Title: Associations between stunting, wasting and body composition: A longitudinal study in 6-15 month-old Kenyan children

Authors: Silvenus O. Konyole[1], Selina A. Omollo[2], John N. Kinyuru[3], Bethwell O. Owuor[4], Benson B. Estambale[5], Christian Ritz[6], Kim F. Michaelsen[7], Suzanne M. Filteau[8,] Jonathan C. Wells[9], Nanna Roos[7], Henrik Friis[7], Victor O. Owino[10], Benedikte Grenov[7]

Affiliations:

[1]Department of Nutritional Sciences, Masinde, Muliro University of Science and Technology, Kakamega, Kenya

[2] Institute of Tropical and Infectious Diseases, University of Nairobi, Nairobi, Kenya

[3]Department of Food Science and Technology, Jomo Kenyatta University of Agriculture and Technology, Juja, Kenya

[4] Biological Sciences Department, Kisii University, Kisii, Kenya

[5] Division of Research, Innovations and Outreach, Jaramogi OgingaOdinga University of Science and Technology, Bondo, Kenya

[6] National Institute of Public Health, University of Southern Denmark, Copenhagen, Denmark

[7] Department of Nutrition, Exercise and Sports, University of Copenhagen, Denmark

[8] Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK

[9] Childhood Nutrition Research Centre, Population, Policy and Practice Research and Teaching Department, University College London (UCL) Great Ormond Street Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK

[10]Nutritional and Health-Related Environmental Studies Section, Division of Human Health, International Atomic Energy Agency, Vienna, Austria

Corresponding author: Silvenus O Konyole, Department of Nutritional Sciences, Masinde, Muliro University of Science and Technology, P.O. Box 190-50100, Kakamega, Kenya, e-mail: konyole2000@yahoo.com, phone: :+254 723 269 286

Sources of support for the work: Danida, the Ministry of Foreign Affairs of Denmark, supported the work through the WINFOOD project (KFM, grant number 57-08 LIFE).

Conflict of interest and funding disclosure: SOK, SAO, JNK, BOO, BBE, CR, KFM, SMF, JCW, NR, HF, VOO, BG all declare no conflict of interest

Running title: Early stunting and body composition

List of abbreviations

BMI	Body mass index
CSB+	Corn-soy-blend plus
FFM	Fat-free mass
FFMI	Fat-free mass index
FM	Fat mass
FMI	Fat mass index
IGF-1	Insulin-like growth factor-1
LAZ	Length-for-age z-score

TBW	Total body water
WLZ	Weight-for-length z-score

ABSTRACT

Background

Early growth and body composition may influence risk of obesity and health in adulthood. Few studies have examined how undernutrition affects body composition in early life.

Objective

We assessed stunting and wasting as correlates of body composition in young Kenyan children.

Methods

Nested in a randomized controlled nutrition trial, this study assessed fat and fat-free mass (FM, FFM) using deuterium dilution technique at age 6 and 15 months. Associations betweenz-score categories of length-for-age Z (LAZ) or weight-for-length Z (WLZ) and FM, FFM, fat mass index (FMI) and fat-free mass index (FFMI) were analyzed by linear mixed models.

Results

Among 499 children enrolled, breastfeeding declined from 99% to 87%, stunting increased from 13% to 32% and wasting remained at 2-3% between 6 and 15 months. Compared to LAZ >0, stunted children had 1.12 (0.88;1.36) kg lower FFM at 6 months increasing to 1.59 (1.25;1.94) kg at 15 months, corresponding to differences of 18% and 17%, respectively. When analysing FFMI, the deficit in FFM tended to be less than proportional to children's height at 6 months ($P \leq 0.060$) and was proportional at 15 months ($P > 0.40$). Stunting was associated with 0.28 (0.09;0.47) kg lower FM at 6 months. The difference lost significance at 15 months, and stunting was not associated with FMI at any time point. Lower WLZ was

associated with lower FM, FFM, FMI and FFMI at 6 and 15 months. Differences in FFM, but not FM, increased with time, while FFMI differences did not change and FMI differences generally decreased with time.

Conclusions

Overall, undernutrition seems to be associated with reduced lean tissue, which may have long-term health consequences.

Trial registration: ISRCTN30012997

Keywords: Body composition, malnutrition, infancy and childhood, fat-free mass, fat mass, infant growth, child growth

INTRODUCTION

Malnutrition remains a challenge in many low-income countries (1). Although stunting has slowly declined over the past decades (1,2), the proportions of wasted and stunted children remain high with 7% of the global population of children below 5 years being wasted and 22% being stunted and with even higher numbers in Africa (1,2). Stunting and wasting are associated with increased mortality and morbidity as well as with long-term consequences including delayed cognitive development in childhood and increased risk of chronic diseases and reduced working capacity in adulthood (1,3,4).

Early growth and specific growth patterns have been associated with risk of obesity and cardio-vascular disease in adulthood (5,6). These associations may be mediated by changes in fat mass (FM) and fat-free mass (FFM) composition (7). For example, low birth weight is associated with lower FFM at birth, in childhood as well as adulthood (7–9), while subsequent high catch-up growth in childhood may result in accumulation of FM and increased risk of later obesity and metabolic syndrome or cardiovascular disease (7,10). Moreover, children exposed to acute malnutrition during childhood have less FFM in adulthood (7,10) and may have greater risk of high blood pressure later in life (7,10).

A narrative review based on a limited number of studies from low- and middle-income countries found that wasting in children with acute malnutrition is associated with very low amounts of both FFM and FM (11). In contrast, stunting is associated with low FFM, which in some cases disappears after adjustment for height, indicating that the low FFM is explained by shorter height (11). Importantly, body composition varies with ethnicity in early life, for example Asian populations tend to have lower levels of lean mass than European or Sub-Saharan African populations from birth onwards (12–14), hence the magnitude of FFM and

FM deficits associated with malnutrition, and the patterns of tissue accretion during supplementary feeding programmes, may also differ between populations.

Building on results from a comparable study in children of similar age in Cambodia (15), the aim of the current study was to assess associations of stunting and wasting with changes in FM, FFM, the height-adjusted indices FMI and FFMI as well as skinfolds in Kenyan children, followed from 6 to 15 months of age. We hypothesized that malnutrition is associated with especially reduced FFM and that reductions in FFM persist or exacerbate with age.

METHODS

Study design, setting and ethical considerations

The current longitudinal study was nested in a randomized controlled trial (RCT) described in detail elsewhere (16). The study was conducted from January 2012 to January 2013 at Makunga, Khaunga and Lusheya health centres of Mumias Sub-county in Kakamega County, Western Kenya. In this region, about a quarter of the children below five years are stunted (17). The area is a rural, malaria-prone, food-insecure and resource-limited locality (18). As part of the intervention, children received one of three study foods at 200-550 kcal/day, with increasing amount from 6 to 15 months of age (16). The foods were designed to meet the energy density and protein recommendations for complementary foods for breastfed children, and the portion size was adjusted to match the maximum daily energy recommended to avoid encouraging substitution of breastmilk (19). The study foods comprised two locally developed flours consisting of germinated amaranth (71-82.5%), maize (10%) and either small fish (3%) and edible termites (10%) or multi-micronutrient premix which were compared against corn-soy blend plus (CSB+).

The study was conducted according to the principles in the Declaration of Helsinki and it was approved by the Kenyatta National Hospital-University of Nairobi Ethics and Research Committee (KNH-UoNERC-P436/12/2010) with a further consultative approval obtained from the Danish National Committee on Biomedical Research Ethics. Before infants were enrolled, caregivers gave written informed consent after oral and written information was provided in the local language or Kiswahili. Permission to implement the study was obtained from relevant government line ministries and local authorities.

Study participant recruitment, participant visits, inclusion and exclusion criteria

When infants were 5 months of age, mother-infant dyads were invited to the study from the health facilities they visited for routine monthly growth monitoring. Trained health workers

screened infants. Inclusion criteria included caregivers' consent to let their child participate and acceptance to prepare and feed their infants with the assigned complementary food according to the parent randomized controlled trial (16). Exclusion criteria were clinical signs of vitamin A deficiency, severe anaemia (haemoglobin< 80g/L) and severe acute malnutrition, defined as weight-for-length z-score (WLZ) <-3, pitting oedema or mid-upper arm circumference (MUAC) <11.5 cm. If any of these exclusion criteria were detected, the infant was referred for treatment as per the Kenya Ministry of Health guidelines. Infants with genetic disorders interfering with growth or chronic illness requiring medication were also excluded. Twins were recruited into the study if both were healthy and met the inclusion criteria. Included twins received the same food supplement. All infants were assessed on the recruitment day (a '6-month visit') and 9 months later at a'15-month visit'.

Body composition assessment

As previously described (16), body composition was assessed using the deuterium dilution technique. This approach represents a 2-component model, dividing body weight into FFM and FM (20). Prior to giving each child an accurately weighed standardized oral dose of deuterium labelled water, a pre-dose sample of about 2 ml of saliva was taken from the child's mouth using a cotton ball (16). Post-dose saliva samples were taken at 2 hours and 3 hours after intake of the deuterium labelled water (20). Saliva samples were collected with a syringe into a tightly closed 1.5 mL cryogenic tube by squeezing the saliva from the wet cotton ball removed from the child's mouth. Samples were kept in an iced cooler box and transported the same day to a central collection point at Lusheya health centre where they were stored at -20°C followed by transfer on dry ice to Kenya Medical Research Institute in Nairobi for analysis (16). A Fourier Transform Infrared spectrophotometer (Shimadzu model 8400s, Shimadzu Corporation, Kyoto, Japan) was used to analyze enrichment of deuterium in the saliva samples and the pre-dose samples were used for background correction. The

intention was to take the higher of the two enrichments at 2 and 3 hours, indicating the attainment of peak enrichment at equilibration. Inadvertently, the 2 and 3 hour values were averaged without retaining the raw data. In addition, cases with >50 ppm difference between the 2 and 3 hour samples were considered as poor agreement and discarded from the dataset. Based on field notes, children were excluded from the dataset if there was uncertainty about their ^2H_2O consumption and if the deuterium enrichment in post-dose saliva was less than 600 mg/kg, indicative of issues with dosing (16). The dilution space and TBW were calculated according to guidelines (20). FFM was calculated by dividing TBW by an age-specific hydration factor: TBW/0.79 for both sexes. FM was calculated as body weight minus FFM (International Atomic Energy Agency, 2010; Skau et al., 2019).The fat-free mass index (FFMI) and the fat mass index (FMI) were calculated by FFM/length^2 (kg/m^2) and FM/length^2 (kg/m^2), respectively (20). These indices express FFM and FM normalized for length and are expressed in the same units as BMI. Negative FM values were removed from the dataset, as they were considered to be due to inadequate consumption of the ^2H_2O dose, or inadequate equilibration with body water. As described by others (21,22), outliers which fitted very poorly with the general association of body water with weight and height were also removed from the dataset. More results were removed at 15 months than 6 months with poor fit likely due to longer equilibration times in older children.

Anthropometry, nutrition and sociodemographic data

Trained assistants with previous experience in growth monitoring took the anthropometric measurements in triplicate using standardized anthropometric techniques and calibrated equipment (23). Length was measured to the nearest 0.1 cm using calibrated length boards, nude weight was measured to the nearest 0.01 kg, using a hanging Seca scale (UniScale) and triceps and subscapular skinfolds were measured to the nearest 0.1 mm using Harpenden skinfold calipers (Crymych, United Kingdom). Head circumference and MUAC were

measured to the nearest 0.1 cm using non-stretchable measuring tape (Harlow Printing Limited). Breast-feeding status was determined both at the 6- and 15- month visit. To estimate whether a child was still breastfed, the caregiver was asked 'Since this time yesterday, has the child been breastfed?' Finally, time of introduction of complementary foods and some socio-demographic variables were obtained at the 6-month visit.

Data analysis

Collected data were monitored daily and double entered within 2 weeks in Microsoft Excel ™. LAZ and WLZ scores were calculated using WHO Anthro™ v3.2.2 based on the WHO's 2006 Child Growth Standards. LAZ and WLZ<-2 were defined as stunting and wasting, respectively.

Statistical analyses were performed with R (R core team, 2017) with the extension packages tukeytrend, lme4 and multcomp. We used χ^2 tests and two-sample t-tests for categorical and continuous variables, respectively, when comparing data by sex and visit. To assessassociations between the independent variables sex z-score categories of LAZ and WLZ with the dependent variables weight, FM, FFM, BMI, FMI, FFMI, triceps and subscapular skinfolds, we used linear mixed models. The models included the following pre-specified co-variates age, sex (except for models with sex as an independent variable), intervention groups of the original trial design, and the interaction between visits and either sex, LAZ or WLZ categories as fixed effects and children and health facility were included as random (intercept) effects. The linear mixed models were used both for extracting cross-sectional differences at 6 and 15 months and for obtaining longitudinal differences from 6 to 15 months. Using the linear mixed models for both types of estimates ensured parsimonious results where all differences agree (using different models for obtaining the cross-sectional and longitudinal differences would come at the risk of getting discrepancies as the estimated means need not

be identical between models). Additionally, there is a gain in efficiency by using a single model as data are used optimally.

We used linear mixed models for FFM and weight to obtain estimates for FM instead of creating a linear mixed model for (weight-FFM). Specifically, estimates for FM were obtained as differences of estimates for FFM and weight. This approach ensures that estimates respect the constraint that FM is entirely determined by FFM and weight. Correlation between estimates for FFM and weight, which were obtained from two separate model fits, was incorporated using an augmented variance-covariance matrix based on score statistics (24,25) For all analyses, model assumptions were checked using residual and normal probability plots. Missing data were not imputed. A significance level of 5% was used. No adjustments for multiple comparisons were applied.

RESULTS

The study enrolled 499 children out of 527 screened (Supplementary Figure 1). The mean (±SD) age of the children was 6.0 ±0.2 months and approximately half were males (Table 1). Almost all children (99%) were breastfed at 6 months of age and 87% were still breastfed at 15 months (Table 2). The stunting prevalence (LAZ<-2) increased from 13% at 6 months to 32% at 15 months (Table 2). In contrast, the wasting prevalence (WLZ<-2) remained low with 3% and 2% at 6 and 15 months of age, respectively. Males showed a trend towards lower LAZ than females at 6 months (P=0.09) and had lower LAZ at 15 months (P=0.03) (data not shown).

Deuterium was dosed to 459 (92%) and 385 (90%) of the children present at 6 and 15 months (Supplementary Figure 1). After laboratory processing and data cleaning, data were available from 442 (89%) of the children at 6 months of age and 288 (67%) at 15 months (Supplementary Figure 1). The children with or without body composition data did not differ with regards to sex, weight, height, MUAC, LAZ, WLZ, BMI or skinfold thickness at 6 or 15 months (P >0.05, Supplementary Table 1). FFM and FFMI increased in both males and females from 6 to 15 months (P <0.001, Table 2). However, FM decreased in males (P =0.002), but not females (P =0.45) and FMI decreased in both sexes (P <0.001, Table 2). The higher weight and FFM in males compared to females persisted when indexed by height as BMI and FFMI (P ≤0.05, Table 2).

The associations between sex decreasing z-score categories of LAZ and WLZ with weight, FM and FFM and their height-adjusted indexes BMI, FMI and FFMI are reported in Tables 3 and 4. At both 6 and 15 months, males weighed around 0.5 kg more than females, which was entirely explained by higher FFM (Table 3). Similarly, males had 0.37 and 0.30 kg/m^2 higher BMI than females at 6 and 15 months, due to 0.44 and 0.43 kg/m^2 higher FFMI (Table 4).

Overall, the lower the LAZ score, the lower the weight, FM and FFM for children at both 6 and 15 months (Table 3). Compared to children with LAZ ≥0, stunting was associated with 1.40 kg and 1.88 kg lower weight at 6 and 15 months, mainly explained by 1.12 kg and 1.59 kg lower FFM (Table 3). The differences in FFM corresponded to 18% and 17% at 6 and 15 months of age, respectively. In contrast, stunted children had 0.28 kg lower FM at 6 months, corresponding to 15%, which did not change as children grew older ($P_{interaction}$ =0.97). The difference in FM between stunted children and children with LAZ ≥0 lost significance at 15 months.(P >0.05, Table 3). At 6 months of age, children with LAZ between 0 and -2, had higher BMI and FFMI, and stunted children tended to have higher FFMI than children with LAZ ≥0 (Table 4). At 15 months of age, BMI, FFMI and FMI differed little among LAZ categories.

Children with low WLZ (<0) had not only lower weight, FFM and FM, but also lower BMI, FFMI and FMI compared to children with WLZ ≥0 (Table 3, 4). Differences in weight and FFM, but not in FM generally increased with time for children with WLZ <0. The BMI difference in children with low WLZ (<0) generally decreased between 6 and 15 months due to a decrease in the FMI difference. FFMI difference did not change with time ($P_{interaction}$>0.05) (Table 4). Sensitivity analyses including FFM and FM results from 345 children at 15 months before outliers were removed from the dataset resulted in similar results (Supplementary tables 2 and 3).

Both triceps and subscapular skinfold thicknesses were lower with lower LAZ and WLZ (Table 5). For stunted children, the triceps was 0.9 mm and 1.4 mm thinner at 6 months and 15 months, respectively, corresponding to differences of 10% and 17% ($P_{interaction}$>0.05). In comparison, the subscapular skinfold was 0.7 mm and 0.6 mm thinner at 6 and 15 months, both corresponding to a difference of 8%.

DISCUSSION

In children from resource-limited rural communities in western Kenya, stunting increased from 13 % at 6 months to 32 % at 15 months, despite a very high prevalence of breastfeeding and supplementation of 200-550 kcal/day, equivalent to WHO recommendations for complementary feeding of breastfed children in the age range (19). The prevalence of wasting remained at 2-3 % throughout the study, which is almost at the level of a normally distributed population following the WHO growth standards. This emphasizes that despite reduction in wasting prevalence, stunting still constitutes a major challenge in some low-income countries.

The results showed that stunting progressed between 6 and 15 months at the expense of FFM while FM was relatively preserved. Other studies have also quite consistently reported that stunted as well as wasted children lack FFM (11,15,26–28). In a parallel Cambodian study, we also found that FFM, but not FM, deficits increased in stunted children between 6 and 15 months of age (15) and corresponded to a lack of approximately 20% of the FFM at both time points. However, stunting was associated with the height-adjusted index, FFMI, differently in Kenyan and Cambodian infants. In the current study, the deficits in FFM were less than proportional to the children's length at 6 months and proportional to their length at 15 months. In the Cambodian study, the FFM deficits were proportional to the children's length at both 6 and 15 months (15). Greater preservation of FFM in Kenyan children is consistent with the overall notion that Asian populations tend to have lower lean mass (12,13).

The observed lower FFM in early life among stunted children in this and other studies may have medium- and long-term consequences (15). In a South-African cohort, stunting at 1 year was associated with lower FM at 10 years (27), while stunting at 2 years was associated with lower FFM but not FM at 10 (27) and 22 years (29). In Nepalese children, stunting at 2 years was associated with reduced amounts of both FM and FFM at 8 years of age (11) and in

Brazilian males, stunting at 2 and 4 years was associated with reduced FMI and FFMI (4 years only) at 18 years (30). Overall, these studies indicate that stunting at an early age is associated with reduced FFM and perhaps reduced fat tissue later on. However, contrasting results were reported in two Brazilian studies in adolescents from slums in Sao Paolo (31,32). The first cross-sectional study found that especially stunted adolescent females had higher weight-for-height than non-stunted adolescents (32). The second, smaller study followed 20 stunted and 30 normal stature 11-15 year-old children for 3 years and showed that stunted males and females gained less FFM and males, but not females, accumulated more FM than controls with normal stature (31). We speculate that differences in associations between early stunting and body composition later in life may be due to nutritional transition from low to high energy-dense food of poor nutritional quality in some populations (33). While nutrient-depleted diets may constrain FFM accretion in early life, at later ages they may potentially promote excess fat accumulation. For example, low-protein diets may drive over-consumption of calories in order to satisfy protein appetite (34). Consequently, the body composition of children who were stunted in early life may vary substantially, depending on what diet they are consuming at later ages.

Undernutrition in early life may also affect the long-term risk of disease. A study that followed up adults exposed to severe undernutrition in early life found that height and FFM were reduced, and though FFMI did not differ, the risk of non-communicable diseases was increased (35,36). These links between undernutrition and growth, whereby FFM is reduced but largely in proportion to height, therefore still appear to be detrimental to long-term health.

Thin children with WLZ<0 also had increasingly lower FFM (measured in absolute kilograms) between 6 and 15 months, while FM deficits did not change and lost significance in all, but two wasted children. In these children, deficits were more severe and persisted after height-adjustment, i.e. FFMI and FMI. FFMI deficits remained, whereas the FMI deficits

generally reduced at 15 months. A similar pattern was seen in the Cambodian study, but more clearly due to a higher number of wasted children (15). This indicates that adipose tissue may be preserved at the expense of fat-free tissue in wasted or mildly wasted children.

Growth in infancy and young childhood is mainly regulated by nutritional status, the growth hormone/insulin-like growth factor-1 (IGF-1) axis and thyroid hormone (37). Lack of one or more growth nutrients, including protein, zinc, magnesium, phosphorus, potassium, and sodium may have resulted in poor accretion of fat-free tissue. As all growth nutrients need to be present in sufficient and balanced amounts to build lean tissue, unbalanced diets with inadequate amounts of one or more growth nutrients will be metabolized and stored as fat tissue (38). In terms of hormonal regulation of growth, studies have found that IGF-1 is downregulated in undernourished children (39) and moreover, IGF-1 is more strongly associated with FFM than FM in children (40). Finally, an evolutionary survival strategy, preserving fat mass at the cost of fat-free tissue, could perhaps explain why FFM appears consistently reduced in undernourished children (41).

Lower LAZ and WLZ was also associated with lower skinfold thicknesses. For LAZ, deficits in triceps seemed to be worse than deficits in subscapular skinfolds, especially at 15 months. This may indicate that central fat (subscapularis) is preserved over peripheral fat (triceps) during chronic malnutrition (11). Although early stunting does not seem to drive adiposity or central fat later in life (42,43), stunted children from Brazil undergoing nutritional transition may be more likely to deposit central fat as they enter puberty (44). Fat is important for immune function. It provides energy for immune response while also secreting leptin which plays a regulatory role in immune function (45), and reduced leptin levels have been associated with increased mortality in severely malnourished children (46,47). However, decreasing WLZ scores was associated with significantly lower both central and limb

skinfolds. As infections are common in wasted children (48), the depletion of both skinfolds in this group is likely to reflect the greater use of fat reserves to fund immune response.

The calculation of TBW and FFM based on the average of the 2 and 3 hours post-doses was a limitation of the current study leading to potential overestimation of FFM and underestimation of FM, if equilibrium was not fully obtained. Although negative FM values were rejected, data may still contain some over-inflated FFM and low FM values. In addition, more implausible FFM and FM results were removed from 15 months than 6 months, which could potentially lead to another overestimation of FFM at 15 months. After the current study was completed, a study in Burkina Faso refined the procedure and found the optimum equilibration time to be 3 hours (49). As there was no difference in any anthropometric measures between children with or without body composition data at 6 or 15 months, and a sensitivity analysis did not find any differences in results before and after removing FFM and FM outlier values, the influence on the study results is considered to be minor. Other limitations include the few wasted children, which affect the validity of these results and lack of biomarkers to enhance interpretation of the study results. Finally, all children received a food intervention, which may influence the generalizability of the results to communities with no supplementation.

Until recently, it was not known how much of weight loss in undernourished children was due to loss of FFM or FM. In a population with high breastfeeding, stunting, but not wasting prevalence, we found that stunting was associated with almost 20% lower FFM at 6 and 15 months of age. The deficit was slightly lower than or proportional to the length of the children at 6 months and 15 months, respectively. Low WLZ was associated with low FFM, FM as well as the height-adjusted indexes FFMI and FMI at both 6 and 15 months. However, the FMI difference reduced between the two time points. Undernutrition, in general, seems to be more strongly associated with lower FFM. As earlier proposed (11), the proportionality of the

FFM deficit with length may vary between stunted populations. Studies are needed to further explore early changes in body composition and how these changes affect growth and health in the longer term.

ACKNOWLEDGEMENTS

Author contribution statement: HF, KFM, NR and VOO designed the study. SMF reviewed the design. SOK, SAO, JNK and BOO collected data under supervision by BBE and VOO. JCW and SOK contributed to data cleaning. BG and CR analysed the data and BG, SOK, SMF, JW, HF and NR interpreted the findings. BG and SOK prepared the first draft of the manuscript. SMF, JW, HF and NR contributed to manuscript writing, and BG finalized the manuscript. All authors have read and approved the final manuscript.

REFERENCES

1. Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet. 2013 Aug 3;382(9890):427–51.

2. UNICEF/WHO/The World Bank Group joint child malnutrition estimates: levels and trends in child malnutrition: key findings of the 2020 edition [Internet]. [cited 2020 Jun 3]. Available from: https://www.who.int/publications-detail/jme-2020-edition

3. Lu C, Black MM, Richter LM. Risk of poor development in young children in low-income and middle-income countries: an estimation and analysis at the global, regional, and country level. Lancet Glob Health. 2016 Dec 1;4(12):e916–22.

4. Prendergast AJ, Humphrey JH. The stunting syndrome in developing countries. Paediatr Int Child Health. 2014 Apr;34(4):250–65.

5. Baird J, Fisher D, Lucas P, Kleijnen J, Roberts H, Law C. Being big or growing fast: systematic review of size and growth in infancy and later obesity. BMJ. 2005 Oct 22;331(7522):929.

6. Barker DJP, Osmond C, Forsén TJ, Kajantie E, Eriksson JG. Trajectories of growth among children who have coronary events as adults. N Engl J Med. 2005 Oct 27;353(17):1802–9.

7. Wells JCK, Chomtho S, Fewtrell MS. Programming of body composition by early growth and nutrition. Proc Nutr Soc. 2007 Aug;66(3):423–34.

8. Admassu B, Wells JCK, Girma T, Andersen GS, Owino V, Belachew T, et al. Body composition at birth and height at 2 years: a prospective cohort study among children in Jimma, Ethiopia. Pediatr Res. 2017 Aug;82(2):209–14.

9. Kuzawa CW, Hallal PC, Adair L, Bhargava SK, Fall CHD, Lee N, et al. Birth weight, postnatal weight gain, and adult body composition in five low and middle income countries. Am J Hum Biol Off J Hum Biol Counc. 2012 Feb;24(1):5–13.

10. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ. 2000 Apr 8;320(7240):967–71.

11. Wells JCK. Body composition of children with moderate and severe undernutrition and after treatment: a narrative review. BMC Med. 2019 25;17(1):215.

12. Yajnik CS, Fall CHD, Coyaji KJ, Hirve SS, Rao S, Barker DJP, et al. Neonatal anthropometry: the thin-fat Indian baby. The Pune Maternal Nutrition Study. Int J Obes Relat Metab Disord J Int Assoc Study Obes. 2003 Feb;27(2):173–80.

13. Jain V, Kurpad AV, Kumar B, Devi S, Sreenivas V, Paul VK. Body composition of term healthy Indian newborns. Eur J Clin Nutr. 2016 Apr;70(4):488–93.

14. Andersen GS, Girma T, Wells JCK, Kæstel P, Leventi M, Hother AL, et al. Body composition from birth to 6 mo of age in Ethiopian infants: reference data obtained by air-displacement plethysmography. Am J Clin Nutr. 2013 Oct;98(4):885–94.

15. Skau JKH, Grenov B, Chamnan C, Chea M, Wieringa FT, Dijkhuizen MA, et al. Stunting, wasting and breast-feeding as correlates of body composition in Cambodian children at 6 and 15 months of age. Br J Nutr. 2019;121(6):688–98.

16. Konyole SO, Omollo SA, Kinyuru JN, Skau JKH, Owuor BO, Estambale BB, et al. Effect of locally produced complementary foods on fat-free mass, linear growth, and iron status among Kenyan infants: A randomized controlled trial. Matern Child Nutr. 2019;15(4):e12836.

17. Kenya National Bureau of Statistics, Ministry of Health/Kenya, National Aids Control Council/Kenya and Development/Kenya. (2015). Kenya demographic and health survey.

18. Desai MR, Terlouw DJ, Kwena AM, Phillips-Howard PA, Kariuki SK, Wannemuehler KA, et al. Factors associated with hemoglobin concentrations in pre-school children in Western Kenya: cross-sectional studies. Am J Trop Med Hyg. 2005 Jan;72(1):47–59.

19. Dewey KG, Brown KH. Update on technical issues concerning complementary feeding of young children in developing countries and implications for intervention programs. Food Nutr Bull. 2003 Mar;24(1):5–28.

20. International Atomic Energy Agency. Introduction to Body Composition Assessment Using the Deuterium Dilution Technique with Analysis of Saliva Samples by Fourier Transform Infrared Spectrometry [Internet]. International Atomic Energy Agency, Vienna, Austria; 2010. Report No.: IAEA human health series no 12. Available from: https://www-pub.iaea.org/MTCD/Publications/PDF/Pub1450_web.pdf

21. Colley RC, Byrne NM, Hills AP. Implications of the variability in time to isotopic equilibrium in the deuterium dilution technique. Eur J Clin Nutr. 2007 Nov;61(11):1250–5.

22. International Atomic Energy Agency. Introduction to body composition assessment using the deuterium dilution technique with analysis of urine samples by isotope ratio mass spectrometry. IAEA Human Health. Series no. 13. (p. 84). Vienna, Austria: International Atomic Energy Agency. 2011.

23. Lohman TG, Roche AF, Burgmeijer RJ. Anthropometric standardization reference manual. Champaign IL: Human Kinetics Books. 1988.

24. Pallmann P, Pretorius M, Ritz C. Simultaneous comparisons of treatments at multiple time points: Combined marginal models versus joint modeling. Stat Methods Med Res. 2017;

25. Ritz C, Laursen RP, Damsgaard CT. Simultaneous inference for multilevel linear mixed models—with an application to a large-scale school meal study. J R Stat Soc Ser C Appl Stat. 2017;66(2):295–311.

26. Bahwere P, Balaluka B, Wells JCK, Mbiribindi CN, Sadler K, Akomo P, et al. Cereals and pulse-based ready-to-use therapeutic food as an alternative to the standard milk- and peanut paste-based formulation for treating severe acute malnutrition: a noninferiority, individually randomized controlled efficacy clinical trial. Am J Clin Nutr. 2016 Apr;103(4):1145–61.

27. Kagura J, Feeley ABB, Micklesfield LK, Pettifor JM, Norris SA. Association between infant nutrition and anthropometry, and pre-pubertal body composition in urban South African children. J Dev Orig Health Dis. 2012 Dec;3(6):415–23.

28. Lelijveld N, Seal A, Wells JC, Kirkby J, Opondo C, Chimwezi E, et al. Chronic disease outcomes after severe acute malnutrition in Malawian children (ChroSAM): a cohort study. Lancet Glob Health. 2016 Sep;4(9):e654-662.

29. Prioreschi A, Munthali RJ, Kagura J, Said-Mohamed R, De Lucia Rolfe E, Micklesfield LK, et al. The associations between adult body composition and abdominal adiposity outcomes, and relative weight gain and linear growth from birth to age 22 in the Birth to Twenty Plus cohort, South Africa. PloS One. 2018;13(1):e0190483.

30. Gigante DP, Victora CG, Horta BL, Lima RC. Undernutrition in early life and body composition of adolescent males from a birth cohort study. Br J Nutr. 2007 May;97(5):949–54.

31. Martins PA, Hoffman DJ, Fernandes MTB, Nascimento CR, Roberts SB, Sesso R, et al. Stunted children gain less lean body mass and more fat mass than their non-stunted counterparts: a prospective study. Br J Nutr. 2004 Nov;92(5):819–25.

32. Sawaya AL, Dallal G, Solymos G, de Sousa MH, Ventura ML, Roberts SB, et al. Obesity and malnutrition in a Shantytown population in the city of São Paulo, Brazil. Obes Res. 1995 Sep;3 Suppl 2:107s–15s.

33. Wells JC, Sawaya AL, Wibaek R, Mwangome M, Poullas MS, Yajnik CS, et al. The double burden of malnutrition: aetiological pathways and consequences for health. Lancet Lond Engl. 2020 04;395(10217):75–88.

34. Simpson SJ, Raubenheimer D. Obesity: the protein leverage hypothesis. Obes Rev Off J Int Assoc Study Obes. 2005 May;6(2):133–42.

35. Mwene-Batu P, Wells J, Maheshe G, Hermans MP, Kalumuna E, Ngaboyeka G, et al. Body composition of adults with a history of severe acute malnutrition during childhood using the deuterium dilution method in eastern DR Congo: the Lwiro Cohort Study. Am J Clin Nutr [Internet]. 2021 Sep 28 [cited 2021 Oct 25];(nqab293). Available from: https://doi.org/10.1093/ajcn/nqab293

36. Mwene-Batu P, Lemogoum D, de le Hoye L, Bisimwa G, Hermans MP, Minani J, et al. Association between severe acute malnutrition during childhood and blood pressure during

adulthood in the eastern Democratic Republic of the Congo: the Lwiro cohort study. BMC Public Health. 2021 May 2;21(1):847.

37. Murray PG, Clayton PE. Endocrine control of growth. Am J Med Genet C Semin Med Genet. 2013 May;163C(2):76–85.

38. Golden MH. Proposed recommended nutrient densities for moderately malnourished children. Food Nutr Bull. 2009 Sep;30(3 Suppl):S267-342.

39. Smith WJ, Underwood LE, Clemmons DR. Effects of caloric or protein restriction on insulin-like growth factor-I (IGF-I) and IGF-binding proteins in children and adults. J Clin Endocrinol Metab. 1995 Feb;80(2):443–9.

40. Grenov B, Larnkjær A, Lee R, Serena A, Mølgaard C, Michaelsen KF, et al. Circulating insulin-like growth factor-1 is positively associated with growth and cognition in 6-9 year-old schoolchildren from Ghana.

41. Wells JCK, Nesse RM, Sear R, Johnstone RA, Stearns SC. Evolutionary public health: introducing the concept. The Lancet. 2017 Jul 29;390(10093):500–9.

42. Wells JCK, Devakumar D, Manandhar DS, Saville N, Chaube SS, Costello A, et al. Associations of stunting at 2 years with body composition and blood pressure at 8 years of age: longitudinal cohort analysis from lowland Nepal. Eur J Clin Nutr. 2019;73(2):302–10.

43. Walker SP, Chang SM, Powell CA. The association between early childhood stunting and weight status in late adolescence. Int J Obes 2005. 2007 Feb;31(2):347–52.

44. Hoffman DJ, Martins PA, Roberts SB, Sawaya AL. Body fat distribution in stunted compared with normal-height children from the shantytowns of São Paulo, Brazil. Nutrition. 2007 Sep 1;23(9):640–6.

45. Lord G. Role of leptin in immunology. Nutr Rev. 2002 Oct;60(10 Pt 2):S35-38; discussion S68-84, 85–7.

46. Bartz S, Mody A, Hornik C, Bain J, Muehlbauer M, Kiyimba T, et al. Severe acute malnutrition in childhood: hormonal and metabolic status at presentation, response to treatment, and predictors of mortality. J Clin Endocrinol Metab. 2014 Jun;99(6):2128–37.

47. Njunge JM, Gwela A, Kibinge NK, Ngari M, Nyamako L, Nyatichi E, et al. Biomarkers of post-discharge mortality among children with complicated severe acute malnutrition. Sci Rep. 2019 Apr 12;9(1):5981.

48. Walson JL, Berkley JA. The impact of malnutrition on childhood infections. Curr Opin Infect Dis. 2018 Jun;31(3):231–6.

49. Fabiansen C, Yaméogo CW, Devi S, Friis H, Kurpad A, Wells JC. Deuterium dilution technique for body composition assessment: resolving methodological issues in children with moderate acute malnutrition. Isotopes Environ Health Stud. 2017 Aug;53(4):344–55.

Table 1: Characteristics of 499 Kenyan 6-month-old children enrolled in a nutrition trial

Child	N	
Males	499	240 (48%)
Age at recruitment, months	499	6.0 ±0.2
Birth order	499	3 [2;5]
Age child introduced to foods or liquid, months	379	3.0 [1.5;5.0]
Breastfed at 6 months[1]	499	494 (99%)
Child morbidity last week		
Cough	499	233 (47%)
Running nost	499	212 (42%)
Diarrhea	499	115 (23%)
Fever	499	197 (39%)
Caregiver		
Mother is primary caregiver	498	486 (97%)
Caregiver age, years	498	24 [21;29]
Married	499	457 (92%)
Education, primary incomplete or less	499	254 (51%)
Religion		
Father		
Living with the family	419	343 (82%)
Age, years	390	30 [26;37]
Education, primary incomplete or less	411	128 (31%)
Household		
Number of people in the household	499	5 [4;7]
Number of children below 5 years	499	2 [1;2]
Household main income source	487	
Farming		238 (49%)
Self employed		136 (28%)
Salaried employed		73 (15%)
Other		40 (8%)
Drinking water		
Protected (well/borehole/pump/tap)	485	273 (56)

Data are presented as N, n (%), mean (±SD) or median [interquartile range]. For some variables the numbers do not add up to 499 due to missing data.
[1]Breastfed children were partially breastfed.

Table 2. Anthropometry and body composition in Kenyan males and females at 6 and 15 months of age

	6 months					15 months				
	N	Males	N	Females	p	N	Males	N	Females	p
Weight, kg	240	7.7±1.0	259	7.2±1.0	<0.001	204	9.9±1.2	224	9.4±1.1	<0.001
Length, cm	240	66.2±2.8	259	64.8±2.8	<0.001	204	75.7±3.0	224	74.5±2.9	<0.001
Mid-upper arm circumference, cm		14.4±1.3	259	14.0±1.2	0.003		14.9±1.2	224	14.7±1.1	0.09
Length-for-age Z, %	240	-0.8 [-1.6; 1.3]	259	-0.7 [-1.4;0.09]	0.09	203	-1.5 [-2.3;-0.8]	224	-1.3 [-2.1;-0.8]	0.03
					0.43					0.14
<-2		37 (15%)		28 (11%)			75 (37%)		60 (27%)	
-2≤ and <-1		64 (27%)		69 (27%)			59 (29%)		80 (36%)	
-1≤ and <0		72 (30%)		89 (34%)			50 (25%)		63 (28%)	
≥0		67 (28%)		73 (28%)			19 (9%)		21 (9%)	
Weight-for-length Z, %	240	0.3 [-0.7;1.0]	259	0.3 [-0.5;1.0]	0.60	203	0.3 [-0.4;-0.9]	224	0.3 [-0.3;1.0]	0.20
					0.19					0.11
<-2		10 (4%)		6 (2%)			6 (3%)		2 (1%)	
-2≤ and <-1		33 (14%)		23 (9%)			16 (8%)		9 (4%)	
-1≤ and <0		57 (24%)		71 (27%)			55 (27%)		72 (32%)	
≥0		140 (58%)		159 (61%)			126 (62%)		141 (63%)	
Body mass index Z		0.07±1.31		0.10±1.12	0.76		0.54±1.05		0.61±0.90	0.43
Fat mass, kg	212	1.6 [1.1;2.0]	230	1.5 [1.1;1.9]	0.17	134	1.3 [0.8;1.7]	154	1.5 [0.8;1.9]	0.21
Fat-free mass, kg	212	6.1±0.8	230	5.7±0.8	<0.001	134	8.5±1.1	154	8.0±1.0	<0.001
Fat mass index, kg/m^2	212	3.6 [2.7;4.6]	230	3.6 [2.6;4.5]	0.69	131	2.3 [1.5;3.1]	150	2.7 [1.3;3.3]	0.24
Fat-free mass index, kg/m^2	212	14.0±1.7	230	13.5±1.5	0.004	131	14.7±1.5	150	14.3±1.5	0.01
Skinfolds										
Triceps, mm	239	8.5 [7.2;10.0]	259	8.1 [6.9;9.5]	0.05	203	7.4 [6.4;8.7]	224	7.3 [6.5;8.4]	0.90
Subscapularis, mm	239	7.8 [6.3;9.2]	259	7.8 [6.7;9.1]	0.80	203	6.7 [5.8;7.8]	224	7.0 [5.9;8.0]	0.06

Data are presented as n (%), mean ± SD or median [IQR] and p-values are calculated using χ^2 tests and two-sample t-tests for categorical and continuous variables. BMI: Body mass index, FM: Fat mass, FFM: Fat-free mass, FMI: Fat mass index, FFMI: Fat-free mass index, LAZ: Length-for-age z-score, MUAC: Mid-upper arm circumference, N: Number of children in each analysis, WLZ: Weight-for-length z-score.

Table 3. Associations of sex, length-for age and weight-for-length z-scores with weight, fat-free mass (FFM) and fat mass (FM) in Kenyan children at 6 and 15 months of age

	6 months								15 months							
		Weight, kg			FFM, kg		FM, kg			Weight, kg			FFM, kg		FM, kg	
	N	Δ.	95% CI	N	Δ	95% CI	Δ	95% CI	N	Δ	95% CI	N	Δ	95% CI	Δ	95% CI
Sex[a]																
Male	240	0.49	0.30;0.69	212	0.46	0.29;0.62	0.04	-0.09;0.17	204	0.53	0.33;0.73	134	0.52	0.32;0.72	0.02	-0.23;0.25
Female	250	-	-	230	-	-	-	-	224	-	-	154	-	-	-	-
Length-for-age Z[b]																
<-2	65	-1.40*	-1.62;-1.18	60	-1.12*	-1.36;-0.88	-0.28	-0.47;-0.09	135	-1.88	-2.14;-1.62	87	-1.59	-1.94;-1.25	-0.29	-0.73;0.16
-2≤ and <-1	133	-0.83	-1.01;-0.65	118	-0.66	-0.86;-0.46	-0.17	-0.33;-0.02	139	-1.03	-1.28;-0.78	93	-0.99	-1.33;-0.65	-0.04	-0.47;0.40
-1≤ and <0	161	-0.41	-0.58;-0.25	141	-0.35	-0.53;-0.16	-0.07	-0.23;0.09	113	-0.67	-0.92;-0.41	71	-0.53	-0.88;-0.18	-0.14	-0.59;0.32
≥0	140	-	-	123	-	-	-	-	40	-	-	25	-	-	-	-
Weight-for-length Z[c]																
<-2	16	-1.75*	-2.06;-1.43	13	-1.17	-1.60;-0.74	-0.57	-0.86;-0.29	8	-2.58	-3.00;-2.16	2	-2.05	-3.10;-1.01	-0.53	-1.00;-0.05
-2≤ and <-1	56	-1.16*	-1.34;-0.97	48	-0.87*	-1.11;-0.64	-0.28	-0.47;-0.10	25	-1.62	-1.87;-1.37	17	-1.41	-1.79;-1.03	-0.21	-0.51;0.09
-1≤ and <0	128	-0.64*	-0.77;-0.51	116	-0.38*	-0.55;-0.22	-0.26	-0.40;-0.11	127	-0.95	-1.08;-0.83	87	-0.88	1.08;-0.69	-0.07	-0.28;0.14
≥0	299	-	-	265	-	-	-	-	267	-	-	170	-	-	-	-

Note. Δ = difference between categories. 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 [a]sex, [b]length-for-age or [c]weight-for-length z-score categories were included as fixed effects and children and health centre were included as random (intercept) effects. Estimates for FM were derived from the corresponding estimates for FFM and weight (with error propagation).

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

Table 4. Associations of sex, length-for-age and weight-for-length z-scores with body mass index (BMI), fat-free mass index (FFMI) and fat mass index (FMI) in Kenyan children at 6 and 15 months of age

	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	
	N	Δ	95% CI	N	Δ	95% CI	Δ	95% CI	N	Δ	95% CI	N	Δ	95% CI	Δ	95% CI
Sex[a]																
Male	240	0.37	0.08;0.66	212	0.44	0.15;0.73	-0.07	-0.32;0.18	204	0.30	0.00;0.60	134	0.43	0.07;0.79	-0.13	-0.49;0.23
Female	250	-	-	230	-	-	-	-	224	-	-	154	-	-	-	-
Length-for-age Z[b]																
<-2	65	0.21	-0.21;0.63	60	0.46	-0.02;0.93	-0.25	-0.66;0.17	135	0.31	-0.18;0.81	87	0.23	-0.45;0.92	0.08	-0.66;0.82
-2≤ and <-1	133	0.38	0.04;0.72	118	0.46	0.07;0.84	-0.07	-0.40;0.25	139	0.61	0.12;1.09	93	0.26	-0.41;0.94	0.34	-0.38;1.06
-1≤ and <0	161	0.45	0.13;0.77	141	0.38	0.01;0.76	0.06	-0.26;0.39	113	0.18	-0.30;0.66	71	0.17	-0.52;0.87	0.01	-0.73;0.74
≥0	140	-	-	123	-	-	-	-	40	-	-	25	-	-	-	-
Weight-for-length Z[c]																
<-2	16	-4.73*	-5.20;-4.26	13	-3.13	-3.87;-2.39	-1.60*	-2.09;-1.11	8	-3.76	-4.41;-3.11	2	-3.55	-5.40;-1.70	-0.21	-0.92;0.50
-2≤ and <-1	56	-3.36*	-3.36;-3.09	48	-2.19	-2.60;-1.78	-1.17	-1.51;-0.83	25	-2.73	-3.11;-2.35	17	-1,94	-2.60;-1.28	-0.79	-1.25;-0.34
-1≤ and <0	128	-2.16*	-2.35;-1.96	116	-1.25	-1.54;-0.96	-0.91*	-1.17;-0.65	127	-1.59	-1.79;-1.40	87	-1.12	-1.46;-0.78	-0.48	-0.78;-0.17
≥0	299	-	-	265	-	-	-	-	267	-	-	170	-	-	-	-

Δ Difference between categories. 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 15 months) and either [a]sex, [b]length-for-age or [c]weight-for-length z score categories were included as fixed effects and children and health centre were included as random (intercept) effects. Estimates for FMI were derived from the corresponding estimates for BMI and FFMI (with error propagation).

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

Table 5. Associations of sex, length-for age and weight-for-length z-scores with triceps and subscapular skin folds in Kenyan children at 6 and 15 months of age, including the difference between 6 and 15 months

	6 months					15 months			
		Triceps skinfold, mm		Subscapularis skinfold, mm			Triceps skinfold, mm		Subscapularis skinfold, mm	
	N	Δ	95% CI	Δ	95% CI	N	Δ	95% CI	Δ	95% CI
Sex[a]										
Male	239	0.36*	0.05;0.67	-0.02	-0.34;0.29	202	-0.03	-0.36;0.30	-0.34	-0.67;-0.01
Female	259	-	-	-	-	224	-	-	-	-
LAZ[b]										
<-2	65	-0.87	-1.35;-0.39	-0.67	-1.16;-0.19	133	-1.42	-2.00;-0.85	-0.55	-1.12;0.03
-2≤ and <-1	132	-1.02	-1.41;-0.63	-0.47	-0.86;-0.08	134	-0.86	-1.42;-0.29	-0.12	-0.68;0.44
-1≤ and <0	161	-0.39	-0.75;-0.02	-0.19	-0.56;0.17	111	-0.84	-1.41;-0.26	-0.04	-0.60;0.53
≥0	140	-	-	-	-	40	-	-	-	-
WLZ[c]										
<-2	15	-1.67	-2.51;-0.84	-1.91	-2.72;-1.11	7	-2.82	-4.02;-1.62	-3.15	-4.30;-2.00
-2≤ and <-1	56	-0.88	-1.34;-0.42	-1.18	-1.62;-0.74	25	-1.64	-2.29;-0.98	-1.57	-2.19;-0.94
-1≤ and <0	128	-0.58*	-0.91;-0.25	-0.89	-1.21;-0.58	126	-1.08	-1.42;-0.74	-1.17	-1.50;-0.85
≥0	299	-	-	-	-	260	-	-	-	-

Δ Difference between categories. 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 [a]sex, [b]length-for-age or [c]weight-for-age z-score categories were included as fixed effects and children and municipality were included as random (intercept) effects.

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