# Association between breast milk intake at 9-10 months of age and growth and development among Malawian young children

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**Contributions:** KGD, MJH, PA and KM designed the breast milk intake study and supervised the research. MA, ELP, JP, JH, and UA coordinated and supervised data collection. CK conducted the research, analysed the data and wrote the draft manuscript. All authors significantly contributed to the manuscript, read and approved the final manuscript.

#### Abstract

WHO recommends exclusive breastfeeding for infants for the first 6 months of life, followed by introduction of nutritious complementary foods alongside breastfeeding. Breast milk remains a significant source of nourishment in the second half of infancy and beyond, however it is not clear whether more breast milk is always better. The present study was designed to determine the association between amount of breast milk intake at 9-10 months of age and infant growth and development by 12-18 months of age. The study was nested in a randomized controlled trial conducted in Malawi. Regression analysis was used to determine associations between breast milk and growth and development. Mean (SD) breast milk intake at 9-10 months of age was 752 (244) g/d. Mean (SD) Length-for-age (LAZ)-score at 12 months and change in LAZ between 12 and 18 months were -1.69 (1.0) and -0.17 (0.6) respectively. At 18 months mean (SD) expressive vocabulary score was 32 (24) words and median (interquartile range) skills successfully performed for fine, gross and overall motor skills were 21 (19-22), 18 (16-19), and 38 (26-40), respectively. Breast milk (g/d) was not associated with either growth or development. Proportion of total energy intake from breast milk was negatively associated with fine motor ( $\beta$ =-0.18, P=0.015) but not other developmental scores in models adjusted for potential confounders. Among Malawian infants, neither breast milk nor percent of total energy intake from breast milk at 9-10 months is positively associated with subsequent growth between 12 and 18 months, or development at 18 months.

# **Key words**

Infant, breast milk intake, Lipid-based Nutrient Supplements, Malawi, growth and development

### Introduction

Exclusive breastfeeding is recommended for the first six months of life (UNICEF & WHO, 2003). After 6 months of age breast milk alone is not sufficient to meet the increasing demand for nutrients and energy of the growing infant, but it continues to be an important source of nutrients and energy during the second half of infancy and beyond (Dewey et al., 2003). Breast milk has immunological functions, contributing towards prevention of infection, protection against inflammation (Walker, 2010) and may also contribute towards promotion of the integrity of the intestinal barrier by regulating the gut microbiota (Rogier et al., 2014). The process of breastfeeding also plays an important role in mother-infant bonding, which may positively affect behaviour and cognitive development by enhancing mother-infant interaction.

Although continued breastfeeding is recommended up to 2 years of age and beyond, there is a lack of information about the association between the quantity of breast milk intake and health outcomes among children during the complementary feeding period. There is a concern that too much energy from complementary foods may displace breast milk (Dewey, 2000), since infants self-regulate their total energy intake (Dewey & Lonnerdal, 1986). In communities where complementary foods are of poor nutritional value, as is the case in developing countries (Dewey, 2013), too much displacement of breast milk could be detrimental to child growth and development. However, whether more breast milk is always "better" is still unclear.

The present study addressed the association between the amount of breast milk consumed at 9-10 months of age and the infant's growth and development by 12-18 months of age. The study also assessed the association between proportion of total energy intake from breast milk and growth and development. The study was conducted in rural Malawi, sub-Saharan Africa, where breastfeeding is almost universal, and a high proportion of mothers continue breastfeeding until their children reach age

18 months or beyond. The objective of this study was therefore to examine whether a greater quantity of breast milk per day, or a greater percentage of infant dietary intake from breast milk as compared to other foods, after age 6 months would be associated with indicators of growth and development.

#### **Materials and Methods**

# Study design, site and participants

This was a prospective cohort study, nested in a randomized controlled single blinded trial, the iLiNS-DOSE trial, conducted in areas surrounding Mangochi District Hospital and Namwera Health Centre in southern Malawi (Maleta et al., 2015). The iLiNS-DOSE trial was designed to test the efficacy of small quantity lipid-based nutrient supplements (SQ-LNS), in doses ranging from 10 to 40 g/day, for supporting infant growth. Healthy infants were eligible for enrollment into the trial if they were 5.50-6.49 months of age, resided in the study area, would be available during the 12-month study period, and were not concurrently participating in any other clinical trial.

Out of 1932 infants enrolled in the iLiNS-DOSE trial, 595 mother-infant pairs were invited to participate in a sub-study designed to assess the impact of SQ-LNS supplementation on breast milk intake at 9-10 months. We did not observe a significant impact of SQ-LNS supplementation on breast milk intake among infants at 9-10 months of age (Kumwenda et al., 2014). The same children were prospectively followed until they turned 18 months, their growth was measured at 12 and 18 months, and motor and language development was assessed at 18 months. We then examined the association between amount of breast milk consumed and growth and development among these children.

Mother-infant pairs were eligible for the breast milk intake sub-study if the infant was enrolled in the main iLiNS-DOSE trial, infant age was between 9.0 and 10.0 months, the mother was breastfeeding the infant on demand, and the mother and infant would be available for the full study period of 2 weeks. Participants were not eligible if the mother was breastfeeding more than one infant or the mother or infant had a severe illness warranting hospital referral. The trial was registered at ClinicalTrials.gov registration as ID: NCT00945698.

# Measurement of breast milk intake and energy from complementary foods

For each infant, breast milk intakes over a 14 day period were measured using the dose-to-mother deuterium oxide dilution technique developed by Coward et al (1982). A comprehensive description of the method is provided elsewhere (Haisma et al., 2003). The details of the assessment of breast milk intake for the present sample have also been described elsewhere (Kumwenda et al., 2014). In brief, on day zero of the sub-study, a baseline saliva sample was taken from both the mother and the infant, followed by a 30 g dose of deuterium given to the mother. Additional saliva samples were collected from both the mother and the infant on study days 1, 2, 3, 4, 13 and 14. Deuterium enrichment in the saliva of both the mother and the infant over the two-week study period was measured by Fourier transform infrared spectroscopy (FTIR 8400 Series; Shimadzu Corporation) (IAEA, 2010). Using the solver function in Excel, a two compartment steady state model (Coward et al., 1982) was run to estimate mean daily breast milk intake over the 14 day period.

Energy intake from breast milk was calculated by multiplying mean breast milk intake in grams by a factor of 0.67 kcal / g, which is the mean energy content per gram of breast milk (Butte et al., 2002). Daily energy intake from non-breast milk sources was measured by 4-pass 24-hour interactive dietary recalls (Ferguson et al., 1995; Hemsworth et al., 2016) on two days, approximately one week apart, during the same 14-day period. Daily energy consumed by infants was then calculated as the average from the two recall days.

### **Anthropometric measurements**

Anthropometric measurements were taken at age 5.9 (0.3) months (baseline), 12.0 (0.5) months, and 18 (0.8) months by trained data collectors, who were routinely supervised and re-trained every 3 months; all measurements were done in triplicate. Measuring equipment was calibrated on a regular

basis. Mothers were weighed in light clothing to the nearest 0.01 kg using an electronic scale (SECA 846; Chasmors Ltd, London England) and height was measured to the nearest 0.1 cm using a stadiometer (Harpenden; Holtain Ltd, Crosswell, UK). Infants were weighed nude to the nearest 0.01 kg using an electronic scale (SECA 735; Chasmors Ltd, London England), and length was measured to the nearest 1 mm using a length board (Harpenden Infantometer, Holtain Limited, Crosswell, Crymych, UK). Length-for-age Z-scores were calculated using the World Health Organization Child Growth Standards (WHO, Multicentre Growth Reference, & Study Group, 2006). Change in LAZ-score was calculated by subtracting Z-score at 12 months from Z-score at 18 months.

# Assessment of development at 18 months of age

Developmental assessment was conducted by trained fieldworkers who were evaluated for reliability and retrained every six mo. Children were assessed 53 weeks after enrollment, at a mean age of approximately 18 months. Motor development was assessed using the Kilifi Developmental Inventory (KDI), which is a tool that was developed in Kenya based on several standard tests originating in high-income countries, including the Griffiths Mental Development Scale and the Merrill-Palmer Scales (Abubakar et al., 2008). Children were evaluated on 35 gross motor skills, such as walking and climbing, and 34 fine motor skills, such as threading beads on a string. The score was the total number of skills the child successfully completed in each of the sub-scales (gross and fine motor) and the total motor score (sum of all 69 skills).

Language development was assessed using an adapted version of the MacArthur-Bates

Communicative Development Inventory (Fenson et al., 2007), based in part on previous adaptations of this tool in Bangladesh (Hamadani et al., 2010) and Kenya (Alcock, 2010). The score was the total number of meaningful words the child was able to say out of a one-hundred-word vocabulary checklist reported through an interview with a caregiver.

# Social-demographic and other independent variables

We measured developmental stimulation from the environment using the Family Care Indicators (FCI) score (Kariger et al., 2012), which was the sum of the source of play materials (3 items), variety of play materials (7 items), whether or not books or magazines were present in the home (2 items), and activities items (6 items) indicating whether any adult has engaged in each of six activities in the past three days (maximum 18 points).

Some social-demographic data (maternal education and age, household assets) were collected during enrolment into the main iLiNS-DOSE trial through interviews using structured questionnaires. The household asset index was constructed using principal components analysis (Vyas & Kumaranayake, 2006) and was standardized with a mean of zero and standard deviation of one. The index reflected baseline ownership of a set of assets (radio, television, refrigerator, cell phone, and stove), drinking water supply, sanitation facilities, and flooring materials.

The Household Food Insecurity Access (HFIA) Scale is a continuous measure of the degree of food insecurity (not actual food quality or intake) in the household based on experiential questions. The HFIA scale is based on a set of questions that captures perceptions and reported experiences of three domains of food insecurity: anxiety and uncertainty about the household food supply; insufficient quality; and insufficient food intake and its physical consequences (Coates et al., 2007). Each household received a score from 0-27 based on a simple sum of the frequency of occurrence of each food insecurity condition. The higher the score, the higher the degree of household food insecurity experienced in the previous four weeks.

# Statistical analysis

This study is based on a total sample size of 358 infants from the sub-study designed to assess the impact of LNS on breast milk intake (Kumwenda et al., 2014). The sample size was calculated to detect a group difference in milk intake of  $\geq$ 86 g/d between the four supplementation groups (0, 10, 20 or 40g). However, the final sample sizes used in different models in this analysis are lower than the total sample size because of missing values. With a sample size of  $\geq$ 158 for each model our study had over 80% power to detect a correlation coefficient of 0.2 at the significance level of 5%.

Data analyses were done using STATA (version 12; STATA Corp, College Station, TX). Continuous variables were assessed for normality to establish the need for data transformation. Fine, gross, and total motor development scores were skewed (skewness>1) therefore were log (k-x) transformed, where k refers to the maximum score, then multiplied by minus one (-1). This transformation reduced skewness to < 1 and preserved the original direction of the score (higher is better). For all other scores, the skewness was < 1 and therefore did not require transformation.

To ascertain if participants in the sub-study were representative of children in the main study, we compared baseline characteristics of children in the sub-study to those of children who were enrolled in the main trial but not included in the sub-study. Differences between means and proportions were compared by t-test and Chi square test respectively.

Bivariate analysis was conducted using simple linear regression to assess the independent association between breast milk intake or % total energy intake from breast milk and each outcome (attained LAZ at 12 months, change in LAZ between 12 and 18 months, motor and language scores). The following covariates were identified *a priori* to be included in the adjusted models based on the known biologically plausible relationship with growth and development: maternal education and

height, household asset score, family care index and household food insecurity. Presence of multicollinearity and adjusted associations between the two dietary variables and growth and development were assessed using multiple linear regressions. The p value of  $\leq 0.05$  was considered statistically significant for all tests.

### **Results**

The study was conducted between March 2010 and November 2011. Data are from a total of 358 mother-infant pairs who participated in the breast milk intake sub-study (**Figure 1**). There were no statistically significant differences in baseline characteristics between the 358 participants in the sub-study and those in the main iLiNS-DOSE trial (Maleta et al., 2015) who were not included in the current analysis (**Table 1**). Complete data for growth and development variables were available for 302 and 309 infants respectively (**Figure 1**). Mean breast milk energy intakes and total daily energy intakes did not differ between those with and those without growth and development data (P>0.05).

The mean (SD) breast milk intake among study participants was 752 (244) g/day or 94 (30) g/kg body weight per day. Breast milk intake ranged from 125 to 1895 g/d (**Figure 2**). The mean (SD) percent of total energy intake from breast milk was 56 (13.3) %. The mean (SD) LAZ-score at 12 months was -1.69 (1.0) and the mean (SD) change in LAZ between 12 and 18 months was -0.17 (0.6). At 18 months the mean (SD) language development score was 32 (24) and the median (interquartile range) untransformed scores for fine, gross and overall motor scores were 21 (19-22), 18 (16-19), and 38 (26-40), respectively.

In bivariate analyses, breast milk in g/d was weakly positively associated with LAZ at age 12 months (standardized  $\beta$ =0.15, P=0.012), while breast milk intake expressed as g/kg body weight per day was weakly negatively associated with LAZ at age 12 months (standardized  $\beta$ =-0.14, P=0.021) but neither was associated with change in LAZ from age 12 to 18 months (**Table 2**). Adjustment of the models for maternal height, education, infant's weight at 9-10 months, household assets, household food insecurity and family care index eliminated the associations between breast milk intake and LAZ-score (Table 2). Percent of total energy intake from breast milk was not associated with either LAZ at

age 12 months or change in LAZ, in both the bivariate analysis as well as the adjusted models (Table 2).

Results of the analysis for the association between breast milk intake or percent of total energy intake from breast milk and developmental outcomes are shown in **Table 3**. Breast milk intake expressed as g/d or g/kg body weight/d was not associated with any of the developmental outcomes in unadjusted or adjusted models (Table 3). For percent of total energy intake from breast milk, there was a small but statistically significant negative association with fine motor scores both before (standardized  $\beta$  = -0.19, P=0.003) and after adjusting (standardized  $\beta$  = -0.18, P=0.015) for maternal height, education, infant's weight at 9-10 months, household assets, household food insecurity and family care index (Table 3). Percent of total energy intake from breast milk was also negatively associated with language development (standardized  $\beta$  = -0.14, P=0.033) in the bivariate analysis, however, the association disappeared in the adjusted model (Table 3). None of the breastfeeding variables was associated with gross or total motor scores.

### **Discussion**

In the present study, we examined whether greater quantity of breast milk per day, or a greater percent of infant dietary intake from breast milk as compared to other foods, after age 6 months is directly associated with indicators of growth and development among Malawian children. We did not observe an association between either breast milk intake or percent of total energy intake from breast milk at 9-10 months and growth between 12 and 18 months among the study population. Breast milk intake was also not significantly associated with any of the developmental outcomes, while percent of total energy intake from breast milk had a weak negative association with fine motor developmental scores both in unadjusted and adjusted models.

Our study has a number of strengths: it had a large sample size, was adequately powered to detect any meaningful associations and was based on measured breast milk intake rather than maternal breastfeeding recalls, as is the case with most other studies. In the present study breast milk intake measurement preceded the outcomes we examined, enabling us to establish a temporal relationship and minimize the potential for reverse causality. The study was based on a randomly selected sample of the eligible children thus enabling generalizability of the results to the larger population of our study group. Finally we controlled for some of the possible confounders of associations between infant diet and growth or development, including maternal height, education and other socio-economic variables.

The study also had some limitations. We did not measure the energy content of breast milk in the present sample, and instead used the published mean of 0.67 kcal/g of breast milk; thus we may have under- or overestimated the true energy content of the breast milk of mothers in the present study. Additionally, breast milk was measured at 9-10 months and growth and development were measured at later ages. It is possible that breast milk intake at age 9-10 months was not indicative of the infant's intake during the entire period from 9 to 18 months and therefore had limited value in predicting

growth and development at later ages. We previously showed that breast milk intake among the present population was largely predicted by infant weight (Kumwenda et al., 2015). Even though all infants gradually reduce breast milk intake as they grow older, it is also likely that the relative ranking (i.e. high vs low consumers) of breast milk intake among children was sustained over a considerable time period. We did not measure birth size in our sample, and thus we were unable to control for size at birth which is also an important determinant of growth during infancy and childhood (Arifeen et al., 2001). Nonetheless, the results for absolute breast milk intake and percent of total energy intake from breast milk were generally similar even though the latter is less likely to be confounded by infant weight and other factors associated with infant size. Overall, our results show that breast milk intake at 9-10 months is not associated with growth between 12 and 18 months or development at 18 months within our study population.

To our knowledge, there are no previous studies investigating associations between the amount of breast milk consumed in the second half of infancy and subsequent growth and development. Previous evidence for associations with other measures of breastfeeding practices is mixed. Some studies and reviews show lack of apparent association between breastfeeding practices (i.e., any breastfeeding, early initiation, exclusive and continued breastfeeding) and growth among infants and younger children (Bhutta et al., 2008; Bork et al., 2012; Jones et al., 2014) and older children (Kramer et al., 2007; Kwok et al., 2010). In a recent systematic review and meta-analysis of breastfeeding promotion studies, Giugliani et al. (2015) also demonstrate a lack of association between promotion of optimal breastfeeding practices and growth among children. On the other hand, some breastfeeding studies from both high (Alvarado et al., 2005; Diaz et al., 1995; Martin et al., 2002) and low (Onyango et al., 1999; Simondon et al., 2001) income countries have demonstrated positive associations between breastfeeding practices (receiving breast milk at any time and duration of breastfeeding) and growth.

Since we could not compare our findings to evidence from studies that used amount of breast milk as an exposure, we cannot comment on the generalizability of our results beyond the study population.

More data are needed to elucidate the relationship between breast milk intake and growth beyond the first six months of exclusive breastfeeding.

We also did not find significant associations between amount of breast milk consumed and developmental outcomes. These findings are inconsistent with published data from younger infants.

Dewey et al. (2001) in two randomized trials showed that infants exclusively breastfed to 6 months crawled earlier and (in one of the trials) were more likely to be walking by 12 months than those who received supplementary food from 4 months. The authors speculated that the observed difference was due to the relatively greater consumption of breast milk in the exclusively breastfed than supplementary fed infants. Similar findings have been reported from studies assessing associations between breastfeeding practices such as initiation, exclusivity and duration and developmental outcomes (Sacker et al., 2006; Thorsdottir et al., 2005; Vestergaard et al., 1999). In these studies, the authors did not specifically attribute the observed associations to breast milk alone, however, they did control for possible confounding and thus it is plausible that their observed associations are explained by breast milk intake.

The inverse relationship between percent of energy intake from breast milk and fine motor developmental scores in our sample is somewhat puzzling, considering that even after 6 months of exclusive breastfeeding, breast milk continues to be an important source of nutrients essential for child growth and development. This finding may be due to the influence of other non-nutrition factors which may have mediated the relationship between amount of breast milk and fine motor development, for instance maternal education. In our previous study, we reported an inverse association between amount of breast milk intake and number of years mothers spend in school (Kumwenda et al., 2015). It has

been reported that maternal education is crucial for fine motor development (Venetsanou & Kambas, 2009) thus demonstrating that other environmental factors may be more influential than amount of breast milk on fine motor development. Sacker et al. (2006) demonstrated that an association between breastfeeding and fine motor delay became insignificant when they adjusted for biological, socioeconomic and psychosocial variables. On the other hand, Torsvik et al. (2015) observed that impairment in motor function, including fine motor skills associated with long-term exclusive breastfeeding was attributed to deficiency of Vitamin B12 among the infants.

The lack of apparent association between breast milk intake and the growth and developmental outcomes in our study may be an indication that there are other unmeasured factors that are more important than the amount of breast milk or that mask the benefits of higher quantities of breast milk for growth and development. It has been hypothesized that factors such as asymptomatic or symptomatic infections, environmental enteropathy, and an unfavorable gut microbiome may significantly impact growth of infants in settings such as those of our study site (Maleta et al., 2015).

# **Key messages**

Breast milk continues to be an important source of nourishment for healthy growth and development during the second half of infancy and beyond.

Neither the amount of breast milk nor the percent of total energy intake from breast milk at 9-10 months was associated with subsequent growth between 12 and 18 months, or gross and total motor scores or language development at 18 months. Percent of energy from breast milk was inversely associated with fine motor scores.

More data are needed to understand the contribution of breast milk to growth and development in the second half of infancy, and beyond.

# **Authorship**

KM, PA, KGD, UA and MA were members of the iLiNS Project Steering Committee, designed the overall study, and contributed to the manuscript. CK analyzed the data and drafted the manuscript. CK and JH supervised the breast milk intake and dietary intake assessment sub-studies. ELP, JP and UA coordinated and supervised anthropometry, socio-demographic and development data collection, respectively. MJH supervised the breast milk intake research. All authors critically commented on drafts and approved the final manuscript.

#### References

- Abubakar, A., Holding, P., van Baar, A., Newton, C. R., & van de Vijver, F. J. (2008). Monitoring psychomotor development in a resource-limited setting: an evaluation of the Kilifi Developmental Inventory. *Ann Trop Paediatr*, 28(3), 217-226. doi: 10.1179/146532808X335679
- Alcock, K. J., Prado, E. L., Rimba, K., Kalu, R., Newton, C. R. J. C., & Holding, P. (2010). *Parent report of language development in illiterate families the CDI in two developing country settings*. Paper presented at the The 21st Congress of the International Society for the Study of Behavioral Development, Lusaka, Zambia, Lusaka, Zambia.
- Alvarado, B. E., Tabares, R. E., Delisle, H., & Zunzunegui, M. V. (2005). [Maternal beliefs, feeding practices and nutritional status in Afro-Colombian infants]. *Arch Latinoam Nutr*, *55*(1), 55-63.
- Arifeen, S. E., Black, R. E., Caulfield, L. E., Antelman, G., & Baqui, A. H. (2001). Determinants of infant growth in the slums of Dhaka: size and maturity at birth, breastfeeding and morbidity. *Eur J Clin Nutr*, 55(3), 167-178.
- Bhutta, Z. A., Ahmed, T., Black, R. E., Cousens, S., Dewey, K., Giugliani, E., . . . Child Undernutrition Study, Group. (2008). What works? Interventions for maternal and child undernutrition and survival. *Lancet*, *371*(9610), 417-440. doi: 10.1016/S0140-6736(07)61693-6
- Bork, K., Cames, C., Barigou, S., Cournil, A., & Diallo, A. (2012). A summary index of feeding practices is positively associated with height-for-age, but only marginally with linear growth, in rural Senegalese infants and toddlers. *J Nutr*, 142(6), 1116-1122. doi: 10.3945/jn.112.157602
- Butte, N.F., Lopez-Alarcon, M.G., & Garza, C. (2002). *Nutrient adequacy of exclusive breastfeeding for the term infant during the first six months of life*. Geneva, Switzerland: World Health Organization.
- Coates, J., Swindale, A., & Bilinsky, P. (2007). *Household Food Insecurity Access Scale (HFIAS) for measurement of food access: indicator guide* (Vol. 3). Washington, DC: FHI 360/FANTA.
- Coward, W. A., Cole, T. J., Sawyer, M. B., & Prentice, A. M. (1982). Breast-milk intake measurement in mixed-fed infants by administration of deuterium oxide to their mothers. *Hum Nutr Clin Nutr*, *36*(2), 141-148.
- Dewey, K. G. (2000). Complementary feeding and breastfeeding. *Pediatrics*, 106(5), 1301.
- Dewey, K. G. (2013). The challenge of meeting nutrient needs of infants and young children during the period of complementary feeding: an evolutionary perspective. *J Nutr*, *143*(12), 2050-2054. doi: 10.3945/jn.113.182527
- Dewey, K. G., Cohen, R. J., Brown, K. H., & Rivera, L. L. (2001). Effects of exclusive breastfeeding for four versus six months on maternal nutritional status and infant motor development: results of two randomized trials in Honduras. *J Nutr*, 131(2), 262-267.
- Dewey, K. G., & Lonnerdal, B. (1986). Infant self-regulation of breast milk intake. *Acta Paediatr Scand*, 75(6), 893-898.
- Dewey, K. G., Pan American Health Organization, & WHO. (2003). Guiding principles for complementary feeding of the breastfed child (pp. 37p). Washington, DC, USA: PAHO, Washington, DC, USA
- Diaz, S., Herreros, C., Aravena, R., Casado, M. E., Reyes, M. V., & Schiappacasse, V. (1995). Breast-feeding duration and growth of fully breast-fed infants in a poor urban Chilean population. *Am J Clin Nutr*, 62(2), 371-376.

- Fenson, L, Marchman, V.A, Thal, D.J, Dale, P.S, Reznick, J.S, & Bates, E. (2007). *MacArthur-Bates Communicative Development Inventories: User's Guide and Technical Manual* (Second Edition ed.). Baltimore: Brookes Publishing.
- Ferguson, E. L., Gadowsky, S. L., Huddle, J. M., Cullinan, T. R., Lehrfeld, J., & Gibson, R. S. (1995). An interactive 24-h recall technique for assessing the adequacy of trace mineral intakes of rural Malawian women; its advantages and limitations. *Eur J Clin Nutr*, 49(8), 565-578.
- Giugliani, E. R., Horta, B. L., Loret de Mola, C., Lisboa, B. O., & Victora, C. G. (2015). Effect of breastfeeding promotion interventions on child growth: a systematic review and meta-analysis. *Acta Paediatr Suppl, 104*(467), 20-29. doi: 10.1111/apa.13160
- Haisma, H., Coward, W. A., Albernaz, E., Visser, G. H., Wells, J. C., Wright, A., & Victora, C. G. (2003). Breast milk and energy intake in exclusively, predominantly, and partially breast-fed infants. *Eur J Clin Nutr*, *57*(12), 1633-1642. doi: 10.1038/sj.ejcn.1601735
- Hamadani, J. D., Baker-Henningham, H., Tofail, F., Mehrin, F., Huda, S. N., & Grantham-McGregor, S. M. (2010). Validity and reliability of mothers' reports of language development in 1-year-old children in a large-scale survey in Bangladesh. *Food Nutr Bull*, *31*(2 Suppl), S198-206.
- Hemsworth, J., Kumwenda, C., Arimond, M., Maleta, K., Phuka, J., Rehman, A. M., . . . Ferguson, E. L. (2016). Lipid-Based Nutrient Supplements Increase Energy and Macronutrient Intakes from Complementary Food among Malawian Infants. *J Nutr, 146*(2), 326-334. doi: 10.3945/jn.115.215327
- IAEA. (2010). Stable isotope technique to assess intake of human milk in breastfed infants. Vienna: IAEA.
- Jones, A. D., Ickes, S. B., Smith, L. E., Mbuya, M. N., Chasekwa, B., Heidkamp, R. A., . . . Stoltzfus, R. J. (2014). World Health Organization infant and young child feeding indicators and their associations with child anthropometry: a synthesis of recent findings. *Matern Child Nutr, 10*(1), 1-17. doi: 10.1111/mcn.12070
- Kariger, P., Frongillo, E. A., Engle, P., Britto, P. M., Sywulka, S. M., & Menon, P. (2012). Indicators of family care for development for use in multicountry surveys. *J Health Popul Nutr*, *30*(4), 472-486.
- Kramer, M. S., Matush, L., Vanilovich, I., Platt, R. W., Bogdanovich, N., Sevkovskaya, Z., . . . Group, Probit Study. (2007). Effects of prolonged and exclusive breastfeeding on child height, weight, adiposity, and blood pressure at age 6.5 y: evidence from a large randomized trial. *Am J Clin Nutr*, 86(6), 1717-1721.
- Kumwenda, C., Dewey, K. G., Hemsworth, J., Ashorn, P., Maleta, K., & Haskell, M. J. (2014). Lipid-based nutrient supplements do not decrease breast milk intake of Malawian infants. *Am J Clin Nutr*, 99(3), 617-623. doi: 10.3945/ajcn.113.076588
- Kumwenda, C., Hemsworth, J., Phuka, J., Arimond, M., Ashorn, U., Maleta, K., . . . Dewey, K. G. (2015). Factors associated with breast milk intake among 9-10-month-old Malawian infants. *Matern Child Nutr.* doi: 10.1111/mcn.12199
- Kwok, M. K., Schooling, C. M., Lam, T. H., & Leung, G. M. (2010). Does breastfeeding protect against childhood overweight? Hong Kong's 'Children of 1997' birth cohort. *Int J Epidemiol*, *39*(1), 297-305. doi: 10.1093/ije/dyp274
- Maleta, K. M., Phuka, J., Alho, L., Cheung, Y. B., Dewey, K. G., Ashorn, U., . . . Ashorn, P. (2015). Provision of 10-40 g/d Lipid-Based Nutrient Supplements from 6 to 18 Months of Age Does Not Prevent Linear Growth Faltering in Malawi. *J Nutr*, 145(8), 1909-1915. doi: 10.3945/jn.114.208181

- Martin, R. M., Smith, G. D., Mangtani, P., Frankel, S., & Gunnell, D. (2002). Association between breast feeding and growth: the Boyd-Orr cohort study. *Arch Dis Child Fetal Neonatal Ed*, 87(3), F193-201.
- Onyango, A. W., Esrey, S. A., & Kramer, M. S. (1999). Continued breastfeeding and child growth in the second year of life: a prospective cohort study in western Kenya. *Lancet*, *354*(9195), 2041-2045. doi: 10.1016/S0140-6736(99)02168-6
- Rogier, E. W., Frantz, A. L., Bruno, M. E., Wedlund, L., Cohen, D. A., Stromberg, A. J., & Kaetzel, C. S. (2014). Secretory antibodies in breast milk promote long-term intestinal homeostasis by regulating the gut microbiota and host gene expression. *Proc Natl Acad Sci U S A*, *111*(8), 3074-3079. doi: 10.1073/pnas.1315792111
- Sacker, A., Quigley, M. A., & Kelly, Y. J. (2006). Breastfeeding and developmental delay: findings from the millennium cohort study. *Pediatrics*, 118(3), e682-689. doi: 10.1542/peds.2005-3141
- Simondon, K. B., Simondon, F., Costes, R., Delaunay, V., & Diallo, A. (2001). Breast-feeding is associated with improved growth in length, but not weight, in rural Senegalese toddlers. *Am J Clin Nutr*, 73(5), 959-967.
- Thorsdottir, I., Gunnarsdottir, I., Kvaran, M. A., & Gretarsson, S. J. (2005). Maternal body mass index, duration of exclusive breastfeeding and children's developmental status at the age of 6 years. *Eur J Clin Nutr*, 59(3), 426-431. doi: 10.1038/sj.ejcn.1602092
- Torsvik, I. K., Ueland, P. M., Markestad, T., Midttun, O., & Monsen, A. L. (2015). Motor development related to duration of exclusive breastfeeding, B vitamin status and B12 supplementation in infants with a birth weight between 2000-3000 g, results from a randomized intervention trial. *BMC Pediatr*, *15*(1), 218. doi: 10.1186/s12887-015-0533-2
- UNICEF, & WHO. (2003). Global strategy for infant and young child feeding. WHO, Geneva, Switzerland.
- Venetsanou, Fotini, & Kambas, Antonis. (2009). Environmental Factors Affecting Preschoolers' Motor Development. *Early Childhood Education Journal*, *37*(4), 319-327. doi: 10.1007/s10643-009-0350-z
- Vestergaard, M., Obel, C., Henriksen, T. B., Sorensen, H. T., Skajaa, E., & Ostergaard, J. (1999). Duration of breastfeeding and developmental milestones during the latter half of infancy. *Acta Paediatr*, 88(12), 1327-1332.
- Vyas, S., & Kumaranayake, L. (2006). Constructing socio-economic status indices: how to use principal components analysis. *Health Policy Plan*, 21(6), 459-468. doi: 10.1093/heapol/czl029
- Walker, A. (2010). Breast milk as the gold standard for protective nutrients. *J Pediatr*, 156(2 Suppl), S3-7. doi: 10.1016/j.jpeds.2009.11.021
- WHO, Multicentre Growth Reference, & Study Group. (2006). WHO Child Growth Standards based on length/height, weight and age. *Acta Paediatr Suppl*, *450*, 76-85.

# Legends to the figures

Figure 1: Participant flow

Figure 2: Histogram of breast milk intake (g/d)

Table 1: Baseline characteristics of the mother-infant pairs<sup>a</sup>

Study group	Breast milk intake sub-	Participants not included in the	P-value	
	study (n 358)	<b>sub-study</b> (n 1337)		
Infant characteristics				
Proportion of males	47%	51%	0.74	
Length-for-age z-score at 5.5-6 months of age	-1.43 (1.03)	-1.39 (1.06)	0.07	
Weight-for-length z-score at 5.5-6 months of age	0.27 (1.10)	0.25 (1.12)	0.81	
Maternal characteristics				
Height <sup>b</sup> (cm)	155.1 (5.4)	155.0 (5.7)	0.27	
Age (y) <sup>c</sup>	26 (17-43)	25 (21-30)	0.77	
Education (y) <sup>c</sup>	4 (0-12)	5 (2-7)	0.32	
Body mass index (kg/m <sup>2</sup> )	21.9 (3.0)	21.9 (2.9)	0.94	
Parity <sup>c</sup>	3 (1-9)	3 (2-4)	0.80	
Household variables				
Household food insecurity score	6 (2-13)	6 (1-10)	0.11	
Household asset Z-scores	-0.17 (-0.72-0.36)	0.04 (-0.72-0.40)	< 0.01	
Family care index	8.9 (3)	9 (3)	0.79	

<sup>&</sup>lt;sup>a</sup>Continuous values are means (SD) and were compared by t-test; proportions were compared by Chi square test. <sup>b</sup>Height and weight were measured at enrollment into the main trial. <sup>c</sup>Household asset Z-scores and maternal age, parity and education are medians (interquartile range).

Table 2: Associations between breast milk variables<sup>a</sup> and LAZ at 12 months and change in LAZ between 12 and 18 months<sup>b,c</sup>

Variables	LAZ-scores at 12 months (	(n 163)	Change in LAZ-scores from 12 to 18 months (n 158)		
	β(95 % CI)	P-value	β(95 % CI)	P-value	
Breast milk intake (g/d)	0.15 (0.03 to 0.27)	0.012	0.01 (-0.12 to 0.14)	0.908	
Breast milk intake (g/d), adjusted model <sup>d</sup>	-0.01 (-0.10 to 0.08)	0.831	0.03 (-0.09 to 0.16)	0.662	
Breast milk intake (g/kg/d)	-0.14 (-0.25 to -0.02)	0.012	0.03 (-0.10 to 0.16)	0.625	
Breast milk intake (g/kg/d), adjusted model <sup>d</sup>	-0.003 (-0.10 to 0.09)	0.952	0.01 (-0.11 to 0.14)	0.871	
% total energy intake from breast milk intake	-0.05 (-0.18 to 0.09)	0.493	0.05 (-0.09 to 0.20)	0.468	
% total energy intake from breast milk intake,	-0.08 (-0.18 to 0.02)	0.185	0.11 (-0.03 to 0.24)	0.182	
adjusted model <sup>d</sup>					

<sup>&</sup>lt;sup>a</sup>Variables measured when infants were 9-10 months old; <sup>b</sup>Analysis was done using linear regression; <sup>c</sup>Standardized regression coefficients;

<sup>&</sup>lt;sup>d</sup>Adjusted for maternal height, education, infants weight at 9-10 months, household assets, household food insecurity and family care index.

Table 3: Associations between breast milk variables<sup>a</sup> and developmental scores at 18 months<sup>b,c</sup>

Variables	18-Month Developmental scores							
	Fine motor (n 188) Gross motor (n 172)			Total motor (n 185)		Language (n 194)		
	β	P-value	β		β	P-value	β	P-value
	(95 % CI)		(95 % CI)	P-value	(95 % CI)		(95 % CI)	
Breast milk intake (g/d)	-0.02	0.771	0.06	0.356	0.06	0.277	0.07	0.204
	(-0.13 to 0.10)		(-0.07 to 0.18)		(-0.05 to 0.18)		(-0.04 to 0.19)	
Breast milk intake (g/d), adjusted	-0.05	0.436	0.04	0.558	0.03	0.652	0.08	0.190
$model^d$	(-0.16 to 0.06)		(-0.08 to 0.16)		(-0.08 to 0.13)		(-0.02 to 0.18)	
Breast milk intake (g/kg/d)	-0.08	0.168	-0.02	0.594	-0.04	0.473	0.05	0.403
	(-0.20 to 0.03)		(-0.15 to 0.11)		(-0.16 to 0.07)		(-0.07 to 0.16)	
Breast milk intake (g/kg/d) adjusted	-0.04	0.553	0.04	0.553	0.04	0.579	0.08	0.194
$model^d$	(-0.14 to 0.07)		(-0.08 to 0.15)		(-0.07 to 0.14)		(-0.02 to 0.18)	
% total energy intake from breast	-0.19	0.003	-0.01	0.865	-0.12	0.068	-0.14	0.033
milk intake	(-0.31 to -0.06)		(-0.15 to 0.13)		(-0.24 to 0.01)		(-0.26 to -0.01)	
% total energy intake from breast	-0.18	0.015	-0.02	0.755	-0.12	0.087	-0.12	0.104
milk intake adjusted model <sup>d</sup>	(-0.29 to -0.06)		(-0.15 to 0.11)		(-0.24 to 0.004)		(-0.23 to 0.01)	

<sup>a</sup>Variables measured when infants were 9-10 months old; <sup>b</sup>Analysis was done using linear regression; <sup>c</sup>Standardized regression coefficients; <sup>d</sup>Adjusted for maternal height, education, infants weight at 9-10 months, household assets, household food insecurity and family care index.

# **Figures**

Figure 1: Participant flow

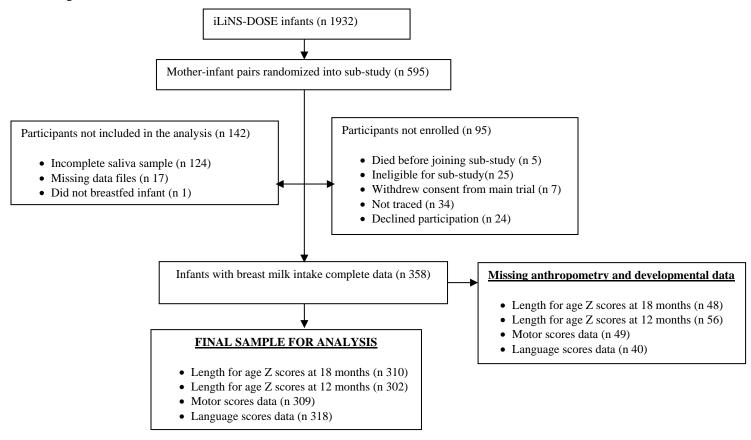


Figure 2: Histogram of breast milk intake (g/d)

