equivalents, WFR: weighed food record

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**Table 3: Percentage agreement for matching foods (items appearing both on the i-24-HR and the WFR) between intervention groups**

	Median (25 <sup>th</sup> , 75 <sup>th</sup> percentile)				
	Control Group (n=25)		Intervention Group (n=106)		p-value <sup>c</sup>
	n <sup>a,e</sup>	Percentage Agreement <sup>b</sup>	n	Percentage Agreement <sup>b</sup>	
Phala, all types (full volume)	25	100.0 (78.5, 122.4)	99	87.5 (68.1, 118.6)	0.457
Nsima, Rice (full volume)	25	78.4 (61.7, 100.0)	98	95.4 (59.5, 141.5)	0.248
Added Sugar	14	141.5 (103.7, 250.0)	69	167.7 (111.2, 295.0)	0.776
Sweetened Snacks	5	61.4 (50.7, 166.0)	45	112.7 (61.1, 195.0)	0.258
Savoury Snacks	8	105.9 (84.6, 137.5)	18	100.0 (56.7, 175.0)	0.683
Meat, Fish and Egg (solid)	7	82.7 (62.9, 294.9)	26	107.8 (62.7, 151.9)	0.735
Legumes, Nuts, Seeds	8	36.1 (26.4, 76.6)	26	76.2 (37.5, 105.3)	0.680
Fruit	4	160.0 (88.1, 231.7)	27	94.0 (66.2, 140.0)	--
Starchy Root and Vegetables	2	29.2 (22.1, 36.3)	20	80.8 (48.2, 145)	--
Milk and Yogurt	3	90.2 (90.0, 103.7)	8	111.0 (53.0, 228.6)	--
Non-dairy beverages	5	115.3 (85.6, 173.7)	15	100.0 (66.8, 142.2)	--
Soup/Broth from Relish	14	239.0 (195.3, 308.3)	54	134.0 (85.7, 240.0)	0.038
LNS	-		65	154.0 (98.8, 298.3) <sup>d</sup>	--

<sup>a</sup> Includes all portion sizes from items that match between the reported and reference values at the same time (i.e.: meal or snack time)

<sup>b</sup> Report percentage = (Reported amount / reference amount) x 100

Reference amount observed during the weighed food record; Reported amount taken from the 24-hour dietary recall.

<sup>c</sup> Mann-Whitney two-sample rank sum test by food group

<sup>d</sup> LNS only present in the diets of the intervention group, which is why there is no between-group comparison. This is descriptive only, looking at the percentage agreement of LNS in the intervention group.

<sup>e</sup> One participant missing in the control group for these analyses

i-24-HR: interactive 24-hour recall, LNS: Lipid-based nutrient supplement, WFR: weighed food record

**Table 4: Number of eating episodes and percentages of matching food groups (items appearing both in the i-24-HR and the WFR), intrusions and omissions by intervention groups**

	Control Group (n=25 <sup>d</sup> )			Intervention Group (n=106)		
	n (%)			n (%)		
	matching <sup>a</sup>	intrusion <sup>b</sup>	omission <sup>c</sup>	matching <sup>a</sup>	intrusion <sup>b</sup>	omission <sup>c</sup>
Phala, all types (full volume)	49 (92.5)	0 (0)	4 (7.6)	166 (94.3)	2 (1.1)	8 (4.6)
Nsima, Rice (full volume)	30 (88.2)	3 (8.8)	1 (2.9)	150 (89.8)	9 (5.4)	8 (4.8)
Added Sugar	22 (73.3)	5 (16.7)	3 (6.7)	105 (68.6)	26 (17.0)	22 (14.4)
Sweetened Snacks	6 (50.0)	2 (16.7)	4 (33.3)	59 (68.6)	15 (17.4)	12 (14.0)
Savoury Snacks	10 (76.9)	2 (15.6)	1 (7.7)	23 (69.7)	5 (15.2)	5 (15.2)
Meat, Fish and Egg (solid)	8 (53.3)	0 (0)	7 (46.7)	34 (56.7)	7 (11.7)	20 (32.8)
Legumes, Nuts, Seeds	13 (76.5)	1 (5.9)	3 (17.6)	39 (68.4)	4 (7.0)	14 (24.6)
Fruit	4 (66.7)	1 (16.7)	1 (16.7)	34 (70.8)	8 (16.7)	6 (12.5)
Starchy Root and Vegetables	2 (40.0)	0 (0)	3 (60.0)	22 (71.0)	4 (12.9)	5 (16.1)
Milk and Yogurt	3 (100)	0(0)	0 (0)	8 (47.1)	6 (35.3)	3 (17.6)
Non-dairy beverages	6 (75.0)	2 (25.0)	0 (0)	20 (62.5)	7 (21.9)	5 (15.6)
Soup/Broth from Relish	18 (62.1)	8 (27.6)	3 (10.3)	68 (64.7)	30 (28.6)	7 (6.7)
LNS	-	--		101 (89.4)	7 (6.2)	5 (4.4)

<sup>a</sup> The total of portions that were matched between the reference (WFR) and reported (i-24-HR), as a percentage of all items in the same group

<sup>b</sup> The total of portions that were reported (i-24-HR) but not observed in the reference data (WFR)

<sup>c</sup> The total of portions that were observed in the reference data (WFR), but not reported (i-24-HR)

<sup>d</sup> One participant missing for these analyses

i-24-HR: interactive 24-hour recall, LNS: Lipid-based nutrient supplement, WFR: weighed food record



**Online supplement Table 1: Average reported (i-24-HR) and reference (WFR) portion sizes by food group**

<b>Median (25th, 75th Percentiles)</b>					
	<b>n<sup>a</sup></b>	<b>Reported amount (g)<sup>b</sup></b>	<b>Reference Amount (g)<sup>c</sup></b>	<b>Percentage agreement<sup>d</sup></b>	<b>P-value<sup>e</sup></b>
Phala, all types (full volume)	125	78.9 (48.5, 112.0)	99.0 (64.7, 136.0)	86.4 (66.1, 114.1)	<0.001
Nsima, Rice (full volume)	124	52.5 (29.1, 80.0)	56.8 (33.5, 89.8)	89.1 (56.6, 135.0)	0.028
Added Sugar	94	5.1 (3.6, 7.9)	3.0 (1.9, 5.5)	143.3 (99.2, 238.9)	<0.001
Sweetened Snacks	64	7.9 (4.1, 15.8)	9.0 (4.0, 15.5)	91.7 (38.0, 158.0)	0.64
Savoury Snacks	34	7.7 (3.5, 11.0)	6.0 (3.0, 10.0)	86.1 (51.9, 157.1)	0.59
Meat, Fish and Egg (solid)	57	6.0 (0, 12.4)	9.2 (4.9, 18.2)	59.7 (0, 110.7)	0.015
Legumes, Nuts, Seeds	50	2.4 (0.4, 5.8)	7.8 (3.9, 16.0)	37.5 (2.4, 83.8)	<0.001
Fruit	38	22.5 (10.0, 35.0)	17.0 (6.0, 32.5)	94.0 (52.0, 136.4)	0.64
Starchy Root and Vegetables	30	18.0 (7.0, 24.0)	15.5 (6.0, 43.0)	50.0 (19.4, 120.0)	0.12
Milk and Yogurt	15	11.8 (5.2, 41.0)	8.0 (1.0, 29.0)	90.1 (36.8, 183.2)	0.82
Non-dairy beverages	33	47.3 (27.5, 76.1)	27.7 (9.0, 86.3)	98.1 (43.8, 123.5)	0.28
Soup/Broth from Relish	94	17.0 (11.7, 26.0)	7.4 (0, 16.9)	138.5 (80.0, 243.1)	<0.001
LNS	68	8.1 (4.5, 11.8)	4.5 (2.0, 9.0)	148.7 (95.0, 274.0)	<0.001

<sup>a</sup> Refers to the number of participants where this food group was present on the WFR, i-24-HR, or both. This includes the average portion size estimation per food group per participant. In the case where one was an intrusion, this resulted in a reference value of zero, and in the case where there is an omission, this resulted in a reported amount of zero. This is the participant average per food group.

<sup>b</sup> median daily average per participant of reported amount derived from i-24-HR

<sup>c</sup> median daily average per participant of reference amount derived from WFR

<sup>d</sup> Percentage agreement: (Reported amount / reference amount) x 100

<sup>e</sup> p-value derived from Wilcoxon signed-rank test for matched pairs

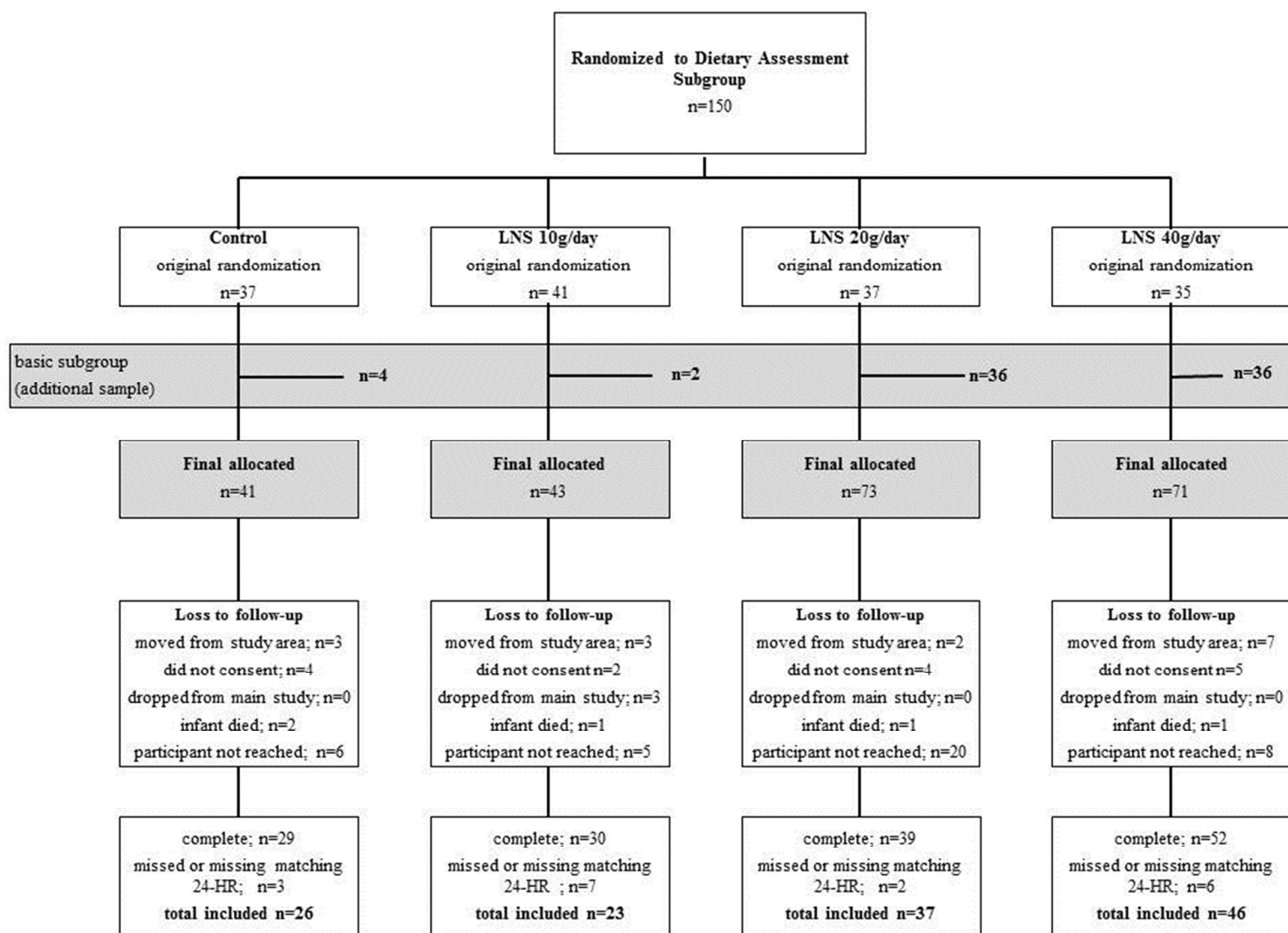
i-24-HR: interactive 24-hour recall, LNS: Lipid-based nutrient supplement, WFR: weighed food record

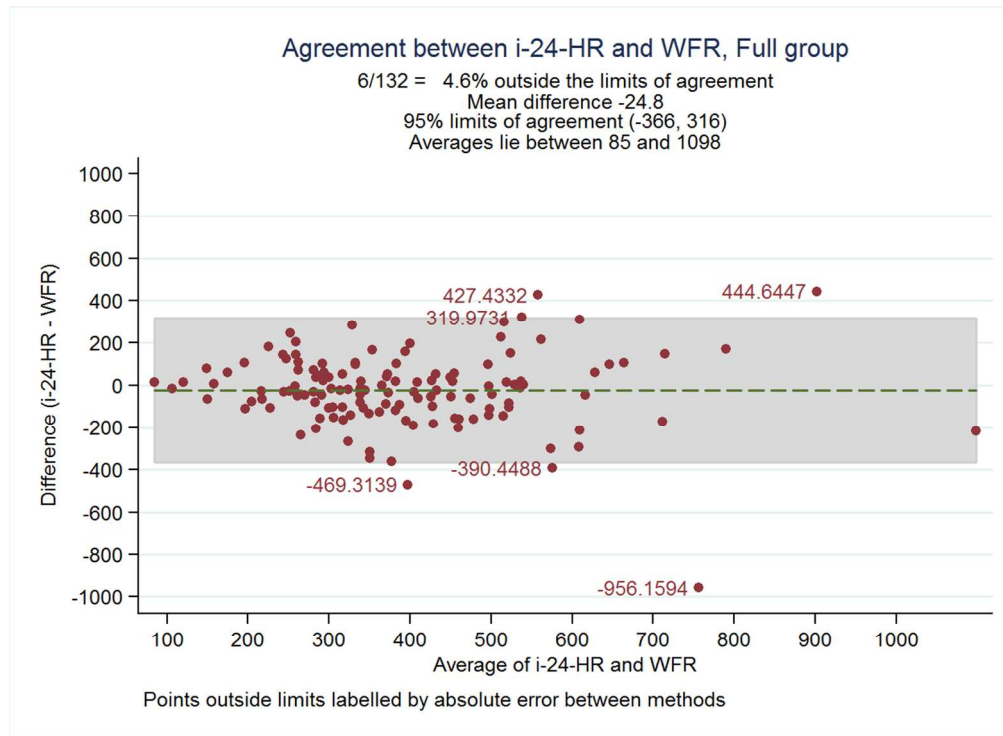
**Online supplement Table 2: Comparison of i-24-HRs that corresponded to and were independent of the WFR. An Assessment of bias in reporting related to the presence of the WFR: the “instrument effect”.**

Nutrient	N=71 Median Intake (25 <sup>th</sup> , 75 <sup>th</sup> percentile)			
	Independent 24-HR Recall	i24-HR WFR	Absolute Difference <sup>a</sup>	p-value <sup>b</sup>
Energy (kcal/d)	375 (273, 553)	327 (246, 463)	-34	0.10
Protein (g/d)	8.8 (5.8, 12.5)	7.6 (5.0, 10.3)	-0.78	0.06
Fat (g/d)	9.8 (5.0, 15.4)	8.1 (4.2, 11.8)	-1.9	0.06
Fe (mg/d)	3.2 (1.9, 5.8)	2.6 (1.7, 5.3)	-0.2	0.50
Zn (mg/d)	2.2 (1.2, 5.9)	2.0 (1.2, 6.1)	-0.1	0.97
Ca (mg/d)	115.9 (41.5, 204.3)	104.9 (34.7, 208.5)	-1.1	0.48
Vitamin A (µg RAE/d)	122.9 (30.3, 262.9)	107.9 (20.5, 292.9)	0	0.79

<sup>a</sup> i-24HR WFR – Independent 24-HR

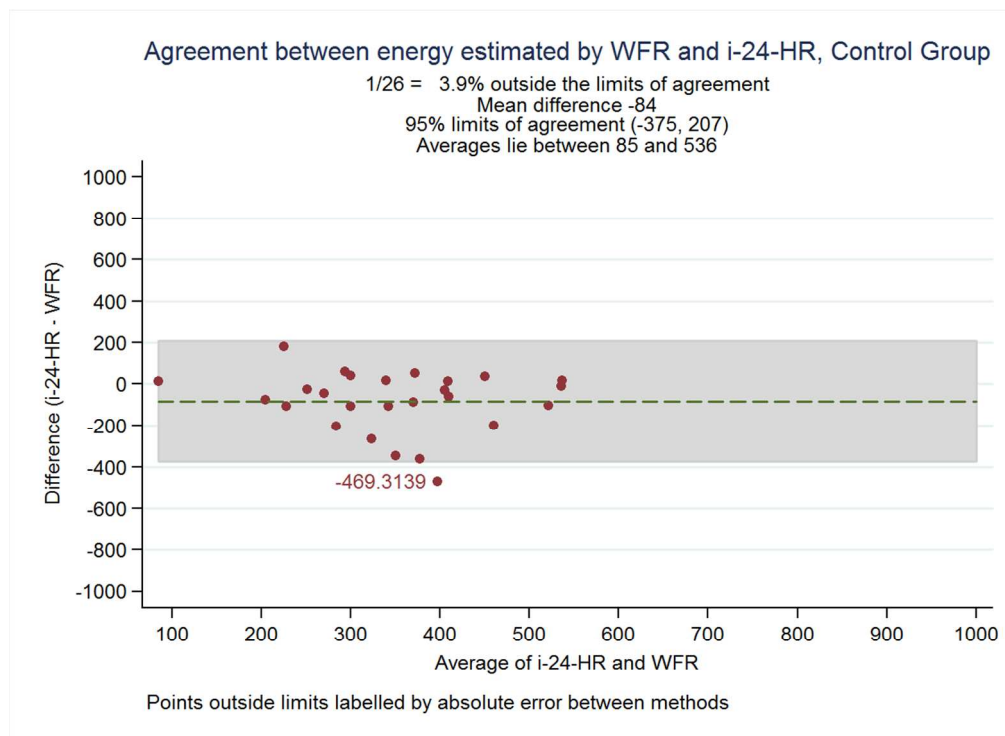
<sup>b</sup> Wilcoxon signed rank matched-pairs test

**Figure 1: Consort Flow Diagram of Participant Enrolment and Inclusion in the Validation Sub-Study**

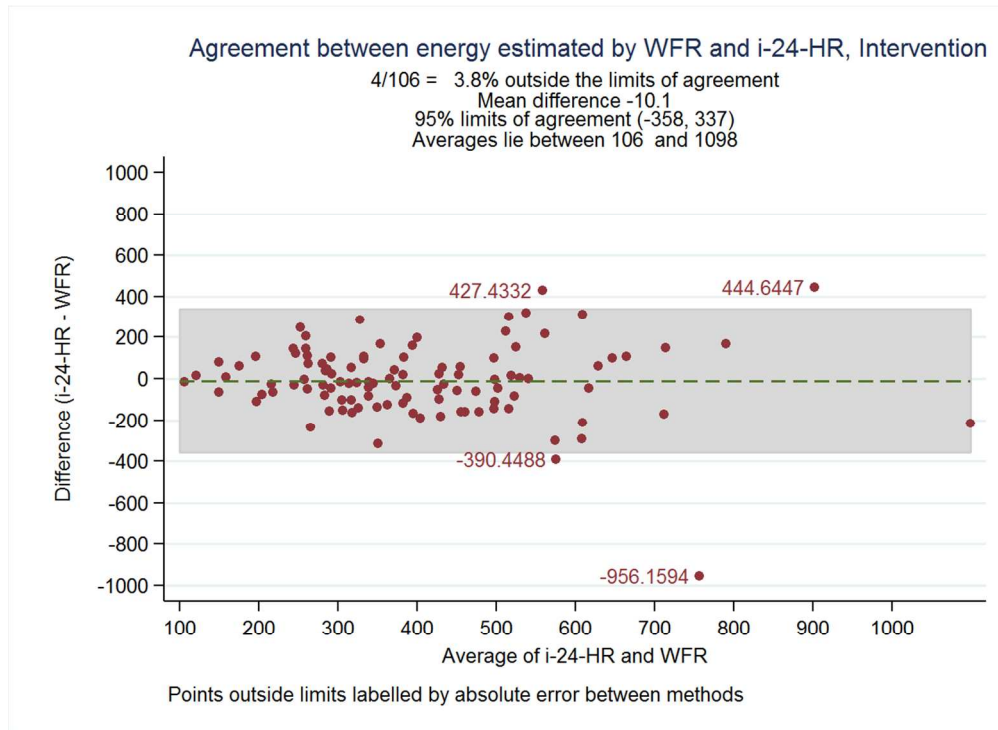
**Online supplement Figure 1: Bland Altman Plot Showing Relative Agreement in energy (kcal/day) estimation between WFR and i-24-HR: Pooled Group**

Review Only

**Online Figure 2a: Bland Altman Plot Showing Relative Agreement in Energy (kcal) estimation between WFR and i-24-HR: Control Group**



ew Only

**Online supplement Figure 2b: Bland Altman Plot Showing Relative Agreement in Energy (kcal) estimation between WFR and i-24-HR: Intervention Group**

New Only

## Comparison of an interactive 24-hour recall and weighed food record for measuring energy and nutrient intakes from complementary foods among 9-10-month-old Malawian infants consuming Lipid-Based Nutrient Supplements

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Running title: Dietary assessment errors of common methods

### Keywords

LNS, weighed record, 24-hr recall, dietary assessment, infants

**Abstract word count: 250, Manuscript body word count: 5 094**

**Number of figures: 1**

**Number of tables: 4**

**Supplementary tables: 2, Supplementary figures: 3**

**Study Funding:** This manuscript is based on research funded by a grant issued to the University of California, Davis from the Bill & Melinda Gates Foundation. The findings and conclusions contained within are those of the authors and do not necessarily reflect positions or policies of the Bill & Melinda Gates Foundation.

All authors declare no conflicts of interest

## 1 Abstract

2 Fortifying complementary foods with lipid-based nutrient supplements (LNS) may improve energy and  
3 nutrient intakes of infants at risk for undernutrition. We aimed to determine the relative validity of an  
4 interactive 24-hour dietary recall (i-24-HR) for assessing the impact of an LNS intervention on dietary  
5 intakes of energy and nutrients among rural Malawian 9-10-month-old infants (n=132) participating in  
6 the iLiNS dose trial. Dietary data were collected for the same day via i-24-HRs and weighed food  
7 records. Inter-method agreements were estimated overall and by intervention group, using Bland-  
8 Altman plots and paired t-tests; measurement error models (differential error); and percentage of food  
9 omissions and intrusions were estimated. Overall, inter-method differences in mean intakes of energy  
10 and most nutrients were not significant. When stratified by group, recalled energy intakes were under-  
11 estimated (-88kcal p=0.01) in the control but not in the intervention group (-10kcal; p=0.6). This  
12 differential reporting error was related to an over-estimation of recalled LNS (8.1g vs 4.5g; p<0.001) in  
13 the intervention group, compensating for an under-estimation of energy and nutrients intakes from  
14 complementary foods. Sources of measurement error in the i-24-HR were under-estimations of starchy  
15 staples, meat/fish/eggs and legumes/nuts/seeds (overall percent agreement overall report rates  
16 betweenranged from 38-89%; p<0.028); and over-estimations of added sugar, soups/broths and LNS  
17 (overall percent agreement betweenoverall report rates ranged from 138-149%; p<0.001). Common  
18 (>30% of eating occasions) omissions were milk/fish/egg, starchy roots/vegetables, and sweetened  
19 snacks. Common intrusions were milk/yogurt. Common (>20% eating occasions) omissions were  
20 meat/fish/eggs, legumes/nuts/seeds and starchy roots/vegetables, and intrusions were milk/ yogurt,  
21 beverages and soup/broths. Starchy staples and LNS were recalled when consumed (>85%) (i.e. well  
22 matched). These results emphasise the importance of considering differential error when interpreting  
23 dietary results in LNS trials.



## 24 **Introduction**

25 Undernutrition is common among young children living in low income countries (1). Both the short-  
26 and long-term adverse effects of under-nutrition impact health and future livelihoods. This underscores  
27 the need for comprehensive intervention packages, including effective dietary strategies. One such  
28 intervention is the use of lipid-based nutrient supplements (LNS) as home fortification of infant foods  
29 (2). Studies of the effectiveness of LNS for reducing undernutrition have shown mixed results (3-5). In  
30 cases where there was no association between LNS intake and growth outcomes (3), low adherence to  
31 the intervention (LNS consumption) and/or the displacement of other foods in the diet might partially  
32 account for the lack of a physiological effect. Thus, to correctly interpret LNS intervention trial results,  
33 accurate measurement of the LNS exposure and its influence on overall dietary intakes is fundamental.  
34 The assessment of infant dietary intakes is complicated for several reasons: 1) infants eat very small  
35 quantities of food; 2) measuring intake includes measuring not only the amount served, but also  
36 amounts left over, spit-up, spilled or dropped; 3) infants are often cared for and fed by multiple people;  
37 and 4) infants are unable to report their own intakes (6). The weighed food record is considered the  
38 “gold standard” dietary assessment method for quantitative estimates of an individual’s dietary intake,  
39 including for young children, because foods are weighed and recorded as they are consumed (7).  
40 However, for large surveys, the 24-hour recall is more practical because it is relatively rapid to  
41 conduct, has a low respondent burden and is less disruptive for low-literacy communities where, for the  
42 weighed food record, research assistants must weigh and record all foods consumed by participants.  
43 The disadvantages of 24-hour recalls are that they are prone to errors of memory, recall bias, errors in  
44 portion size reporting and potentially a social-desirability bias (8). The interactive multiple pass 24-  
45 hour recall (i-24-HR) was developed specifically for areas with low literacy rates, and includes a  
46 pictorial chart to prospectively record dietary intakes and reduce errors of memory (9).  
47 Previous studies, in Malawi, Ghana, Sweden and the United States, have assessed the validity of the  
48 24-hour dietary recall method relative to weighed food records (WFR) for estimating the energy and  
49 nutrient intakes of young children (10-13). They show recalled compared to weighed energy intakes are  
50 generally over-estimated (10, 12, 14), which for rural Malawian 15-m olds was by 13% (10). This  
51 pattern of over-estimation of energy intakes might be more pronounced for toddlers than infants, if  
52 accurate reporting becomes more difficult as the diet becomes more complex (12, 15). To our  
53 knowledge no study has validated the 24-hour recall for African infants under 12-months of age.  
54 There is also evidence that certain foods are more accurately reported than others (16, 17). Such  
55 differences become important when assessing dietary exposures in a LNS intervention trial because  
56 LNS, which is an energy and nutrient dense food, is not present in the diet of the control group.

3

57 Systematic under- or over-estimation of LNS intakes would bias between-group comparisons by either  
58 exaggerating or attenuating the observed effect of LNS on infant dietary intakes, of energy and  
59 nutrients. An accurate assessment of dietary exposure is essential in dietary intervention trials to  
60 properly understand the association between dietary exposure and outcome (18-20). To our knowledge,  
61 the i-24-HR has not been validated for use among infants who are participating in an LNS intervention  
62 trial.

63 This study, therefore, aimed to assess the relative validity of the i-24-HR used in an LNS intervention  
64 trial, the iLiNS study (3). The iLiNS study aimed to evaluate the efficacy of three doses of LNS for the  
65 prevention of stunting among infants supplemented from 6 to 18 months of age. In this trial, inter-  
66 group differences in dietary intakes of energy and nutrients were assessed when the infants were 9-10  
67 months of age (21). The specific objectives of the current study were to 1) assess the relative validity of  
68 the i-24-HR method for estimating dietary intakes of energy, protein, fat, iron, zinc, calcium and  
69 vitamin A from complementary foods using a 1-day WFR as the reference method; 2) assess whether  
70 there is a differential bias in i-24-HR measures of energy intake between the control group and  
71 intervention groups, and 3) describe potential sources of measurement error in the i-24-HR, including  
72 errors in the types or amounts of LNS and complementary foods reported.

## 73 **Methods**

### 74 **Design and Study Population**

75 A cross-sectional validation study was nested within a dietary assessment sub-study of infants  
76 participating in a 12-month LNS randomised control trial (iLiNS-DOSE trial) conducted in Mangochi  
77 district, Malawi from November 2009+0 and July 2012. [Data collection for the dietary assessment sub-](#)  
78 [study took place between March 2010 and October 2011\\*\\*\\* when the infants were 9-10 m of age. Data](#)  
79 [collection](#) ~~Data collection~~ for the dietary validation study took place between October 2010 and  
80 October 2011. The main trial was designed to assess the impact of three different doses of LNS (10g,  
81 20g and 40g) on linear growth; which was delivered bi-weekly to households in the intervention  
82 groups. The objectives and methods of the iLiNS-DOSE trial (n=1980) and the dietary assessment  
83 sub-study (n=688) are described in more detail in Maleta, et.al. (3) and Hemsworth, et.al. (21),  
84 respectively. In the dietary assessment sub-study, two i-24-HRs were done exactly 7-days apart when  
85 the infants were between 9 and 10 months of age. One i-24-HR was done during the week -LNS was  
86 delivered, and the other in the subsequent week. In the validation study the WFRs which were done  
87 one-day prior to a corresponding i-24-HR, were done just after the LNS delivery day to maximize

88 capturing the presence of LNS in the child's diet. The other i-24-HR was collected either 7-days before  
89 or 7-days after the i-24-HR that corresponded with the WFR day.

## 90 Sampling

91 A ~~stratified random~~ ~~random~~-sample of 228 infant-mother dyads was ~~obtained~~ ~~calculated~~ ~~selected~~ for the  
92 validation study (~~i.e.~~, 56 in each of the control, 10g, 20g, and 40g LNS groups). ~~The~~ ~~is~~-sample size ~~for~~  
93 ~~the validation study~~ was ~~chosen~~ ~~calculated~~ to allow detection of a difference of 55kcal (one 10g dose of  
94 LNS) between ~~each of~~ the four intervention groups with power of 80% and  $\alpha=0.05$ , assuming a  
95 standard deviation of the difference between the methods (WFR minus i-24-HR) of 138 kcal (derived  
96 from a pilot study), and a 10% attrition rate (e.g. missed i-24-HR following the WFR).

97 The original inclusion criterion was participation in the dietary assessment sub-study of the iLiNS-  
98 DOSE trial. The validation study, however, began seven months after the trial began, which meant that  
99 one third of participants had already completed the dietary sub-study and were no longer eligible for  
100 the validation study. As a result, to meet our target sample size of 228 age-eligible infants, we selected  
101 additional infants (n=78) at random from the basic sub-study group (i.e., not randomised to any  
102 additional sub-study at baseline to minimise respondent burden) to reach the intended sample size. It  
103 introduced an imbalance in the number of infants from the control and 10g LNS groups versus the 20g  
104 and 40g LNS groups. As such, more infants were in the 20g LNS and 40g LNS groups than the other  
105 two groups in this validation study.

## 106 Ethical Approval

107 Ethical approval for this sub-study ~~study~~ was granted by the London School of Hygiene and Tropical  
108 Medicine Research Ethics Board as well as by the College of Medicine Research Ethics Board in  
109 Malawi. Informed written consent was obtained from all participating caregivers in this study. The trial  
110 was registered at clinicaltrials.gov with the identifier: NCT00945698

## 111 Dietary Assessment

### 112 *Interactive 24-hour Recall (i-24-HR)*

113 Dietary data were collected using a 4-pass i-24-HR, developed for use in a rural African context (9).  
114 The method was modified specifically for a similar population ~~to~~ ~~and~~ ~~included~~ pictorial charts  
115 (intended to reduce intrusions and omissions), bowls/cups/plates, and measured portion sizes using real  
116 food replicas and salted models. In the dietary assessment sub-study, caregivers were given the  
117 pictorial food chart and a plastic cup and bowl 2-days before the i-24-HR was done. On the day before  
118 the i-24-HR, ~~caregiver~~ ~~they~~ were asked to prospectively record on the pictorial chart all foods,

5

119 beverages, and LNS (if appropriate) when given to the child to minimise memory errors; and to feed  
120 their child from the cup and bowl provided to minimise portion size estimation errors. In the first pass,  
121 during the i-24-HR interview, from memory, the caregiver was asked to serially recall all foods,  
122 supplements and beverages that their child had consumed in the previous 24 hours. In the second pass,  
123 information about the time, place, and description of the food or beverage was collected. In the third  
124 pass, portion sizes were estimated by the [caregivers/parents](#) showing the amount served and the  
125 amount left-over using real food replicas (with or without excess salt to preserve them) and unit  
126 descriptions (e.g. package of biscuits). The amounts were weighed [by the interviewers](#) using digital  
127 kitchen scales (Home Elegance, accurate to  $\pm 1$ g), and recorded. The amount consumed was calculated  
128 as the amount served minus the amount left-over. LNS portion sizes were measured using a pot of  
129 LNS, which was weighed before and after the caregiver had removed the amount of LNS used at each  
130 eating occasion. Left-overs were subtracted from the amount of LNS served. If LNS was mixed with  
131 other foods, the amount left over was calculated by multiplying the amount served by the proportion of  
132 the mixed dish that was consumed, assuming uniform mixing. The consumption of LNS was not  
133 specifically probed to prevent errors of intrusion (i.e. items listed but not actually consumed). To  
134 reduce potential differences in recording, interviewers were given extensive training and used  
135 standardised operating procedures, including a portion size estimation manual, detailing the specific  
136 methods for portion size estimations and probing. At the end of the third pass, [interviewers data](#)  
137 [collectors](#) asked for the pictorial chart. Any discrepancies between the pictorial chart and the food list  
138 of the i-24-HR were discussed. In the final pass, the data collector summarised and confirmed the food  
139 and drinks recorded in the i-24-HR.

#### 140 ***Weighed Food Record (WFR)***

141 All foods and beverages consumed by the child from 6 a.m. until the final meal of the day were  
142 weighed and recorded by a data collector, using digital kitchen scales (Home Elegance, accurate to  $\pm$   
143 1g). Left-over foods were weighed either individually, if they could be separated on the plate, or as a  
144 mixture, assuming uniform mixing. Recipe data were collected by weighing all raw ingredients and the  
145 final cooked dish. The WFR data collector was not involved in the collection of the i-24-HR data.

#### 146 **Questionnaires**

147 Socio-demographic background characteristics [of the infants were collected within two weeks of](#)  
148 [baseline enrolment in the iLiNS study, when the infants were 6 months old, using an interviewer-](#)  
149 [administered questionnaire. analysed\(maternal occupation, maternal education level, household size,](#)  
150 [head of household, and presence of other child under 5 years in the household\) of the infants were](#)

151 | ~~collected using an interviewer administered questionnaire within two weeks of baseline enrolment~~  
152 | ~~(when infants were 6 months of age).~~

### 153 | **Data processing**

154 | Conversion factors were developed for the i-24-HR, and used to estimate the grams of food consumed.  
155 | Average recipes were calculated for cooked dishes using the individual recipes collected from each  
156 | household. These data were used to calculate intakes of ingredients from cooked dishes in the i-24-  
157 | HRs. Intakes of energy and nutrients from the WFR and i-24-HRs were estimated, using a food  
158 | composition table developed for this study (21).  
159 | The time each item was consumed was also recorded, and it was used to match the corresponding  
160 | eating occasions for inter-method portion size comparisons. Meals and snacks consumed after 19:00  
161 | were removed from both the WFR and i-24-HR (i.e. a 12-hour WFR and recall were created) because  
162 | there were occasions during the collection of the WFR when the final meal was consumed after the  
163 | data collector had left the household.

### 164 | **Statistical Analysis**

165 | All data analyses were performed using Stata version 12 (StataCorp LLC, College Station, Texas). The  
166 | three LNS intervention groups were collapsed to form one large group, for all analyses, because there  
167 | were no significant inter-group differences in energy and nutrient intakes from complementary foods  
168 | (including LNS), and the group sample sizes were small (21). In all analyses, except the analyses for an  
169 | instrument effect (see below), data from only one of the two i-24-HR were used, which was the i-24-  
170 | HR collected for the same day as the WFR. Energy and nutrient intake distributions from the WFR and  
171 | i-24-HRs were mathematically transformed, when necessary, for the analyses.

172

### 173 | **Sociodemographic variables**

174 | A composite variable for socioeconomic status was calculated using principal component analysis  
175 | (PCA), and the PCA scores were divided into quintiles using the first principal component. The  
176 | following variables were used as part of the composite variable: maternal occupation, household  
177 | crowding, source of electricity, source of water, sanitary facilities, material of roofing, and material of  
178 | house walls.

179 | Chi-squared tests, for categorical socio-demographic variables, and two-sample t-tests, for non-  
180 | categorical socio-demographic variables, were used to check for variables associated with  
181 | “missingness” of WFRs and for differences between intervention groups (control vs. LNS) in the  
182 | validation study.

7

183 ***Assessment of agreement between dietary assessment methods***

184 Paired t-tests were used to compare mean intakes of energy and nutrients from the corresponding i-24-  
 185 HR and WFR. Absolute differences (“error”) in amounts of energy and nutrients between the two  
 186 methods were calculated as follows: i-24-HR – WFR. A two-sample t-test with equal variances was  
 187 used to compare the absolute differences between the control and intervention groups. Bland-Altman  
 188 plots were used to estimate, for energy intakes, the level of agreement between the two methods and  
 189 the 95% limits of agreement.

190 ***Assessment of differential error***

191 Measurement error modelling was used to investigate whether error in the i-24-HR differed by  
 192 treatment group. We let  $S_1$  denote the i-24-HR measurement (square-root transformed) made at the  
 193 same time as the WFR, and  $W_1$  denote the WFR measurement itself (square-root transformed). The  
 194 second independent i-24-HR measurement (square-root transformed) was denoted ~~the square-root~~  
 195 ~~transformed measure~~  $S_2$ . The true, but unobserved, intakes at time points 1 and 2 were denoted  $Y_1$  and  
 196  $Y_2$  respectively. At time point  $j$  ( $j = 1,2$ ) the relationships between the observed measurements of  
 197 dietary intake and the unobserved underlying true intake were assumed to be of the following forms,  
 198 where we allowed separate model parameters for individuals in the control (C) and combined  
 199 intervention (T) groups,

200 **Equation 1**

201

$$\text{Combined intervention group: } S_j = \gamma_{0T} + \gamma_{1T}Y_j + \epsilon_{Tj}$$

$$\text{Control group: } S_j = \gamma_{0C} + \gamma_{1C}Y_j + \epsilon_{Cj}$$

202

$$\text{Combined intervention group: } W_1 = Y_j + \delta_{Tj}$$

$$\text{Control group: } W_1 = Y_j + \delta_{Cj}$$

203 The  $\epsilon$  and  $\delta$  terms are random errors with mean zero and constant variance. The WFR is assumed to  
204 provide an unbiased estimate of true intake in both the control and intervention groups. The intercept  
205 parameters  $\gamma_{0T}$  and  $\gamma_{0C}$ , and slope parameters  $\gamma_{1T}$  and  $\gamma_{1C}$ , represent systematic error in the i-24-HR  
206 measurement. We assessed evidence for differential error based on [estimates of bootstrap confidence](#)  
207 [intervals](#) for the differences  $\gamma_{1T} - \gamma_{1C}$  and  $\gamma_{0T} - \gamma_{0C}$  [and corresponding bootstrap confidence intervals](#).  
208 [The parameters of the measurement error model in Equation 1 were estimated via a method of](#)  
209 [moments approach](#).

### 210 ***Sources of disagreement between the i-24-HR and WFR***

211 To identify possible sources of disagreement between the two dietary assessment methods, we  
212 categorised each food and drink item (for composite dishes, we matched the individual ingredients) as  
213 an omission (present on WFR, absent on i-24-HR), an intrusion (absent on WFR, present on i-24-HR)  
214 or a match (present on both methods at matching meal/snack times). We calculated the frequency of  
215 each category across food groups (i.e., phala; nsima and rice; added sugar; sweetened snacks; savoury  
216 snacks; meat, fish and egg; legumes, nuts, and seeds; fruit; starchy roots and vegetables; milk and  
217 yogurt; non-dairy beverages; soup/broth from relish; and LNS), a method previously described by  
218 Smith, et.al. (22). We compared the median percentage agreement for each food group, (i.e. 100\*  
219 reported amount (i-24-HR) / reference amount (WFR)), for the intervention and control groups, using  
220 Mann-Whitney rank sum test when the sample was at least five consumers. In the case where one food  
221 within a food group of these is an intrusion, this resulted in a reference amount of zero (at the  
222 individual food level only), and in the case where there is an omission, this resulted in a reported  
223 amount of zero. We also compared the overall inter-method differences, in the grams of food consumed  
224 in each food group, using the Wilcoxon signed-rank test.

### 225 ***Instrument Effect***

226 We tested for an “instrument effect”, because the presence of a data collector on the day of the WFR  
227 might have influenced the [caregivers/respondent's](#) ability to recall dietary intakes during its  
228 corresponding i-24-HR. This “instrument effect” was assessed using the Wilcoxon signed-rank test, by  
229 comparing the median intakes of energy and nutrients estimated using the i-24-HR corresponding to the  
230 WFR day and the i-24-HR collected on a day independent of the WFR (i.e., collected one week before  
231 or after the WFR). [For this analysis, n=71 matched records were available](#).

## 232 Results

### 233 Participants

234 A total of 228 infants were selected to participate in the validation study. However, 78 were lost to  
235 follow-up and 18 did not have a matching WFR and i-24-HR. The final sample size analysed was 132  
236 matching i-24HRs and WFRs (**Figure 1**). There were no significant differences in socio-demographic  
237 characteristics comparing those with missing data and those who completed the WFR (data not shown).  
238 Likewise, there were no differences in baseline characteristics between the intervention and control  
239 group (**Table 1**).

### 240 Agreement between dietary assessment methods

241 The reported energy intakes were lower in the i-24-HR compared to the WFR, although the difference  
242 was not statistically significant ( $p=0.09$ ) (**Table 2**). Reported protein intake was significantly  
243 underestimated and calcium intake was significantly over-estimated by the i-24-HR compared to the  
244 WFR ( $p<0.001$ ). There were no significant between-method differences in intakes of fat, iron, zinc or  
245 vitamin A. The Bland-Altman plot showed a systematic bias for under-reporting recalled energy  
246 intakes compared to the WFR and poor agreement at the individual level, with 95% limits of agreement  
247 of -3668 kcal to 3167 kcal (Online supplement **Figure 1**).

248 When stratified by intervention group, however, there was a significant under-estimation of recalled  
249 energy intakes in the control group ( $p=0.010$ ) but not in the intervention group ( $p=0.60$ ) (**Table 2**).  
250 Recalled intakes of protein, fat, iron and zinc were also significantly underestimated in the control  
251 group. In the intervention group, recalled intakes of protein were significantly under-estimated,  
252 whereas recalled intakes of calcium and zinc were significantly overestimated (Table 2). **Further, after**  
253 **comparing the absolute differences (“error”) calculated between the WFR and i-24-HR in the control**  
254 **and intervention groups, we found significant differences ( $p\leq 0.05$ ) for energy (kcal) and iron, and all**  
255 **other nutrients were considered non-significant ( $p>0.05$ ).** The Bland-Altman plot by intervention  
256 group (Online supplement **Figures 2a and 2b**) showed poor 95% limits of agreement (**LOA**) for  
257 energy at an individual level, for both the intervention (**95% LOA -358, 337 kcal**) and control (**95%**  
258 **LOA -375 to 207 kcal**) groups; and a mean systematic under-estimation of energy intakes in the control  
259 group only (**-84 kcal, 95% LOA -375 to 207 kcal**).

260  
261 By fitting the measurement error models in equation 1, we found that  $\hat{\gamma}_{1C} = -2.4$  (95% CI (-24.9,  
262 29.7)) and  $\hat{\gamma}_{1T} = 2.6$  (95% CI (-20.0, 20.2)),  $\hat{\gamma}_{0C} = 63.2$  (95% CI (58.8, 67.3)) and  $\hat{\gamma}_{0T} = -32.5$  (95%  
263 CI (-34.5, -30.6)). The confidence intervals were obtained from the 2.5 and 97.5 percentiles of 1000



264 bootstrap estimates, using bootstrap samples stratified by intervention group. The expected i-24-HR  
 265 measure of energy intake ( $S$ ) given the true intake ( $Y$ ) is therefore  $E(S|Y) = -32.5 + 2.6Y$  in the  
 266 combined intervention group, and  $E(S|Y) = 63.2 - 2.4Y$  in the control group. The estimates of the  
 267 slope are in opposite directions in the intervention and control groups because the correlation between  
 268 the independent i-24 and the WFR is positive; in the intervention group, but negative in the control  
 269 group; however the CIs are very wide and the 95% bootstrap CI for the difference  $\gamma_{1T} - \gamma_{1C}$  was (-  
 270 46.6, 56.5). However, there was strong evidence for a difference in the intercepts; the 95% bootstrap CI  
 271 for the difference  $\gamma_{0T} - \gamma_{0C}$  was (-100.1, -90.7) The model-based approach, therefore, **provides**  
 272 **suggests that the relationship between the i-24-HR measure of energy intake and the true intake may be**  
 273 **different in the intervention and groups, i.e. indication of a indicates a potential evidence of** differential  
 274 error.

#### 275 Sources of **disagreement between the measurement error in the i-24-HR and WFR**

##### 276 *LNS intakes*

277 In the intervention group, there was a significant between-method difference in estimated LNS intakes.  
 278 The median intake was significantly higher for the recalled (i-24-HR) than reference (WFR) amount  
 279 (i.e., 8.1g (4.5, 11.8) vs 4.5g (2.0, 9.0);  $p < 0.001$ ) (**Online Supplement Table 1**). The median (IQR)  
 280 percentage agreement (matched LNS portions) indicates recalled LNS consumption was over-estimated  
 281 by over 50% compared to the WFR (**Table 3**). Close to 90% of the eating occasions matched on both  
 282 the WFR and i-24-HR; and rates of intrusions and omissions were similar and low (**Table 4**).

##### 283 *Complementary food intakes*

284 At the pooled group level, phala, legumes, nuts and seeds, and meat, fish and eggs were significantly  
 285 under-estimated; whereas, soups/broths from relish and added sugar were significantly over-estimated  
 286 in the i-24-HR compared to the WFR (**Online Supplement Table 1**). There were no significant  
 287 differences between intervention- and control groups in reporting accuracy (i.e., percentage agreement  
 288 for food groups), except for soups/broths from relish, where the control group showed a higher over-  
 289 reporting rate than the intervention group. These comparisons, for four of the 12 food groups, were  
 290 limited by the small sample size of the control group (Table 3).

291 In both the intervention and control groups, a comparison of food group matches, intrusions and  
 292 omissions showed the highest reporting agreement for staples, where over 88% of the phala and nsima  
 293 eating occasions matched between the two methods (Table 4). Episodically consumed foods such as  
 294 meat, fish and eggs (which were frequently misreported as soup/broth from relish), starchy roots and

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295 vegetables, and sweetened snacks had poor reporting matching, with a higher tendency for respondents  
296 to omit (i.e. forget) as opposed to intrude (i.e. add in error).

### 297 **The “instrument-effect”**

298 There was no evidence of an “instrument effect”. There were no significant differences in estimated  
299 intakes of energy or nutrients comparing the independent i-24-HR (performed either one week before  
300 or after the WFR) and the corresponding i-24-HR (i.e., for the same day as the WFR). The absolute  
301 differences ranged from zero RAE/d to 34 kcal/d (**Online supplement Table 2**).

### 302 **Discussion**

303 In the context of a LNS supplementation trial, we found there was no significant difference comparing  
304 energy intakes measured using the i-24-HR to the WFR when all groups were pooled. This comparison  
305 was not biased towards agreement by the weighing process, because the independent and  
306 corresponding i-24-HRs provided similar estimates of energy and nutrients intakes. However, this  
307 pooled comparison masked a difference between the intervention and control group. When stratified by  
308 intervention group, the i-24-HR systematically under-estimated dietary energy intakes compared with  
309 the WFR in the control group but not in the intervention group. The significant difference in the “error”  
310 or absolute difference between the methods in control and intervention groups suggest a differential for  
311 recalled energy intakes. This differential error, **for estimating median energy intakes**, primarily is the  
312 result of an over-estimation of the energy-dense supplement (LNS), which was only consumed by the  
313 intervention group. It compensated for the under-estimation of energy intakes from complementary  
314 foods because most **caregiversrespondents** were able to report whether their infant had consumed it. **In**  
315 **contrast, when using dietary data collected via i-24-HRs to examine associations, the 95% LOA**  
316 **indicate poor agreement at the individual level, in both groups, which will attenuate associations.**  
317 These results highlight ~~the importance, when aiming to~~ of estimating differential measurement error to  
318 ~~correctly interpret estimate inter-group differences in the impact of an energy and nutrient dense~~  
319 ~~supplement on~~ **median intakes of energy and nutrients dietary intakes (and growth outcomes) in an**  
320 **intervention trial, the importance of examining whether systematic measurement error when**  
321 **quantifying intervention food consumption, contributes to a differential bias. In studies aiming to**  
322 **examine associations between dietary intakes and functional outcomes (e.g., growth), the i-24-HR is**  
323 **inferior to more accurate methods of dietary assessment.** In our study considerable effort was made to  
324 accurately estimate LNS consumption. The **caregiversrespondentscaregivers** were asked to spoon out  
325 the amount of LNS served to the infant and estimate the amount left-over, which were both weighed  
326 and recorded.

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327 There were few differences, comparing the intervention and control group, for between-method  
328 agreement in the estimation of complementary foods intakes. In the pooled group analyses, the main  
329 sources of between-method disagreement were under-estimated recalled portion sizes of dietary staples  
330 (phala, rice and nsima by between 11 and 14%), meat, fish and eggs and legumes, nuts and seeds.  
331 Energy-dense foods, such as added sugar, were overestimated by over 40% compared with the WFR;  
332 but it did not compensate for the under-estimation of energy from staples (phala, nsima and rice). This  
333 result is not surprising because dietary staples provide a high percentage of daily energy intakes for  
334 rural infants in Malawi.

335 Underestimation of certain food groups is not unique and has been reported among women in Malawi  
336 (9) as well as preschool aged children in Ghana (11). However, the underestimation in energy intakes  
337 relative to the WFR, in the control group of our study, is in contrast to results from a study of 10-13  
338 month old Senegalese infants (n=45), which showed the 24-hour recall was a relatively good measure  
339 of intake compared to WFR (23, 24); and a study of 15-month old rural Malawian infants (n=169),  
340 which showed a systematic over-estimation in energy and nutrient intakes (10). The sources of  
341 measurement error, in the previous Malawian study, is-are unknown. These inter-study differences  
342 could be a function of inter-method or age group differences. In our study, we probed for left-overs  
343 and adjusted the portion sizes in the i-24-HR based on recalled left-overs. This adjustment was not  
344 reported in the other studies. It has been suggested that as a diet becomes more complex (as the infant  
345 ages), the reporting accuracy changes (12) and perhaps the direction of the error also changes.

346 The results of this validation study suggest that a differential error might be present when an i-24-HR  
347 is used to measure group median dietary intakes, which is related to a systematic over-estimation of  
348 the exposure (LNS). Linear calibration techniques could be used to correct the systematic under-  
349 estimation of energy intakes from non-LNS foods. Previous studies have developed correction factors  
350 using the WFR as the reference standard to adjust i-24-HR energy intakes for a systematic  
351 overestimation of energy intakes compared to the WFR. This technique is not recommended for the  
352 current study because the reference method is subject to the same errors as the test method (19, 25), e.g.  
353 both the WFR and i-24-HR are subject to mis-estimation of items that were spilled or spit up. The  
354 linear calibration equations would only have been appropriate if we had used a biomarker, such as the  
355 stable isotope technique to measure total energy expenditure, which is an unbiased and independent  
356 measure of long-term energy intake (6, 20).

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### 357 **Study Limitations and Advantages**

358 The main study limitations were the relatively low sample size and high rate of attrition. The study was  
359 underpowered to detect differential error in the i-24-HR between control vs. intervention groups. The  
360 high rate of attrition occurred because of the logistical demands of this validation study in a large  
361 catchment area (i.e. transportation, communication with households, etc.). No observed background  
362 characteristics were associated with missing the visit.

363 Another limitation was the reference method used. The WFR is the most common reference standard  
364 for comparison with ~~the a~~ 24-hour dietary recall because it is less resource-intensive than collection of  
365 biomarkers, and it provides useful robust information about portion size estimation, intrusions and  
366 omissions. However, it does not meet the strict criteria for a valid reference method (26). To validate  
367 the i-24-HR (repeated to provide an estimate of usual intakes), for estimating energy intakes alone, the  
368 doubly labelled water method is the preferred reference method (25, 27). Further, the modelling  
369 approach we used to assess evidence for differential error (equation 1), relies on an assumption that the  
370 WFR provides an unbiased measure of intake, as well as additional assumptions about the form of the  
371 systematic errors.

372 This study also had many advantages. It was carried out several3-months after the start of the  
373 intervention, which meant that the children were habituated to the intervention food. It was also  
374 conducted over a long period of time which allowed for seasonal variation in dietary patterns and  
375 episodically consumed foods to be captured. This study is also the first study that we are aware of that  
376 has assessed the relative validity of the i-24-HR for estimating the dietary intakes of rural African  
377 infants under 12 months of age who are participating in an LNS intervention trial. Such trials are  
378 important because the process of stunting predominantly occurs before 15 months of age in rural Africa  
379 (28). Detailed and accurate dietary intake information will contribute to an improved understanding of  
380 direct causes of stunting and undernutrition. The study results emphasise the importance of considering  
381 a potential differential bias to avoid the misinterpretation of intervention results.

### 382 **Conclusions**

383 At the pooled group level, the i-24-HR showed relatively good agreement to the WFR. However, there  
384 was an apparent differential bias whereby the meandian intakes of energy and some nutrients were  
385 under-estimated compared with the WFR in the control group but not in the intervention group.

386 Considering the cost and logistical implications of the WFR, the i-24-HR could be used in its place, for  
387 estimating meandian intakes, but careful attention should be made during the design stage to the  
388 objectives of the study and whether only measures of absolute intakes or overall between-group

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389 differences are required. Absolute intakes might be under-estimated, if the i-24-HR is used to estimate  
 390 dietary energy intakes of 9-10-month-old infants who are not consuming an energy dense supplement,  
 391 such as LNS. Future interventions evaluating differential dietary exposures (such as LNS) should  
 392 consider, when comparing groups, whether a systematic error in intervention food measurement  
 393 introduced a differential bias. When designing the study, they should put effort into developing an  
 394 accurate method of quantifying intervention food consumption;- and where possible, evaluate it in a  
 395 pilot study before commencing data collection. For researchers aiming to examine associations  
 396 between dietary intakes and functional outcomes, such as growth, if resources permit, they should  
 397 include a dietary assessment validation study, ~~preferably~~ with a biomarker reference method (or using a  
 398 gold-standard reference method) to understand the dietary assessment method's measurement error  
 399 structure ~~and~~ to help avoid misinterpretation of dietary intakes in relation to final growth outcomes.

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#### 400 **Acknowledgements**

401 We are grateful for the skilled and dedicated efforts of the data collection team: Mayamiko Banda,  
 402 Hamsa Banda, Zikomo Chipatso, Reuben Mbwana, Tony Kansilanga, Mike Njaya, and Yacinta Stima.  
 403 We are thankful to Jimmy Ngwaya who carefully prepared the food models which formed the basis of  
 404 the data collection tools. A special thank you to Kathryn Dewey and Per Ashorn for their guidance and  
 405 leadership in developing the protocol for this study, and expert advice throughout the study  
 406 implementation and analysis. We are grateful for the vision, wisdom and professional guidance of the  
 407 whole iLiNS study Steering Committee ([http://ilins.org/about-ilins/who-we-are/ilins-steering-](http://ilins.org/about-ilins/who-we-are/ilins-steering-committee)  
 408 [committee](http://ilins.org/about-ilins/who-we-are/ilins-steering-committee)).

#### 409 **Author contributions**

410 J.H, C.K., K.M., U.A., M.A., & E.L.F designed the research and significantly contributed to the aim  
 411 and structure of manuscript; J.H. & C.K. conducted the research; A.M.R. & R.K. provided statistical  
 412 guidance and assistance with methods; J.H, R.K. & E.L.F analysed data and performed statistical  
 413 analyses; J.H drafted the paper with inputs from R.K. & E.L.F; J.H., R.K. & E.L.F had primary  
 414 responsibility for the final content. R.K. & E.L.F have equal contribution to senior authorship. All  
 415 authors have read and approved the final manuscript.

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**Table 1 Characteristics of participants at enrolment into the main study (at 6 months of age)**

	<b>Control</b>	<b>Intervention</b>	<b>p-value</b>
Participants (n)	26	106	
Female n (%)	14 (54)	49 (47)	0.50 <sup>a</sup>
<b>Socio-demographic Background Characteristics (n)</b>	<b>24</b>	<b>105</b>	
Maternal age; mean (SD) years	28.8 (7.3)	26.6 (5.9)	0.12 <sup>b</sup>
Maternal Education; mean (SD) years	3.9 (3.4)	4.4 (3.6)	0.52 <sup>b</sup>
Female-headed household n (%)	2 (8.3)	12 (11.9)	0.78 <sup>a</sup>
More than one child under 5 years old in household n (%)	11 (45.8)	44 (41.9)	0.06 <sup>a</sup>
<b>Maternal occupation n (%)</b>			0.64 <sup>a</sup>
Farming/Fishing	17 (77.3)	66 (66.0)	
House wife	3 (16.6)	27 (27.0)	
Indoor / office work	1 (4.6)	3 (3.0)	
Other	1 (4.6)	3 (3.0)	
Unknown	0 (0)	1 (1)	
<b>Information collected during time of visit (n)</b>	<b>26</b>	<b>106</b>	
Season (rainy: October - March) n (%)	12 (46.1)	56 (52.8)	0.80 <sup>a</sup>
Infant Breastfeeding n (%)	25 (100) <sup>c</sup>	104 (98.1)	0.49 <sup>a</sup>

a Chi-square

b Two-sample t-test

[c n=25 breastfed, n=1 missing value in this control group](#)

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**Table 2: Estimated intakes of energy and selected nutrients (Mean and 95 % Confidence Interval)<sup>a</sup> using the i-24-HR compared to WFR between the hours of 06:00 and 18:00 by intervention group and pooled group**

Nutrient	Control Group (n=26)				Intervention Group- LNS (n=106)					Pooled Group (n=132)			
	WFR	i-24-HR Recall	Abs. Diff <sup>b</sup>	p-value <sup>c</sup>	WFR	i-24-HR Recall	Abs Diff <sup>b</sup>	p-value <sup>c</sup>	p-value <sup>d</sup>	WFR	i-24-HR Recall	Abs Diff <sup>b</sup>	p-value <sup>c</sup>
Energy (kcal/d)	376 (317, 437)	293 (246, 345)	-88	0.010	388 (352, 424)	379 (346, 412)	-10	0.60	0.052	385 (355, 416)	361 (333, 390)	-25	0.09
Protein (g/d)	9.6 (7.7, 11.6)	7.1 (5.8, 8.4)	-2.9	0.009	9.4 (8.4, 10.5)	8.2 (7.3, 9.0)	-1.6	0.007	0.36	9.5 (8.5, 10.4)	8.0 (7.3, 8.6)	-1.8	<0.001
Fat (g/d)	7.3 (5.3, 9.8)	5.3 (4.0, 6.8)	-2.8	0.05	10.0 (8.7, 11.5)	10.4 (9.1, 11.7)	0.1	0.62	0.10	9.6 (8.3, 10.7)	9.2 (8.2, 10.4)	-0.4	0.65
Iron (mg/d)	2.6 (2.1, 3.2)	1.8 (1.4, 2.2)	-0.1	<0.001	3.7 (3.3, 4.2)	4.0 (3.4, 4.5)	0.3	0.25	0.020	3.5 (3.1, 3.9)	3.5 (3.0, 3.9)	0.03	0.68
Zinc (mg/d)	1.6 (1.2, 1.9)	1.1 (0.9, 1.4)	-0.5	<0.001	3.3 (2.8, 3.8)	3.8 (3.1, 4.4)	0.6	0.020	0.07	2.9 (2.5, 3.3)	3.1 (2.6, 3.7)	0.4	0.18
Calcium (mg/d)	38 (25, 54)	53 (33, 77)	21.6	0.20	94 (77, 113)	128 (107, 152)	38.3	<0.001	0.41	81 (68, 96)	111 (93, 130)	35.1	<0.001
Vitamin A (µg RAE/d)	39 (18, 67)	24 (9, 46)	-	0.19	143 (113, 176)	164 (130, 202)	24.1	0.10	0.23	117 (93, 144)	125 (99, 156)	15.9	0.37

<sup>a</sup> Data back-transformed from square root transformation for presentation

<sup>b</sup> Absolute mean difference - i-24HR Recall – WFR

<sup>c</sup> Matched pairs T-test

<sup>d</sup> Two-group t-test with equal variances between intervention and control group absolute differences

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i-24-HR: interactive 24-hour recall, LNS: Lipid-based nutrient supplement, RAE: retinol activity equivalents, WFR: weighed food record

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**Table 3: Percentage agreement for matching foods (items appearing both on the i-24-HR and the WFR) between intervention groups**

	Median (25 <sup>th</sup> , 75 <sup>th</sup> percentile)				p-value <sup>c</sup>
	Control Group (n=25)		Intervention Group (n=106)		
	n <sup>a,e</sup>	Percentage Agreement <sup>b</sup>	n	Percentage Agreement <sup>b</sup>	
Phala, all types (full volume)	25	100.0 (78.5, 122.4)	99	87.5 (68.1, 118.6)	0.457
Nsima, Rice (full volume)	25	78.4 (61.7, 100.0)	98	95.4 (59.5, 141.5)	0.248
Added Sugar	14	141.5 (103.7, 250.0)	69	167.7 (111.2, 295.0)	0.776
Sweetened Snacks	5	61.4 (50.7, 166.0)	45	112.7 (61.1, 195.0)	0.258
Savoury Snacks	8	105.9 (84.6, 137.5)	18	100.0 (56.7, 175.0)	0.683
Meat, Fish and Egg (solid)	7	82.7 (62.9, 294.9)	26	107.8 (62.7, 151.9)	0.735
Legumes, Nuts, Seeds	8	36.1 (26.4, 76.6)	26	76.2 (37.5, 105.3)	0.680
Fruit	4	160.0 (88.1, 231.7)	27	94.0 (66.2, 140.0)	--
Starchy Root and Vegetables	2	29.2 (22.1, 36.3)	20	80.8 (48.2, 145)	--
Milk and Yogurt	3	90.2 (90.0, 103.7)	8	111.0 (53.0, 228.6)	--
Non-dairy beverages	5	115.3 (85.6, 173.7)	15	100.0 (66.8, 142.2)	--
Soup/Broth from Relish	14	239.0 (195.3, 308.3)	54	134.0 (85.7, 240.0)	0.038
LNS	-		65	154.0 (98.8, 298.3) <sup>d</sup>	--

<sup>a</sup> Includes all portion sizes from items that match between the reported and reference values at the same time (i.e.: meal or snack time)

<sup>b</sup> Report percentage = (Reported amount / reference amount) x 100

Reference amount observed during the weighed food record; Reported amount taken from the 24-hour dietary recall.

<sup>c</sup> Mann-Whitney two-sample rank sum test by food group

<sup>d</sup> LNS only present in the diets of the intervention group, which is why there is no between-group comparison. This is descriptive only, looking at the percentage agreement of LNS in the intervention group.

<sup>e</sup> [One participant missing in the control group for these analyses](#)

i-24-HR: interactive 24-hour recall, LNS: Lipid-based nutrient supplement, WFR: weighed food record

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**Table 4: Number of eating episodes and percentages of matching food groups (items appearing both in the i-24-HR and the WFR), intrusions and omissions -by intervention groups**

	Control Group (n=25 <sup>d</sup> )			Intervention Group (n=106)		
	n (%)			n (%)		
	matching <sup>a</sup>	intrusion <sup>b</sup>	omission <sup>c</sup>	<u>matching</u> <sup>a</sup> matching	<u>intrusion</u> <sup>b</sup> intrusion	<u>omission</u> <sup>c</sup> omission
Phala, all types (full volume)	49 (92.5)	0 (0)	4 (7.6)	166 (94.3)	2 (1.1)	8 (4.6)
Nsima, Rice (full volume)	30 (88.2)	3 (8.8)	1 (2.9)	150 (89.8)	9 (5.4)	8 (4.8)
Added Sugar	22 (73.3)	5 (16.7)	3 (6.7)	105 (68.6)	26 (17.0)	22 (14.4)
Sweetened Snacks	6 (50.0)	2 (16.7)	4 (33.3)	59 (68.6)	15 (17.4)	12 (14.0)
Savoury Snacks	10 (76.9)	2 (15.6)	1 (7.7)	23 (69.7)	5 (15.2)	5 (15.2)
Meat, Fish and Egg (solid)	8 (53.3)	0 (0)	7 (46.7)	34 (56.7)	7 (11.7)	20 (32.8)
Legumes, Nuts, Seeds	13 (76.5)	1 (5.9)	3 (17.6)	39 (68.4)	4 (7.0)	14 (24.6)
Fruit	4 (66.7)	1 (16.7)	1 (16.7)	34 (70.8)	8 (16.7)	6 (12.5)
Starchy Root and Vegetables	2 (40.0)	0 (0)	3 (60.0)	22 (71.0)	4 (12.9)	5 (16.1)
Milk and Yogurt	3 (100)	0(0)	0 (0)	8 (47.1)	6 (35.3)	3 (17.6)
Non-dairy beverages	6 (75.0)	2 (25.0)	0 (0)	20 (62.5)	7 (21.9)	5 (15.6)
Soup/Broth from Relish	18 (62.1)	8 (27.6)	3 (10.3)	68 (64.7)	30 (28.6)	7 (6.7)
LNS	-	--		101 (89.4)	7 (6.2)	5 (4.4)

<sup>a</sup> The total of portions that were matched between the reference (WFR) and reported (i-24-HR), as a percentage of all items in the same group

<sup>b</sup> The total of portions that were reported (i-24-HR) but not observed in the reference data (WFR)

<sup>c</sup> The total of portions that were observed in the reference data (WFR), but not reported (i-24-HR)

<sup>d</sup> One participant missing for these analyses

i-24-HR: interactive 24-hour recall, LNS: Lipid-based nutrient supplement, WFR: weighed food record

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**Online supplement Table 1: Average reported (i-24-HR) and reference (WFR) portion sizes by food group**

Median (25th, 75th Percentiles)					
	n <sup>a</sup>	Reported amount (g) <sup>b</sup>	Reference Amount (g) <sup>c</sup>	Percentage agreement <sup>d</sup>	P-value <sup>e</sup>
Phala, all types (full volume)	125	78.9 (48.5, 112.0)	99.0 (64.7, 136.0)	86.4 (66.1, 114.1)	<0.001
Nsima, Rice (full volume)	124	52.5 (29.1, 80.0)	56.8 (33.5, 89.8)	89.1 (56.6, 135.0)	0.028
Added Sugar	94	5.1 (3.6, 7.9)	3.0 (1.9, 5.5)	143.3 (99.2, 238.9)	<0.001
Sweetened Snacks	64	7.9 (4.1, 15.8)	9.0 (4.0, 15.5)	91.7 (38.0, 158.0)	0.64
Savoury Snacks	34	7.7 (3.5, 11.0)	6.0 (3.0, 10.0)	86.1 (51.9, 157.1)	0.59
Meat, Fish and Egg (solid)	57	6.0 (0, 12.4)	9.2 (4.9, 18.2)	59.7 (0, 110.7)	0.015
Legumes, Nuts, Seeds	50	2.4 (0.4, 5.8)	7.8 (3.9, 16.0)	37.5 (2.4, 83.8)	<0.001
Fruit	38	22.5 (10.0, 35.0)	17.0 (6.0, 32.5)	94.0 (52.0, 136.4)	0.64
Starchy Root and Vegetables	30	18.0 (7.0, 24.0)	15.5 (6.0, 43.0)	50.0 (19.4, 120.0)	0.12
Milk and Yogurt	15	11.8 (5.2, 41.0)	8.0 (1.0, 29.0)	90.1 (36.8, 183.2)	0.82
Non-dairy beverages	33	47.3 (27.5, 76.1)	27.7 (9.0, 86.3)	98.1 (43.8, 123.5)	0.28
Soup/Broth from Relish	94	17.0 (11.7, 26.0)	7.4 (0, 16.9)	138.5 (80.0, 243.1)	<0.001
LNS	68	8.1 (4.5, 11.8)	4.5 (2.0, 9.0)	148.7 (95.0, 274.0)	<0.001

<sup>a</sup> Refers to the number of [participantsrespondents](#) where this food group was present on the WFR, i-24-HR, or both. This includes the average portion size estimation per food group per participant. In the case where one was an intrusion, this resulted in a reference value of zero, and in the case where there is an omission, this resulted in a reported amount of zero. This is the [participantrespondent](#) average per food group.

<sup>b</sup> median daily average per participant of reported amount derived from i-24-HR

<sup>c</sup> median daily average per participant of reference amount derived from WFR

<sup>d</sup> Percentage agreement: (Reported amount / reference amount) x 100

<sup>e</sup> p-value derived from Wilcoxon signed-rank test for matched pairs

i-24-HR: interactive 24-hour recall, LNS: Lipid-based nutrient supplement, WFR: weighed food record

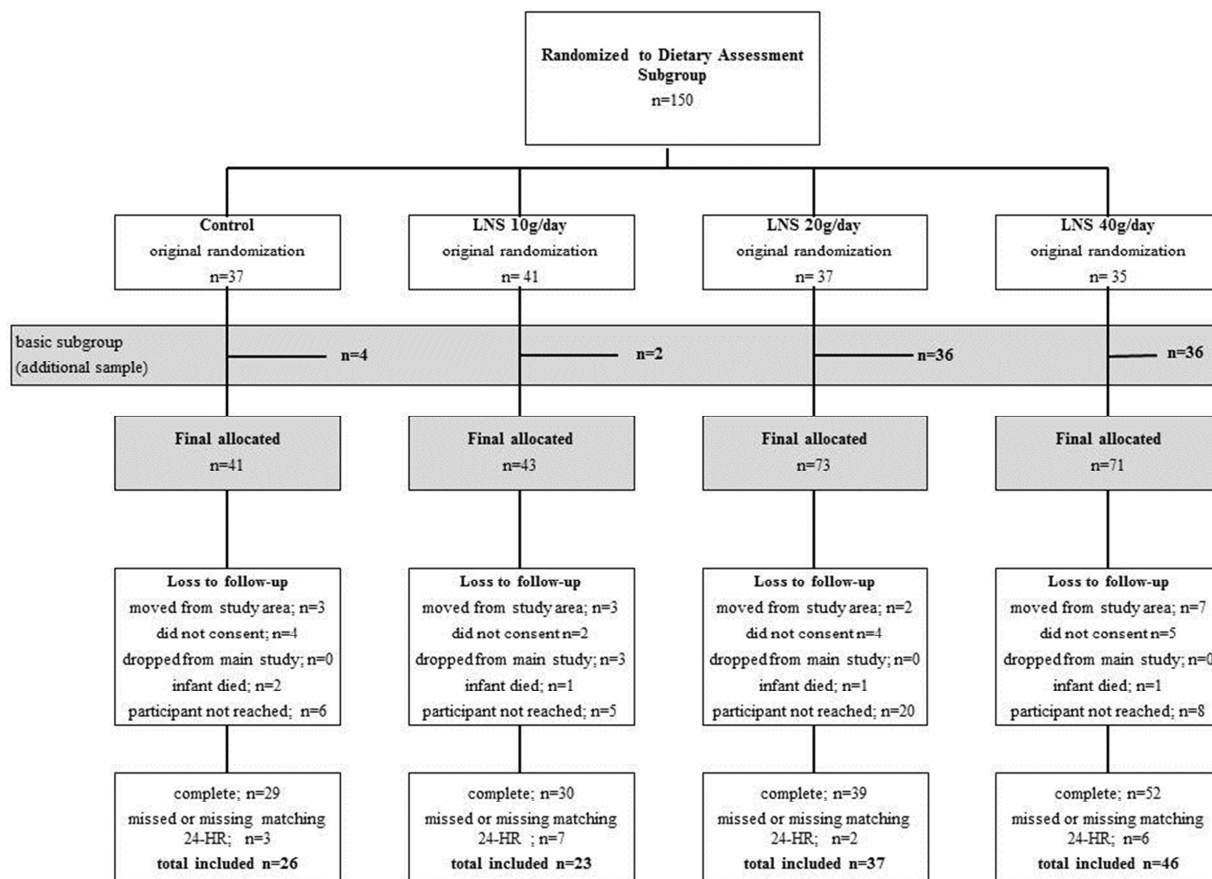
**Online supplement Table 2: Comparison of i-24-HRs that corresponded to and were independent of the WFR. An Assessment of bias in reporting related to the presence of the WFR: the “instrument effect”.**

Nutrient	N=71 Median Intake (25 <sup>th</sup> , 75 <sup>th</sup> percentile)			
	Independent 24-HR Recall	i24-HR WFR	Absolute Difference <sup>a</sup>	p-value <sup>b</sup>
Energy (kcal/d)	375 (273, 553)	327 (246, 463)	-34	0.10
Protein (g/d)	8.8 (5.8, 12.5)	7.6 (5.0, 10.3)	-0.78	0.06
Fat (g/d)	9.8 (5.0, 15.4)	8.1 (4.2, 11.8)	-1.9	0.06
Fe (mg/d)	3.2 (1.9, 5.8)	2.6 (1.7, 5.3)	-0.2	0.50
Zn (mg/d)	2.2 (1.2, 5.9)	2.0 (1.2, 6.1)	-0.1	0.97
Ca (mg/d)	115.9 (41.5, 204.3)	104.9 (34.7, 208.5)	-1.1	0.48
Vitamin A (µg RAE/d)	122.9 (30.3, 262.9)	107.9 (20.5, 292.9)	0	0.79

<sup>a</sup> i-24HR WFR – Independent 24-HR

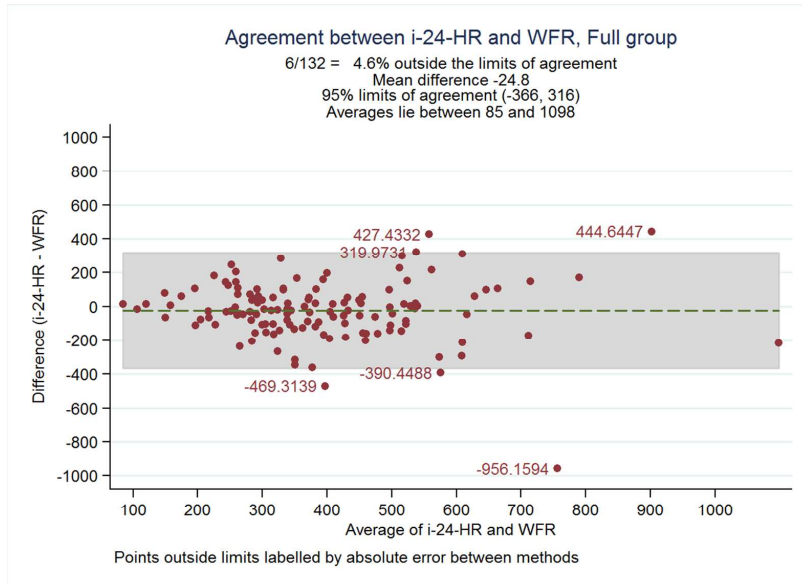
<sup>b</sup> Wilcoxon signed rank matched-pairs test

Figure 1: Consort Flow Diagram of Participant Enrolment and Inclusion in the Validation Sub-Study

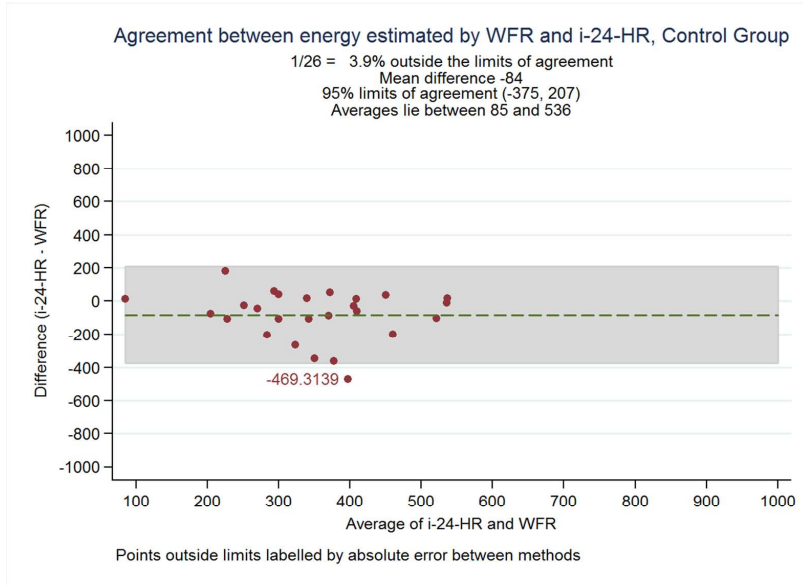




**Online supplement Figure 1: Bland Altman Plot Showing Relative Agreement in energy (kcal/day) estimation between WFR and i-24-HR: Pooled Group**



**Online Figure 2a: Bland Altman Plot Showing Relative Agreement in Energy (kcal) estimation between WFR and i-24-HR: Control Group**



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**Online supplement Figure 2b: Bland Altman Plot Showing Relative Agreement in Energy (kcal) estimation between WFR and i-24-HR: Intervention Group**

