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Stunting, wasting and breastfeeding as correlates of body composition in Cambodian children at 6 and 15 months of age

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Stunting, wasting and breastfeeding as correlates of body composition in Cambodian children at 6 and 15 months of age

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Footnotes to the title:

- i) The “Online Supporting Material” is available from the link in the online posting of the article and from the same link in the online table contents at <http://jn.nutrition.org>.
- ii) Abbreviations: CDHS, Cambodian Demographic Health Survey; FM, fat mass; FFM, fat-free mass; FMI, fat mass index; FFMI, fat-free mass index; FM%, percentage fat mass; IAEA, International Atomic Energy Agency; LAZ, length-for-age z-score; SAM, severe acute malnutrition; TBW, total body water; WLZ, weight-for-length z-score.

For Review Only

1 **Abstract**

2 The study aimed at assessing stunting and wasting as correlates of body composition in Cambodian
3 children. As part of a nutrition trial (ISRCTN19918531), fat (FM) and fat-free mass (FFM) were
4 measured using ^2H dilution at 6 and 15 months of age. Linear mixed-effects models were used to
5 assess associations of stunting, wasting, sex and breastfeeding with FM and FFM and height-adjusted
6 indexes FMI and FFMI. Of 419 infants enrolled, 98% were breastfed, 15% stunted and 4% wasted at
7 6 months. At 15 months, 78% were breastfed, 24% stunted and 11% wasted. Those not breastfed had
8 lower FMI at 6 months (-1.22; 95% CI -2.05;-0.39) but not at 15 months (-0.23, -0.51;0.051). Stunted
9 children had lower FM at 6 months and lower FFM at 6 and 15 months compared to children with
10 length-for-age ≥ 0 Z. Stunting was not associated with FMI or FFMI. Wasted children had lower FM,
11 FFM, FMI and FFMI at 6 and 15 months compared to children with weight-for-length Z (WLZ) ≥ 0 .
12 Generally, FFM and FFMI deficits increased with age, whereas FM and FMI deficits decreased,
13 reflecting interactions between age and WLZ. For example, the FFM deficits were -0.99 (-1.26;-0.72)
14 kg at 6 months, and -1.44 (-1.69; -1.19) kg at 15 months (interaction, $p < 0.05$), while the FMI deficits
15 were -2.12 (-2.53;-1.72) kg/m^2 at 6 months and -1.32 (-1.77;-0.87) kg/m^2 at 15 months (interaction,
16 $p < 0.05$). This indicates that undernourished children preserve body fat at the detriment of fat-free
17 tissue, which may have long-term consequences for health and working capacity.

18 **Keywords:** Body composition, fat-free mass, fat mass, infancy, childhood, breastfeeding, stunting,
19 wasting

20 **Introduction**

21 The period from conception to 24 months of age (first 1000 days) represents a window of opportunity
22 for nutritional interventions promoting healthy growth ^(1,2). In order to design interventions that
23 prevent growth faltering and support health in low- and middle-income settings, it is important to
24 understand the associations between nutritional status, mode of feeding and body composition in early
25 life.

26 While anthropometric markers of growth (weight, height, mid-upper arm circumference (MUAC))
27 are widely used, few studies are able to differentiate the accretion of fat mass (FM) versus fat-free
28 mass (FFM). FFM accretion indexes development of organ and muscle tissue, and infancy represents
29 a critical period in this context, as the structure and function of organs and tissues is strongly
30 contingent on the magnitude of hyperplastic growth. A study from Ethiopia, using air-displacement
31 plethysmography to measure body composition from birth to 6 months, found low birth weight to be
32 associated with low FFM at birth. A large variation in fat accretion was observed during the first 6
33 months ^(3,4). Follow-up of the same cohort showed positive correlations between FFM at birth and
34 height and child development at 2 years of age ^(5,6). In general, greater weight gain in infancy is
35 associated with greater adult height and FFM, whereas from early childhood, greater weight gain is
36 primarily associated with greater adult FM ^(7,8). Muscle mass may potentially contribute both to
37 immediate survival ⁽⁹⁾, as well as to long-term cardio-metabolic health ⁽¹⁰⁾.

38 The accretion of fat may also be important for short-term survival of children. A cross-sectional study
39 reported that 3-18 months old Gambian infants overall had less FFM and FM than UK infants as
40 measured by the ²H dilution technique. However, whereas the reduction in FFM of Gambian vs UK
41 children increased with age, the FM difference decreased with age, suggesting that growth faltering
42 affects FFM more than adiposity ⁽¹¹⁾. In line with this, a study from Uganda found low levels of leptin,
43 indicative of low FM, to be a predictor of mortality in children hospitalized with severe acute
44 malnutrition (SAM) ⁽¹²⁾.

45 The influence of breastfeeding on body composition has mainly been studied in high-income
46 countries. A meta-analysis showed that breastfed infants accumulated more fat than formula-fed
47 infants did during the first 8-9 months. However, at 12 months, formula-fed infants had a higher FM
48 than breastfed infants ⁽¹³⁾. There is also an increasing number of studies showing that the composition
49 of breastmilk has an effect on body composition in the infant, and thereby suggesting mechanisms

50 for an effect on growth ⁽¹⁴⁾. Differences in dietary protein content and quality are also suggested to
51 play a role in the effects on early growth via stimulation of IGF-1 and insulin ⁽¹⁵⁾.

52 In Cambodia, the prevalence of undernutrition among pre-school children has remained largely
53 unchanged for the past 10 years. The 2014 Cambodian Demographic and Health Survey (CDHS)
54 concluded that 32% of children under 5 years of age are stunted; 24% are underweight and 10% are
55 wasted ⁽¹⁶⁾. Thus, Cambodia still has an urgent need to prevent undernutrition in pre-school children.
56 The main objective of this paper was to assess the role of stunting, wasting and breastfeeding as
57 correlates of FFM and FM in Cambodian children at the ages of 6 and 15 months. The hypothesis
58 was that associations of stunting, wasting and breastfeeding with body composition changes with age.

For Review Only

59 **Methods**

60 *Study design and ethics.* This longitudinal study was nested in a randomized trial investigating the
61 effect of four nutrition interventions on prevention of malnutrition (the WinFood study,
62 ISRCTN19918531 <http://www.isrctn.com/ISRCTN19918531>)⁽¹⁷⁾. Children received the
63 interventions from 6 to 15 months of age. The aim of the current study was to assess stunting, wasting
64 and breastfeeding as correlates of body composition, and to test if such associations change with age.
65 The four intervention groups were merged for this study and statistical analyses were adjusted for the
66 interventions, as described below. The study was conducted according to the guidelines laid down in
67 the Declaration of Helsinki and all procedures involving human subjects were approved by the
68 National Ethics Committee for Health Research, Ministry of Health, the Royal Government of
69 Cambodia (151 NEHR) and a consultative approval was obtained from the National Committee of
70 Health Research Ethics in Denmark. Written informed consent was obtained from all caregivers of
71 participating infants and the caregivers were informed that their child could leave the study whenever
72 they wanted to.

73 *Study site.* The study was conducted from March 2011 to March 2012 in seven rural municipalities
74 located in two Operational Districts (PeaReang and Sithor Kandal) in the Prey Veng province, south-
75 east of Phnom Penh. Prey Veng is agricultural low-land, bordering the Mekong River and recognized
76 to be vulnerable to food insecurity. Prevalence of stunting and wasting was similar to the national
77 level.

78 *Study subjects.* All parents of single-born infants were given an invitation for their child to participate
79 in the WinFood study if their child was born between 15 August and 15 December 2010. Children
80 were recruited when they had reached the age of approximately 5.5 months. On the day of
81 recruitment, infants were seen by a paediatrician and screened for severe wasting (weight-for-length
82 z-score (WLZ) <-3), pitting oedema, clinical signs of vitamin A deficiency or anaemia (Hb < 80 g/L).
83 If any of these signs were found, the infant was excluded and referred for treatment. Infants with a
84 history of acute or persistent diarrhoea at recruitment were also given a treatment referral and invited
85 for a new screening 2 to 4 weeks later. All infants were assessed on the recruitment day (a “6-month
86 visit”) and 9 months later at a “15-month visit”.

87 *Body composition.* Body composition was assessed using the ²H dilution technique to measure total
88 body water (TBW), and hence FFM and FM, following the protocol developed by the International

89 Atomic Energy Agency (IAEA) ⁽¹⁸⁾. Each infant was given an accurately weighed oral dose of 7 g ²H
90 oxide (99.8% ²H₂O) (Cambridge Isotope Laboratories Inc., USA), which was kept at 4°C until use.
91 The ²H kit consisted of a pre-weighed ²H₂O dose in a 5 ml tube, a 10 ml syringe and a needle to draw
92 the ²H₂O from the tube to the syringe. The weight of the ²H kit prior to dosing was recorded to the
93 nearest 0.01 g. Furthermore, two pre-weighed paper towels were used to absorb any spilled ²H₂O.
94 With the 10 ml syringe, the ²H₂O dose was administered by one of the authors (JKHS) to the child
95 while it was sitting on the lap of the mother. The ²H kit and the paper towels, if used, were weighed
96 immediately after administration of the dose and the weight was recorded to determine the dose
97 consumed, subtracting the weight of any dose spilled on the tissues. Saliva samples were collected
98 by two nurses. A cotton ball was put in the mouth of the child for 3 to 5 minutes. A sewing thread
99 was tightened around the cotton ball and hung out of the mouth of the child to prevent the child from
100 swallowing the cotton ball. The wet cotton ball was removed from the child's mouth and put into a
101 syringe barrel, and the saliva was pushed into a 1.5 ml cryotube. Saliva samples were stored at -20°C
102 until they were analysed for ²H₂O enrichment at St. Johns Research Institute, Bangalore, India. A pre-
103 dose saliva sample was collected before giving the ²H₂O dose and a post-dose was collected 3 hours
104 after the ²H₂O dose was given. ²H enrichment was measured by Fourier transformed infrared
105 spectrometer (FTIR) (Shimadzu Corporation, Kyoto, Japan) and analysed by the software developed
106 at MRC Dunn Nutrition Unit, Cambridge, UK. The instrument was calibrated with ²H₂O standards,
107 prepared in tap water, ranging from 100 – 2000 ppm. Saliva samples were centrifuged before analysis
108 and the clear sample loaded on a calcium fluoride cell (path length of 100 µm) without air bubbles.
109 Enrichment of the pre-dose sample from the child was used for background correction of post-dose
110 samples. The samples were measured in duplicate and the coefficient of variation (CV) was <1%.
111 Inter-assay CV (of 1000 ppm standard) for the method was also <1%. The calculations of FFM and
112 FM based on ²H₂O enrichment results were undertaken by calculating the TBW in kg ⁽¹⁸⁾:

113
$$\text{TBW (kg)} = \text{dilution space}/1.041;$$

114
$$\text{Dilution space} = \text{{}^2\text{H dose given to child (mg) / enrichment in saliva (mg/kg)}$$

115 The constant 1.041 was used to correct the isotope dilution space for non-aqueous proton exchange,
116 when calculating total body water ⁽¹⁸⁾. To calculate the FFM and FM:

117
$$\text{FFM} = \text{TBW}/\text{hydration factor}; \quad \text{FM} = \text{weight of child (kg)} - \text{FFM (kg)}$$

118 The FFM and FM were calculated individually and adjusted with specific hydration factors for sex
119 and age of the child ⁽¹⁹⁾. All children with percentage fat mass (FM%) <5%, were further reviewed
120 and checked with field notes regarding any problems during the act of administering the ²H₂O to the
121 child. In case of any uncertainty regarding the amount of ²H₂O dose consumed by the child, the child's
122 results were excluded from the analyses.

123 The FFM index (FFMI) and the FM index (FMI) were calculated by FFM/length² (kg/m²) and
124 FM/length² (kg/m²), respectively. These indices express FFM and FM normalized for length and are
125 expressed in the same unit as BMI. Both FFM and FM and the corresponding indices FFMI and FMI
126 were reported as body composition outcomes. It was anticipated that stunting, which only affects
127 length, and wasting affecting weight-for-length could show differential correlations to body
128 composition indicators with and without normalization for length.

129 *Correlates.* All anthropometric measures were recorded by the same four field assistants who had
130 been trained according to the WHO child growth guidelines ⁽²⁰⁾. All measurements were performed
131 once by two different assistants and the mean measurement was used in the analysis. Weight was
132 measured by using an electronic scale (SECA scale, Hamburg, Germany), and recorded to the nearest
133 100 g. Length was measured to the nearest millimetre on locally produced length boards. Both SECA
134 scales and length boards were borrowed from World Food Programme, Cambodia. Anthropometric
135 z-scores, length-for-age z-score (LAZ) and WLZ were calculated based on WHO's 2006 Child
136 Growth Standards ⁽²¹⁾, using WHO Anthro v.3.1 ⁽²²⁾. Additionally, triceps and subscapular skinfolds
137 were measured with a Harpenden skinfold caliper (Baty International, UK) to the nearest millimetre.
138 If MUAC or skinfold measurements differed by more than 5 mm and 2 mm respectively, both
139 assistants had to repeat the measurement.

140 Breastfeeding status was determined both at the 6- and 15-month visit. To estimate if the child was
141 still breastfed, the question: "*Since this time yesterday, has the child been breastfed?*" was asked.
142 Additionally, a few socio-demographic variables were obtained at the 6- month visit.

143 *Statistical analysis.* Data were double-entered in Epidata v.3.1 (The EpiData Association, Odense,
144 Denmark) and analysed using STATA 12 for Windows (StataCorp, Texas, USA) and R (R Core
145 Team, 2017) with the extension packages lme4, multcomp and turkeytrend. Comparison of data for
146 boys and girls at the 6- and 15-month visits was carried out using chi-square tests and two-sample t-
147 tests for categorical and continuous variables, respectively. Changes in FM, FFM, FMI and FFMI
148 from 6 to 15 months were analysed by paired t-tests.

149 Separate linear mixed-effects models were fitted to FFM and weight. Age, sex, intervention groups
150 of the original trial design, and the interaction between visit (6 or 15 months) and either sex,
151 breastfeeding, length-for-age or weight-for-length z score categories, were included as fixed effects
152 and children and municipality were included as random (intercept) effects. Specifically, differences
153 between categories at 6 months and 15 months and changes in differences (between categories)
154 from 6 to 15 months were estimated; the latter corresponded to test for interaction. The
155 corresponding estimates for FM were derived from the estimates for FFM and weight using a
156 marginal models approach ⁽²³⁾. Similarly, models were fitted to BMI and FFMI and estimates were
157 derived for FMI. For triceps and subscapular skin folds, similar linear mixed-effects models were
158 fitted using the same fixed and random effects as for weight and FFM. For all analyses, model
159 assumptions were checked using residual and normal probability plots, respectively. A significance
160 level of 5% was used. No adjustment for multiple comparisons was applied.

161 Results

162 Of 514 infants screened, 419 (82%) were recruited for the nutrition intervention trial (**Figure 1**). The
163 mean age was 5.9 months [range: 5.0-7.9], and 53% were boys (**Table 1**). At the 6-month visit, 98%
164 were breastfed, 15% were stunted (LAZ<-2) and 4% were wasted (WLZ <-2). At 15 months, these
165 figures had changed to 78% breastfed, 24% stunted and 11% wasted children, with no differences
166 between boys and girls. Data on FFM and FM were available on 389 (93%) infants at the 6-month
167 visit and on 293 (82%) at the 15-month visit (Figure 1). In total, 413 children had FFM and FM data
168 from at least one visit. At the 6 month visit, children without body composition data had slightly
169 lower WLZ (0.4 vs. 0.7 z-scores, $p < 0.05$), but not LAZ. There were no differences in WLZ or LAZ
170 at the 15 months visit between children with vs. without body composition data. Boys had higher
171 weight, length, MUAC, BMI, FM, FFM and FFMI, but not FMI than girls at the 6-month visit. At
172 15 months, the sex differences remained except that boys did not have a higher FM than girls (**Table**
173 **2**). FM and FMI decreased between 6 and 15 months ($p < 0.001$), whereas FFM ($p < 0.001$) but not
174 FFMI ($p = 0.09$) increased. In adjusted analyses, higher weight in boys compared to girls both at the
175 6- and 15-month visits was mainly due to higher FFM (**Table 3a**); similarly higher BMI in boys was
176 mainly due to higher FFMI (**Table 3b**). At the 6-month visit, the 0.59 (95% CI 0.35; 0.83) kg/m^2
177 higher BMI in boys was due to 0.49 (95% CI 0.29; 0.68) kg/m^2 higher FFMI, and at the 15-month
178 visit, the 0.56 (95% CI 0.31; 0.81) kg/m^2 higher BMI in boys was due to 0.45 (95% CI 0.23; 0.66)
179 kg/m^2 higher FFMI (Table 3b).

180 Breastfed and non-breastfed children did not differ in weight or BMI at the 6- or the 15-month visits
181 (Table 3a and 3b). However, FMI was reduced by 1.22 (0.39;2.05) kg/m^2 at the 6 month visit in non-
182 breastfed infants ($n = 6$, 1.4%). At 15 months, non-breastfed infants ($n = 79$, 22%) no longer had lower
183 FMI compared to breastfed infants (-0.23, -0.51;0.051 kg/m^2). FFMI tended to be higher in non-
184 breastfed infants at 6 months (0.74, -0.002;1.48 kg/m^2) and was higher in non-breastfed compared to
185 breastfed infants at 15 months (0.28, 0.03;0.53 kg/m^2).

186 Deficits in weight/FM/FFM (Table 3a) and BMI/FMI/FFMI (Table 3b) increased as LAZ and WLZ
187 categories declined, i.e. higher deficits were observed for LAZ and WLZ <-2 than LAZ and WLZ
188 between -1 and -2 or -1 and 0. Stunting (LAZ <-2) was associated with reduced FM at 6 months and
189 lower FFM at both 6 and 15 months compared to children with LAZ ≥ 0 (Table 3a). Stunting was not
190 associated with FFMI or FMI at 6 or 15 months (Table 3b).

191 The BMI deficits associated with wasting at both 6- and 15-month visits were explained by lower
192 FFMI as well as FMI. FMI deficits improved between 6 and 15 months for all z-score categories
193 (interaction, $p < 0.05$, table 3b and **Online supporting material**) whereas FFMI deficit point estimates
194 worsened for all WLZ-score categories. However, worsening of FFMI deficit was only significant
195 for WLZ between -1 and 0 (-0.4 kg/m^2 [-0.8; -0.03], $p < 0.05$) (Table 3b and online supporting
196 material). By comparison, the FFM deficits worsened in all z-score categories ($p \leq 0.05$) and FM
197 deficits improved for WLZ between -2 and 0 ($p < 0.05$) from 6 to 15 months (Table 3a and online
198 supporting material).

199 Compared to children with $\text{LAZ} \geq 0$, stunted children had thinner triceps and subscapular skinfolds at
200 6 months but not at 15 months. Wasted children had thinner triceps and subscapular skinfolds both at
201 the 6 and 15 months visits (Online supporting material).

202

203 **Discussion**

204 This study showed that stunting, wasting, sex and breastfeeding status were associated with body
205 composition in early life. Stunting was associated with a lower FMI at 6 months, but at 15 months
206 there were no difference in FMI and FFMI compared to children with LAZ >0. For wasted children
207 at 6 and 15 months, both FFMI and FMI were lower than in children with WLZ >0. The non-breastfed
208 infants had lower FMI at 15 months compared to infants being breastfed.

209 The study population consisted of infants from rural Cambodia who, despite a very high prevalence
210 of breastfeeding, had stunting and wasting rates of 14% and 4% respectively at 6 months of age. At
211 15 months, these were further deteriorated to 24% and 11% of children being stunted and wasted,
212 although they all had received one of the four types of nutritional supplementation between the 6 and
213 15 month visits, and the majority continued breastfeeding until 15 months. This pattern of worsening
214 stunting during the complementary feeding period has been seen in many low income countries ⁽²⁴⁾.
215 It was apparently difficult to stop the decrease in LAZ and WLZ during this critical period. One
216 possible reason could be a high prevalence of environmental enteric dysfunction ⁽²⁵⁾.

217 The decrease in fat mass and especially in fat mass index seen from 6 to 15 months in this study is
218 expected and similar to the decline observed in infants from two reference groups from the USA
219 ^(19,26). This decrease is also reflected in the decrease in skinfolds and BMI during this age period seen
220 in the WHO growth Standards ⁽²¹⁾.

221 Boys had, as expected, a higher weight and BMI at both 6 and 15 months, as is also seen in the WHO
222 growth standards ⁽²¹⁾. The higher weight and BMI were explained mainly by a higher FFM and FFMI
223 which has also been shown in many other studies showing higher lean mass in boys ^(27,28).

224 **Stunting/wasting**

225 Both stunting and wasting were associated with absolute deficits in FFM and FM, with the deficits
226 increasing with the magnitude of malnutrition. Furthermore, the deficits in FFM increased with age,
227 whereas those for FM decreased with age in children with WLZ <0. This indicates that malnutrition
228 disproportionately affects FFM, with this impact increasing with age.

229 However, after adjusting for length, i.e. when using FMI and FFMI, the impact of stunting on fat-free
230 tissue decreased and lost significance. The fat-free tissue deficit associated with stunting is thus fully

231 explained by the reduced length, indicating that stunted children are gaining fat-free tissue in the same
232 proportion to their length as non-stunted children.

233 Regarding wasting, in contrast, deficits in fat-free and fat tissue remained apparent after adjusting for
234 length. There was a subtle worsening of the fat-free tissue deficit with age, but a notable decrease in
235 the magnitude of fat deficit. Again, in association with age, wasted children increasingly seem to
236 preserve fat at the expense of FFM.

237 Preservation of fat in infants and undernourished children was also reported in other studies. A small,
238 cross-sectional study from the Gambia assessed FFM and FM in 3-18 months old Gambian infants
239 against a reference group of healthy British infants ⁽¹¹⁾. Data indicated that FFMI deficit in the
240 Gambian infants initially increased, whereas the FMI deficit initially decreased with age. A study
241 followed-up a cohort of 320 Malawian children (median age 9.3 years, inter-quartile range 8.1-10.3)
242 that had been treated for SAM at a younger age. Compared to their nearest sibling and age and sex-
243 matched community controls, the previous SAM cases had lower FFM (estimated by bio-electrical
244 impedance analysis), lower anthropometric measurements, weaker hand grip and shorter endurance
245 in an exercise test ⁽²⁹⁾. Another study in children with SAM in the Democratic Republic of Congo
246 analysed FM and FFM in a subgroup of children with an average age of 3 years ⁽³⁰⁾. After recovery
247 from SAM, these children had a lower FFM than non-wasted children from the same community.
248 However, FFMI was not different between the two groups ⁽³⁰⁾.

249 Mechanistically, the preferential accumulation of fat in undernourished children may be due to the
250 lack of specific type II nutrients, such as zinc, which are required for synthesis of FFM ⁽³¹⁾. However
251 this scenario may also be viewed as an evolved survival strategy, stimulated by malnutrition during
252 infancy ^(11,32). Developmental trade-offs between FM and FFM accretion are predicted by
253 evolutionary life history theory in association with various ecological factors, and future nutritional
254 interventions might benefit from considering this theoretical issue ⁽³³⁾. The involvement of fat reserves
255 in acute malnutrition was discussed by Bartz et al ⁽¹²⁾. They conducted a comprehensive metabolomic
256 analysis of children admitted with SAM and found that low levels of leptin, a marker of adipose tissue
257 reserve, was the major biochemical factor predicting mortality. The authors further suggested that
258 fatty acid metabolism has an important role in adaptation to acute malnutrition.

259 To reduce the risk of lower muscle mass and strength in wasted children, the development of new
260 nutritional interventions should be aimed at increasing accretion of FFM. In a multi-factorial study in

261 1,609 malnourished children aged 6-23 months, twelve different nutritional supplements were given
262 over a period of 12 weeks. FFM constituted 93.5% of the weight increase and lipid-based nutrition
263 supplements resulted in a 0.083 kg/m² higher FFMI than corn-soy-blends ⁽³⁴⁾. This indicates that
264 energy-dense lipid-based nutrition supplements may reduce a detrimental decline in FFM in
265 malnourished children.

266 In stunted children, preservation of FM over FFM has shown conflicting long-term results. Two
267 studies from Brazil found an association between stunting and risk of obesity in children ^(35,36). A
268 subsequent study at one of the sites found lower fat oxidation, a risk marker of obesity, in stunted
269 compared to non-stunted children ⁽³⁷⁾. However, a birth cohort study in 2000 Brazilian boys found
270 low height-for-age z-score at ages 2 and 4 years to be associated with low FMI but not FFMI at 18
271 years of age, and low weight-for-height z-score at 2 and 4 years to be strongly associated with both
272 low FMI and FFMI ⁽³⁸⁾ at 18 years. Thus, the birth cohort study indicated that stunting and wasting
273 in childhood was not associated with overweight in late adolescence. In sub-Saharan Africa a large
274 birth cohort study found no association between stunting and later obesity ^(39,40) and children who had
275 been stunted at 2 years of age had lower FFM at 22 years than non-stunted children ⁽⁴¹⁾. However,
276 few studies have had access to accurate body composition methodologies, and most published studies
277 have addressed older age groups, when linear growth is relatively canalized.

278 **Breastfeeding**

279 In the present study, we found that non-breastfed compared to breastfed children did not differ in
280 BMI at the 6- or the 15-month visit. At the 6-month visit, a lower FMI was observed in non-
281 breastfed infants, which reduced in magnitude and only remained as a trend at 15 months. FFMI
282 was higher in non-breastfed infants at both 6 and 15 months. At the 6 month visit only six infants
283 were non-breastfed, and these results therefore have to be interpreted with caution. A meta-analysis
284 found that formula-fed infants had a lower FM compared to breastfed infants at the age of 6 months,
285 but from 6-12 months the formula-fed infants were gaining more FM ⁽¹³⁾. Formula-fed infants had
286 higher FFM compared to breastfed infants throughout infancy. The non-breastfed infants of the
287 current study seem to have similar changes in FM and FFM as formula-fed compared to breastfed
288 infants in the meta-analysis.

289 A trial in Iceland, where infants were randomized to be exclusively breastfed until 6 months of age
290 or to be introduced to complementary food at 4 months of age, showed that the latter had a 0.33 kg

291 (95% CI: -0.77; 0.11) lower FM at 6 months of age⁽⁴²⁾. At the 15-month visit, we found that the non-
292 breastfed children had a lower FMI compared to the children who were still being breastfed. These
293 findings may suggest that breast milk supports the accretion of FM, which is likely to be an important
294 energy buffer to young children at risk of undernutrition during this period of rapid growth. In the
295 Lancet Breastfeeding series, it was concluded that there is suggestive evidence for breastfeeding
296 protecting against later obesity⁽⁴³⁾. Interestingly, the protective effect was of the same magnitude in
297 low- and middle income countries as in high income countries⁽⁴⁴⁾. Studies on how composition of
298 breastmilk is related to later body composition of the infant is also giving new insight into the
299 mechanisms by which early diet influence body composition. One study showed that human milk
300 leptin content at 1 months was inversely related to total body fat, percent fat mass and trunk fat at the
301 age of 6 months⁽⁴⁵⁾ and in another study several aspects of human milk oligosaccharides pattern at 1
302 months was associated with body composition at 6 months⁽⁴⁶⁾. Furthermore, a study found that human
303 milk adiponectin was positively associated with body weight and sum of skinfolds up to 2 years⁽⁴⁷⁾.

304 **Strengths and limitations**

305 Measurement of body composition in relation to stunting and wasting in infancy and early
306 childhood is a major strength of this paper. The ²H dilution technique is considered a valuable
307 method to quantify whole body FM and FFM in research⁽⁴⁸⁾. Most earlier studies have used
308 anthropometric measures to examine body composition, but this was questioned in several studies
309⁽⁴⁹⁻⁵⁰⁾. The ²H dilution technique can be applied in field studies; however, in addition to being
310 expensive, it is a technically challenging method. If the practical work of administering ²H to
311 children and/or collecting the saliva from children is not performed properly, it can introduce under-
312 or overestimation of FM. Meticulous administration of ²H and recording of spillage by one person
313 resulting in more accurate measurements of FM and FFM is therefore a strength of this study. It is a
314 limitation that data on breastfeeding only included information about “any breastfeeding the
315 previous 24 hours”. The duration of exclusive breastfeeding and frequency of breastfeeding after
316 exclusive breastfeeding stopped may have influenced growth and body composition at 15 months
317 and especially at 6 months of age. The children in the study received nutritional intervention. This
318 may have implications on the generalizability of the results to similar children in the community
319 who will not receive nutritional supplementation.

320 **Conclusion**

321 The study has shed some light on how the body deals with malnutrition in infants and young
322 children in a food insecure setting with a high prevalence of breastfeeding. Boys had an expected
323 higher FFMI than girls at both the 6- and 15-month visits. Breastfeeding seemed to support
324 accretion of FM as indicated by a lower FMI in children who stopped breastfeeding before 15
325 months. Stunting at 6 months was associated with lower FMI, but not FFMI, whereas wasting at
326 both 6 and 15 months was associated with both lower FFMI and FMI. The FFMI deficit increased
327 slightly with age, but the FMI deficit decreased. Malnourished children seem to preserve body fat
328 for immediate survival at the expense of FFM accretion. This may have long-term consequences
329 with reduced functional outcomes and higher risk of non-communicable diseases. There is a need
330 for further studies from middle- and low-income countries on how nutrition intake influences body
331 composition, and how changes in body composition in early life influence growth, development and
332 long term health.

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338 **Conflict of interest**

339 None

340 **Authorship**

341 J.K.H.S., T.B., C.C., C.H., S.F., F.T.W., M.A.D., J.B., H.F., K.F.M. and N.R. designed the research
342 project. J.K.H.S., T.B., C.C., and C.M. conducted the research. J.K.H.S., B.G., J.C.W., K.F.M., N.R.
343 C.R. and H.F. analysed the data. J.K.H.S., B.G., K.F.M., J.C.W and H.F. wrote the first draft of the
344 manuscript. All authors have read and approved the final manuscript.

345

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Table 1: Baseline characteristics of 419 Cambodian children

Data are presented as n (%) or mean and SD

Child characteristics	n	%	Mean	SD
Boys	220	53		
Age at recruitment, months			5.9	0.6
Household size				
Number of people in the household			5.3	1.7
Number of children below 5 years			1.3	0.6
Water and sanitation				
Source of drinking water				
Unprotected well	232	55		
Protected well ^a	186	44		
Toilet facility				
Flush or pour flush toilet	321	77		
Pit latrine	88	21		
Socio-economic status				
Education				
Caregiver education, years			5.3	2.5
Household head education, years			6.7	2.8
Primary income				
Farming	237	57		
Employment/salary/daily labour	110	26		
Other	72	17		
Households owning land	390	93		

^aProtected well is defined as a well with a lid

Table 2. Breastfeeding status, anthropometry, fat mass and fat free mass in Cambodian boys and girls at 6 and 15 months of age.

	6 months							15 months						
	N	Boys	SD	N	Girls	SD	p	N	Boys	SD	N	Girls	SD	p
Breastfeeding, %														
Not breastfed	3	1.4		4	2.0		0.89	33	17.3		46	27.7		0.025
Breastfed	216	98.6		194	98.0			158	82.7		120	72.3		
Age at introduction to complementary food, months	220	5.6	0.8	199	5.6	0.7	0.14							
Weight, kg	220	7.1	0.9	199	6.5	0.8	<0.001	192	8.9	0.9	166	8.2	0.8	<0.001
Length, cm	220	65.2	2.3	199	63.5	2.3	<0.001	192	75.7	2.4	166	74.1	2.7	<0.001
MUAC, cm	219	14.1	1.0	199	13.7	1.0	<0.001	192	13.9	0.9	166	13.4	0.8	<0.001
LAZ, %							0.47							0.27
<-2	36	16.4		26	13.1		0.42	47	24.6		38	23.0		0.82
-2≤ and <-1	74	33.6		59	29.6		0.44	75	39.3		52	31.5		0.16
-1≤ and <0	78	35.4		84	42.2		0.19	58	30.3		60	36.4		0.28
≥0	32	14.5		30	15.1		0.99	11	5.8		15	9.1		0.32
WLZ, %							0.31							0.31
<-2	12	5.5		6	3.0		0.32	22	11.5		17	10.2		0.84
-2≤ and <-1	39	17.7		47	23.6		0.17	80	41.7		61	36.8		0.40
-1≤ and <0	99	45.0		82	41.2		0.49	70	36.5		76	45.8		0.09
≥0	70	31.8		64	32.2		>0.99	20	10.4		12	7.2		0.39
BMI, kg/m ²	220	16.7	1.4	199	16.1	1.4	<0.001	192	15.5	1.0	166	15.0	1.0	<0.001
FM, kg	205	1.6	0.5	184	1.4	0.5	0.013	161	1.3	0.5	132	1.2	0.5	0.24
FFM, kg	205	5.6	0.6	184	5.1	0.6	<0.001	161	7.6	0.8	132	7.0	0.8	<0.001
FMI, kg/m ²	205	3.6	1.1	184	3.6	1.1	0.46	161	2.3	0.9	132	2.2	0.8	0.52
FFMI, kg/m ²	205	13.1	0.9	184	12.6	1.0	<0.001	161	13.2	0.9	132	12.7	1.0	<0.001

Results are shown as mean, SD, and N (number of children in each analysis). MUAC, Mid-upper arm circumference; LAZ, Length-for-age z-score; WLZ, Weight-for-length z-score; BMI, Body mass index; FM, Fat mass; FFM, Fat-free mass; FMI, Fat mass index; FFMI, Fat-free mass index.

Table 3a. Estimated mean differences in weight, fat-free mass, and fat mass within sex, breastfeeding, length-for-age and weight-for-length z score categories among Cambodian children at 6 and 15 months of age (n=413).

	6 months						15 months					
	Weight, kg		FFM, kg		FM, kg		Weight, kg		FFM, kg		FM, kg	
	Diff.	95% CI	Diff.	95% CI	Diff.	95% CI	Diff.	95% CI	Diff.	95% CI	Diff.	95% CI
Sex^a												
Boy	0.63	0.47;0.78*	0.49	0.36; 0.61*	0.14	0.05;0.23*	0.66	0.50; 0.83*	0.57	0.42;0.71*	0.10	-0.05; 0.25
Girl	-	-	-	-	-	-	-	-	-	-	-	-
Breastfeeding^b (n=412)												
Not breastfed	-0.30	-0.68;0.09	0.22	-0.24;0.69	-0.52	-0.87;-0.17*	-0.05	-0.17;0.07	0.15	0.002;0.30*	-0.20	-0.34;-0.06*
Breastfed	-	-	-	-	-	-	-	-	-	-	-	-
Length-for-age Z^c												
<-2	-1.40	-1.58;-1.23**	-1.13	-1.31;-0.96**	-0.27	-0.43;-0.12*	-1.74	-1.95;-1.53*	-1.57	-1.80;-1.35*	-0.17	-0.40; 0.07
-2≤ and <-1	-0.90	-1.04;-0.75*	-0.78	-0.93;-0.63*	-0.12	-0.25;0.02	-1.08	-1.28;-0.88*	-1.00	-1.22;-0.79*	-0.08	-0.30; 0.15
-1≤ and <0	-0.45	-0.59;-0.31*	-0.39	-0.54;-0.25*	-0.06	-0.19;0.08	-0.53	-0.72;-0.34*	-0.49	-0.71;-0.28*	-0.04	-0.26; 0.19
≥0	-	-	-	-	-	-	-	-	-	-	-	-
Weight-for-length Z^d												
<-2	-1.54	-1.75; -1.34*	-0.99	-1.26;-0.72**	-0.55	-0.78;-0.33*	-1.66	-1.84; -1.47*	-1.44	-1.69; -1.19*	-0.22	-0.51; 0.08
-2≤ and <-1	-1.03	-1.14; -0.91*	-0.48	-0.63;-0.33**	-0.54	-0.67;-0.42**	-1.14	-1.29;-0.98*	-1.07	-1.28; -0.86*	-0.07	-0.33; 0.19
-1≤ and <0	-0.61	-0.70; -0.52*	-0.30	-0.42;-0.18**	-0.31	-0.40;-0.22**	-0.52	-0.66; -0.37*	-0.70	-0.90; -0.50*	0.18	-0.08; 0.45
≥0	-	-	-	-	-	-	-	-	-	-	-	-

FFM, Fat-free mass; FM, Fat mass.

Separate linear mixed-effects models were fitted to FFM and weight. Age, sex, intervention groups of the original trial design, and the interaction between visit (6 or 15 months) and either ^asex, ^bbreastfeeding, ^clength-for-age or ^dweight-for-length z-score categories were included as fixed effects and children and municipality were included as random (intercept) effects. Estimates for FM were derived from the corresponding estimates for FFM and weight (with error propagation).

* Significantly different (p< 0.05) from the reference category.

Significant interaction i.e., change in difference between 6 and 15 months (p<0.05).

Table 3b. Estimated mean differences in body mass index, fat-free mass index, and fat mass index within sex, breastfeeding, length-for-age and weight-for-length z score categories among Cambodian children at 6 and 15 months of age (n=413).

	6 months						15 months					
	BMI, kg/m ²		FFMI, kg/m ²		FMI, kg/m ²		BMI, kg/m ²		FFMI, kg/m ²		FMI, kg/m ²	
	Diff.	95% CI	Diff.	95% CI	Diff.	95% CI	Diff.	95% CI	Diff.	95% CI	Diff.	95% CI
Sex^a												
Boy	0.59	0.35;0.83*	0.49	0.29; 0.68*	0.10	-0.13;0.34	0.56	0.31; 0.81*	0.45	0.23;0.66*	0.11	-0.18; 0.40
Girl	-	-	-	-	-	-	-	-	-	-	-	-
Breastfeeding^b (n=412)												
Not breastfed	-0.48	-1.16;0.21	0.74	-0.002;1.48	-1.22	-2.05;-0.39**	0.05	-0.16;0.26	0.28	0.03;0.53*	-0.23	-0.51;0.051
Breastfed	-	-	-	-	-	-	-	-	-	-	-	-
Length-for-age Z^c												
<-2	-0.46	-0.82;-0.11*	-0.16	-0.50;0.18	-0.30	-0.65;0.05	-0.17	-0.59;0.26	-0.15	-0.59;0.30	-0.02	-0.47;0.42
-2≤ and <-1	-0.21	-0.51;0.08	-0.19	-0.48;0.10	-0.03	-0.32;0.27	-0.01	-0.40;0.39	-0.12	-0.54;0.30	0.12	-0.30;0.53
-1≤ and <0	-0.07	-0.35;0.20	-0.03	-0.31;0.25	-0.04	-0.32;0.24	0.07	-0.31;0.45	0.01	-0.41;0.43	0.06	-0.35;0.46
≥0	-	-	-	-	-	-	-	-	-	-	-	-
Weight-for-length Z^d												
<-2	-4.06	-4.34;-3.79**	-1.94	-2.36;-1.52*	-2.12	-2.53;-1.72**	-3.37	-3.63; -3.11*	-2.05	-2.45;-1.65*	-1.32	-1.77;-0.87*
-2≤ and <-1	-2.95	-3.10;-2.80**	-1.14	-1.37;-0.90*	-1.82	-2.09;-1.54**	-2.39	-2.60; -2.17*	-1.47	-1.80;-1.14*	-0.91	-1.31;-0.51*
-1≤ and <0	-1.83	-1.95;-1.70**	-0.70	-0.88;-0.51**	-1.13	-1.35;-0.91**	-1.39	-1.60; -1.17*	-1.09	-1.42;-0.77*	-0.29	-0.71;0.12
≥0	-	-	-	-	-	-	-	-	-	-	-	-

BMI, Body mass index; FFMI, Fat-free mass index; FMI, Fat mass index.

Separate linear mixed-effects models were fitted to BMI and FFMI. Age, sex, intervention groups of the original trial design, and the interaction between visit (6 or 15 months) a either ^asex, ^bbreastfeeding, ^clength-for-age or ^dweight-for-length z score categories were included as fixed effects and children and municipality were included as random (interce effects. Estimates for FMI were derived from the corresponding estimates for BMI and FFMI (with error propagation).

* Significantly different (p< 0.05) from the reference category.

Significant interaction i.e., change in difference between 6 and 15 months (p<0.05).

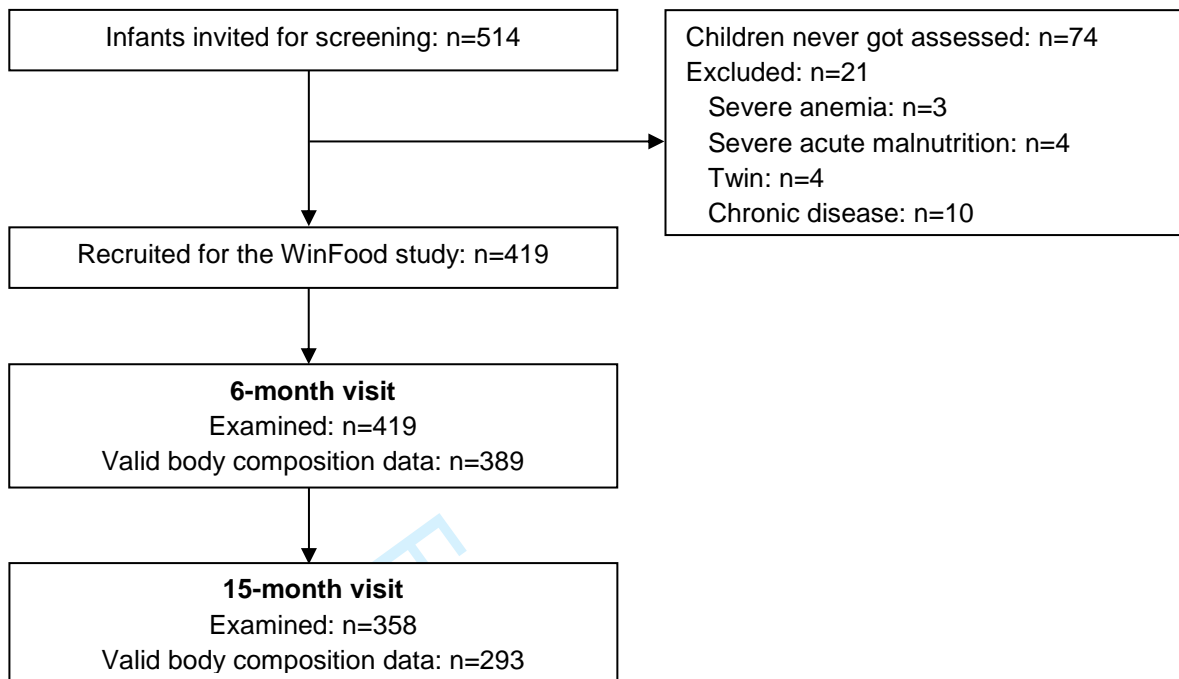
Figure legends

Figure 1: Flow diagram of study participants

Figure 1 footnote: Definition of “valid body composition data”: Body composition calculated from children with no uncertainty about ^2H spillage, resulting in more precise calculations of fat mass and fat-free mass.

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Figure 1



Online supporting material

Table 1. Estimated mean differences in weight, fat-free mass, and fat mass within sex, breastfeeding, length-for-age and weight-for-length z score categories among Cambodian children at 6 and 15 months of age (n=413)

	6 months						15 months						Interaction						
	Weight, kg		FFM, kg		FM, kg		Weight, kg		FFM, kg		FM, kg		Weight, kg		FFM, kg		FM, kg		
	Diff	95% CI	Diff	95% CI	Diff	95% CI	Diff	95% CI	Diff	95% CI	Diff	95% CI	Diff	95% CI	Diff	95% CI	Diff	95% CI	
Sex^a																			
Boy	0.63	0.47;0.78*	0.49	0.36; 0.61*	0.14	0.05;0.23*	0.66	0.50; 0.83*	0.57	0.42;0.71*	0.10	-0.05; 0.25	0.04	-0.06;0.14	0.08	-0.06;0.21	-0.04	-0.16;0.09	
Girl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Breastfeeding^b (n=412)																			
Not breastfed	-0.30	-0.68;0.09	0.22	-0.24;0.69	-0.52	-0.87;-0.17*	-0.05	-0.17;0.07	0.15	0.002;0.30*	-0.20	-0.34;-0.06*	0.25	-0.14;0.64	-0.07	-0.55;0.40	0.32	-0.06;0.70	
Breastfed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
LAZ^c																			
<-2	-1.40	-1.58;-1.23*	-1.13	-1.31;-0.96*	-0.27	-0.43;-0.12*	-1.74	-1.95;-1.53*	-1.57	-1.80;-1.35*	-0.17	-0.40; 0.07	-0.34	-0.54;-0.13#	-0.44	-0.70;-0.18#	0.10	-0.15;0.35	
-2≤ and <-1	-0.90	-1.04;-0.75*	-0.78	-0.93;-0.63*	-0.12	-0.25;0.02	-1.08	-1.28;-0.88*	-1.00	-1.22;-0.79*	-0.08	-0.30; 0.15	-0.18	-0.37;0.01	-0.22	-0.46;0.02	0.04	-0.19;0.27	
-1≤ and <0	-0.45	-0.59;-0.31*	-0.39	-0.54;-0.25*	-0.06	-0.19;0.08	-0.53	-0.72;-0.34*	-0.49	-0.71;-0.28*	-0.04	-0.26; 0.19	-0.08	-0.27;0.11	-0.10	-0.34;0.15	0.02	-0.21;0.26	
≥0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
WLZ^d																			
<-2	-1.54	-1.75; -1.34*	-0.99	-1.26;-0.72*	-0.55	-0.78;-0.33*	-1.66	-1.84; -1.47*	-1.44	-1.69; -1.19*	-0.22	-0.51; 0.08	-0.11	-0.35; 0.13	-0.45	-0.79;-0.11#	0.34	-0.02;0.69	
-2≤ and <-1	-1.03	-1.14; -0.91*	-0.48	-0.63;-0.33*	-0.54	-0.67;-0.42*	-1.14	-1.29;-0.98*	-1.07	-1.28; -0.86*	-0.07	-0.33; 0.19	-0.11	-0.27; 0.05	-0.59	-0.82;-0.35#	0.48	0.20;0.75#	
-1≤ and <0	-0.61	-0.70; -0.52*	-0.30	-0.42;-0.18*	-0.31	-0.40;-0.22*	-0.52	-0.66; -0.37*	-0.70	-0.90; -0.50*	0.18	-0.08; 0.45	0.09	-0.06; 0.25	-0.40	-0.62;-0.18#	0.49	0.21;0.77#	
≥0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

FFM, Fat-free mass; FM, Fat mass, LAZ, Length-for-age Z, WLZ, Weight-for-length Z
 Separate linear mixed-effects models were fitted to FFM and weight. Age, sex, intervention groups of the original trial design, and the interaction between visit (6 or 15 months) and either ^asex, ^bbreastfeeding, ^cLAZ or ^dWLZ categories were included as fixed effects and children and municipality were included as random (intercept) effects. Estimates for FM were derived from the corresponding estimates for FFM and weight (with error propagation).

* Significantly different (p< 0.05) from the reference category.

Significant interaction i.e., change in difference between 6 and 15 months

Table 2. Estimated mean differences in body mass index, fat-free mass index, and fat mass index within sex, breastfeeding, length-for-age and weight-for-length z score categories among Cambodian children at 6 and 15 months of age (n=413)

	6 months						15 months						Interaction					
	BMI, kg/m ²		FFMI, kg/m ²		FMI, kg/m ²		BMI, kg/m ²		FFMI, kg/m ²		FMI, kg/m ²		BMI, kg/m ²		FFMI, kg/m ²		FMI, kg/m ²	
	Diff	95% CI	Diff	95% CI	Diff	95% CI	Diff	95% CI	Diff	95% CI	Diff	95% CI	Diff	95% CI	Diff	95% CI	Diff	95% CI
Sex^a																		
Boy	0.59	0.35;0.83*	0.49	0.29;0.68*	0.10	-0.13;0.34	0.56	0.31;0.81*	0.45	0.23;0.66*	0.11	-0.18;0.40	-0.03	-0.21;0.15	-0.04	-0.28;0.20	0.01	-0.24;0.26
Girl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Breastfeeding^b (n=412)																		
Not breastfed	-0.48	-1.16;0.21	0.74	-0.002;1.48	-1.22	-2.05;-0.39*#	0.05	-0.16;0.26	0.28	0.03;0.53*	-0.23	-0.51;0.051	0.53	-0.16;1.22	-0.46	-1.23;0.32	0.98	0.11;1.86#
Breastfed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LAZ^c																		
<-2	-0.46	-0.82;-0.11*	-0.16	-0.50;0.18	-0.30	-0.65;0.05	-0.17	-0.59;0.26	-0.15	-0.59;0.30	-0.02	-0.47;0.42	0.29	-0.11;0.70	0.02	-0.48;0.52	0.28	-0.19;0.74
-2≤ and <-1	-0.21	-0.51;0.08	-0.19	-0.48;0.10	-0.03	-0.32;0.27	-0.01	-0.40;0.39	-0.12	-0.54;0.30	0.12	-0.30;0.53	0.21	-0.17;0.58	0.06	-0.39;0.52	0.14	-0.28;0.56
-1≤ and <0	-0.07	-0.35;0.20	-0.03	-0.31;0.25	-0.04	-0.32;0.24	0.07	-0.31;0.45	0.01	-0.41;0.43	0.06	-0.35;0.46	0.14	-0.23;0.52	0.05	-0.42;0.51	0.10	-0.32;0.52
≥0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WLZ^d																		
<-2	-4.06	-4.34;-3.79*	-1.94	-2.36;-1.52*	-2.12	-2.53;-1.72*	-3.37	-3.63;-3.11*	-2.05	-2.45;-1.65*	-1.32	-1.77;-0.87*	0.69	0.32;1.07#	-0.11	-0.68;-0.46	0.80	0.20;1.40#
-2≤ and <-1	-2.95	-3.10;-2.80*	-1.14	-1.37;-0.90*	-1.82	-2.09;-1.54*	-2.39	-2.60;-2.17*	-1.47	-1.80;-1.14*	-0.91	-1.31;-0.51*	0.56	0.30;0.82#	-0.34	-0.73;0.06	0.90	0.43;1.38#
-1≤ and <0	-1.83	-1.95;-1.70*	-0.70	-0.88;-0.51*	-1.13	-1.35;-0.91*	-1.39	-1.60;-1.17*	-1.09	-1.42;-0.77*	-0.29	-0.71;0.12	0.44	0.20;0.67#	-0.39	-0.76;-0.03#	0.84	0.37;1.30#
≥0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

BMI, Body mass index; FFMI, Fat-free mass index; FMI, Fat mass index; LAZ, Length-for-age Z; WLZ, Weight-for-length Z.

Separate linear mixed-effects models were fitted to BMI and FFMI. Age, sex, intervention groups of the original trial design, and the interaction between visit (6 or 15 months) and either ^asex, ^bbreastfeeding, ^cLAZ or ^dWLZ categories were included as fixed effects and children and municipality were included as random (intercept) effects. Estimates for FMI were derived from the corresponding estimates for BMI and FFMI (with error propagation).

* Significantly different ($p < 0.05$) from the reference category.

Significant interaction i.e., change in difference between 6 and 15 months ($p < 0.05$).

Table 3. Estimated mean differences in triceps and subscapular skin folds within sex, breastfeeding, length-for-age and weight-for-length z-score categories among Cambodian children at 6 and 15 months of age. Data are regression coefficient (B) and 95% confidence interval (95% CI)

	6 months				15 months				Interaction			
	Triceps skinfold, mm		Subscapularis skinfold, mm		Triceps skinfold, mm		Subscapularis skinfold, mm		Triceps skinfold, mm		Subscapularis skinfold, mm	
	B	95% CI	B	95% CI	B	95% CI	B	95% CI	B	95% CI	B	95% CI
Sex^a												
Boy	0.13	-0.05; 0.32	0.14	-0.08; 0.35	0.33	0.13; 0.53*	0.10	-0.12; 0.32	0.19	-0.01; 0.39	-0.03	-0.21; 0.15
Girl	-	-	-	-	-	-	-	-	-	-	-	-
Breastfeeding^b												
Not breastfed	-0.72	-1.37; -0.08*	-0.79	-1.44; -0.15*	-0.36	-0.57; -0.15*	-0.08	-0.28; 0.13	0.36	-0.30; 1.03	0.72	0.06; 1.38#
Breastfed	-	-	-	-	-	-	-	-	-	-	-	-
LAZ^c												
<-2	-0.31	-0.63; 0.009	-0.41	-0.74; -0.08*	0.004	-0.39; 0.40	-0.25	-0.65; 0.15	0.31	-0.11; 0.74	0.16	-0.23; 0.55
-2 _≤ and <-1	-0.28	-0.55; -0.01*	-0.34	-0.62; -0.06*	0.031	-0.34; 0.40	-0.15	-0.52; 0.22	0.31	-0.08; 0.71	0.19	-0.17; 0.55
-1 _≤ and <0	-0.16	-0.41; 0.10	-0.22	-0.47; 0.04	0.10	-0.26; 0.47	-0.02	-0.38; 0.34	0.25	-0.14; 0.66	0.20	-0.16; 0.57
≥0	-	-	-	-	-	-	-	-	-	-	-	-
WLZ^d												
<-2	-1.28	-1.70; -0.87*	-1.99	-2.38; -1.60*	-1.02	-1.41; -0.64*	-1.74	-2.10; -1.39*	0.26	-0.27; -0.79	0.24	-0.23; 0.72
-2 _≤ and <-1	-0.96	-1.19; -0.73*	-1.49	-1.70; -1.27*	-0.78	-1.09; -0.46*	-1.19	-1.48; -0.90*	0.18	-0.18; 0.55	0.29	-0.03; 0.62
-1 _≤ and <0	-0.65	-0.84; -0.47*	-0.92	-1.09; -0.75*	-0.29	-0.60; 0.02	-0.66	-0.95; -0.38*	0.36	0.02; 0.71#	0.26	-0.05; 0.57
≥0	-	-	-	-	-	-	-	-	-	-	-	-

Linear mixed-effects models were fitted for triceps and subscapular skinfolds. Age, sex, intervention groups of the original trial design, and the interaction between visit (6 or 15 months) and either ^asex, ^bbreastfeeding, ^clength-for-age or ^dweight-for-age z-score categories were included as fixed effects and children and municipality were included as random (intercept) effects.

* Significantly different from the reference category (p< 0.05)

Significant interaction