Exploring linear growth retardation in Rwandan children:

Ecological and Biological factors

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Exploring linear growth retardation in Rwandan children:

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Eric Matsiko

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Chapter 1

General Introduction

Undernutrition in Rwanda

Stunted linear growth and anaemia are both major public health concerns in developing countries, with a disproportionate burden affecting Sub-Saharan Africa. Although the global prevalence of stunting among children under five years of age has declined from 40% in 1990 to 23.2% in 2015, progress has been small in Africa, with a decrease from 45% in 1990 to 31.7% in 2015, and the Eastern African region still being hit the hardest with an estimated prevalence of 37% [1]. Moreover, in the East Africa region, the anaemia prevalence among under five years children declined from 74% in 1995 to 55% in 2011 [2]. Despite this observable decline, the prevalence of both stunting and anaemia is still high in Eastern African countries. For example in Rwanda, stunting prevalence reduced from 44% in 2010 to 38% in 2015 [3], which is still higher than the average stunting prevalence in Africa (31.7%) and slightly greater than the prevalence in the Eastern Africa region (37%) [1]. In Rwanda, stunting prevalence climaxed to 49.4% in children aged 18-23 months, though 21% of children were already stunted before their first anniversary [3]. In addition, stunting has been consistently higher amongst boys compared to girls, and the sex disparity increases as stunting prevalence declines: in 2010, 47% of boys vs. 41% of girls were stunted and in 2015, 43% of boys vs. 33% of girls were stunted [3, 4]. However, no studies have tried to explain the causes of this sex disparity. As for anaemia, its prevalence remained nearly unchanged (38% in 2010 vs. 37% in 2015), and it is greatly prevalent in children of 6-8 months of age (72%) [3]. Little is known about the causes of anaemia including the contribution of iron deficiency and other context-specific causes and the magnitude of anaemia or iron deficiency during the breastfeeding period is largely unknown in Rwanda. As opposed to stunting and anaemia, other forms of child undernutrition, notably underweight and wasting, are at much lower levels (9.3% and 2.2%, respectively) in Rwanda [3].

Despite progress in reducing its magnitude, undernutrition still costs US $820 million (504 billion Rwandan Francs) annually; this is equivalent to 11.5% of Rwanda's annual gross domestic product [5]. Therefore, it is crucial to further reduce undernutrition, particularly stunting and anaemia. To achieve this, it is necessary to deeply understand the context-specific aetiology of these two forms of undernutrition.

Determinants and health effects of stunting and anaemia

Stunting and anaemia result from the same modifiable factors. These factors include poor nutrition and morbidity at the immediate level. The immediate factors are strongly underlain by household and family factors [6] such as food insecurity, poverty, unsanitary home environment, and lack or low maternal or caregiver education [7-10]. In turn, child nutrition is mainly influenced by feeding practices. For example, inadequate breastfeeding practices mainly in the first 6 months of age, including late breastfeeding initiation, non-exclusive breastfeeding, or not breastfeeding at all, deprive the child of adequate intake of energy and nutrients, and protective components from breastmilk [9, 11, 12]. This, therefore, increases a child's susceptibility to undernutrition and morbidity, which may eventually lead to stunted linear growth [13]. To optimise child nutrition, the World Health Organization recommends feeding the child a nutritious, diverse, and safe diet at adequate frequencies from 6 months onwards [14]. However, some children mostly in developing countries are not fed accordingly disposing them to an increased risk of undernutrition [15]. It was found that inadequate complementary feeding practices played a role in the genesis and continuity of delayed child growth and in the occurrence of anaemia [16-18]. For instance, studies in developing countries reiterate that the likelihood of delayed growth and anaemia was higher in children whose diet was not diverse and whose feedings were below the minimal frequency [18, 19-21].

Recurrent infections such as diarrhoea, respiratory tract infections, intestinal parasites, malaria, and fever lead to impaired child growth and anaemia by causing inflammation, reducing food intake due to lost appetite, and diverting nutrients for their own use [22-27]. Checkley et al. (2008) reported that the risk of becoming stunted before 2 years of age increases by 25% when children have had five or more episodes of diarrhoea in the first 2 years [24]. Poor sanitation of the homes, inadequate hand hygiene, unimproved drinking water sources, and untreated drinking water underlie the occurrence of diarrhoea and worm infections [28-31]. This is a path linking water, sanitation, and hygiene to child undernutrition.

These determinants of child undernutrition may depend on the context. For example, Rwanda has a high rate of exclusive breastfeeding (81%) compared to the neighbouring countries such as Uganda (66%) [32] and Tanzania (59%) [33], yet Rwandan children are more affected by stunting (38%) compared to Uganda (29%) [32], or Tanzania (34%) [33]. Therefore, context-specific studies are necessary to better understand the aetiology of child undernutrition.

Subsequent to child stunting and anaemia, various immediate and long-term consequences occur [34]. These include susceptibility to infection, poor physical growth, inadequate cognitive and behaviour development, reduced economic productivity, and chronic diseases in later life [35-38].

Assessment of breastfeeding practices and breastmilk quantification

The World Health Organisation recommends to breastfeed exclusively for the first six months of infancy and to continue breastfeeding until no less than two years of age [39]. However, figures on exclusive breastfeeding are prone to overestimation due to social desirability of answers to questionnaires, and differences in interpretation of exclusive breastfeeding practices (e.g. feeding of drinking water, herbal tea, or traditional medicine). In addition, the information on human milk composition and maternal nutrition for sustaining infant nutritional

requirements throughout the exclusive breastfeeding period is still scare [40, 41]. This gap in knowledge is partly due to the incapacity of methods to reliably quantify breastmilk intake and to assess breastfeeding practices objectively without relying on self-reported data [42, 43]. Following recent development in the use of stable isotopes for nutritional assessment, the deuterium oxide dose-to-mother technique allows to objectively measure breastmilk output and to truly know the exclusivity of breastfeeding at the time of measurement [44]. The application of this method, in combination with analysis of breastmilk nutrient content, may permit to assess the dietary intake of young infants more accurately [40]. To determine the adequacy of breastmilk quantity and nutrient intake and to link breastfeeding patterns to children's growth, accurate quantification of breastmilk intake is essential. Studies using the deuterium oxide dose-to-mother technique have been sampling either saliva or urine to measure breastmilk output. However, saliva and urine may produce different quantities of the intended outcome as has been shown for doubly labelled water studies aiming to assess body composition and energy expenditures [45]. Therefore, it would be valuable to assess whether such differences between saliva and urine sampling also exist for the measurement of breastmilk output.

Brief description of the study country

Rwanda is a landlocked country with a surface area of 26,338 km^2 and is inhabited by around 12,756,625 million people, with a majority (75%) living in rural areas [46]. The country is divided into five administrative provinces, i.e. the City of Kigali, Eastern, Western, Northern, and Southern provinces. Each province is subdivided into districts and in total, Rwanda counts 30 districts.

A big majority of the population relies on agriculture as their main source of food and income. Staple foods of the Rwandan population are tubers, cereals, roots, legumes, and green vegetables. Certain segments of the population face the problem of food insecurity i.e. 28% of

households were at the borderline of the food consumption score in 2012 [47]. This segment of the population had a dietary pattern dominated by roots or tubers, and sometimes legumes, mainly beans. Food insecurity, poverty, and a monotonous diet make particularly the rural population susceptible to undernutrition.

Sites of the studies

To investigate the sex-specific predictors of linear growth retardation, we analysed data from a cross-sectional study that was conducted in eight districts across Rwanda (**Figure 1**).

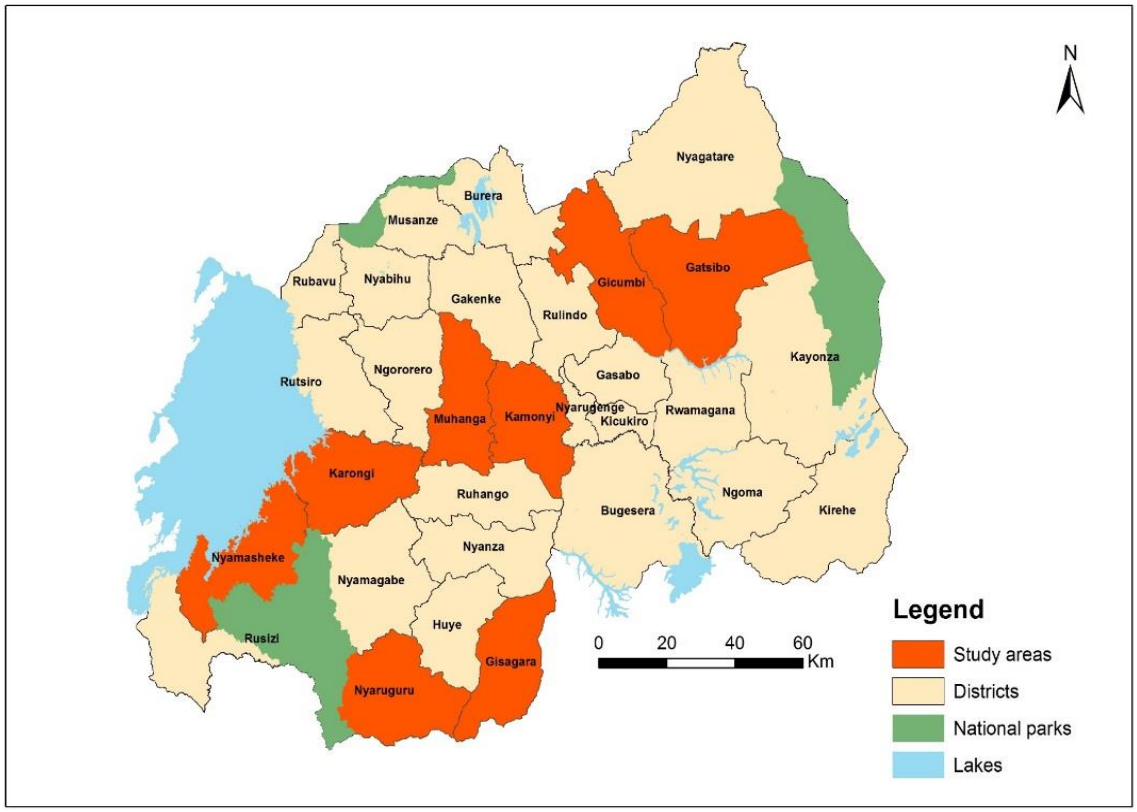

Figure 1: Districts for the cross-sectional study

Then we conducted a longitudinal study in the district of Muhanga (**Figure 2**), one of the eight districts of the cross-sectional study. The district of Muhanga is located in the southern

province and was among the districts highly affected by stunting (47%) in 2010, food insecurity (26%) in 2012, and poverty (54%) in 2011 [4, 47, 48].

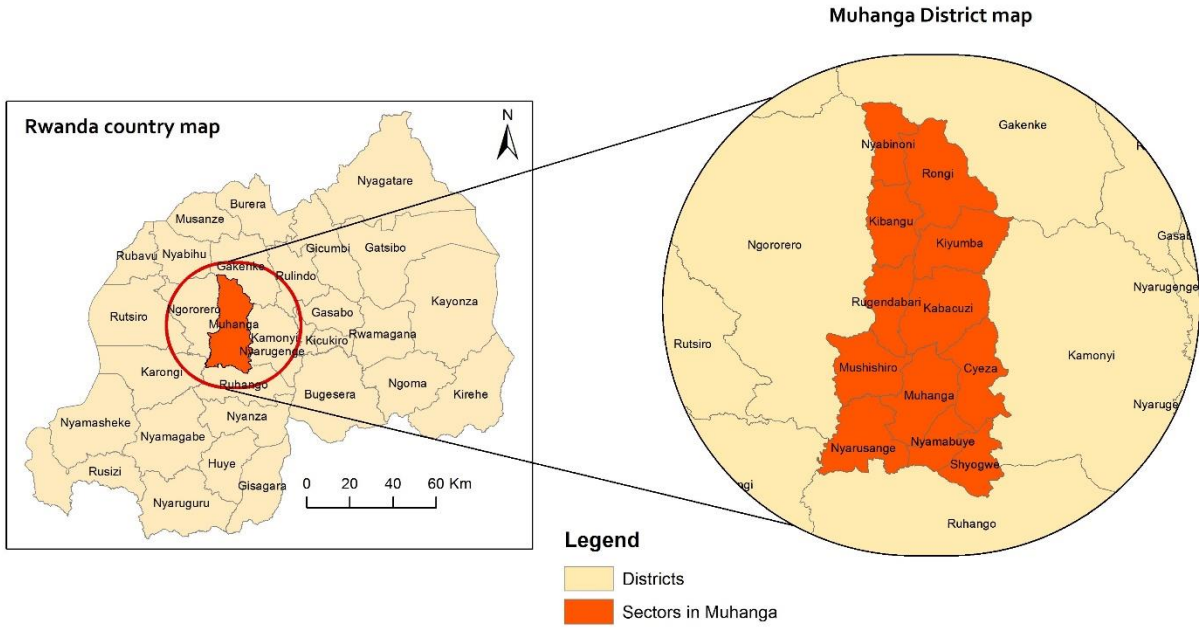

Figure 2: District of the longitudinal study.

Research questions and thesis outline

The current studies aimed to explore immediate and underlying factors of child linear growth, anaemia, and iron status among children of 0-23 months of age in Rwanda. The following research questions guided the studies:

1. What are the sex-specific factors of stunting among children aged 6-23 months in Rwanda?
2. What are the patterns and factors determining linear growth in the first year of life (0-12 months)?
3. How is dietary iron intake related to the occurrence of anaemia, iron deficiency, and iron deficiency anaemia at 12 months of age?
4. Is breastmilk intake measured based on saliva samples comparable to breastmilk intake measured based on urine samples?

Thesis Outline

The findings presented in this thesis focus on linear growth, anaemia, and iron status, and breastmilk intake quantification for children aged 0 to 23 months. **Chapter 1** gives the background and rationale of the studies presented in this thesis**. Chapter 2** describes factors that explain the disparity in stunting between boys and girls of 6-23 months of age in Rwanda. C**hapter 3** describes the patterns and predictive factors of linear growth from birth to 12 months of age and c**hapter 4** shows the magnitude of anaemia and iron deficiency among children and their mothers at 4 and 12 months postpartum. Chapter 4, in addition, discusses predictors of anaemia, iron deficiency, and iron deficiency anaemia in children of 12 months of age. **Chapter** 5 compares breastmilk intake quantified using saliva or urine samples from children of 2-4 months of age. **Chapter 6** presents a general discussion of the studies' findings, their public health importance, and recommendations for further research.

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Chapter 2

Sex-specific predictors of stunted linear growth among children of 6-23 months of age in Rwanda:

A cross-sectional study

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Abstract

***Background*:** Stunted linear growth remains a public health problem in Rwanda and it disproportionately affects more boys compared to girls. This study aimed to identify factors that explain the difference in stunting between boys and girls aged 6-23 months in Rwanda.

***Methods*:** We analysed cross-sectional data of 1228 children aged 6-23 months from eight districts with a high burden of malnutrition in Rwanda collected in 2014-2015 as the baseline survey of a nutrition program. Predictors of stunting prevalence, such as feeding practices, morbidity, and their underlying factors, were modelled using Cox proportional hazard regression with robust variance and stratified for child's sex

Results: Stunting significantly affected more boys than girls with a prevalence of 43.3% vs. 28.0%, respectively. For exposure to any risk factor, boys had a higher prevalence of stunting compared to girls. The multivariate analysis showed that being fed porridge as first weaning food as opposed to cow's milk was a significant factor for stunting prevalence in boys solely (PR=1.44, 95% CI=1.07-1.94, p-interaction=0.048) while discontinued breastfeeding was a significant factor in girls only (PR=1.49, 95% CI=1.05-2.11, p-interaction=0.017).

***Conclusions*:** The prevalence of stunting among boys is higher than among girls in Rwanda. This seems to be most strongly related to the nutritional quality of foods during the complementary feeding period. More in-depth research is required to compare feeding habits and dietary intake in relation to nutritional requirements of Rwandan boys and girls during the vulnerable second stage of the first 1000 days of their lives.

Introduction

Stunting is a major public health concern in developing countries, with the highest prevalence occurring in Sub-Saharan Africa [1]. Stunting is defined as length or height for age z-scores less than minus two standard deviations when compared to the WHO Child Growth Standard median [2]. Among the consequences of stunting, child mortality, and impaired cognitive and motor development are documented [3, 4]. Children who are stunted at 2 years are more likely to perform poorly in school, not to attain their full adult height, and to have lower economic productivity and higher morbidity [5-7]. Although the global prevalence of stunting in children younger than five has declined from 40% in 1990 to 22.3% in 2017, limited progress has been made in Eastern Africa, with a current estimated prevalence of 37% [1]. In Rwanda, the prevalence of stunting in children younger than five years recently decreased from 44% in 2010 to 38% in 2015 as assessed by Demographic Health Surveys [8, 9]. As reported for other countries [10-12], child linear growth retardation in Rwanda increases sharply from 6 months of age, coinciding with the introduction of complementary feeding and comes to a peak prevalence of 49% in children aged 18-23 months [8, 9].

Stunting is caused by multiple factors ranging from factors linked to intake of food such as food security and poverty, factors linked to inappropriate care such as level of maternal education, and factors related to childhood illnesses such as exposure to infectious diseases, access to clean water, improved sanitation, and adequate hygiene practices [3, 13, 14]. Childhood illnesses may affect stunted growth disproportionately in boys since it has been postulated that the male sex is more vulnerable to infectious diseases both at young and at old age [15-17]. Furthermore, children's diet in the areas of scarcity may provide too little energy and nutrients to fulfil the higher nutritional demands of boys [18], which poses boys to an increased risk of stunting. Although a disparity in childhood stunting to the disadvantage of

boys has been reported for the African region previously, it is not universal across countries [19-20]. However, what is striking, in the case of Rwanda, is the large disparity in the reported prevalence of stunting between boys (43%) and girls (33%) [9]. Understanding the context-specific determinants of stunting is key to accelerating stunting reduction. Therefore, in this study, we aim to identify the sex-specific household and proximal factors related to stunting among a sample of children aged 6 to 23 months of age in Rwanda by investigating differences in exposure and differences in associations.

Methods

Study site and subjects

We analysed data from the baseline survey of a nutrition program funded by the Embassy of the Kingdom of the Netherlands (EKN) and implemented by UNICEF Rwanda and its non-governmental organization partners. The program aimed at accelerating stunting reduction among children under two years old in Rwanda. Based on the prevalence of stunting, food insecurity, and poverty, ten districts out of 30 were included in the EKN nutrition program. The University of Rwanda was mandated to conduct a baseline survey in only eight districts namely: Gatsibo, Gicumbi, Gisagara, Kamonyi, Karongi, Muhanga, Nyamasheke, and Nyaruguru. The two remaining districts received additional support through another program, including a separate baseline, so they were excluded from the current baseline survey. Four of the selected districts are located in the Southern province, two in the Western province, and one district each in the Northern and Eastern provinces. The only province with no district included was the City of Kigali as stunting levels are much lower here compared to the rest of the country.

The survey had a cross-sectional study design and its primary units were administrative cells. The administrative structure in Rwanda consists of 5 provinces, 30 districts, 416 sectors, 2,148

cells, and 14,837 villages. From each district, 30 cells were selected by a probability proportionate to size sampling method. In each selected cell, exhaustive lists of all children under two years along with their mothers were compiled. Based on the lists, a sample of mother-child pairs was randomly selected. Data were collected at the households of participants, from October 2014 to January 2015, using a structured questionnaire programmed in an Open Data Kit software program (developed by the University of Washington, USA) and installed on tablets (Samsung, GT-P5220). Trained field staff took anthropometric measures of the children. Because children were younger than 24 months of age, we took their recumbent length using wooden length boards (UNICEF model) at the participants' homes. The child's length was measured in duplicate, and a third measurement was taken in case the first two measurements differed more than 0.5 cm. The survey protocol and data collection were approved by the Rwandan National Ethics Committee (No.255/RNEC/2014). The ethical guidelines as laid down in the declaration of Helsinki and its amendments were followed. In accordance, the study staff explained the study objective and procedures to the parents both verbally and in writing before they gave their written consent at the recruitment. For the child to participate, both parents signed the consent form. Because the questionnaires were administered to the mothers, they were requested to take part voluntarily in the study and also signed a consent form.

Exposure and outcome variables

The dependent outcome variable for this analysis was dichotomized into stunted and not stunted. Stunting was defined as children whose length-for-age z-scores (LAZs) were below -2 standard deviations in relation to the median LAZ of the World Health Organization (WHO) child growth standards [2]. LAZs were generated using the WHO Anthro software version 3.2.2.

The explanatory variables were selected based on the UNICEF conceptual framework of undernutrition [13]. Variables linked to immediate causes of child malnutrition included child feeding practices such as timely initiation of breastfeeding; current breastfeeding status; the age of introduction of soft, semi-solid, or solid foods; meal frequency, dietary diversity, and minimum acceptable diet. The daily meal frequencies were dichotomized into "below minimum meal frequency" or "achieved minimum meal frequency". Minimum meal frequency was defined as 2 and 3 meals a day for children of 6-8 months of age and 9-23 months of age respectively, in addition to breastmilk feeds, and 3 meals a day for non-breastfed children of 6-23 months of age [21]. The daily dietary diversity was categorized into "below minimum dietary diversity" or "achieved minimum dietary diversity" and minimum was defined here as consumption of foods from at least 4 food groups a day excluding breastmilk [21]. Accordingly, the variable of acceptable diet was also categorized into "below minimum acceptable diet" or "achieved minimum acceptable diet" and when a child concurrently achieved a daily minimum meal frequency and a daily minimum dietary diversity [21]. In addition to this, the type of first complementary foods was included. Child morbidity included illnesses such as diarrhoea, vomiting, fever, malaria, continuous coughing, and runny nose during the last two weeks. Variables linked to underlying causes of child malnutrition at the household and family level included sex of the household head, household size, agricultural land possession, livestock possession, kitchen garden availability, household wealth, household hunger indicators, mother's occupation, mother's marital status, mother's education level, and mother's ability to read and write. The household hunger indicators were calculated according to Ballard et al. [22]. Based on household living standards and economy, the Rwandan government classified its households into five wealth categories such as the poorest, poorer, poor, rich, and richest. These wealth categories were included in the questionnaire, but they were regrouped into two categories, i.e. "low" for the two lowest categories and "high"

for the three remaining categories for the purpose of the analysis. Questions on water, sanitation, and hygiene consisted of the water source, water treatment, and mothers' reported handwashing practices.

Statistical analysis

Statistical analysis was executed in Statistical Analysis Software (SAS) 9.4. The generated dataset was checked for duplicates, missing values, and outliers. All the missing observations in the explanatory variables were replaced by automatically generated values through multiple imputations. For this, all the variables were entered in the imputation model and 10 imputations were carried out with 20 iterations under a fully conditional specification imputation method [23].

All the analyses stratified for the child's sex were performed on the imputed explanatory variables. The variables with a p-value of ≤ 0.10 in Cox univariate regression analysis were included in the Cox multiple regression analysis. The Cox regression models contained a constant time set to 1 and a robust variance to calculate prevalence ratios and their 95% confidence intervals [24]. The models were adjusted for the child's age and the mother's age. We checked for multi-collinearity among variables, and the diagnostics showed that the values for variance inflation factor (VIF) and tolerance were ~1.02 and ~0.9 respectively (multi-collinearity exists when VIF values are > 4 and tolerance values are < 0.2). For confirmation that we did not miss any important variables, we ran a sex-specific principal component analysis to select variables that explained the most variability in the data. This did not lead to any changes in the selection of variables that were retained in the multiple regression models.

Results

Missing data analysis

Nineteen percent (19%) of children had a missing value in the outcome variable. Some of the explanatory variables were significantly different between children with or without missing values in the outcome variable. Variables with higher frequencies in missing data were female household head; no treatment of drinking water; mothers reporting not to wash hands before child feeding; breastfeeding not being initiated within one hour; daily meal frequency below the minimum number; and more frequent illnesses except for malaria.

Study participants

Exposure variables are equally distributed across the child sexes, but stunting prevalence differs (**Table 1**). Both total stunting (HAZ <-2SD; 43.3 vs. 28.0 %, p<0.001) and severe stunting (HAZ <-3SD; 17.3 vs. 11.6 %, p=0.003) affected more boys than girls. This disparity in stunting prevalence between boys and girls substantially widened from 12 to 17 months of age (**Figure 1**).

Concerning household and family characteristics, 12% of the boys and 11% of the girls lived in households that did not possess agricultural land; however, the prevalence of stunting was significantly higher among girls who lived in such households compared to those living in households possessing agricultural land (38.5% vs. 26.8%, p=0.039). This was not the case for the boys in whom stunting prevalence was not associated with possession of agricultural land (43.3% vs. 40.5%, p=0.64). Although the proportion of boys and girls whose mothers were illiterate was nearly the same (23% vs. 25%), girls whose mothers were unable to read or write were more soften stunted than those whose mothers could read and write (34% vs. 26%, p=0.049). However, the mother's ability to read and write did not significantly affect stunting

occurrence in boys (42.1% vs. 47.2%, p=0.31). No significant differences in stunting prevalence in boys or in girls were seen in relation to the rest of household and family characteristics.

Table 1: Characteristics of the study participants

Factor	**Boys (N=621)**	**Girls (N=607)**
Stunting prevalence		
Total (LAZ <-2SD),	43.3 (39.4, 47.2)[a]	28.0 (24.5-31.6) [a]
Severe (LAZ <-3SD)	17.3 (14.4, 20.3) [a]	11.6 (9.0-14.0) [a]
Gender of household head: *Female*	49 (7.8)	28 (4.6)
Household size: ≥ *4 people*	498 (80.2)	489 (80.6)
Agricultural land possession: *No*	75 (12.0)	65 (10.7)
Livestock possession: *No*	221 (35.6)	209 (34.4)
Kitchen garden availability: *No*	489 (78.7)	466 (76.7)
Household hunger indicators: *Moderate to Severe*	283 (45.6)	255 (42.0)
Household wealth category: *Low*	186 (30.0)	170 (28.0)
Mother education: *None*	128 (20.6)	134 (22.1)
Mother literacy: *No*	143 (23.0)	152 (25.0)
Mother occupation: *Off-farm*	78 (12.5)	152 (12.0)
Mother marital status: *No partner*	82 (13.2)	65 (10.7)
Water source: *Unimproved*	201 (33.8)	192 (31.6)
Drinking water treatment: *No*	342 (55.0)	340 (56.0)
Mother's handwashing before child feeding: *No*	270 (43.5)	277 (45.6)
Initiation of breastfeeding: *After one hour*	101 (16.3)	82 (13.5)

Table 1 continues...

Current breastfeeding status: *No*	44 (7.0)	49 (8.3)
Age at introduction of complementary foods: *< 6 months*	78 (12.6)	89 (14.7)
Type of first complementary food: *Porridge*	505 (81.4)	497 (81.8)
Meal frequency [b]: *< Minimum*	314 (50.6)	308 (50.7)
Dietary diversity scores [c]: *< Minimum*	512 (82.4)	486 (80.0)
Acceptable diversity [d]: *< Minimum*	530 (85.3)	500 (82.3)
Diarrhoea: *Yes*	232 (37.4)	214 (35.3)
Vomiting: *Yes*	119 (19.2)	126 (20.7)
Fever: *Yes*	214 (34.4)	126 (32.4)
Malaria: *Yes*	37 (6.0)	36 (6.0)
Continuous cough: *Yes*	305 (49.1)	299 (49.2)
Runny nose: *Yes*	319 (51.4)	327 (53.8)

Note: *The values in tables are frequencies and percentage in brackets. [a] Values in brackets are the 95% Confidence Intervals. [b] Minimum is defined as 2 and 3 meals a day for the children of 6-8 months and 9-23 months of age respectively, in addition to breastmilk feeds, and 3 meals a day for the non-breastfeed children of 6-23 months of age. [c] Minimum is defined as the consumption of foods from at least 4 food groups a day. [d] Minimum is defined as achieving minimum meal frequency and minimum dietary diversity. SD = Standard deviation.*

With regard to water, sanitation, and hygiene, 43.5% of boys' mothers and 45.6% of girls' mothers reported not washing their hands before feeding their children (**Table 1**). Stunting occurred significantly more often in boys in case mothers reported not washing their own hands before child feeding (48% vs. 39%, p=0.034); this was not the case for girls (28% vs. 27.4%, p=0.86). The distribution of stunting prevalence in boys and girls did not differ significantly according to the type of water source. Nevertheless, lack of treatment of drinking water was

significantly associated with a higher prevalence of stunting in boys (39% vs. 47%, p=0.043) but not in girls.

Porridge was predominantly reported to be fed as the first complementary foods and this was a similar percentage for boys (81%) and girls (82%) (**Table 1**). Stunting occurred significantly more often in boys who were fed porridge as opposed to cow's milk (46% vs. 31%, p=0.001) but not in girls (29% vs. 27.4%, p=0.39).

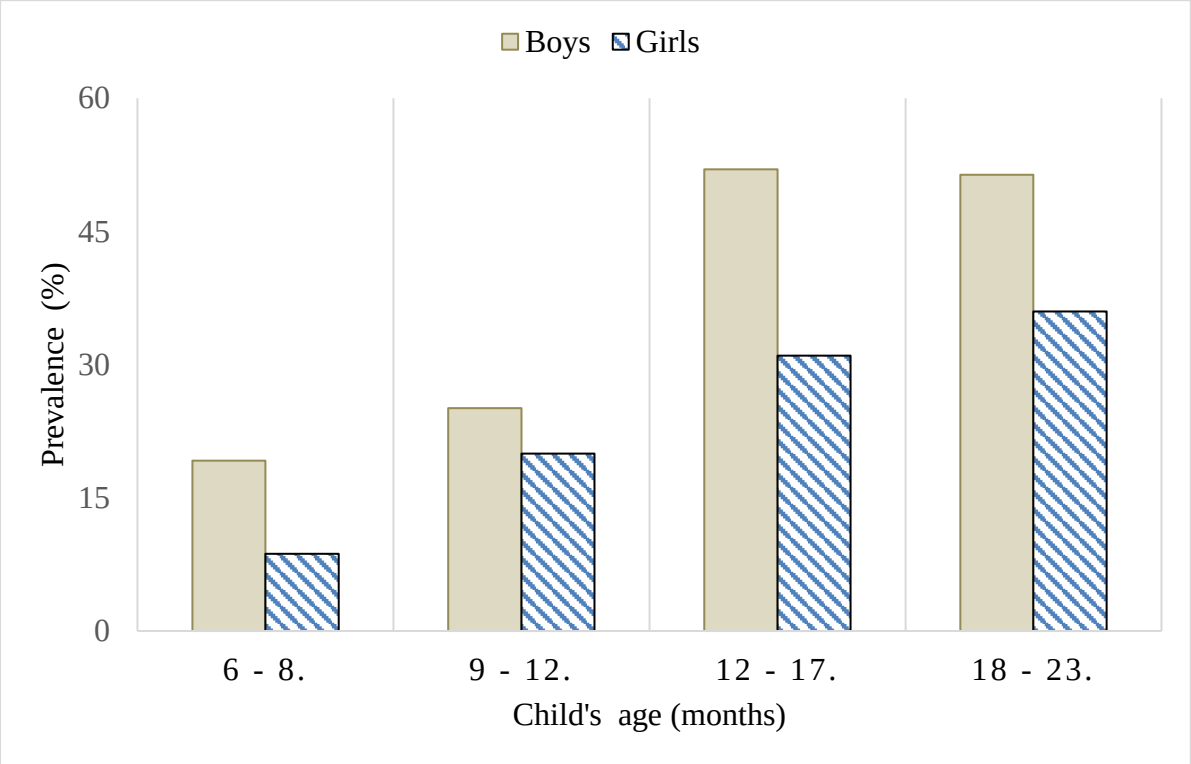

Figure 1: Disparity in stunting prevalence between boys and girls per age group

At the time of data collection, 7% of the boys and 8.3% of the girls were not breastfed anymore (**Table 1**). For the girls, discontinued breastfeeding before the age of 23 months was significantly related to a higher stunting prevalence than continued breastfeeding (46% vs. 26%, p=0.025), while for the boys no apparent difference was observed (42% vs. 43%, p=0.72). Achieving minimum dietary diversity, or a minimum meal frequency, or a minimum acceptable diet was not significantly associated with stunting prevalence either in the boys or in the girls.

Some children were reported to have suffered from different types of illnesses during the two weeks prior to the survey (**Table 1**). Runny nose, reported in 51.4% of the boys and in 54% of the girls, was the most frequent illness together with continuous coughing reported in 49% of boys and girls. Although malaria was reported in a small number (6%) of the boys and girls, it was the only illness significantly related to the occurrence of stunting in the boys (61% vs 42%, p=0.023).

Sex-specific risk factors of stunting

Table 2 presents the results of the bivariate analysis on the association of factors with stunting in boys and girls. The type of the first complementary food and lack of maternal handwashing before child feeding were specific significant correlates of stunting in the boys while unimproved water source and maternal illiteracy were specific factors of stunting prevalence in the girls.

For the boys, the prevalence of stunting increased by 50% when they were fed porridge as the first complementary food compared to when they were fed cow's milk. The prevalence of stunting increased by 30% when the boys had been sick with malaria in the previous two weeks and by 20% when the mothers reported not to wash their own hands before feeding the child. For girls, the prevalence of stunting increased by 30% when their mother was illiterate or when their households fetched water from an unsafe source (**Table 2**).

Table 2: Bivariate analysis of potential determinants of stunting for boys and girls adjusted for the child's age and mother's age

Variables	**Boys**			**Girls**		
	PR	**95% CI**	**P-value**	**PR**	**95% CI**	**P-value**
Gender of household head: *Male(R), Female*	1.28	0.68,2.38	0.444	0.97	0.55,1.69	0.907
Household size: *< 4 people (R), ≥4 people*	1.22	0.95,1.55	0.117	1.19	0.79,1.69	0.334
Land availability: *Yes (R), No*	0.90	0.68,1.20	0.465	1.39	1.00,1.95	0.053
Livestock possession: *Yes (R), No*	0.95	0.78,1.14	0.597	1.12	0.86,1.46	0.393
Possession of kitchen garden: *Yes (R), No*	1.04	0.83,1.30	0.749	1.06	0.78,1.44	0.702
Household hunger: Moderate-Severe, Little-None *(R)*	1.08	0.91,1.29	0.373	1.22	0.95,1.57	0.122
Household wealth: *Low, High(R)*	1.19	0.96,1.47	0.103	1.27	0.96,1.68	0.096
Mother education: *≥ Primary (R), None*	0.98	0.80,1.21	0.878	1.25	0.95,1.66	0.116
Mother literacy: *Yes (R), No.*	1.08	0.89,1.31	0.459	1.34	1.03,1.75	0.030
Mother occupation: *Agriculture(R), Off farm*	1.19	0.94,1.51	0.144	1.02	0.69,1.51	0.916
Mother marital status: *With partner (R), No partner*	1.06	0.83,1.36	0.636	1.25	0.89,1.76	0.194
Water source: *Improved (R), Unimproved*	1.09	0.91,1.30	0.385	1.31	1.01,1.70	0.049
Drinking water treated: *Yes (R), No*	1.16	0.97,1.39	0.385	1.27	0.98,1.65	0.074

Table 2 continues...

Mother's handwashing before child feeding: *Yes (R), No*	1.20	1.01,1.42	0.041	0.98	0.76,1.26	0.874
Initiation of breast feeding: *Within 1 hour (R), After 1 hour*	0.97	0.74,1.25	0.792	1.05	0.69,1.62	0.806
Current breast feeding: *Yes (R), No*	0.72	0.49,1.06	0.094	1.33	0.94,1.89	0.103
Type of first drink: *Cow's milk (R), Porridge*	1.46	1.10,1.94	0.010	0.96	0.69,1.32	0.795
Age of introduction of CF*: ≥6 months (R), Before 6 months*	0.85	0.60,1.25	0.444	1.15	0.77,1.72	0.497
Meal frequencies: ≥ *Minimum (R),* < *Minimum* [1]	1.17	0.95,1.44	0.150	0.93	0.65,1.32	0.671
Dietary diversity scores: ≥ *Minimum (R),* < *Minimum* [2]	1.09	0.76,1.58	0.510	1.48	0.93,2.38	0.100
Acceptable diet: ≥ *Minimum (R),* < *Minimum* [3]	1.13	0.68,1.88	0.729	1.56	0.93,2.62	0.093
Diarrhoea: *No (R), Yes*	1.10	0.92,1.31	0.281	1.02	0.78,1.33	0.881
Vomiting: *No (R), Yes*	1.18	0.96,1.45	0.121	1.06	0.78,1.44	0.697
Fever: *No (R), Yes*	1.10	0.92,1.32	0.311	1.06	0.81,1.39	0.677
Malaria: *No (R), Yes*	1.31	0.99,1.73	0.059	1.05	0.63,1.76	0.856
Continuous coughing: *No (R), Yes*	1.14	0.95,1.36	0.148	1.08	0.84,1.40	0.529
Runny nose: *No (R), Yes*	1.07	0.89,1.27	0.468	1.07	0.83,1.38	0.589

Note: PR*: Prevalence ratio,* ***CI****: Confidence interval, (****R****): Reference group. [1] The minimum is defined as 2 and 3 meals a day for the children of 6-8 months and 9-23 months of age, respectively, in addition to breastmilk feeds, and 3 meals a day for the non-breastfeed children of 6-23 months of age. [2] The minimum is defined as the consumption of foods from at least 4 food groups a day. [3] The minimum is defined as achieving the minimum meal frequency and the minimum dietary diversity.*

In the multivariate analysis, porridge as the first complementary food emerged as the only significant predictor of stunting prevalence in boys (PR=1.44, 95% CI=1.07-1.93, p-interaction with child's sex=0.048) (**Table 3**). In girls, discontinuation of breastfeeding before the age of 24 months was a significant predictor of increased stunting prevalence (PR=1.52, 95% CI=1.07-2.16, p-interaction with child's sex=0.017) (**Table 4**).

Table 3: Prevalence ratios for the association of variables with stunting in boys adjusted for the child's age and mother's age

Variables	**PR**	**95% CI**	**P-value**
Current breast feeding: *Yes (R), No*	0.75	0.52, 1.09	0.137
Type of first complementary food: *Cow's milk (R), Porridge*	1.44	1.07, 1.94	0.015
Malaria: *No (R), Yes*	1.22	0.92, 1.63	0.169
Household wealth: *High (R), Low*	1.09	0.90, 1.33	0.379
Hands washed prior to child feeding: *Yes (R), No*	1.16	0.97, 1.39	0.096

***Note**: **PR**: Prevalence ratio, **CI**: Confidence interval, (**R**): reference group*.

Table 4: Prevalence ratios for the association of variables with stunting in girls adjusted for the child's age and mother's age

Variables	**PR**	**95% CI**	**P-value**
Current breast feeding: *Yes (R), No*	1.52	1.07, 2.16	0.019
Household wealth: *High (R), Low*	1.20	0.90, 1.60	0.206
Drinking water treated: *Yes (R), No*	1.21	0.93, 1.56	0.152
Mother literacy: *Yes (R), No.*	1.24	0.94, 1.62	0.123
Water source: *Improved (R), Unimproved*	1.27	0.97, 1.64	0.075
Acceptable diet: ≥ *Minimum (R), < Minimum* [1]	1.40	0.85, 2.28	0.176

***Note: PR**: Prevalence ratio, **CI**: Confidence interval, **(R)**: Reference group.* [1] *The minimum is defined as achieving the minimum meal frequency and the minimum dietary diversity.*

Discussion

In our analysis, the sex-specific factors determining stunting prevalence among children of 6 to 23 months old were identified from data recently collected in eight districts in Rwanda with a high prevalence of stunting, food insecurity, and poverty. We found that exposure variables did not differ between the sexes, but that boys are profoundly more often stunted than girls (43% vs. 28%). This finding reiterates earlier Rwanda demographic and health survey reports of a higher stunting prevalence in boys [8, 9]. Our observations are also consistent with reports from other East African countries, such as Tanzania [25, 26], Kenya [27, 28], Uganda [30] and Ethiopia [29, 31]. However, none of these studies explains the disparity in the undernourishment between the sexes. In the current study, however, feeding porridge as the first complementary food compared to cow's milk as well as discontinued breastfeeding were the two factors that independently explained the sex-based disparity in stunting between boys and girls.

A similar proportion of boys and girls was fed porridge as their first complementary food as opposed to cow's milk, but only for boys, this practice was associated with an increased prevalence of stunting. Furthermore, discontinued breastfeeding before the age of 24 months, which occurred in less than 10% of boys and girls, was associated with an increased prevalence of stunting in girls, while for boys this tended to be the other way around. One of the explanations for this may be that the combination of continued breastfeeding with a cereal-based porridge provided too little energy and nutrients to fulfil the higher nutritional demands of boys, whereas this was less of an issue for girls [18]. Porridge usually is the first and common complementary food throughout childhood in Sub-Saharan Africa [32], but is often of a watery consistency and poor nutritional quality [25, 33, 34], posing children at risk of malnutrition [32, 35, 36]. In Rwanda, porridge fed to children is mainly based on cereals, and a recent study confirms that complementary foods provide insufficient nutrients [37]. Contrary to porridge, cow's milk was linked to reduced prevalence of stunting in boys in the current study. Feeding cow's milk may also have trade-offs since it has been associated with increased risk of anaemia and allergy in children [38, 39]. Similar to our observation in girls, Onyango et al. (1999) reported that a shorter duration of breastfeeding was associated with decreased vertical growth [40]. The lack of a similar association in boys may in part be explained by reverse causality that triggers mothers to continue breastfeeding children who do not thrive well [41, 42].

The question arises whether the above-described feeding practices sufficiently explain the observed disparity in stunting between boys and girls. Although in multivariate analysis, none of the morbidity indicators were significantly associated with stunting, it is known that frequent infections can contribute to interrupted or retarded growth [43-45]. This would explain the bivariate associations of malaria and lack of handwashing of mothers with stunting in boys, the

latter exposing them to harmful pathogens [46, 47]. However, both associations were not significant anymore in the multivariate model. Since our data only reflected morbidity in the two weeks prior to data collection, we may have failed to fully capture its association to stunting. Data on inflammation and other biomarkers related to morbidity could have given additional evidence but this was not collected as part of this study. Morbidity may affect stunted growth disproportionately in boys since it has been postulated that the male sex is more vulnerable to infectious diseases both at young and at old age [15-17]. This may be related on the one hand to later lung maturation in male neonates [48] and on the other hand to a weaker immune response in comparison to girls due to protective hormones related to the X chromosome [49]. A higher male neonatal and infant mortality has been observed rather universally across populations, despite a higher male birth rate. It has been postulated that in the areas with a high level of deprivation, parents may consciously or unconsciously prioritize girls over boys. This is explained from an evolutionary perspective by reasoning that to maximize future reproductive success it is more critical for girls to survive than for boys, regardless of the girl's health condition [15]. This natural selection process may be extended up till late in childhood [15]. Alternatively, in a case of better conditions and a higher male-to-female birth ratio, parents may invest more in the rarer gender. Both explanations may play a role in Rwanda, dependent on the level of deprivation households are facing year by year.

Even though some studies found that socioeconomic characteristics are associated with stunting [28, 50-53], surprisingly, in the present study, we did not find any independent socio-economic factor that was significantly associated with stunting either in boys or in girls. Similar to our findings, Wamani et al. (2007) found that there was no significant interaction between household socio-economic status and child sex for the risk of stunting [19].

The main strength of this study is that we analysed a large sample of children from districts scattered across Rwanda. However, a major limitation of this study is that the analysis was based on cross-sectional data and therefore we cannot establish any causal relationships to explain fully why the sex disparity exists. In addition, with these data, we cannot explore if there are any sex-based parental care practices or preferences in favour of girls or to the disadvantage of boys. We also did not collect data on quantities of foods consumed so to further explore dietary adequacy between boys and girls. Additionally, data collection relied on the respondent's memory, thus it might have caused recall bias. Furthermore, the study was conducted in only 8 out of 30 districts in Rwanda, and for this reason, the generalizability of our findings is limited. The missing values in the outcome variable for explanatory factors might have biased their association with stunting. Finally, we cannot exclude the effects of unmeasured variables, for example, biological factors and parental care practices, despite including an extensive set of variables in our analysis.

In conclusion, this study shows that the prevalence of stunting among young children is high in Rwanda but remains substantially higher among boys. Although the higher prevalence of stunting among boys is prominently related to the type of their first complementary food, boys are more stunted than girls in relation to any of the factors. It is still to be elucidated further whether the disparity in stunting prevalence between the sexes is explained by dietary adequacy, or also by differentials in morbidity pattern and parental care practices during the vulnerable second stage of the first 1000 days of their lives.

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Chapter 3

Exploring patterns and predictors of linear growth from birth to 12 months of age in Rwanda:

A longitudinal investigation

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Abstract

***Background*:** In Rwanda, linear growth retardation is the prevailing form of undernutrition during childhood. With the current study, we aimed to longitudinally investigate the influence of feeding practices on child linear growth among Rwandan children from birth to 1 year of age.

***Methods*:** We conducted a longitudinal study on 192 mother-child pairs living in a rural area in Rwanda. From birth to 1 year of age, anthropometric measures and feeding data were collected monthly. To identify predictors of linear growth, the child's length and its potential predictors were analysed using a linear mixed model. We stratified the analysis by age ranges of 0-5, 6-12, and 0-12 months to consider the differences in the start of feeding practices.

***Results*:** The study indicated that the children were born with length deficits (mean length deficit of -1.4 cm compared to the normative median length of the 2006 WHO child growth standard), which gradually deteriorated with the child's age (mean length deficit of -2.7 cm at one year compared to the median normative length of the 2006 WHO child growth standard). Early introduction of complementary feeding had a negative effect on linear growth (-0.69 cm, 95% CI: -0.90, -0.49), as well as the duration of diarrhoeal disease (-0.02 cm, 95% CI: -0.04, -0.00) from 0-12 months of age. Late initiation of breastfeeding (-0.73 cm, 95% CI: -1.45, -0.01) and the duration of malaria infection (-0.02 cm, 95% CI: -0.05, -0.00) led to diminished growth in the second half of infancy. High frequency of breastfeeding (-0.01 cm, 95% CI: -0.02, -0.00) and duration of diarrhoeal disease (-0.02 cm, 95% CI; -0.04, -0.00) affected linear growth negatively in the first half of infancy.

Conclusion: Late initiation of breastfeeding, high breastfeeding frequency, early introduction of complementary feeding, and the duration of malaria and diarrhoea predicted diminished postnatal linear growth. However, since growth retardation appears to have manifested already

at birth, improving maternal nutritional status may be even more important to ensure that children are born with adequate body dimensions.

Introduction

Linear growth retardation is the most prevailing form of malnutrition during childhood and it is estimated that globally 149 million children under five years of age are stunted (height-for-age z-score < -2 standard deviations) [1]. Most children under five years of age who experience stunted growth live in less developed countries (146 million) with 59 million living in Africa. Large differences in stunting patterns exist between countries in Africa [2-4], and East Africa counts a higher number of stunted children (24 million) than any other African region [1]. Furthermore, Africa and particularly Eastern Africa are off the desired trajectory for achieving the 2025 targets in stunting reduction [1]. This shows that linear growth retardation is still a major public health problem in the East Africa. In addition, based on the normative median height set by the 2006 WHO child growth standards, there are still children with deficient growth despite not being stunted. While linear growth deficit usually deteriorates sharply from birth to two years of age, it starts already earlier in life during the foetal period [3]. Leroy et al. (2014) show that the length deficit accumulated during foetal life and in infancy contributes 62.5% to the total height deficit accrued at 5 years of age in the countries of Sub-Saharan Africa [5].

Once manifested in the first two years of life, retarded linear growth profoundly affects the realisation of full physical and developmental potential [6-8]. In the short term, linear growth retardation pre-disposes a child to poor psycho-motor development [7], which delays mental developmental maturity and may eventually reduce the child's cognitive ability [9]. In the long term, reduced cognitive ability subsequently affects a child's schooling performance, which is one of the risk factors of reduced work productivity in adulthood [9, 10]. Moreover, retarded linear growth is associated with increased risk of chronic diseases in adulthood [11].

The causes of linear growth deficit are multifactorial and they comprise, amongst many others, modifiable factors such as nutrition and morbidity at a proximal level [12, 13]. Studies in different settings have shown that inadequate breastfeeding and complementary feeding practices impair linear growth in early life [14, 15]. These risky feeding practices include late initiation or early cessation of breastfeeding [4, 16, 17], early or late introduction of complementary feeding [18], insufficient feeding frequency, poor dietary diversity, and inadequate nutrient content of complementary foods [19, 20]. Suboptimal feeding practices, together with unsanitary home environment and unsafe foods, may increase the child's risk to infectious diseases such as diarrhoea and other morbidities [21-23], which in turn, may lead to stunted linear growth through causing nutrient losses, decreasing nutrient uptake due to reduced appetite, and causing inflammation [21, 24, 25].

Concerning Rwanda, the level of linear growth retardation among children under five years of age has been slowly declining over the years, with a reported stunting prevalence of 44% in 2010 and 38% in 2015 [26]. Within the Eastern African region, Rwanda has a higher linear growth retardation compared to Tanzania, Kenya, and Uganda [3]. However, Rwanda has a higher rate of exclusive breastfeeding (87%) [26] compared to the neighbouring countries such as Uganda (66%) [27] and Tanzania (59%) [28]. Therefore, it seems that there are context-specific determinants, which drive the observable disparity in the prevalence of stunted growth among neighbouring countries. Most of the studies that tried to explain the causes of stunted growth were of the cross-sectional design. Studies with a longitudinal design are, however, better placed to give insight into cause-effect relationships [29]. Therefore, the current study aimed to longitudinally investigate the patterns of early linear growth and to assess the influence of feeding practices on child linear growth among Rwandan children from birth to one year of age.

Methods

Study site and subjects

We conducted a community-based longitudinal study from December 2016 to May 2018 in the district of Muhanga, one of the districts affected by high levels of linear growth retardation in Rwanda. This study was specifically implemented in the catchment areas of Rutobwe and Buramba health centres located in a rural part of the district. After getting positive advice from the medical ethical committee of Wageningen University and Research, the Rwanda National Ethics Committee approved the study (No. 734/RNEC/2016). We followed the ethical guidelines as laid down in the declaration of Helsinki and its amendments. In accordance, the study objective and procedures were explained to parents both verbally and in writing before they gave their written consent to participate. Each expectant mother was pre-contacted to inform her about the research before she came to the health centre to wait for delivery and her child and herself were enrolled shortly after birth (within 12 hours of birth). The informed consent for the child was obtained from both parents in case a child had both parents, otherwise only the mother consented for her child. We enrolled a convenience sample of 192 mother-child pairs into the study.

Data collection

Before the start of the fieldwork, research assistants were trained on the research procedures, questionnaires, and anthropometrics, and all study procedures were pre-tested. Shortly after birth, we measured the weight and length of the new-borns and while still confined at the health centres, the mothers were interviewed to collect data on their pregnancy and on breastfeeding practices. Two weeks later, at their homes, the mothers were interviewed again to collect data on the characteristics of their households. From then, mothers were interviewed monthly to collect data on breastfeeding and complementary feeding practices and on morbidity of their

children in the previous 30 days. We started collecting data on meal frequency and dietary diversity at 6 months of age. Child length was measured using wooden length boards (UNICEF model) at birth and on a monthly basis until 12 months of age and was recorded to the nearest 0.1 cm. The child's weight was taken using an electronic scale (Seca 878) and recorded to the near 0.1 kg. Weight and length were measured and recorded in duplicate and averaged.

Exposure and outcome variables

Length of the child was used as an outcome variable in the bivariate and multivariate analysis while indicators of breastfeeding and complementary feeding practices were the main independent predictors of child linear growth. For the breastfeeding indicators, we included variables such as the initiation of breastfeeding dichotomised into "within one hour of birth" or "after one hour of birth", and breastfeeding frequency in the last 24 hours (self-reported by the mothers). For the complementary feeding indicators, we included variables such as the age of introduction of complementary feeding, meal frequencies, dietary diversity, and acceptable diet. The age of introduction of complementary feeding was dichotomised into "early introduction" i.e. before 6 months of age or "timely introduction" i.e. between 6-8 months of age. There were no children introduced to complementary feeding later than 8 months of age. The daily meal frequencies were dichotomised into "below minimum meal frequency" or "achieved minimum meal frequency". The minimum meal frequency was defined as 2 and 3 meals a day for the children of 6-8 months of age and 9-12 months of age respectively, in addition to breastmilk feeds, and 3 meals a day for the non-breastfeed children of 6-12 months of age [31]. The daily dietary diversity was categorised into "below minimum dietary diversity" or "achieved minimum dietary diversity" and the minimum was defined here as the consumption of foods from at least 4 food groups a day excluding breastmilk [30]. Accordingly, the variable of acceptable diet was also categorised into "below minimum acceptable diet" or "achieved minimum acceptable diet" and the minimum acceptable diet was

defined as concurrently achieving a daily minimum meal frequency and a daily minimum dietary diversity [30].

Childhood illnesses are considered as proximal predictors of linear growth [12]. Child's illnesses that we considered were diarrhoea, acute respiratory tract infections (comprising coughing, runny nose, and wheezing), and malaria. All the morbidities were self-reported by the mothers based on the symptoms except for malaria, which was based on community health worker diagnosis (malaria is routinely diagnosed and treated by the community health workers in Rwanda). For linear mixed modelling, we summed up the number of days a given child had been ill with the disease each month from birth to 12 months of age to get the illness duration (cumulative days). Furthermore, we also considered the following factors in the models: child's sex, maternal age, maternal height, maternal education, household wealth index calculated based on household assets and utilities, and household hunger scores calculated according to Ballard et al., 2011 [31]. The causal relationships among variables relative to the outcome variable are shown in **Figure 1,** which was constructed using DAGitty software [32].

Statistical analysis

We calculated length-for-age differences by subtracting the measured length of each child from the median length of the sex and age-specific WHO child growth standard. Further, we generated z-scores of length-for-age using Anthro software version 3.2 to estimate the prevalence of stunting [33]. Because the complementary feeding normally starts at 6 months of age, we stratified the analysis into age ranges of 0-5, 6-12, and 0-12 months and based on these age ranges, we ran the analysis for all children and for boys and girls separately. All statistical analyses were executed in a statistical analysis system (SAS) software, version 9.4 (SAS Institute Inc, USA). Variables with a p-value of ≤ 0.10 in the bivariate modelling qualified for the multivariable modelling. To avoid multi-collinearity the identified potential

independent predictors were checked for correlation and predictors with correlation coefficients < 0.3 were used in the final multivariable models.

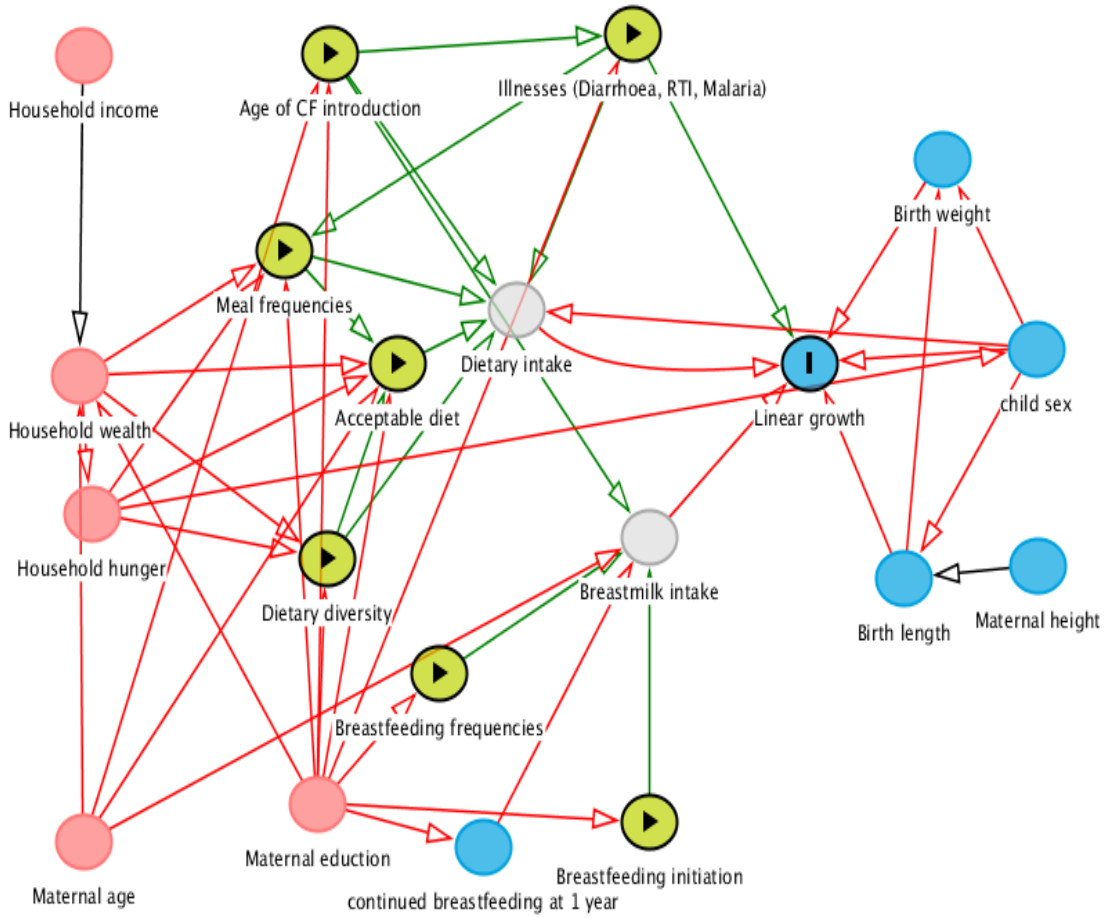


Figure 1: The causal relationships among variables relative to the outcome variable.

Exposure *Outcome* *Ancestor of outcome* *Ancestor of exposure and outcome* *Unobserved (latent)* *Causal path* *Biasing path.*

Based on model fit, the linear mixed models that we used contained a polynomial quadratic equation for time (months), three random effects i.e. intercept, slope, and curvature, and the unstructured covariance matrix. The model estimation method was the maximum likelihood. Statistical significance was based on P-values being less than 0.05. Based on the DAGitty analysis (**Figure 1**), we adjusted the multivariable models for the mother's age and education and for the household wealth.

Results

Study participants

Of 192 enrolled mother-child pairs, 12 did not complete the study including six who moved to different locations, two children who died, and four who voluntarily ended their participation. From birth to 12 months of age, the mean length of the children increased by 24 cm (95% CI: 23.7, 24.4). **Table 1** shows the participant characteristics based on study completers (n=180) in relation to length increment from birth to 12 months of age. The characteristics are classified into child feeding variables, child illnesses, and other variables.

Regarding child feeding practices, 13.3% of new-borns were initiated to breastfeeding late (after one hour of birth), which was associated with a significantly smaller mean annual length increment as compared to those who were timely initiated to breastfeeding (22.8 cm vs. 24.2 cm, p=0.006) (**Table 1**). Some study children were early introduced to complementary foods (under 6 months of age) and their number increased with age to become 49.5% at 5 months of age (**Figure 2**). The rest of the children (50.5%) were timely introduced to complementary foods (6-8 months of age). Comparing boys and girls, while at 3 months of age, boys were more often already introduced to complementary foods compared to girls (11.4% vs. 4%), at 5 months of age more girls than boys (53% vs. 37%) already received complementary foods (**Appendix 1**). However, the mean length increase from birth to 12 months of age did not differ between those with early and timely introduction of complementary feeding (**Table 1**). Seventy-seven percent (77%) of the mothers indicated that perceived inadequacy of breastmilk production was the main reason that triggered them to the early introduction of complementary feeding.

Table 1: Participant characteristics and their association with length increment from 0 to 12 months of age

Participants' characteristics	**n, %** **Median (IQR)**	**Length increment (0-12 months)**	
		Mean±SD	**P-value** [1]
Feeding practices			
Initiation of breastfeeding			
Early (within one hour)	156 (86.7)	24.2±2.2	0.006
Late (after one hour)	24 (13.3)	22.8±2.1	
Breastfed at 12 months	178 (98.8)		
Breastfeeding frequency	15 (10, 20)		
Age of introduction of complementary feeding	6 (5, 6)		
Timely (6-8 months)	91 (50.5)	24.1±2.4	0.640
Early (< 6 months)	89 (49.5)	23.9±2.0	
Child meal frequency [2, 3]	2 (2, 3)		
≥ *Minimum*	73 (40.6)	9.3±1.2	0.851
< *Minimum*	107 (59.4)	9.2±1.8	
Child dietary diversity score [2, 4]	3 (2, 4)		
≥ *Minimum*	86 (47.8)	9.4±1.5	0.213
< *Minimum*	94 (52.2)	9.1±1.7	
Child acceptable diet [2, 5]	1 (1, 2)		
≥ *Minimum*	41 (22.8)	9.3±1.4	0.939
< *Minimum*	137 (77.2)	9.3±1.6	

Table 1 continues...

Child illness [6]			
Diarrhoeal episodes	2.5 (1, 4)		
None	15 (8.0)	24.1±2.1	0.884
Any	165 (92.0)	24.0±2.2	
Duration (days till 12 months of age	14 (7, 24)		
Malarial episodes	0.0 (0, 1)		
None	128 (71.0)	24.3±1.8	0.010
Any	52 (29.0)	23.3±2.2	
Duration (days till 12 months of age)	7 (3, 10)		
Respiratory tract infection [7] episodes	8.0 (6, 10)		
< 6 episodes	22 (12.0)	23.8±2.3	0.159
≥ 6 episodes	158 (88.0)	24.1±2.2	
Duration (days till 12 months of age	36 (21, 55)		
Other variables			
Child Sex			
Boys	83 (46.0)	24.4±2.1	0.026
Girls	97 (54.0)	23.7±2.3	
Childbirth weight	2900 (2635, 3145)		
Low birth weight (< 2,500g)	25 (14.0)	24.5±2.2	0.300
Normal birth weight (≥ 2,500g)	155 (86.0)	23.9±2.2	
Mather's age at birth	31.0 (25.0, 36.0)		
Mather's height	157.4 (153.6, 160.7)		
< 145 cm	2 (1.2)	23.7±1.2	0.844
≥ 145 cm	178 (98.8)	24.0±2.2	

Table 1 continues...

Mother education			
None	6 (3.3)	22.9±2.8	0.419
Primary incomplete	77 (42.8)	23.9±2.3	
Primary complete	77 (42.8)	24.1±2.1	
Any secondary	20 (11.1)	24.3±2.1	
Household wealth			
Low	72 (40.0)	24.0±2.3	0.843
High	108 (60.0)	24.0±2.4	
Household income per month			
< 10,000Rwf	102 (57.0)	24.3±2.2	0.079
≥ 10,000Rwf	78 (43.0)	23.7±2.2	
Household hunger indicators	0 (0.0, 2.0)		
No Hunger (Little to none)	132 (73.0)	23.9±2.4	0.310
Hunger (Moderate to Severe)	48 (27.0)	24.3±1.2	

Note: *For the continuous variables, the median values with their interquartile range (1st and 3rd quartiles) in brackets are reported while for the categorical variables, the frequency and the percentages in brackets are reported.* [1] *The p-value for the independent T-Test to compare means of the variables.* [2] *as assessed at 12 months of age and the mean length increment was from 6 to 12 months of age.* [3] *The minimum is defined as 2 and 3 meals a day for the children of 6-8 months and 9-12 months respectively, in addition to breastmilk feeds, and 3 meals a day for the non-breastfeed children of 6-12 months of age.* [4] *The minimum is defined as the consumption of foods from at least 4 food groups a day.* [5] *The minimum is defined as achieving the minimum meal frequency and the minimum dietary diversity concurrently.* [6] *Illness occurrences were reported over the last 30 days from 0 to 12 months of age (greatest number of episodes is 12 and the duration is the days of having illness summed up from 0 to 12 months of age).* [7] *included coughing, runny nose, and wheezing. SD = Standard Deviation.*

From 6 to 12 months of age, the proportion of children who were fed below the daily minimum meal frequency fluctuated with age from 69% at 6 months of age through 25% at 8 months of age to 59.4% at 12 months of age (**Figure 2**). The proportion of children who ate a diet below the minimum diversity decreased from 91% at 6 months of age to 50% at 8 months of age and

then it remained relatively the same (47%-52%) until 12 months of age (**Figure 2**). Children who did not eat a minimally acceptable diet also varied with age from 75.3% at 6 months of age through 86.7% at 9 months of age to 77.2% at 12 months of age (**Figure 2**). Moreover, a similar fluctuation in the proportion of children who did not achieve a minimal meal frequency, a minimal dietary diversity, and a minimally acceptable diet occurred in boys and in girls (**Appendix 1**).

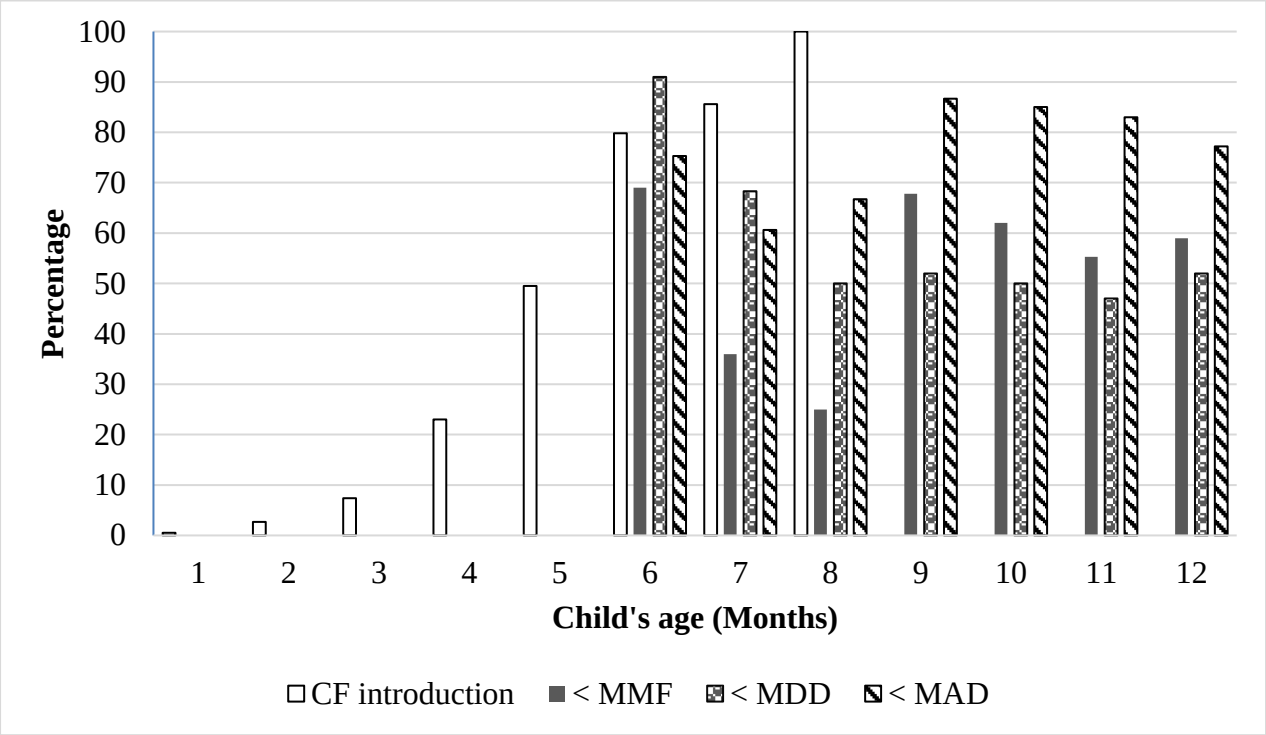


Figure 2: Distribution of child feeding indicators by child's age (month)

Note: ***CF****: Complementary Feeding (cumulative percentage).* ***MMF*** *is the minimum meal frequency and is defined as 2 and 3 meals a day for the children of 6-8 months and 9-12 months of age respectively, in addition to breastmilk feeds and 3 meals a day for the non-breastfeed children of 6-12 months of age.* ***MDD*** *is the minimum dietary diversity and is defined as the consumption of foods from at least 4 food groups a day.* ***MAD*** *is the minimum acceptable diet and is defined as concurrently achieving the MMF and the MDD.*

However, achieving these indicators of complementary feeding practices or not was not significantly associated with mean length increment (**Table 1).** Conversely, based on child's

sex, the mean length increment from 6 to 12 months of age in boys who did not eat a diet of a minimal diversity tended to be smaller than the mean length increment in boys who ate a diet of a minimal diversity (8.9 cm vs. 9.6 cm, p=0.082).

From 0 to 12 months of age, the illnesses that affected children the most was acute respiratory tract infection (runny nose, coughing and wheezing), reported in almost all children (99.5% had at least one episode a year or 88% had at least six episodes a year). The duration that children suffered from acute respiratory tract infection was 36 days (the median value) a year. Ninety-two percent (92%) of the children were reported to have had at least one diarrhoeal episode and the duration of diarrhoeal disease was 14 days (the median value) a year. Twenty-nine percent (29%) of the children were reported to have had at least one malarial episode and the duration of malarial infection was 7 days (the median value) a year. When malaria was reported, the children had a significantly smaller mean annual length increment compared to when it was not reported (23.3 cm vs. 24 cm, p=0.010) for all children (**Table 1**) and (23.4 cm vs. 24.8 cm, p=0.007) for boys (**Appendix 2**).

The prevalence of low birth weight was 14%, although linear growth increment among these children was not significantly different from those born with adequate birth weight. In general, girls had a significantly smaller annual length increment compared to boys (23.7 cm vs. 24.4 cm, p=0.026) while the mean length increment did not significantly differ between categories of the rest of the other variables for all children and for boys and girls separately (**Table 1** and **Appendix 2**).

Linear growth pattern in the first of child's life

The prevalence of stunted linear growth (length-for-age z-score < -2 SD) among study children increased from 14.6% at birth to 24.4% at 12 months of age. However, based on length-for-age difference < 0 cm, 85.6%, 91.6%, and 80.4% among all children, boys, and girls

respectively were shorter compared to the normative median length of 2006 WHO child growth standards at 12 months of age (**Figure 3**). Moreover, children were born with a length deficit of -1.4 cm, which continually widened to -2.7 cm at 12 months of age and boys consistently had a larger length deficit compared to girls, though this was not significantly different (**Appendix 3**).

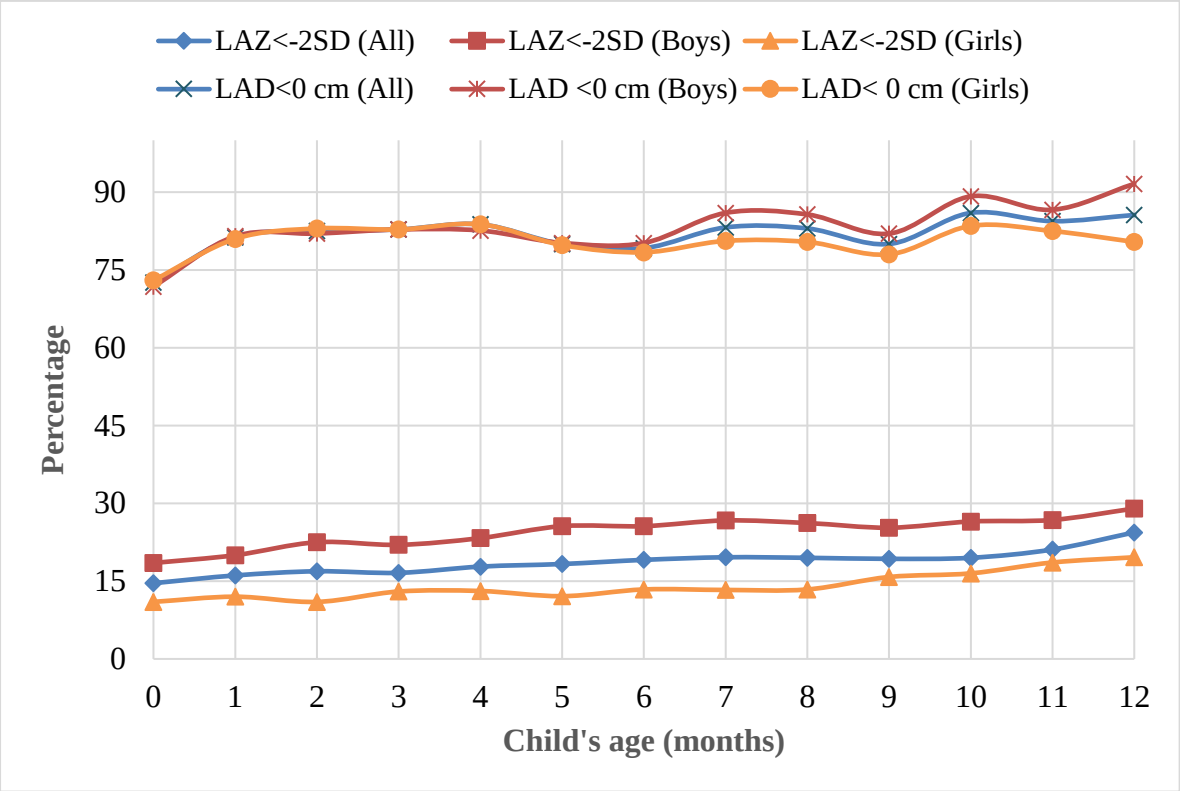


Figure 3: Trend of the prevalence of stunted linear growth (length-for-age Z-scores < – 2 SD) and linear growth deficit (length-for-age deference < 0 cm) calculated based on WHO child growth standards of 2006 [34]. ***Note****: SD: Standard deviation. LAZ: Length-for-age z-scores. LAD: Length-for-age difference.*

Predictors of linear growth

Bivariate analysis of predictors of linear growth

In bivariate analysis, late initiation of breastfeeding was associated with a nearly significant length decline of -0.83 cm (95% CI: -1.82, 0.14) from 6 to 12 months of age (**Table 3**). With each extra breastfeeding episode per day, a significant decrease in length of -0.02 cm (95% CI: -0.03, -0.00) was noted; the median value of daily breastfeeding frequency was 15 (IQR: 11, 20) in the first six months. The breastfeeding frequency was also associated with length decrease from 0 to 12 months of age, though this decrease was not significant anymore (-0.01 cm, 95% CI: -0.00, 0.00). None of the complementary feeding indicators was significantly related to linear growth in the age range of 6-12 months (**Table 3)**. However, the early introduction of complementary feeding was significantly associated with decreased linear growth from 0-12 months of age (-0.76 cm, 95% CI: -0.95, -0.55) (**Table 3**). Concerning child's illnesses, we observed a significant decreased linear growth with increase in the duration that children had suffered from diarrhoea in the first 6 months of age (-0.02 cm, 95% CI: -0.03, -0.00 per day with illness) and from birth to 12 months of age (-0.01 cm, 95% CI: -0.02, -0.00 per day with illness). From 6 to 12 months of age, there was also a significant decelerated linear growth with an increase in the duration that children had suffered from malaria infection (-0.03 cm, 95% CI: -0.05, -0.00 per day with illness) (**Table 3)**.

Turning to the category of other variables, we observed that birth weight, birth length, Mather's height, and Mather's education were strong predictors of length increments of the children during the first and second halves of infancy and from birth to 12 months of age (**Table 3)**. Boys had larger length increments of, respectively, 0.54 cm, (95% CI: 0.03, 1.05), 1.22 cm (95% CI: 0.34, 2.08), and 0.57 cm (95% CI: 0.06, 1.08) from 0-5, 6-12, and 0-12 months of

age (**Table 3**). Lastly, children born in households that experienced hunger had a significant smaller length increment of 0.33 cm (95% CI: 0.63, -0.03) from 0-12 months (**Table 3**).

Looking at sex-based bivariate analysis, we found that late breastfeeding initiation resulted in a significant decrease of -1.70 cm (95% CI: -3.28, -1.11) in only boys in the second half of infancy (**Appendix 4**). However, the breastfeeding frequency was significantly related to a declined linear growth of -0.02 cm (95% CI: -0.03, -0.0) in both boys and girls in the first half of infancy (**Appendices 4** and **5**). The duration of suffering from diarrhoea and acute respiratory tract infections were significant predictors of a decreased linear growth respectively in girls and boys, during the first half of infancy and from birth to 12 months of age (**Appendices 4** and **5**).

Table 3: Bivariate analysis for factors associated with length for the children aged from 0-5, 6-12, and 0-12 months of age

Variables	**0-5 months** (n=180 children)			**6-12 months** (n=180 children)			**0-12 months** (180 children)		
	Est	**95 CI**	**P-value**	**Est**	**95 CI**	**P-value**	**Est**	**95 CI**	**P-value**
Feeding practices									
Late initiation of breastfeeding	0.02	-0.72, 0.77	0.954	-0.83	-1.82, 0.14	0.095	-0.09	-0.84, 0.65	0.809
Daily breastfeeding frequency	-0.02	-0.03, -0.00	0.000	-0.00	-0.00, 0.00	0.952	-0.01	-0.01, 0.00	0.057
Early introduction of CF	-0.04	-0.19, 0.27	0.752	0.37	-0.99, 0.23	0.229	-0.76	-0.97, -0.55	<.0001
Below minimum meal frequency [1]				-0.06	-0.14, 0.02	0.156			
Below minimum dietary diversity [2]				-0.01	-0.09, 0.07	0.780			
Below minimum acceptable diet [3]				-0.06	-0.16, 0.03	0.187			
Child illness [4]									
Diarrhoea - duration (days)	-0.02	-0.03, -0.00	0.043	-0.00	-0.02, 0.00	0.222	-0.01	-0.02, -0.00	0.004
Acute RT infection - duration (days) [5]	-0.00	-0.01, 0.00	0.450	0.00	-0.00, 0.01	0.471	-0.01	-0.01, -0.00	0.002
Malaria - duration (days)	0.03	-0.14, 0.20	0.727	-0.03	-0.05, -0.00	0.027	-0.01	-0.04, 0.02	0.504

Table 3 continues…

Other variables									
Birth weight (g)	3.20	3.75, 3.64	<.0001	3.18	3.48, 3.88	<.0001	3.20	3.75, 3.64	<.0001
Birth length (cm)	0.93	0.89, 0.98	<.0001	0.73	0.59, 0.87	<.0001	0.88	0.83, 0.93	<.0001
Mather's age	0.00	-0.03, 0.04	0.807	-0.00	-0.05, 0.04	0.744	0.00	-0.02, 0.04	0.62
Mother's height (cm)	0.05	0.01, 0.09	0.017	0.07	0.01, 0.13	0.012	0.05	0.01, 0.10	0.009
Child's sex - Boys	0.54	0.03, 1.05	0.037	1.22	0.34, 2.08	0.005	0.57	0.06, 1.08	0.026
Mother's education-*None*	-1.26	-2.70, 0.17	0.085	-2.44	-4.30, -0.59	0.009	-1.26	-2.70, 0.17	0.085
Household wealth	-0.02	-0.19, 0.15	0.823	0.20	0.01, 0.47	0.034	0.02	-0.13, 0.19	0.747
Household with hunger	-0.37	-0.94, 0.18	0.288	0.00	-0.24, 0.24	0.998	-0.33	-0.63, -0.03	0.026

***Note: Est**: Estimate. **CI**: Confidence interval. **CF**: Complementary feeding. **RT**: Respiratory tract. The outcome variable was length. The models were fitted with a polynomial equation and three random effects: intercept, slope, and curvature of time with the unstructured covariance matrix. [1] The minimum is defined as 2 and 3 meals a day for the children of 6-8 months and 9-12 months respectively, in addition to breastmilk feeds and 3 meals a day for the non-breastfeed children of 6-12 months of age. [2] The minimum is defined as the consumption of foods from at least 4 food groups a day. [3] The minimum is defined as achieving concurrently the minimum meal frequency and the minimum dietary diversity. [4] Duration is the cumulative number of days for each illness as reported by the mothers in the previous 30 days each month from 1 to 12 months of age. [5] included coughing, runny nose, and wheezing.*

Multivariate analysis of predictors of linear growth

Late initiation of breastfeeding was associated with a nearly significant length decrease of -0.19 cm (95% CI: -0.39, 0.00) in the age range of 0-5 months and with a significant length decline of -0.73 cm (95% CI: -1.45, -0.01) in the age range of 6-12 months (**Table 4**). Higher breastfeeding frequency significantly decreased the length by -0.01 cm (95% CI: -0.02, -0.00) both in the age ranges of 0-5 months and 0-12 months (**Table 4**). Even though none of the indicators of complementary feeding practices was significantly associated with linear growth in the age range of 6-12 months, early introduction of complementary feeding was significantly associated with a decrease in length of -0.69 cm (95% CI: -0.90, -0.49) (**Table 4**). Regarding child's illnesses, we observed a significant decreased linear growth of -0.02 cm (95% CI: -0.04, -0.00) and -0.01 cm (95% CI: -0.02, -0.00) in the first 6 months of age and from 0-12 months of age, respectively, when the duration of suffering from diarrhoea increased. From 6 to 12 months of age, there was also a significant decelerated linear growth of -0.03 cm (95% CI: -0.05, -0.00) with increasing duration of malaria infection (**Table 4**)

From the multivariate analysis, birth weight and birth length also emerged as independent predictors of length increments of the children during the first and second halves of infancy and from birth to 12 months of age. When considering all exposure variables, boys only had a significantly larger length increment compared to girls in the age range of 6-12 months (+1.01 cm, 95% CI: 0.51, 1.51) (**Table 3)**.

The sex-specific analyses showed that late initiation of breastfeeding was independently associated with a close to significant length decrease of -0.20 cm (95% CI: -0.40, 0.02) among girls in the age range of 0-5 months and of -1.16 cm (95% CI: -2.38, 0.05) in boys in the age range of 6 - 12 months. For both boys and girls, the breastfeeding frequency was related to a significant declined linear growth only in the first half of infancy (-0.02 cm, 95% CI: -0.03, -

0.00). The early introduction of complementary feeding was associated with a significant decreased linear growth from birth to 12 months of age -0.47 cm (95% CI: -0.73, -0.12) in boys and -0.88 cm (95% CI: -1.15, -0.60) in girls. For only girls, we saw a significant decreased linear growth with an increase in the duration of suffering from diarrhoea in the age ranges of 0-5 and 0-12 months. Finally, we noticed a similar effect of birth weight and birth length on length increment in boys and girls for all age ranges except that birth weight did not significantly predict linear growth from 6-12 months of age in girls (**Appendices 6** and **7**).

Table 4: Multivariate analysis of the association between length and its predictors in the children aged from 0-5, 6-12, and 0-12 months of age

Variables	0-5 months (n=180)			6-12 months (n=180)			0-12 months (n=180)		
	Est	95% CI	P-value	Est	95% CI	P-value	Est	95% CI	P-value
Late initiation of breastfeeding	-0.19	-0.39, 0.00	0.057	-0.73	-1.45, -0.01	0.044	-0.22	-0.50, 0.04	0.104
Daily breastfeeding frequency	-0.01	-0.02, -0.00	0.002	-0.00	-0.01, 0.00	0.531	-0.01	-0.01, -0.00	0.030
Early introduction of CF	-0.03	-0.25, 0.19	0.777	0.15	-0.32, 0.63	0.531	-0.69	-0.90, -0.49	<.0001
Below minimum meal frequency [1]				-0.04	-0.13, 0.03	0.244			
Below minimum dietary diversity [2]				0.01	-0.10, 0.07	0.768			
Diarrhoea - duration (days) [3]	-0.02	-0.04, -0.00	0.016	-0.00	-0.01, 0.00	0.395	-0.01	-0.02, -0.00	0.020
Malaria - duration (days) [3]	-0.02	-0.18, 0.13	0.775	-0.02	-0.05, -0.00	0.040	-0.00	-0.04, 0.02	0.617
Birth weight	0.62	0.39, 0.84	<.0001	1.22	0.38, 2.05	0.004	0.83	0.53, 1.14	<.0001
Birth length	0.83	0.78, 0.87	<.0001	0.50	0.33, 0.67	<.0001	0.76	0.70, 0.82	<.0001
Boys	0.06	-0.08, 0.19	0.420	1.01	0.51, 1.51	<.0001	-0.00	-0.19, 0.17	0.937

***Note: Est**: Estimate. **CI**: Confidence interval. **CF**: Complementary feeding. The outcome variable was length. Each separate model for each age group was fitted with a polynomial equation and 3 random effects: intercept, slope, and curvature of time with the unstructured covariance matrix. The models were adjusted for the mother's education, mother's age, and household wealth. [1] The minimum is defined as 2 and 3 meals a day for the children of 6-8 months and 9-12 months of age respectively, in addition to breastmilk feeds, and 3 meals a day for the non-breastfeed children of 6-12 months of age. [2] The minimum is defined as the consumption of foods from at least 4 food groups a day. [3] Duration is the cumulative number of days for illness as reported by the mothers in the previous 30 days each month from 1 to 12 months of age.*

Discussion

This study aimed to assess the patterns of linear child growth and to determine the influence of feeding practices on linear growth in the first year of a child's life. The study shows that linear growth was compromised during infancy among the study children and that children are already born with a length deficit, which gradually deteriorated further as they grew. Besides, it reveals that the predictors of poor linear growth included late initiation of breastfeeding, high frequency of breastfeeding episodes, and early introduction of complementary feeding, as well as the duration of diarrhoea and malaria diseases. However, none of the indicators of complementary feeding practices predicted linear growth in the second half of infancy.

A smaller monthly length increment compared to the 2006 WHO child growth standards resulted in absolute length deficits for the study children, who accrued a mean deficit of 2.7 cm at one year of age. With analysis of 51 national survey data from low and middle income countries, Leroy et al. (2015) found a similar linear growth trend whereby at 8 months of age, children in Ethiopia had accumulated a mean length deficit of 2 cm and children in Peru had accrued a deficit of 3 cm [34]. A similar linear growth-decelerating pattern from birth to one year of age and beyond was reported in other studies, which used the z-scores [3-5]. Comparing our findings with those of other studies confirms that linear growth does not catch-up during early childhood; instead, it continually deteriorates [2-5, 34]. Even though linear growth decelerates in the comparable patterns, its steepness in decline differs amongst countries or regions; with Sub-Saharan Africa and Eastern Africa showing the steepest declines[1]. Moreover, the length deficit in the first two years of a child's life contributes more than half to the total length deficit accrued at 5 years of age in many low and middle-income countries, with a slightly higher contribution (64.2%) seen in Sub-Saharan Africa [5]. Therefore, context-

specific studies, like ours, are crucial to better understand the causes of linear growth retardation.

Considering all the accrued length deficits at one year of age, it is interesting to note that the majority (85.6%) of the study children were shorter compared to children of the 2006 WHO child growth standard based on median length. Furthermore, more boys are affected than girls (91.6% vs. 80.4%). However, the length deficit between boys and girls starts to widen at 6 months of age to the disadvantage of boys, which coincides with the introduction of complementary feeding. This is probably related to the slightly higher nutritional requirements of boys, which are not met by the complementary diet.

We saw that, at 1 year of age, 24.4% of the children were stunted, i.e. had a height-for-age z score < -2, and that boys (29%) were more often stunted than girls (19.6 %). These results confirm what we reported in chapter 2 of this thesis and reflect results of the two last Rwandan demographic and health surveys, which both found that stunting affected more boys than girls in Rwanda [26, 35]. Generally, boys in many Sub-Saharan African countries show more linear growth retardation than girls [36] and this was recently confirmed by a study in Kenya, which showed that boys grew slower than girls [37].

Linear growth deficit in early childhood has multifactorial causes. Exposure to inadequate environmental factors that include diet and its underlying feeding practices play a paramount role [12]. The findings of this study indicate that late breastfeeding initiation, high breastfeeding frequency, and early introduction of complementary feeding were independently associated with decelerated linear growth. Contrary to the WHO recommendation to initiate breastfeeding within one hour after birth, in the current study, 13.3% of the children were initiated to breastfeeding later than recommended. This late initiation of breastfeeding resulted in a significant decelerated linear growth in the second half of infancy. This finding

corroborates the finding of Jones et al. (2014) [38]. A possible explanation for this might be that the late initiation of breastfeeding deprives the child of early immunity-boosting compounds, which are abundant in breastmilk colostrum and this may subsequently increase a child's susceptibility to infectious diseases [39]. Particularly, in the second half of infancy, the child's susceptibility to infectious diseases increases because of the introduction of complementary foods in addition to the child starting to explore the home environment resulting in increased exposure to pathogens [40]. Therefore, a significant effect of late breastfeeding initiation on linear growth observed in the second half of infancy might have been mediated through infections [41] since these are known immediate causes of impaired linear growth [12, 13].

Our study shows that the frequency of breastfeeding was inversely related to linear growth. Although counterintuitive at first sight, this finding is likely related to the fact that with frequent breastfeeds, the child might drink small amounts of breastmilk, which in addition may be less dense in energy and nutrients. This breastfeeding behaviour is common in rural areas because being mostly confined with their children; mothers tend to often nurse their children regardless if they are hungry or not. Another possible explanation for this observation could be reverse causality. Some studies reported that mothers who perceived that their children did not grow well tended to continue breastfeeding them [42, 43]. Likewise, mothers may increase the frequency of breastfeeding to improve their child's growth status.

The current study found that nearly half of the children were introduced to complementary feeding before 6 months of age. This practice caused a significant deceleration of a child's linear growth. The inadequacy of the introduced fluids or foods might explain this finding. As it was reported in Rwanda [20] and in other countries in Sub-Saharan Africa [14, 44, 45], the complementary foods, especially in rural settings, are of poor nutritional quality. Poor nutritional quality diets are mostly found in poor families, which often experience hunger, and

consequently, children from such families are at greater risk of growth retardation [14, 38, 46-48]. However, another possible explanation to this finding is that the early introduced fluids and foods may be unsafe and when coupled to poor personal hygiene might cause infections such as diarrhoea [49, 50]. In their longitudinal study, Checkley et al. (2008) showed that the risk of stunted linear growth increased with episodes of diarrhoea before two years of age [51]. In line with this, the current study also shows that the duration in days for which a child had suffered from diarrhoea caused a deceleration of linear growth. Diarrhoea indicates that a child is infected with intestinal worms, viruses or malignant bacteria, which in turn reduce the absorption of nutrients, divert nutrients for their own use, cause nutrient losses or decrease appetite [25, 25]. In this way, diarrhoea might cause a decrease in linear growth [24, 51, 52].

Another important finding related to infectious diseases is that 29% of the children were found to have had at least one episode of malaria in infancy and that the duration of suffering from malaria significantly predicted poor linear growth in the second half of infancy. This result contrasts those of Genton et al. (1998) who indicated an increased risk of malaria in children with better linear growth [53] and of other studies that reported no association [54, 55]. However, our finding corroborates the findings of earlier longitudinal studies in The Gambia, which found that malaria infection was significantly associated with the risk of linear growth retardation among young children [56, 57]. A study in Kenya reported that suffering from malaria increased the risk of stunting among children under two years of age [58]. Similarly, by controlling for unmeasured confounders using matching to control, Kang et al. (2013) provided strong evidence for the effect of malaria on linear growth. They indicated that for each episode of malaria, stunting risk increased by 32% among children under two years of age [59].

Regarding the complementary feeding indicators, notably meal frequencies and dietary diversity scores in the current study did not predict linear growth. This finding is consistent

with the findings of other studies, which indicated no association between complementary feeding practices and linear growth [38, 60]. However, this is not universal because some other studies did find associations between complementary feeding practices and child linear growth [61-65]. This inconsistency may be due to low sensitivity or specificity of complementary feeding indicators making them a poor measure for the causal relationship between dietary intake and linear growth as was discussed by Jones et al. (2014) [38].

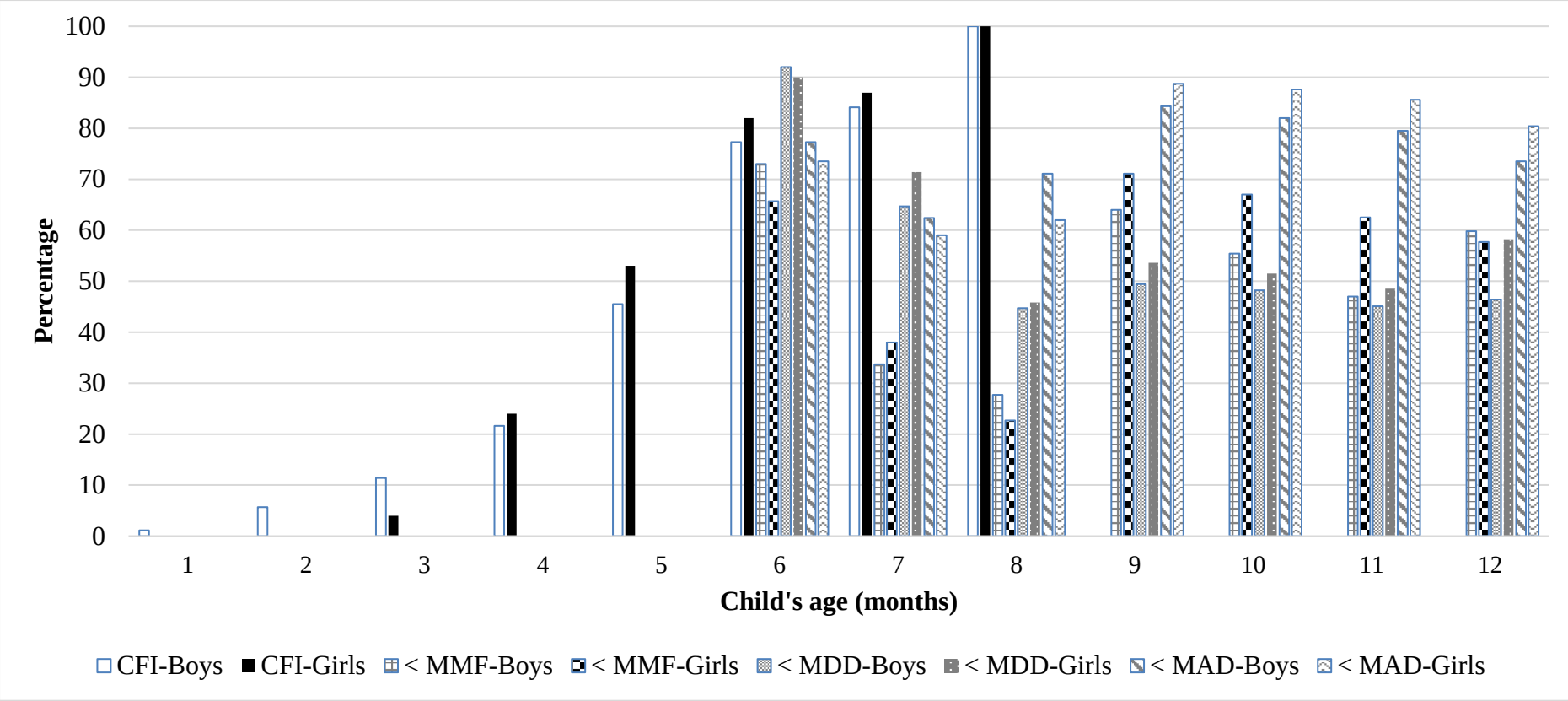
While our aim was to evaluate the influence of feeding practices on linear growth, our study confirms a strong effect of biological factors, including birth weight and birth length, which is consistent with previous studies [66-68]. Inadequate anthropometric measures at birth reflect the poor intrauterine environment that restricts foetal growth. Maternal undernutrition is an important modifiable factor that contributes to poor pregnancy outcome [68, 69]. Therefore, to increase the chances for a good pregnancy outcome, it is crucial to prevent maternal undernutrition throughout pregnancy.

The great strength of our study lies in its longitudinal design that allowed measuring the length and collecting data on feeding practices repeatedly from the same subjects. This design allowed creating trust between participants and field assistants and this trust, therefore, positively contributed to the collection of unbiased data. In addition, it allows interpreting associations in terms of cause and effect. However, with its small sample size and non-random inclusion of subjects, caution must be applied, as the findings may not be generalized or extrapolated to the whole of Rwanda. In addition, for the feeding and morbidity data, we relied on the mother's memory to recall what happened in the previous 30 days, which might have introduced recall errors.

To conclude, the current study has revealed that children in this study were born with length deficits, which gradually deteriorated with the child's age. Moreover, the trend of linear growth

showed no signs of catch-up during infancy. Significant predictors of linear growth that emerged from this study were late initiation of breastfeeding, high breastfeeding frequency, early introduction of complementary feeding, and the duration in days for having suffered from diarrhoea and malaria infections. Moreover, the study confirmed the strong positive effect of birth weight and birth length on linear growth. Therefore, to enhance postnatal growth, it is crucial to address these factors. However, since growth retardation appears to have manifested already at birth, improving maternal nutritional status may be even more important to ensure that children are born with adequate body dimensions. Finally, further investigations should explore the reasons why mothers perceive to have inadequate breastmilk production, which triggered them to early introduce complementary feeding and the reasons for late initiation of breastfeeding among some mothers.

Appendices



Appendix 1: Distribution of child feeding indicators by child's age (month) and sex.

Note: ***CFI*** *is the Complementary feeding introduction (cumulative percentage).* ***MMF*** *is the minimum meal frequency and is defined as 2 and 3 meals a day for the children of 6-8 months and 9-12 months respectively, in addition to breastmilk feeds and 3 meals a day for the non-breastfeed children of 6-12 months of age.* ***MDD*** *is the minimum dietary diversity and is defined as the consumption of foods from at least 4 food groups a day.* ***MAD*** *is the minimum acceptable diet and is defined as concurrently achieving the MMF and the MAD.*

Appendix 2: Participant characteristics and their association with length increments from 0-12 months of age by child's sex

Participants' characteristics	**Boys (n=83)**			**Girls (n=97)**		
	n, % **Median (IQR)**	**Length increment (0-12 months)**		**n, %** **Median (IQR)**	**Length increment (0-12 months)**	
		Mean±SD	**P-value**		**Mean±SD**	**P-value**
Feeding practices						
Initiation of breastfeeding						
Early (within one hour)	76 (91.5)	24.6±2.1	0.057	80 (82.5)	23.8±2.3	0.209
Late (after one hour)	7 (8.5)	22.9±2.0		17 (17.5)	23.0±2.2	
Breastfed at 12 months	81 (97.6)			97 (100)		
Breastfeeding frequencies	15 (10, 20)			15 (10, 20)		
Age of introduction of CF	6 (5, 6)			5 (5.0, 6.0)		
Timely (6-8 months)	46 (55.4)	24.4±2.4	0.861	44 (45.3)	23.8±2.5	0.632
Early (< 6 months)	37 (54.6)	24.5±1.7		53 (54.7)	23.6±2.1	
Child meal frequency [1, 2]	2 (2, 3)			2 (2, 3)		
≥ *Minimum*	32 (38.6)	9.4±1.9	0.712	41 (42.2)	9.3±1.4	0.523
< *Minimum*	51 (61.4)	9.2±1.1		56 (57.8)	9.1±1.7	

Appendix 2 continues….

Child dietary diversity score [1, 3]	4 (3, 4)			3 (2, 4)		
≥ *Minimum*	45 (54.2)	9.6±1.2	0.082	41 (42.3)	9.2±1.7	0.975
< *Minimum*	38 (45.8)	8.9±2.0		56 (57.7)	9.2±1.4	
Child acceptable diet [1, 4]						
≥ *Minimum*	22 (26.5)	9.5±1.0	0.620	19 (19.6)	9.1±1.7	0.634
< *Minimum*	61 (73.5)	9.2±1.9		77 (80.4)	9.3±1.5	
Child illness [5]						
Diarrhoea episodes	2.5 (1, 4.7)			2.5 (1.2, 4)		
None	7 (9.2)	24.1±1.3	0.630	8 (8.2)	24.1±2.8	0.546
Any	76 (90.8)	24.5±2.2		89 (91.8)	23.6±2.2	
Duration (days till 12 months of age)	15 (7, 26)			13 (9, 22.7)		
Malaria episodes	0 (0, 0.7)			0 (0, 1)		
None	61 (73.4)	24.8±2.1	0.007	67 (69.0)	23.8±2.4	0.304
Any	22 (26.6)	23.4±1.9		30 (31.0)	23.3±1.9	
Duration (days till 12 months of age)	8 (7, 11)			6.5 (3, 7)		

Appendix 2 continues…

Respiratory tract infection [6] episodes	8 (6, 10)			8 (7, 9)		
< 6 episodes	7 (8.4)	24.3±1.6	0.885	15 (15.4)	22.9±2.5	0.188
≥ 6 episodes	76 (91.6)	24.4±2.2		82 (84.6)	23.8±2.2	
Duration (days till 12 months of age)	36 (21, 54)			35 (20, 59)		
Other variables						
Child birth weight (g)	3,000 (2,670, 3,257)			2,860 (2,630, 3,075)		
Low birth weight (< 2,500g)	11 (13.8)	24.6±2.2	0.850	14 (12.0)	24.4±2.3	0.211
Normal birth weight (≥ 2,500g)	72 (86.2)	24.4±2.1		83 (88.0)	23.6±2.3	
Mother's age at birth	32.0 (25.2, 36.7)			30.0 (25.0, 35.0)		
Mother's height (cm)	157.5 (154, 160.4)			157.1 (53.2, 160.8)		
< 145 cm	2 (2.4)	23.7±1.2	0.635			
≥ 145 cm	81 (97.6)	24.5±2.1				
Mother's education						
None	3 (3.6)	24.5±3.3	0.716	3 (3.1)	20.6±0.1	0.181
Primary incomplete	35 (42.2)	24.2±2.1		42 (43.3)	23.6±2.6	
Primary complete	34 (41.0)	24.8±2.2		43 (44.3)	23.6±2.2	
Any secondary	11 (13.2)	24.7±2.5		9 (9.3)	24.2±2.1	

Appendix 2 continues…

Household wealth						
Low	33 (39.7)	24.3±2.1	0.428	39 (40.2)	23.8±2.5	0.341
High	50 (60.3)	24.7±2.3		58 (59.8)	23.4±2.3	
Household income per month						
< 10,000Rwf	49 (59.0)	24.8±2.3	0.088	53 (54.6)	23.8±2.1	0.453
≥ 10,000Rwf	34 (41.0)	24.0±1.8		44 (45.4)	23.5±2.4	
Household hunger indicators						
No Hunger (Little to none)	59 (71.0)	24.3±2.2	0.108	73 (75.2)	23.6±2.5	0.828
Hunger (Moderate to Severe)	24 (29.0)	25.2±2.0		24 (24.8)	23.5±2.1	

***Note**: For the continuous variables, the median values with its interquartile range (1st and 3rd quartiles) were reported while for the categorical variables, the frequency and the percentages are reported. [1] assessed at 12 months of age and the mean length increment was from 6 to 12 months of age. [2] The minimum is defined as 2 and 3 meals a day for the children of 6-8 months and 9-12 months of age respectively, in addition to breastmilk feeds, and 3 meals a day for the non-breastfeed children of 6-12 months of age. The minimum is defined as the consumption of foods from at least 4 food groups a day. [4] The minimum is defined as concurrently achieving the minimum meal frequency and the minimum dietary diversity. [5] Illness occurrences were reported over the last 30 days from 1 to 12 months of age (maximum number of episodes is 12 and the duration (days) of having illness were summed up from 0 to 12 months of age). [6] included coughing, runny nose, and wheezing. **SD** = Standard Deviation. **CF**: Complementary feeding.*

Appendix 3: Means and standard deviations for the monthly length increment and monthly length-for-age difference

Age, month (n)	Length monthly increment			Length-for-age difference [1]		
	All	Boys	Girls	All	Boys	Girls
0 (192)	-	-	-	-1.46±1.88	-1.52±1.98	-1.36±1.72
1 (190)	4.25±1.16	4.21±1.16	4.28±1.17	-1.91±1.93	-2.11±2.10	-1.70±1.72
2 (189)	3.45±1.26	3.54±1.31	3.38±1.21	-1.97±1.99	-2.25±2.16	-1.72±1.79
3 (186)	3.01±1.17	3.22±1.14	2.82±1.18	-1.81±2.08	-2.04±2.22	-1.60±1.95
4 (186)	2.09±0.99	2.11±1.04	2.07±0.95	-2.10±2.20	-2.41±2.34	-1.83±2.02
5 (185)	1.93±0.94	1.99±0.93	1.87±0.96	-2.12±2.26	-2.43±2.58	-1.85±1.92
6 (183)	1.66±0.87	1.74±0.93	1.59±0.93	-2.16±2.26	-2.38±2.52	-2.16±2.02
7 (184)	1.55±0.84	1.63±0.91	1.47±0.76	-2.22±2.18	-2.35±2.28	-2.10±2.10
8 (180)	1.36±0.81	1.26±0.76	1.45±0.83	-2.27±2.27	-2.52±2.36	-2.06±2.18
9 (178)	1.42±0.81	1.45±0.75	1.38±0.87	-2.24±2.24	-2.45±2.38	-2.05±2.45
10 (180)	1.17±0.84	1.22±0.70	1.14±0.94	-2.44±2.40	-2.55±2.50	-2.32±2.41
11 (179)	1.15±0.85	1.08±0.77	1.21±0.91	-2.52±2.32	-2.60±2.31	-2.40±2.27
12 (180)	1.02±0.85	1.01±0.89	1.02±0.82	-2.68±2.37	-2.80±2.41	-2.57±2.42

***Note**: [1] Length-for-age difference was calculated as follows: measured length minus the median length of the 2006 WHO child growth standards.*

Appendix 4: Bivariate analysis of the associated between length and its predictors in boys aged from 0-5, 6-12, and 0-12 months of age

Variables	**0-5 months (n=83)**			**6-12 months (n=83)**			**0-12 months (n=83)**		
	Est	**95 CI**	**P-value**	**Est**	**95 CI**	**P-value**	**Est**	**95 CI**	**P-value**
Feeding practices									
Late initiation of breastfeeding	-0.19	-1.53, 1.14	0.778	-1.70	-3.28, -1.11	0.036	-0.71	-1.98, 0.55	0.269
Daily breastfeeding frequency	-0.02	-0.03, -0.00	0.028	0.00	-0.00, 0.01	0.554	-0.00	-0.01, 0.00	0.260
Early introduction of CF	-0.02	-0.38, 0.34	0.917	0.79	-0.01, 1.60	0.052	-0.45	-0.76, -0.13	0.004
Below minimum meal frequency [1]				0.00	-0.11, 0.13	0.904			
Below minimum dietary diversity [2]				-0.03	-0.15, 0.09	0.654			
Below minimum acceptable diet [3]				0.02	-0.11, 0.16	0.769			
Child illness [4]									
Diarrhoea - duration (days)	-0.01	-0.03, 0.01	0.473	-0.00	-0.02, 0.01	0.823	-0.01	-0.02, 0.00	0.320
Acute RT infection - duration (days) [5]	-0.01	-0.03, -0.00	0.035	-0.00	-0.01, 0.00	0.341	-0.01	-0.02, -0.00	0.000
Malaria - duration (days)	-0.07	-0.33, 0.19	0.600	-0.02	-0.06, 0.01	0.166	-0.00	-0.05, 0.03	0.737

Appendix 4 continues…

Other variables									
Birth weight, g	3.19	2.57, 3.81	<.0001	2.78	1.89, 3.67	<.0001	3.03	2.37, 3.57	<.0001
Birth length, cm	0.96	0.89, 1.02	<.0001	0.75	0.57, 0.93	<.0001	0.89	0.82, 0.96	<.0001
Mother's age at birth	0.01	-0.04, 0.06	0.733	-0.02	-0.09, 0.04	0.486	0.00	-0.05, 0.05	0.975
Mother's height, cm	0.06	-0.00, 0.13	0.063	0.08	0.00, 0.16	0.042	0.06	0.00, 0.13	0.039
Mother education - *None*	-2.35	-4.54, -0.17	0.034	-2.61	-5.13, -0.09	0.041	-1.96	-4.09, 0.11	0.063
Household wealth	-0.25	-0.55, 0.03	0.087	0.12	-0.21, 0.46	0.461	-0.14	-0.42, 0.13	0.299
Households with hunger	-0.46	-1.34, 0.45	0.311	0.13	-0.21, 0.47	0.459	-0.13	-0.59, 0.29	0.512

***Note: Est**: Estimate. **CI**: Confidence interval. **CF**: Complementary feeding. **RT**: Respiratory tract. The outcome variable was length. The models were fitted with a polynomial equation and three random effects: intercept, slope, and curvature of time with the unstructured covariance matrix. [1] The minimum is defined as 2 and 3 meals a day for the children of 6-8 months and 9 - 12 months respectively, in addition to breastmilk feeds and 3 meals a day for the non-breastfeed children of 6-12 months of age. [2] The minimum is defined as the consumption of foods from at least 4 food groups a day. [3] The minimum is defined as achieving concurrently the minimum meal frequency and the minimum dietary diversity. [4] Duration is a cumulative number of days for illnesses as reported by the mothers in the previous 30 days each month from 1 to 12 months of age. [5] included coughing, runny nose, and wheezing.*

Appendix 5: Bivariate analysis of the associated between length and its predictors in girls aged from 0-5, 6-12, and 0-12 months of age

Variables	**0-5 months (n=97)**			**6-12 months (n=97)**			**0-12 months (n=97)**		
	Est	**95% CI**	**P-value**	**Est**	**95% CI**	**P-value**	**Est**	**95% CI**	**P-value**
Feeding practices									
Late initiation of breastfeeding	0.34	-0.46, 1.15	0.398	-0.02	-1.10, 1.05	0.959	0.38	-0.42, 1.19	0.352
Daily breastfeeding frequency	-0.02	-0.03, -0.00	0.008	-0.00	-0.01, 0.00	0.383	-0.01	-0.02, 0.00	0.072
Early introduction of CF	-0.06	-0.36, 0.24	0.691	0.07	-0.72, 0.87	0.858	-0.98	-1.26, -0.70	<.0001
Below minimum meal frequency [1]				-0.11	-0.22, -0.00	0.044			
Below minimum dietary diversity [2]				0.00	-0.11, 0.12	0.930			
Below minimum acceptable diet [3]				-0.14	-0.27, -0.01	0.031			
Child illness [4]									
Diarrhoea - duration (days)	-0.02	-0.04, -0.00	0.011	-0.02	-0.04, 0.00	0.059	-0.02	-0.03, -0.01	0.001
Acute RT infection - duration (days) [5]	0.00	-0.00, 0.01	0.572	0.00	-0.00, 0.01	0.801	-0.00	-0.01, 0.00	0.168
Malaria - duration (days)	0.13	-0.08, 0.35	0.238	-0.03	-0.06, 0.00	0.101	-0.01	-0.05, 0.03	0.629

Appendix 5 continues…

Other variables									
Birth weight, g	2.99	2.36, 3.67	<.0001	2.87	1.85, 3.89	<.0001	3.01	2.36, 3.67	<.0001
Birth length, cm	0.90	0.84, 0.95	<.0001	0.55	0.35, 0.75	<.0001	0.86	0.79, 0.94	<.0001
Mather's age	-0.00	-0.04, 0.03	0.704	-0.01	-0.07, 0.04	0.615	-0.00	-0.04, 0.03	0.821
Mother's height, cm	0.04	-0.00, 0.09	0.099	0.06	-0.01, 0.13	0.099	0.04	-0.01, 0.09	0.116
Mother education- *None*	-0.39	-2.13, 1.34	0.655	-1.79	-4.08, 0.49	0.123	-0.36	-2.11, 1.39	0.685
Household wealth	0.08	-0.10, 0.27	0.354	0.21	-0.05, 0.47	0.113	0.08	-0.09, 0.26	0.341
Household with hunger	-0.26	-0.93, 0.40	0.440	-0.13	-0.46, 0.20	0.438	-0.48	-0.86, -0.10	0.011

***Note: Est**: Estimate. **CI**: Confidence interval. **CF**: Complementary feeding. **RT**: Respiratory tract. The outcome variable was length. The models were fitted with a polynomial equation and three random effects: intercept, slope, and curvature of time with the unstructured covariance matrix. [1] The minimum is defined as 2 and 3 meals a day for the children of 6-8 months and 9-12 months of age, respectively, in addition to breastmilk feeds and 3 meals a day for the non-breastfeed children of 6-23 months of age. [2] The minimum is defined as the consumption of foods from at least 4 food groups a day. [3] The minimum is defined as achieving concurrently the minimum meal frequency and the minimum dietary diversity. [4] Duration is a cumulative number of days for illness as reported by the mothers in the previous 30 days each month from 1 to 12 months of age. [5] included coughing, runny nose, and wheezing.*

Appendix 6: Multivariate analysis of the association between length and its predictors in boys aged from 0-5, 6-12, and 0-12 months of age

Variables	**0-5 months (n=83)**			**6-12 months (n=83)**			**0-12 months (n=83)**		
	Est	**95 CI**	**P-value**	**Est**	**95 CI**	**P-value**	**Est**	**95 CI**	**P-value**
Late initiation of breastfeeding	-0.21	-0.33, 0.25	0.265	-1.16	-2.38, 0.05	0.061	-0.31	-0.77, 0.14	0.179
Daily breastfeeding frequency	-0.01	-0.02, -0.00	0.022	-0.00	-0.01, 0.01	0.809	-0.01	-0.02, 0.00	0.109
Early introduction of CF	0.02	-0.31, 0.37	0.873	0.46	-0.19, 1.12	0.165	-0.47	-0.73, -0.12	0.005
Below minimum meal frequency [1]				0.02	-0.10, 0.14	0.762			
Below minimum dietary diversity [2]				-0.00	-0.13, 0.12	0.957			
Diarrhoea - duration (days) [3]	-0.01	-0.03, 0.01	0.391	-0.00	-0.01, 0.01	0.924	-0.00	-0.02, 0.00	0.414
Malaria - duration (days) [3]	-0.12	-0.36, 0.11	0.289	-0.03	-0.06, 0.01	0.162	-0.00	-0.05, 0.04	0.763
Birth weight	0.69	0.35, 1.03	<.0001	0.78	0.30, 1.87	0.159	0.89	0.49, 1.30	<.0001
Birth length	0.82	0.75, 0.89	<.0001	0.61	0.39, 0.83	<.0001	0.75	0.66, 0.83	<.0001

***Note: Est**: Estimate. **CI**: Confidence interval. **CF**: Complementary feeding. The outcome variable was length. Each separate model for each age group was fitted with a polynomial equation and 3 random effects: intercept, slope, and curvature of time with the unstructured covariance matrix. The models were adjusted for the mother's education, mother's age, and household wealth. [1] The minimum is defined as 2 and 3 meals a day for the children of 6-8 and 9-12 months of age respectively, in addition to breastmilk feeds, and 3 meals a day for non-breastfeed children of 6-12 months of age. [2] The minimum is defined as the consumption of foods from at least 4 food groups a day. [3] Duration is a cumulative number of days for illness was reported by the mothers in the previous 30 days each month from 1 to 12 months of age.*

Appendix 7: Multivariate analysis of the association between length and its predictors in girls aged from 0-5, 6-12, and 0-12 months of age

Variables	**0-5 months (n=97)**			**6-12 months (n=97)**			**0-12 months (n=97)**		
	Est	**95 CI**	**P-value**	**Est**	**95 CI**	**P-value**	**Est**	**95 CI**	**P-value**
Late initiation of breastfeeding	-0.20	-0.42, 0.02	0.074	-0.45	-1.34, 0.43	0.315	-0.20	-0.55, 0.14	0.242
Daily breastfeeding frequency	-0.01	-0.01, -0.00	0.033	-0.00	-0.01, 0.00	0.252	-0.01	-0.01, 0.00	0.102
Early introduction of CF	-0.08	-0.36, 0.20	0.577	-0.05	-0.75, 0.65	0.881	-0.88	-1.15, -0.60	<.0001
Below minimum meal frequency [1]				-0.10	-0.22, 0.00	0.068			
Below minimum dietary diversity [2]				0.03	-0.08, 0.15	0.599			
Diarrhoea - duration (days) [3]	-0.02	-0.04, -0.00	0.009	-0.01	-0.03, 0.00	0.220	-0.02	-0.03, -0.00	0.009
Malaria - duration (days) [3]	0.09	-0.11, 0.30	0.386	-0.03	-0.06, 0.00	0.144	-0.00	-0.05, 0.03	0.766
Birth weight	0.54	0.24, 0.84	0.000	1.67	0.46, 3.04	0.007	0.76	-0.66, 0.85	0.001
Birth length	0.83	0.77, 0.89	<.0001	0.32	0.07, 0.58	0.010	0.75	-0.64, 1.02	<.0001

***Note: Est**: Estimate. **CI**: Confidence interval. **CF**: Complementary feeding. The outcome variable was length. Each separate model for each age group was fitted with a polynomial equation and 3 random effects: intercept, slope, and curvature of time with the unstructured covariance matrix. The models were adjusted for the mother's education, mother's age, and household wealth. [1] The minimum is defined as 2 and 3 meals a day for the children of 6-8 months and 9-12 months of age, respectively, in addition to breastmilk feeds, and 3 meals a day for the non-breastfeed children of 6-12 months of age. [2] The minimum is defined as the consumption of foods from at least 4 food groups a day. [3] Duration is a cumulative number of days for illness was reported by the mothers in the previous 30 days, each month from 1 to 12 months of age.*

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Chapter 4

Anaemia and iron status among children of 4 and 12 months of age in Rwanda:

Insights into nutritional anaemia and predictors

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Abstract

Background: In Rwanda, anaemia affects 72% and 61% of children aged 6-8 and 9-11 months respectively. However, the contribution of nutritional anaemia is not known. This study, therefore, aimed to assess iron status among children and their mothers at 4 and 12 months postpartum and to examine if dietary iron intake predicts the occurrence of anaemia, iron deficiency (ID), and iron deficiency anaemia (IDA) in 12 months-old children.

***Methods*:** A longitudinal study of 192 children was conducted from 2016 to 2018 in Rwanda. We measured haemoglobin concentration and collected blood samples from the mother-child pairs at birth and at 4 and 12 months postpartum. Ferritin, sTfR, CRP, and AGP concentrations were measured using a sandwich ELISA technique. Haemoglobin and ferritin values were adjusted for altitude and inflammation, respectively. Dietary iron intake data were collected using a full 24-hour recall. Predictors of anaemia, ID, and IDA at 12 months of age were modelled using Cox proportional hazard regression with constant time.

Results: In infants, anaemia, ID, and IDA occurred in 73%, 10%, and 8% at 4 months of age, and in 48%, 28%, and 18% at 12 months of age. The contribution of ID to anaemia cases increased from 10% at 4 months of age to 36.5% at 12 months of age. In their mothers, anaemia, ID, and IDA occurred in 12%, 3%, and 1% at both 4 and 12 months. The child's dietary iron intake was inversely associated with ID at 12 months of age (PR=0.79, 95% CI: 0.62, 1.03), but not with anaemia or IDA. Malaria predicted anaemia (PR=1.05, 1.00-1.10), ID (PR=1.09, 1.04-1.15) and IDA (PR=1.15, 1.06-1.25), and the presence of inflammation was associated with anaemia [(PR=1.01 (1.01-1.10). A high body iron store at 4 months of age was associated with reduced prevalence of ID at 12 months of age (PR=0.73, 95% CI: 0.59, 0.89).

***Conclusions**:* While dietary iron intake does not significantly predict anaemia, 36.5% of anaemia cases coincided with ID at 12 months of age. Malaria and inflammation are the two

most important predictors of anaemia and IDA at this age. Therefore, the prevention of inflammatory diseases is crucial to make dietary measures effective.

Introduction

Globally, anaemia is one of the major public health problems and its magnitude varies across the globe with developing countries being mostly affected and children under five years of age being highly vulnerable. Among children under five years of age, it was estimated that 43% globally and 62.3% in Africa were affected by anaemia in 2011 [1]. Anaemic children are at increased risk of morbidity and mortality due to reduced activity of the immune system [2], and in addition, they are at increased risk of impaired cognitive and motor development during infancy and childhood [3].

The causes of anaemia in children during their first year of life have not been extensively studied in the African context. Anaemia has multifactorial causes that can broadly be divided into nutrient deficiencies [4-6] and infectious diseases [7]. It has been estimated that nutritional deficiencies, particularly iron deficiency, contribute ~50% to anaemia cases globally [8]. To meet the physiological needs during the first 6 months of exclusive breastfeeding as recommended by WHO [9], infants rely on only two iron sources; 1) iron reserves built during their foetal life, and 2) breastmilk, which supplies a low quantity of well absorbable iron [10]. Some studies demonstrated that exclusive breastfeeding in addition to the iron reserves endowed at birth could suffice to maintain physiological iron needs during the first six months of life [11-13]. Hoverer, other studies have reported depleted iron reserves by four to six months of age, which may lead to iron deficiency and anaemia at that age, and eventually to anaemia in the second half of infancy [14, 15]. This is particularly true for infants born under unfavourable conditions, such as those who are born pre-term, have low birth weight or whose mothers are anaemic [16-18]. Since such unfavourable conditions are commonly found in Sub-Saharan Africa, the depletion of iron reserves at the age of six months is expected to be commonly found.

At six months of age, the child's physiological iron needs increase considerably [19], and can no longer be met by exclusive breastfeeding. Therefore, additional iron has to be supplied by the child's diet. However, starchy staples and other plant-based foods, which dominate complementary diets of children in Sub-Saharan Africa [20-23], are poor sources of bioavailable iron and pose children to an even greater risk of iron deficiency [24]. Together with a poor diet, the occurrence of anaemia is further aggravated by infectious diseases, which disturb iron homeostasis, destroy erythrocytes, and restrict or suppress erythropoiesis, leading to anaemia of inflammation [25-27].

In Rwanda, a demographic and health survey of 2015 shows that 37% of all children of 6-59 months of age are anaemic, and that anaemia is more prevalent among children of 6-11 months of age (72% at 6-8 months of age and 61% at 9-11 months of age). It also shows that children living in rural areas are more often anaemic compared to those living in urban areas (38% vs. 30%) [28]. However, it is not known to what extent the depletion of iron reserves during the exclusive breastfeeding period, dietary factors, and exposure to infections contribute to anaemia. Therefore, we longitudinally followed children from birth onwards and aimed to assess anaemia and iron status among infants and their mothers at birth and at 4 and 12 months postpartum in a rural area of Rwanda. We specifically aimed to examine the contribution of dietary iron intake to the occurrence of anaemia, iron deficiency, and iron deficiency anaemia at 12 months of age.

Methods

Study site and subjects

The current study took place in the rural area of Muhanga district, Southern Province, Rwanda, under the approval of the Rwanda National Ethics Committee (No 734/RNEC/2016). We followed the ethical guidelines as laid down in the declaration of Helsinki and its amendments. In accordance, the study objective and procedures were explained to parents both verbally and in writing before they gave their written consent. One hundred and ninety-two (192) apparently healthy mother-child pairs were recruited for the study shortly after birth and followed until 12 months postpartum. During the recruitment, the study staff explained the purpose of the study to both parents of the child and requested the mother to participate voluntarily. For the child, both parents had to agree on participation. Upon acceptance, both parents signed a consent form.

Blood collection and processing

Haemoglobin was measured at birth and at 4 and 12 months postpartum. At birth, blood samples were obtained by heel prick for the baby and by finger prick for the mother. The pricking site was disinfected using an alcohol swab and was allowed to dry before puncturing the skin. The skin puncture was done with a 2.25 mm lancet for the baby and with a 2.4 mm lancet for the mother. After pricking, two drops of blood were wiped off and a third drop was filled into a micro-cuvette. At 4 and 12 months of age, immediately after drawing venous blood, a drop of blood was pipetted into a micro-cuvette. A blood-filled micro-cuvette was placed in a haemoglobin photometer (HemoCue 201^{+} machine) that displayed the haemoglobin concentration, which was recorded in a datasheet to the nearest 0.1 g/dl.

At 4 and 12 months of age, an experienced phlebotomist drew 5 ml of venous blood from each child and 9 ml from each mother. At 4 months of age, the child's blood was collected in EDTA tubes for haemoglobin measurement and plasma separation, while at 12 months of age; it was collected into trace element free clot activating tubes. The latter tubes were also used for the mothers at 4 and 12 months postpartum. For blood clotting, filled tubes were kept in a closed container to avoid direct exposure to sunlight for about 2 hours. Tubes were then centrifuged at 2000 rpm for 10 minutes, and the serum or plasma supernatant was pipetted, using trace element free pipette tips, into 1-ml cryogenic vials. Aliquots were transported to the University of Rwanda to be stored at -25^{o}C. At the end of the study, all plasma and serum samples were transported frozen to the Wageningen University and Research, the Netherlands, where they were stored at -25^{o}C until analysed.

Laboratory analysis

Serum and plasma samples were transported on dry ice to an accredited laboratory (VitMin Lab, Willstaett, Germany) for further analysis. With the use of a sandwich ELISA technique [29], iron status indicators of interest such as ferritin and soluble transferrin receptors (sTfR), and inflammation indicators such as C-Reactive Protein (CRP) and α-Acid Glycoprotein (AGP) were simultaneously measured.

Haemoglobin values were adjusted for altitude using a formula suggested by Sullivan et al. (2008) [30]. WHO recommended cut-offs were used to define anaemia as adjusted haemoglobin values <11 g/dl for the children, and <12 g/dl for the mothers [31]. Ferritin values were adjusted for inflammation using correction factors according to Thurnham et al. (2010) [32]. Presence of inflammation was defined as CRP values >5 mg/l or AGP values > 1 g/l. The adjusted ferritin values <12 µg/l for the children, and <15 µg/l for the mothers [33] indicated iron deficiency. Concurrent presence of iron deficiency and anaemia showed iron deficiency

anaemia. We calculated body iron reserves using the ratio of soluble transferrin receptors (R) and adjusted ferritin (F) (in µg/l) as follows: - [Log(R/F ratio) – 2.8229]/0.1207, and we expressed the body iron reserves as mg/kg body weight [34].

Predictors of anaemia, iron deficiency, and iron deficiency anaemia

To explain the causes of anaemia, iron deficiency, and iron deficiency anaemia at 12 months of age, dietary iron intake (naturally in foods or added to food in the form of micronutrient powder) was considered as the most proximal independent factor. The intake of iron and other nutrients were derived from dietary data collected using a 24-hour recall method with multiple pass approach [35]. The amounts of nutrient intake were calculated based on the food composition table of Uganda and using COMPLEAT software (Version 1.0, Wageningen University and Research, The Netherlands). Feeding practices underlying dietary iron intake including meal frequency, dietary diversity, and acceptable diet were also assessed. On their turn, these feeding practices were influenced by household food security measured according to Ballard et al. (2011) [36], household wealth (calculated based on household assets and utilities), and maternal education. Presence of inflammation (based on CRP and AGP) and the duration (days) of illnesses (diarrhoea, fever, and malaria in the previous 30 days) at 12 months of age were also included as proximal factors which, on the one hand, affect iron status directly, and, on the other hand, may interfere with dietary iron intake. We also assessed the influence of haemoglobin concentration and iron status at birth and at 4 months postpartum on iron status indicators at 12 months of age.

Statistical analysis

All analyses were carried out in SAS version 9.4 (SAS Institute Inc, USA). Categorical variables were expressed as frequencies and their corresponding percentages, and continuous variables as median (IQR= first and third quartiles). We used Cox regression models containing

a constant time and a robust variance to calculate prevalence ratios and their 95% confidence intervals [37]. We used bivariate Cox regression to identify potential predictors to model in the multivariate cox regression. If two independent variables were correlated (correlation coefficient ≥ 0.30), the one that was most likely to be related to the outcome or more comprehensive was kept in the multivariate analysis. For example, we dropped serum ferritin from the model to the favour of body iron stores. Moreover, independent variables, which correlated with the independent variable of interest (iron intake) as well as with other independent variables in the models, were removed from the multivariate models. The significance level was set at $p < 0.05$. For assessing the net effect of iron intake and biological factors on anaemia, iron deficiency, and iron deficiency anaemia, we adjusted the models for the age of introduction of complementary foods or drinks, birth weight, sex, and for the intake of other uncorrelated micronutrients (folate, calcium, vitamin B12, vitamin C, and beta-carotene).

Results

Study participants

Table 1 presents the characteristics of the study participants, morbidity, feeding practices, and nutrient intake among children at 12 months of age. Fourteen percent (14%) of children were of low birth weight, while 24.4% and 4% were stunted and wasted at 12 months of age, respectively. Twenty-seven percent (27%) of the children were reported to have suffered from diarrhoea or fever, and 4% from malaria during the 30 days preceding the interview at 12 months of age. None of the participant characteristics and child illnesses differed significantly between anaemic and non-anaemic children.

Nearly half of the children (49.5%) were introduced to complementary foods or drinks before the appropriate age (6 months). Almost all children (98.8%) were still breastfed at 12 months

of age and the median value of breastfeeding frequencies was 13 times per day. About 60% of the children were fed meals below the minimum meal frequency, and slightly over half (52%) ate a diet below a minimum required diversity, resulting in a majority (77%) of children not achieving a minimally acceptable diet. Based on seven food groups by WHO [38], the two main food groups mostly consumed by children in the previous 24 hours were the group of grains, tubers, roots, and plantains (95%), and the group of legumes and nuts (81%), while the group of eggs was consumed the least (1.1%). Vitamin A rich fruits and vegetables were consumed by 58.2%, and 66.7% had consumed other fruits and vegetables. Eleven percent (11%) of children had consumed meats or other flesh foods, and 16.4% had consumed dairy products (mainly cow's milk) in the previous 24 hours. Twelve percent (12%) of the children had eaten micronutrient powders added to their food in the previous 7 days. Based on the median values, the children had consumed 576 kcal, 20 mg of protein, 13.3 mg of fibres, 4.8 mg of iron, 172.4 ug of folate, 0.4 mg of vitamin B6, 16.4 mg of vitamin C, and 106.5 mg of calcium in the previous 24 hours (**Table 1**).

None of the feeding indicators or nutrient intake differed significantly between anaemic and non-anaemic (**Table 1**), iron deficient, and non-iron deficient (results not presented), and between iron-deficient anaemic and non-iron deficient anaemic children (results not presented).

Table 1: Participant characteristics according to child anaemia status at 12 months of age [1]

Characteristics	**Total (**N=177**)**	**Anaemic (**N= 85**)**	**Non-anaemic (**N= 92**)**
Boys	83 (*46.0)*	39 (*46.0)*	42 (*45.7)*
Birth weight *(kg)*	2900 (2635, 3145)	2910 (2100, 3900)	2890 (1600, 3900)
Low birth weight (<2500 g)	*25 (14.0)*	*9 (10.6)*	*16 (17.4)*
Length for age z-scores	-0.8 (-1.6, -0.2)	-1.2 (-1.8, -0.6)	-0.8 (-1.6, -0.1)
< -2 SD	*43 (24.4)*	*23 (27.1)*	*20 (21.7)*
< -3 SD	*5 (2.8)*	*3 (3.5)*	*2 (2.2)*
Weight for height z-scores	-0.1 (-0.8, 0.6)	0 (-0.8, 0.6)	-0.2 (-1., 0.2)
< -2 SD	7 (4.0)	4 (4.7)	3 (3.3)
Household wealth			
Low	*71 (40.0)*	*35 (41.2)*	*36 (39.0)*
High	*108 (60.0)*	*50 (58.8)*	*56 (61.0)*
Household hunger score	0.0 (0.0, 2.0)	0.0 (0.0, 2.0)	0 (0.0, 2.0)
No hunger (Little to None)	*130 (73.4)*	*68 (73.0)*	*62 (74.0)*
Hunger (Moderate and Severe)	*47 (26.6)*	*23 (27.0)*	*24 (26.0)*

Table 1 continues...

Maternal education			
Not educated	*6 (3.4)*	*3 (3.5)*	*3 (3.3)*
Primary Incomplete	*74 (41.8)*	*31 (36.5)*	*43 (46.7)*
Primary Complete	*77 (43.5)*	*33 (51.8)*	*44 (36.0)*
Any Secondary	*20 (11.3)*	*7 (8.2)*	*13 (14.0)*
Child morbidity			
Diarrhoea, duration (days) [4]	4 (3, 7)	4 (3, 7)	3.5 (3, 7)
Any episode	*48 (27.0)*	*20 (23.5)*	*28 (30.4)*
Fever, duration (days) [4]	3 (2, 4)	3 (2, 4.5)	3 (2, 4)
Any episode	*49 (27.7)*	*21 (24.5)*	*28 (30.4)*
Malaria, duration (days) [4]	4 (3, 7)	7 (3, -)	3.5 (2.2, 6.2)
Any episode	*7 (4.0)*	*3 (3.5)*	*4 (4.3)*
Feeding practices			
Age at introduction of complementary feeding (months)	6.0 (5, 6)	5.0 (4.5, 6.0)	6.0 (5.0, 6.0)
< 6 months	*88 (49.5)*	*43 (48.3)*	*46 (51.7)*
> 9 months	*0 (0.0)*	*0 (0.0)*	*0 (0.0)*

Table 1 continues…

Continued breastfeeding at 12 months of age	*176 (98.8)*	*84 (98.8)*	*91 (99.0)*
Breastfeeding frequencies in 24 hours	*13 (10, 20)*	*12 (10, 20)*	*13 (10, 20)*
Daily meal frequency[3]	2 (2, 3)	2 (2, 3)	2 (2, 3)
Daily dietary diversity[4]	3 (2, 4)	4 (2, 4)	3 (2, 4)
< *Minimum daily meal frequency*	*105 (59.3)*	*54 (63.5)*	*51 (55.5)*
< *Minimum dietary diversity*	*91 (51.4)*	*42 (49.4)*	*49 (53.3)*
< *Minimum* acceptable diet[5]	*136 (77.0)*	*68 (80.0)*	*68 (74.0)*
Meat and flesh foods consumed	20 (11.0)	13 (16.0)	7 (8.2)
Dairy products consumed	29 (16.4)	14 (16.5)	15 (16.3)
Use of micronutrient powders	21 (12.0)	10 (11.8)	11 (12.0)
Energy and nutrient intake			
Energy intake (*kcal/day)*	576.0 (370.5, 950.2)	575.5 (340.5, 1012.8)	576.5 (371.2, 923.2)
Protein (*g/day)*	20.0 (10.3, 31.2)	20.0 (10.5, 28.6)	19.8 (10.2, 32.2)
Fibres (*g/day)*	13.3 (6.0, 21.4)	13.9 (6.9, 20.5)	12.5 (6.0, 22.3)

Table 1 continues…

Iron (naturally in foods) (*mg/day)*	4.6 (2.3, 7.1)	4.6 (2.3, 6.5)	4.2 (1.8, 7.5)
Haem iron (mg/day)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)
Iron intake [(micronutrient powders (MNPs)] *(mg/day)*	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)
Total iron intake (foods and MNPs) *(mg/day)*	4.8 (2.3, 7.6)	4.9 (2.3,7.4)	4.5 (2.1, 7.9)
Retinol *(ug/day)*	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)
Vitamin B12 *(ug/day)*	0.0 (0.0, 0.3)	0.0 (0.0, 0.1)	0.0 (0.0, 0.2)
Riboflavin *(mg/day)*	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)
Folate *(ug/day)*	172.4 (59.0, 358.2)	176.4 (62.7, 325.0)	165.0 (43.0, 386.5)
Vitamin B6 *(mg/day)*	0.4 (0.2, 0.7)	0.4 (0.2, 0.7)	0.4 (0.2, 0.6)
Vitamin C *(mg/day)*	16.4 (7.0, 34.5)	18.5 (8.2, 33.0)	14.8 (5.0,35.7)
B-Carotene *(ug/day)*	177.5 (57.4, 408.0)	221.2 (69.0, 444.0)	153.0 (33.5, 372.8)
Calcium *(mg/day)*	106.5 (50.0, 173.5)	108.5 (56.5, 174.2)	106.0 (46.2, 173.0)
Zinc *(mg/day)*	2.4 (1.2, 3.4)	2.4 (1.2, 3.4)	2.2 (1.0, 3.9)

Note: *[1] Values are frequencies (%), and median (IQR) where applicable. [2] Minimum 3 meals a day for the children aged 9-12 months. [3] The minimum is defined as the consumption of foods from at least 4 food groups per day. [4] The minimum is defined as achieving the minimum meal frequency and minimum dietary diversity. [5] The illness occurrence and duration (days) in the previous 30 days were reported by the mother. SD = Standard Deviation.* None of the participants' characteristics differed between anaemic and non-anaemic children.

Haematological characteristics of children and their mothers

Indicators of iron status and inflammation for the children and their mothers at 4 and 12 months postpartum are presented in Figures 1 and 2, and in Table 2. Among children, the prevalence of anaemia was 73% at 4 months of age of but it dropped to 48% at 12 months of age (**Figure 1**).

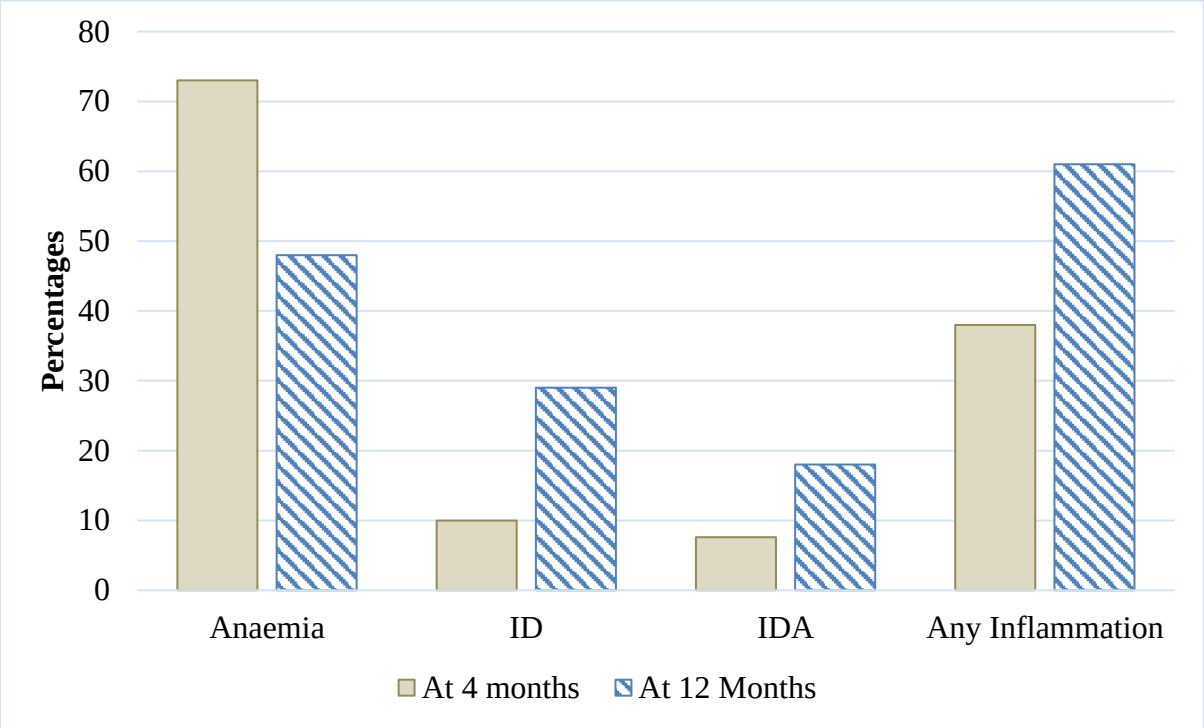

Figure 1: Prevalence of anaemia, ID, IDA, and inflammation among children at birth and 4 and 12 months postpartum. ***Note:*** *Anaemia is defined as an adjusted haemoglobin concentration < 11 g/dl. ID is Iron deficiency and is defined as corrected ferritin values < 12 ug/l. IDA is iron deficiency Anaemia and is defined as the concurrence of anaemia and iron deficiency. Inflammation is based on C-reactive protein values > 5 mg/l or α-Acid Glycoprotein values > 1 g/l. Anaemia is based on 182 and 177 children, ID is based on 160 and 178 children, IDA is based on 160 and 178 children and inflammation is based on 160 and 180 children at 4 and 12 months of age, respectively.*

Table 2: Iron status and infection or inflammation indicators of children and their mothers at 4 and 12 months postpartum

Characteristics	**Child**		**Mother**	
	At 4 months	**At 12 months**	**At 4 months**	**At 12 months**
	(N=185)	(N=180)	(N=185)	(N=180)
Haemoglobin (Hb)[1, 2] *(g/dl)*	10.4 (9.5, 11.0)	11.0 (10.1, 11.8)	13.3 (12.6, 14.1)	13.7 (12.8, 14.8)
Ferritin [3, 4], *µg/l*	41.5 (22.5, 84.7)	19.2 (10.5, 31.4)	60.5 (35.4, 87.8)	70.5 (41.4, 95.2)
Soluble Transferrin receptors (sTfR) (*mg/l)*[3]	8.4 (6.8, 10.7)	12.1 (9.9, 14.9)	8.8 (4.3, 31.4)	8.3 (4.1, 27.4)
Body iron stores (BIS)[3, 5] *(mg/kg)*	4.3 (3.5, 5.0)	3.3 (2.3, 3.8)	0.7 (0.6, 0.8)	-
BIS < 0 mg/kg body weight	*0 (0.0)*	*4 (2.1)*	*0 (0.0)*	-
C-Reactive protein (CRP)[3] *(mg/l)*	0.8 (0.2, 4.0)	0.8 (0.2, 4.7)	3.0 (2.3, 3.7)	0.7 (02, 2.0)
Prevalence of inflammation (CRP ≥ 5 mg/l)	*35 (22.0)*	*42 (23.2)*	*0 (0.0)*	*7 (4.0)*
α-Acid Glycoprotein (AGP)[4], *g/l*	0.7 (0.3, 3.8)	0.7 (0.5, 1.1)	0.8 (0.6, 1.2)	0.8 (0.6, 1.1)
Prevalence of inflammation (AGP ≥ 1 g/l)	*53 (33.0)*	*95 (53.4)*	*74 (41.0)*	*64 (35.8)*

Note: *Values are median (IQR), or frequency (%). [1] n = 182 at 4 months and 177 at 12 months postpartum for both children and mothers. [2] Adjusted for altitude; [3] n = 160 and 178 children at 4 and 12 months of age respectively, and 182 and 176 mothers at 4 or 12 months postpartum respectively. [4] Inflammation-adjusted ferritin values; [5] At 12 months postpartum, body iron stores were not calculated for mothers due to lack of their body weight.*

The median value of haemoglobin concentration that was 18 g/dl at birth dropped to 10.4 g/dl at 4 months of age and then slightly increased to 11 g/dl at 12 months of age (**Table 2**). Iron deficiency based on inflammation-adjusted ferritin was 10% at 4 months of age, and this increased to 28% at 12 months of age (**Figure 1**), while the median value of ferritin concentration declined from 41.5 µg/l at 4 months of age to 19.2 µg/l at 12 months of age (**Table 2**). The contribution of iron deficiency to anaemia cases increased from 10% at 4 months of age to 36.5% at 12 months of age. No child had depleted body iron reserves at 4 months of age, while 2% of the children had depleted body iron reserves at 12 months of age. The prevalence of inflammation increased from 38% at 4 months of age to 61% at 12 months of age based on CRP and AGP combined (**Figure 1**).

Concerning mothers, the prevalence of anaemia, iron deficiency, and iron deficiency anaemia was much lower as compared to their children and did not differ much over time points (**Figure 2**). Moreover, concentrations of haemoglobin, ferritin, and sTfR were similar over time (**Table 2**). At 4 months postpartum, none of the mothers had depleted body iron reserves (not measured at 12 months postpartum). Iron deficiency contributed 4.5% and 9.5% to anaemia cases at 4 and 12 months postpartum, respectively. Inflammation was present in 41% and 36.3% of mothers at 4 and 12 months postpartum, respectively, based on CRP and AGP combined.

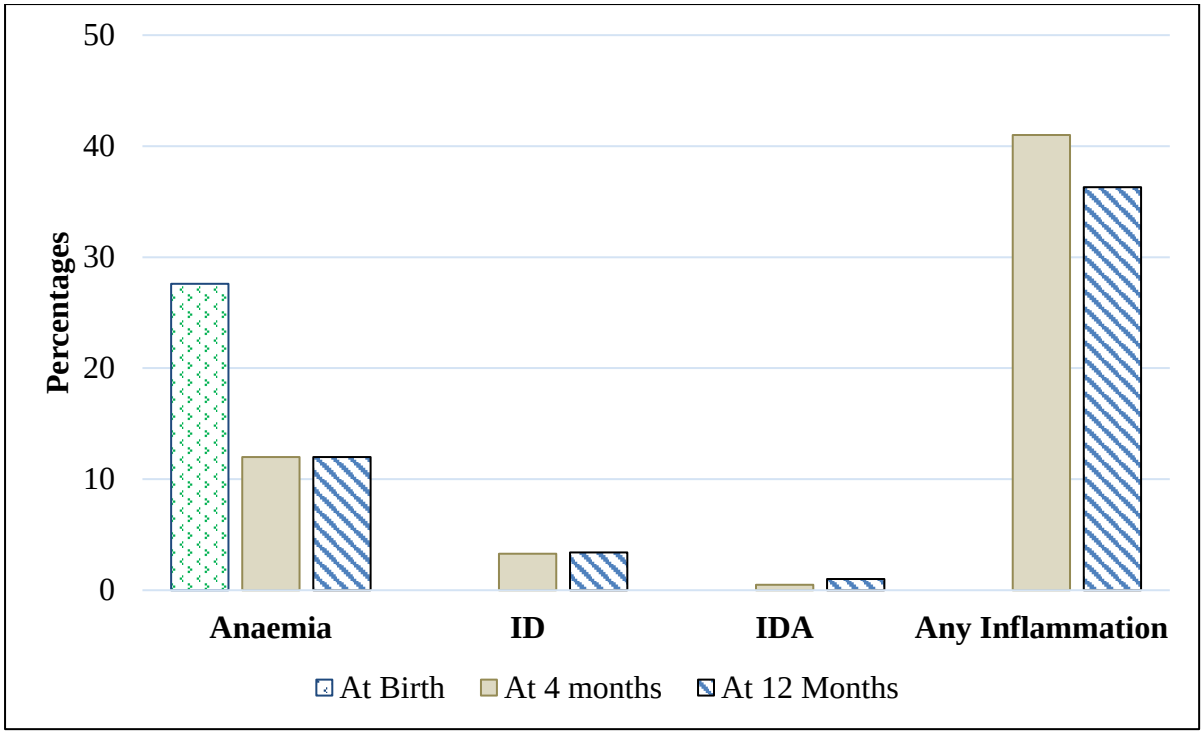

Figure 2: Prevalence of Anaemia, ID, IDA, and inflammation among mothers at 4 and 12 months postpartum. ***Note:*** *Anaemia is defined as haemoglobin concentration < 11 g/dl at birth or < 12 g/dl at 4 and 12 months. ID = Iron deficiency (corrected ferritin < 15 ug/L). IDA = Iron Deficiency Anaemia (concurrence of anaemia and iron deficiency). Inflammation is based on C-reactive protein values > 5 mg/l or α-Acid Glycoprotein values > 1 g/l. Anaemia is based on 192 mothers at birth, and on 182 and 177 mothers at 4 and 12 months respectively. ID and IDA were based on 182 and 176 at 4 and 12 months respectively mothers, and inflammation is based on 182 and 179 mothers at 4 and 12 months respectively.*

Predictors of anaemia, iron deficiency, and iron deficiency anaemia

Table 3 shows the results of the bivariate analysis for the associations between predictors and anaemia, ID, and IDA. The results indicate that neither dietary iron intake nor indictors of complementary feeding practices were significantly related to child anaemia, child iron deficiency, and child iron deficiency anaemia at 12 months of age. However, malaria, fever, and inflammation at 12 months of age and iron status at birth and at 4 months significantly determined child iron status at 12 months of age.

Table 3: Bivariate association of variables with anaemia, iron deficiency, and iron deficiency anaemia among children of 12 months of age.

Variables	**Anaemia** [1]			**Iron deficiency** [2]			**Iron deficiency anaemia** [3]		
	PR	**95% CI**	**P-value**	**PR**	**95% CI**	**P-value**	**PR**	**95% CI**	**P-value**
Energy intake *(kcal/day)*	1.00	1.00, 1.00	0.531	1.00	0.99, 1.00	0.938	1.00	0.99, 1.00	0.511
Proteins *(g/day)*	1.00	0.98, 1.01	0.991	0.99	0.98, 1.01	0.751	0.99	0.97, 1.02	0.811
Fibres *(g/day)*	1.00	0.98, 1.01	0.981	1.00	0.97, 1.02	0.996	0.99	0.96, 1.02	0.554
Iron intake (foods) *(mg/day)*	0.99	0.94, 1.04	0.726	0.99	0.92, 1.06	0.803	0.97	0.88, 1.06	0.559
Non-haem iron (mg/day)	0.98	0.94, 1.03	0.601	0.99	0.92, 1.06	0.836	0.97	0.88, 1.07	0.604
Haem iron (mg/day)	1.02	0.76, 1.37	0.875	0.86	0.53, 1.39	0.554	0.94	0.53, 1.68	0.848
Iron intake (MNPs) *(mg/day)*	1.02	0.94, 1.20	0.598	0.75	0.56, 1.01	0.057	0.77	0.53, 1.12	0.175
Total iron intake *(mg/day)*[4]	0.99	0.95, 1.04	0.912	0.96	0.90, 1.03	0.309	0.95	0.86, 1.04	0.286
Retinol *(ug/day)*	1.00	0.99, 1.01	0.849	1.01	1.00, 1.02	0.020	1.01	1.01, 1.03	0.002
Beta-Carotene *(ug/day)*	1.00	1.00, 1.00	0.125	1.00	1.00, 1.00	0.976	1.00	1.00, 1.00	0.898
Vitamin B12 *(ug/day)*	1.02	0.97, 1.06	0.369	0.99	0.89, 1.10	0.916	1.03	0.93, 1.14	0.561
Riboflavin *(mg/day)*	1.16	0.93, 1.45	0.169	0.87	0.44, 1.72	0.701	0.94	0.47, 1.88	0.869
Folate *(ug/day)*	1.00	0.99, 1.00	0.641	1.00	0.99, 1.00	0.847	0.99	0.99, 1.00	0.343
Vitamin B6 *(mg/day)*	1.35	0.88, 2.07	0.165	1.36	0.68, 2.72	0.373	1.96	0.80, 4.77	0.137

Table 3 continues…

Vitamin C *(mg/day)*	1.00	1.00. 1.00	<.0001	1.00	0.99, 1.00	0.694	1.00	0.99, 1.00	0.283
Calcium *(mg/day)*	1.00	0.99, 1.00	0.692	1.00	0.99, 1.00	0.190	1.00	0.99, 1.00	0.146
Zinc *(mg/day)*	1.02	0.99, 1.05	0.116	0.99	0.91, 1.07	0.800	0.99	0.89, 1.10	0.830
Breastfeeding frequencies	0.99	0.96, 1.03	0.773	0.98	0.94, 1.03	0.592	0.98	0.92, 1.05	0.688
Age of introduction of foods or drinks	0.99	0.89, 1.10	0.942	1.05	0.89, 1.22	0.544	1.14	0.92, 1.44	0.225
Meal frequencies	0.93	0.78, 1.09	0.376	0.91	0.72, 1.15	0.442	0.77	0.56, 1.08	0.135
Dietary diversity scores	1.04	0.92, 1.17	0.517	1.14	0.95, 1.37	0.146	1.12	0.86, 1.47	0.392
Dairy products not consumed	1.69	0.93, 3.08	0.083	0.65	0.38, 1.10	0.111	1.21	0.46, 3.19	0.691
Meat and flesh foods not consumed	0.71	0.49, 1.03	0.073	0.95	0.45, 1.95	0.893	0.69	0.29, 1.59	0.385
Diarrhoea, duration *(days)*	0.99	0.93, 1.05	0.720	0.99	0.91, 1.07	0.759	0.98	0.87, 1.12	0.848
Fever, duration *(days)*	0.99	0.92, 1.08	0.882	1.10	1.03, 1.17	0.001	1.05	0.92, 1.20	0.458
Malaria, duration *(days)*	1.03	0.96, 1.10	0.445	1.08	1.02, 1.14	0.004	1.13	1.06, 1.19	<.0001
Inflammation based on CRP *(mg/l)*	1.01	1.00, 1.01	<.0001	0.97	0.93, 1.01	0.156	0.95	0.90, 1.00	0.075
Inflammation based on AGP *(g/l)*	1.04	1.02, 1.06	<.0001	0.97	0.70, 1.35	0.864	1.13	0.75, 1.71	0.546
Haemoglobin at birth *(g/dl)*	0.93	0.87, 0.98	0.015	0.96	0.86, 1.06	0.402	0.88	0.79, 0.99	0.036
Haemoglobin at 4 mo. *(g/dl)*	0.78	0.69, 0.89	0.003	0.90	0.74, 1.09	0.304	0.77	0.59, 1.01	0.060

Table 3 continues...

Serum ferritin at 4 mo. *(µg/l)*	1.00	1.00, 1.01	0.050	0.99	0.98, 1.00	0.101	0.99	0.98, 1.00	0.081
Serum sTfR at 4 mo. *(mg/l)*	1.03	0.99, 1.07	0.106	1.05	0.99, 1.11	0.088	1.09	1.06, 1.12	<.0001
Body iron stores at 4 mo. *(mg/kg body weight*)	1.03	0.90, 1.18	0.592	0.79	0.64, 0.97	0.026	0.76	0.57, 1.01	0.057
Anaemia at 4 mo.	1.60	1.04, 2.47	0.030	0.95	0.57, 1.60	0.861	1.04	0.50, 2.17	0.902
ID at 4 mo.	0.85	0.48, 1.53	0.600	1.35	0.68, 2.69	0.384	1.45	0.57, 3.64	0.432
IDA at 4 mo.	1.01	0.56, 1.82	0.965	1.46	0.72, 2.99	0.292	1.42	0.50, 4.02	0.503
Maternal anaemia at birth *(g/dl)*	0.96	0.90, 1.07	0.732	1.06	0.92, 1.21	0.405	1.07	0.92, 1.24	0.398
Maternal anaemia at 4 mo. *(g/dl)*	0.92	0.82, 1.03	0.152	1.07	0.90, 1.28	0.421	1.06	0.83, 1.35	0.643
Sex-Boys	1.00	0.74, 1.36	0.975	1.06	0.67, 1.69	0.791	1.28	0.67, 2.42	0.448
Birth weight *(g)*	1.32	0.92, 1.91	0.113	0.84	0.49, 1.46	0.543	1.00	0.50, 2.01	0.998
Weight at 12 mo. *(g)*	1.05	0.91, 1.22	0.460	1.20	0.97, 1.49	0.088	1.20	0.90, 1.59	0.195
Household wealth	0.99	0.89, 1.11	0.942	1.00	0.95, 1.18	0.986	0.92	0.73, 1.15	0.451
Household hunger scores	1.05	0.94, 1.18	0.373	0.80	0.63, 1.03	0.080	0.98	0.96, 1.28	0.913
Mather's education	1.04	0.84, 1.28	0.724	0.63	0.90, 1.12	0.247	0.86	0.60, 1.24	0.429

Note: *Bivariate models were unadjusted. [1] defined as haemoglobin concentration < 11 g/dl. [2] defined as inflammation-adjusted ferritin < 12 ug/l. [3] defined as simultaneously and iron deficiency. [4] Iron naturally in food plus iron added to food via micronutrient powders (MNPs).* ***PR****= Prevalence Ration.* ***CI*** *= Confidence Interval.* ***mo****= month.* ***MNPs*** *= Micronutrients powders.* ***CRP*** *= C-reactive protein.* ***AGP*** *= α-Acid Glycoprotein.*

The presence of inflammation at 12 months of age was associated with increased anaemia at that age based on both CRP (PR=1.01, 95% CI: 1.00, 1.01, p<.0001) and AGP (PR=1.04, 95% CI: 1.02, 1.06). However, anaemia prevalence at 12 months of age significantly decreased when children had higher haemoglobin concentrations at birth (PR=0.93, 95% CI: 0.87, 0.98), and at 4 months of age (PR=0.78, 95% CI: 0.69, 0.89). Furthermore, childbirth haemoglobin concentrations were related to a lower iron deficiency anaemia at 12 months of age (PR=0.88, 95% CI: 0.79, 0.99) (**Table 3**). The prevalence of iron deficiency at 12 months of age decreased when children had higher body iron reserves at 4 months of age (PR=0.79, 95% CI: 0.64, 0.97). Conversely, the prevalence of iron deficiency increased with increasing duration (days) for which children had suffered from fever (PR=1.10, 95% CI: 1.03, 1.17) or from malaria (PR=1.08, 95% CI: 1.02, 1.14). The duration (days) for which children had suffered from malaria likewise increased the prevalence of iron deficiency anaemia (PR=1.13, 95% CI: 1.06, 1.19) (**Table 3**).

Factors that emerged from the multivariate models as independent and significant predictors of child's anaemia, in addition to haemoglobin at birth and 4 months of age, were the presence of inflammation (PR=1.01, 95% CI: 1.01, 1.02) and the duration of suffering from malaria at 12 months of age (PR=1.05, 95% CI: 1.00, 1.10) (**Table 4**). Malaria also predicted increase in both iron deficiency (PR=1.09, 95% CI: 1.04, 1.15) and iron deficiency anaemia (PR=1.15, 95% CI: 1.06, 1.25). Lastly, the prevalence of iron deficiency at 12 months of age was lower when children had better body iron reserves at 4 months of age (PR=0.73, 95% CI: 0.59, 0.89), and tended to be lower with higher total iron intake (PR=0.79, 95% CI: 0.62, 1.03) (**Table 4**).

Table 4: Multivariate analysis for the association of variables with anaemia, iron deficiency, and iron deficiency anaemia among children of 12 months of age.

Variables	**Anaemia** [1]			**Iron deficiency** [2]			**Iron deficiency anaemia** [3]		
	PR	**95% CI**	**P-value**	**PR**	**95% CI**	**P-value**	**PR**	**95% CI**	**P-value**
Total iron intake (*mg/day)*[4]	0.99	0.91, 1.08	0.851	0.79	0.62, 1.03	0.083	0.90	0.69, 1.18	0.458
C-reactive protein (*mg/L)*	1.01	1.01, 1.02	0.037				0.96	0.91, 1.00	0.076
Malaria, duration (days)	1.05	1.00, 1.10	0.043	1.09	1.04, 1.15	0.000	1.15	1.06, 1.25	0.000
Haemoglobin at birth *(g/dL)*	0.94	0.86, 1.01	0.103				0.90	0.78, 1.04	0.169
Haemoglobin at 4 mo. *(g/dL)*	0.85	0.70, 1.02	0.094				0.92	0.69, 1.27	0.580
Body iron stores at 4 mo. *(mg/kg body weight)*				0.73	0.59, 0.89	0.002	0.81	0.60, 1.08	0.163

***Note**: Dietary iron intake was kept in each model plus significant (univariate analysis, p<0.10) and uncorrelated (r<0.30) variables. Each of the three models was adjusted for child's health centre of residence, birth month, birth weight, age of introduction of food or drinks, sex, folate, calcium, vitamins B12 and C, and Beta-Carotene. [1] defined as haemoglobin concentration < 11 g/dl. [2] defined as corrected ferritin < 12 ug/l. [3] defined as simultaneously anaemia and iron deficiency. [4] Iron naturally in food plus iron added to food via micronutrient powders (MNPs). PR= Prevalence Ration. CI = Confidence Interval. mo= month. CRP = C-reactive protein.*

Discussion

The current study aimed to examine the contribution of dietary iron intake to the occurrence of anaemia, iron deficiency, and iron deficiency anaemia among children at 12 months of age. The study shows that at 4 months of age, anaemia was more prevalent, while iron deficiency and iron deficiency anaemia prevailed at 12 months of age. From 4 to 12 months of age, the anaemia prevalence decreased by 1.5 times, whereas the prevalence of iron deficiency almost tripled. The present study shows that dietary iron intake tended to be weakly related to the iron status indicators assessed among children at 12 months of age. However, the presence of inflammation and duration of malaria at 12 months of age, and body iron stores at 4 months of age significantly predicted anaemia and iron status indicators at 12 months of age.

Iron status is infrequently assessed in infants younger than 6 months of age. For example, in Rwanda, there is no data on haemoglobin and iron status in children younger than 6 months of age. However, comparing our findings with those of other studies confirms that iron deficiency and anaemia already occur in children younger than 6 months of age [16, 39]. Some children in resource-limited settings are likely to have insufficient endowed birth iron reserves [40], which are unlikely to maintain physiological iron requirements during the first 6 months of age [11, 39, 41]. Therefore, poor iron status in these children can partly be explained by a depletion of body iron reserves putting them to an increased risk of anaemia [42]. In our study, 38% of children were already affected by inflammation at 4 months of age. Therefore, the presence of inflammation might have contributed to the higher prevalence of anaemia at this age [25].

A declining pattern of anaemia prevalence with a child's age was expected and confirms what is consistently reported in the last two demographic and health surveys in Rwanda [28, 43]. However, this finding contrasts that of Siegel et al. (2006) who reported an increasing anaemia prevalence with age till 17 months of age in Nepal [44]. Contrary to anaemia, we found iron deficiency to increase with the child's age. This compares well to the finding of Rawat et al

(2014) who reported an increasing prevalence of ID from 6 to 11 months of age in Bangladeshis infants [45]. Nevertheless, iron deficiency contributed more than one third to anaemia cases in the current study.

The literature states that the causes of poor iron status during the second half of infancy are related to complementary foods poor in iron amongst other factors [24]. However, in the current study, dietary iron intake, and even the indicators of complementary feeding practices did not significantly predict anaemia or iron-deficiency anaemia at 12 months of age although dietary iron intake weakly associated with iron deficiency. The lack of significant associations between dietary iron intake and any of the iron status indicators can firstly be attributed to the small amount of food consumed, and the low variability in iron intake among children at this young age [45]. Eating a small quantity of foods that in addition contain poorly absorbable iron may have added to the absence of a significant association. As shown before in a rural area in Rwanda [20], and in other countries [21, 23, 42], and confirmed by our study, plant-based foods, which contain less bioavailable iron, dominate the diet of the majority of the children. This diet is high in phytates and low in iron absorption enhancers, for example, meat and vitamin C. For the effect of vitamin C on iron absorption, Cook et al. (1977) found that supplementing a diet with 25 to 1,000 mg of vitamin C increased the iron absorption by 0.8% to 7.1% [46]. Later, Teucher et al. (2004) indicated that a diet containing low to medium levels of iron inhibitors required 20 mg of vitamin C for 3 mg of iron [47]. Therefore, it seems that the intake of vitamin C (16.4 mg of vitamin C vs. 4.8 mg of iron, median values) and low consumption of meats or other flesh foods (11% of children) in the current study did not suffice to adequately enhance iron absorption from a highly plant-based diet. Moreover, we measured iron intake based on foods consumed by a child only on a single day rather than on multiple days to reflect the usual intake. This may have led to a misclassification of iron intake. Nonetheless, like our study, other studies have also shown lack of a significant association

between dietary iron intake or feeding practice indictors and anaemia among study children [42, 45].

Anaemia and poor iron status have multifactorial causes and our study confirms some of these. The presence of inflammation significantly increased the risk of anaemia among study children at 12 months of age. This finding is in line with what other studies report [25, 45]. Inflammatory diseases play a role in the genesis of anaemia and iron deficiency [7] by destroying erythrocytes, suppressing erythropoiesis, and restricting iron mobilisation or absorption [25-27], and thus causing anaemia of inflammation. In the current study, the duration (days) that children had suffered from malaria in the previous 30 days was significantly associated with anaemia, iron deficiency, and iron deficiency anaemia. Malaria has previously been linked to anaemia and poor iron status in preschool children because it causes haemolysis, limits red blood cell formation and reduces uptake of dietary iron [5]. Therefore, by restricting iron uptake from diets that are already low in bioavailable iron, as was the case in our study, malaria may accentuate the occurrence of anaemia and poor iron status in many rural populations in low-income countries [24].

Our study showed that better body iron reserves at 4 months of age are significantly associated with a reduced prevalence ratio of iron deficiency anaemia at 12 months of age. This confirms previous studies showing that adequacy of iron status in the first half of infancy has important implications for iron status in the second half of infancy [14, 15, 18]. As shown in other settings, improving maternal iron status during pregnancy [48] and the practice of delayed cord clamping could help to increase the amount of iron endowment to the child at birth [49, 50]. Delayed cord clamping has not yet been implemented routinely in rural areas of Rwanda and therefore holds promise. In addition, early screening and treatment of anaemia and iron deficiency may be undertaken among children at 4-6 months of age [51]. This would allow

children to better cope with their considerable physiological iron needs experienced from 6 months of age onwards.

The strength of the current study lies in its longitudinal design, in which we assessed iron status for the same children and their mothers at 4 and 12 months postpartum. It, therefore, adds to the scarce data on iron status among infants. The limitation is that the sample size is insufficient to allow generalisation of the finding to larger areas. In addition, dietary iron intake was collected on a single day, which hampered the estimation of habitual dietary iron intake.

In conclusion, the current study shows that infectious diseases importantly contribute to the increased prevalence of anaemia and iron deficiency among children during infancy. Therefore, to make dietary interventions effective, infectious diseases must be controlled.

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Chapter 5

Comparing saliva and urine samples for measuring breastmilk intake with the deuterium oxide dose-to-mother technique among children 2-4 months old

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Abstract

***Background*:** Saliva and urine are the two main body fluids sampled when breastmilk intake is measured with the deuterium oxide dose-to-mother technique. However, these two body fluids may generate different estimates of breastmilk intake due to differences in isotope enrichment. Therefore, we aimed to assess how the estimated amount of breastmilk intake differs when based on saliva and urine samples and to explore whether the total energy expenditure of the mothers is related to breastmilk output.

Methods: We used a convenience sample of 13 pairs of mothers and babies aged 2 to 4 months who were exclusively breastfed and apparently healthy. To assess breastmilk intake, we administered doubly labelled water to the mothers, and collected saliva samples from them, while simultaneously collecting both saliva and urine from their babies over a 14-day period. Isotope ratio mass spectrometer was used to analyse the samples for deuterium and oxygen-18 enrichments. Enrichment data were fitted to a model for water turnover in mothers and babies with a multipoint protocol using the solver function in Microsoft Excel to calculate breastmilk intake.

Results: Estimated mean breastmilk intake based on saliva samples was significantly higher than that based on urine samples (854.5 g/day vs. 812.8 g/day, p=0.029). This can be attributed to slightly higher isotope enrichments in saliva and to a poorer model fit for urine samples as shown by its square root of the mean square error higher than that for saliva samples (14.6 mg/kg vs. 10.4 mg/kg, p=0.001). Maternal energy expenditure did not correlate with breastmilk output.

Conclusion: Our study suggests that saliva sampling generates slightly higher estimates of breastmilk intake and is more precise as compared to urine and that breastmilk output is not related to maternal energy expenditure.

Introduction

The World Health Organization recommends exclusively breastfeed children during the first 6 months of life [1]. In this period, breastmilk should preferably be the sole source of nourishment for the child's growth and development [2]. Exclusive breastfeeding imparts the child with health benefits and reduces the risk of childhood morbidity and mortality [3-5]. To determine the adequacy of breastmilk quantity and nutrient intake and to link breastfeeding patterns to children's growth, and development, accurate quantification of breastmilk intake is essential.

Breastmilk intake used to be quantified by test weighing methods, for which the child's weight is taken before and after each breastfeed, and the difference between these two weights amounts to breastmilk ingested by the child [6, 7]. However, this method is time-consuming and disturbs the breastfeeding routine. Another disadvantage is the inability to assess if a child is exclusively breastfed because the test weighing method does not capture water intake from other sources [8]. In surveys, the prevalence of exclusive breastfeeding is usually based on maternal recalls, which are often associated with recall bias and socially desirable responses that lead to over-estimation of the true prevalence [9].

To overcome these challenges, a more objective technique called "deuterium oxide dose-to-mother technique" involving the use of stable isotopes was developed [8, 10]. The deuterium oxide dose-to-mother technique, first described by Coward in 1980 [11], was found to give comparable estimates of breastmilk intake to the test weighing method [10]. The major advantage of the stable isotope technique is that daily breastmilk intake is estimated over a 14-day period without interfering with the breastfeeding routine or being too much of a burden to the mothers [8]. Additionally, with this technique, a child can either be classified as exclusively breastfed or not. The technique is based on the deuterium enrichment of body fluids of both

the mother and the child. For the easiness of collection, either saliva or urine is usually preferred as body fluid [12, 13]. However, in studies with labelled water, the level of isotope enrichment has been found to differ between types of body fluids, with saliva samples having a slightly higher isotopic enrichment than urine samples [12-15]. Therefore, the use of either of these types of body fluids is likely to result in different outcomes [16]. To this end, Rieken et al. (2011) assessed the comparability of saliva and urine samples to quantify energy expenditure and body composition, and they concluded that both types of body fluids gave comparable estimates [16]. However, Jankowski et al. (2004) and Schierbeek et al. (2009) found that, as opposed to urine, saliva provided a more accurate estimate of the intended outcome [12, 13].

Although both saliva and urine have been sampled in studies to measure breastmilk intake with the deuterium oxide dose-to-mother technique [6, 17-19], it is not known how the type of body fluid affects the estimate for breastmilk intake. Therefore, we aimed to quantify and compare estimates of breastmilk intake with the deuterium oxide dose-to-mother technique when based on either saliva or urine samples among 2 to 4-month-old children. In addition, since we expected large differences in physical activity patterns between mothers, we simultaneously measured maternal energy expenditure by using doubly labelled water and explored how this was related to breastmilk output.

Methods

Study site and subjects

The study was conducted in a rural area of Muhanga District, Southern Province, Rwanda, and in the town of Wageningen, the Netherlands. The Rwandan National Ethics Committee and the Medical Ethics Committee of Wageningen University and Research approved this study. We followed the ethical guidelines as laid down in the declaration of Helsinki and its amendments.

In accordance, the study objective and procedures were explained to parents both verbally and in writing before they gave their written consent.

A convenience sample of 13 mother-child pairs (5 Dutch and 8 Rwandans) participated in the study. The Rwandan participants were recruited through the Rutobwe health centre and Dutch participants were recruited at the local swimming pool during baby swimming sessions and at child consultation clinics. The recruited mothers were exclusively breastfeeding (reported by themselves), apparently healthy, and were willing to stay in the study areas for the next two weeks. The children were aged 2 to 4 months, full-term, singleton, and were apparently healthy. **Figure 1** summarizes of the main steps of the study and their sequence.

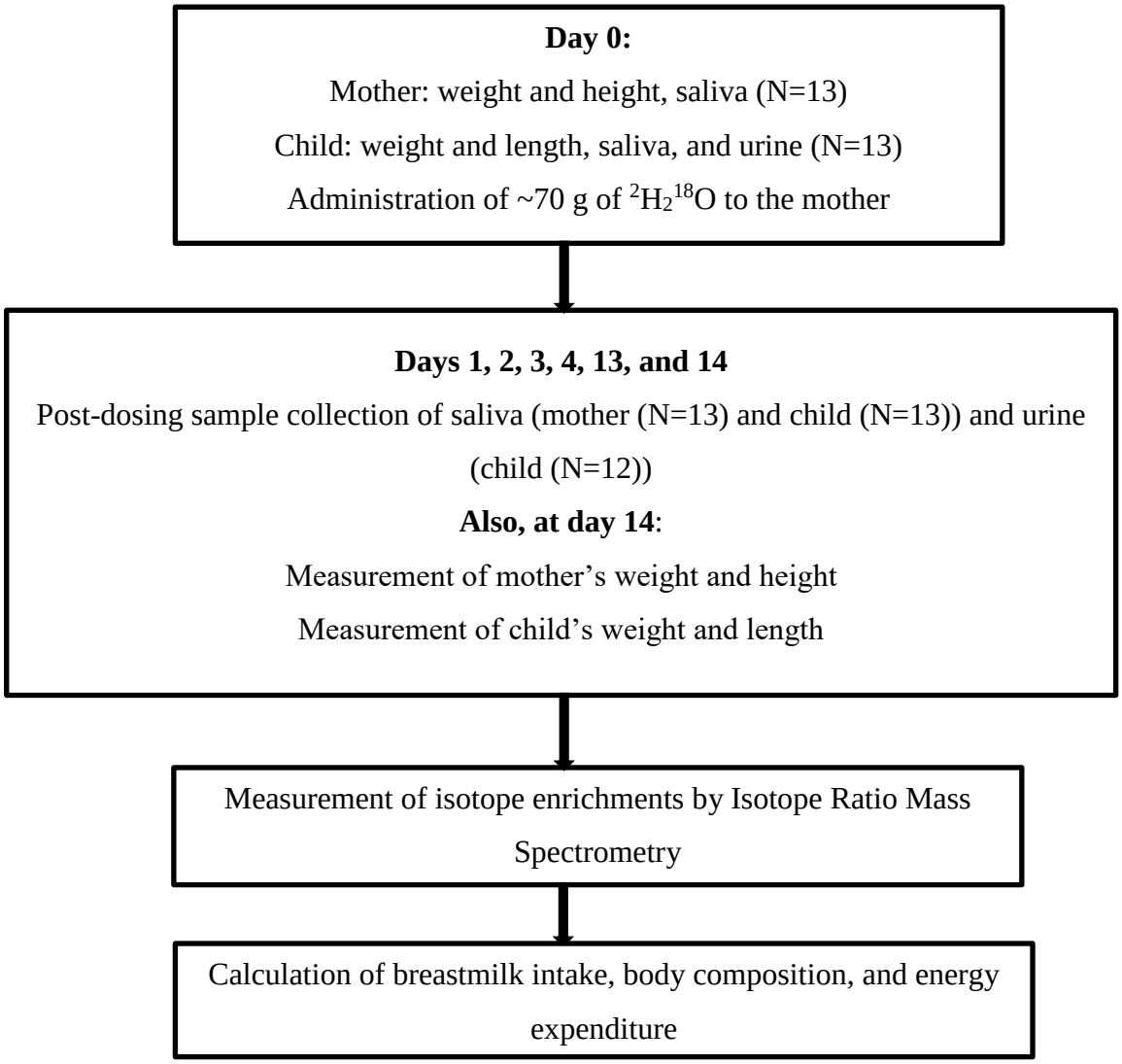

Figure 1: Study flow diagram

Preparation and administration of doubly labelled water doses

A multipoint protocol was used for concurrently estimating breastmilk intake of the children and energy expenditure of the mothers. The doubly labelled water mixture was prepared ahead of the start of the dosing. Before aliquoting individual doses of approximately 70 g, doubly labelled water was filtered using Whatman puradisc FP 30/0.2 syringe filters (GE Healthcare Europe GmbH, Eindhoven, The Netherlands), and dispensed into a 250 ml polyethylene bottle. The prepared doses were stored overnight in a refrigerator until administered to the mothers. Mothers received a mixture of 1.8 g 10% enriched $H_2{}^{18}O$ (Centre for Molecular Research Ltd, Moscow, Russia) and 0.3 g 99% enriched D_2O (Cambridge Isotope Laboratories, Inc, Andover, MA, USA) per kg body water. It was assumed that the bodyweight of females comprised 50% of body water [20]. In human studies for breastmilk output and body composition, the deuterium consumed by study participants enriches the body water to a maximum of 0.1%, which is safe and far lower than 15% at which harmful effects can occur [21, 22].

Each mother drank an accurately weighted dose of ~70 g from the polyethylene bottle using a straw. Immediately after drinking the dose, we rinsed the bottle with approximately 100 ml drinking water and the mother drank this water rinse as well using the same straw. The time when the mother drank the dose was recorded on data collection sheets.

Data and sample collection

We used the UNICEF electronic scale (Seca 878) and length or height wooden boards (UNICEF model) to measure weight and length (child) or height (mother) respectively. These anthropometric measures were collected in duplicate from mothers and children at baseline (day 0) and at day 14 of the study (**Figure 1**). Weight was recorded to the nearest 0.1 kg and height or length to the nearest 0.1 cm.

After baseline anthropometric measures and before the mother drank the dose (day 0), we collected 2 ml of saliva from both mother and child, and 5 ml of urine only from the child. Subsequent urine and saliva samples were collected on 1, 2, 3, 4, 13, and 14 days post-dosing (**Figure 1**).

To collect maternal saliva samples, the mother held a sterile cotton ball in her mouth until saturated with saliva. The saliva saturated cotton ball was then transferred into a 20 ml syringe and saliva was squeezed into a 2 ml cryogenic vial. Cotton swabs were used to collect saliva samples from the child. We kept the cotton swabs moving in the child's mouth for about 2 minutes. The saliva sodden cotton swabs were then placed into a syringe to express saliva into a 2 ml cryogenic vial. This process was repeated 2 to 3 times to collect enough saliva (2 ml) from the child.

We collected urine samples from children only. After cleaning the genital parts, the child wore a diaper fitted with a cotton inlay pad. Once a child urinated, we removed the cotton inlay pad and placed it into a plastic bag. After cutting a corner tip from the plastic bag, we manually squeezed out urine into a cup. Of this, 5 ml of urine was immediately transferred into a cryogenic vial. We recorded the sampling time of all samples on data collection sheets.

Storage of samples

The sealing caps of the cryogenic vials were wrapped with parafilm for tight closure. On the day of collection, the cryogenic vials were transported in a cooler box to the local laboratory at University of Rwanda and stored in a freezer at -20^{o}C until they were transported frozen to the laboratory of the Division of Human Nutrition and Health, Wageningen University and Research, The Netherlands.

Analysis of samples for the isotope enrichment

All samples were flame-sealed in 25 ul pre-calibrated pipettes (Vitrex Medical A/S, Herlev, Denmark). Isotopic enrichment of the saliva and urine samples and diluted doses were analysed at the Centre for Isotope Research, Groningen, The Netherlands, as described elsewhere [23]. Briefly, the urine samples in the capillaries were subjected to a micro-distillation procedure to obtain pure distilled water. Next, a volume of 0.12 µl of distilled water was injected using an auto-sampler (CTC PAL, CTC Analytics, Zwingen, Switzerland) through a heated septum into a high-temperature pyrolysis unit consisting of a glassy carbon reactor with a temperature > 1300 °C (Hekatech, Wegberg, Germany). The reaction products of the pyrolysis process such as H_2 and CO_2 gasses were led by a continuous flow of helium gas on a GC, where the gasses were separated and led into a continuous flow Isotope Ratio Mass Spectrometry (IRMS) system (Isoprime, GV Instruments, Manchester, UK). Each sample was injected six times for δ^2H analysis followed by three more injections for $\delta^{18}O$ analysis. Ratios (R) of $C^{18}O/C^{16}O$ and $^3H/^2H$ relative to reference water (VSMOW, Vienna Standard Mean Ocean Water) were expressed in δ units of ‰ after correction for the memory effects. The relative difference between sample isotope ratio and the isotope ratio of the international standard was expressed as delta units using this formula: $\delta^{18}O$ or δ^2H (‰) = 1000 * (Rs – Rvsmow)/Rvsmow. Enrichments expressed as delta units were converted into ppm excess [24, 25]. ^2H and ^{18}O dilution spaces were calculated using the intercept method.

Reference water (biomedical enriched waters gravimetrically prepared from VSMOW) was analysed for quality control and showed analytical variations of < 0.5% for both isotopes, and accuracy defined as deviation from the certified values were < 1% for δ^2H and < 0.3% for $\delta^{18}O$.

Calculations of breastmilk intake and water intake from other sources

We calculated breastmilk intake according to Haisma et al. [26]. The calculations were based on fitting the deuterium enrichment data to a model for water turnover in the mother and child. We used solver function in Microsoft excel to fit data of enrichment to the following equations:

Data from the mother: $\frac{\mathrm{Em(t)}}{\mathrm{Em(0)}} = e^{-k_{mm}t}$ where $\boldsymbol{E_{m(t)}}$ is the deuterium enrichment in the mother's body water at time t, in mg/kg; *t* is the time since the dose was taken; $\boldsymbol{E_{m(0)}}$ is the deuterium enrichment in the mother's body water at time zero, mg/kg; $\boldsymbol{k_{mm}}$ is the fractional water turnover in the mother (kg/day).

Data from the child:

$E_{b(t)} = E_{m(o)} \left(\frac{F_{bm}}{V_b}\right) \left(\frac{e^{-k_{mm}t} - e^{-(F_{bb}/V_b)t}}{(F_{bb}/V_b) - k_{mm}}\right)$ where: $\boldsymbol{E_{b(t)}}$ is the deuterium enrichment in the baby's body water at time t, in $mg.kg^{-1}$; $\boldsymbol{t}$ is the time since the dose was taken by the mother, in days; $\boldsymbol{E_{m(0)}}$ is the deuterium enrichment in the mother's body water at time zero mg/kg; $\boldsymbol{F_{bm}}$ is the transfer of water from the mother to the child via breastmilk (kg/day); $\boldsymbol{V_b}$ is the baby's total ^{2}H distribution space (kg). V_b was assumed to change linearly with weight over the study period, $V_b = 0.84\ W^{0.82}$; $\boldsymbol{k_{mm}}$ is the fractional water turnover in the mother (kg/day); $\boldsymbol{F_{bb}}$ is the total water loss in the child (kg/day).

The amount of breastmilk intake was calculated from the water flow from the mother to the child assuming that 87.1% of breastmilk is water [27]. Therefore, breastmilk intake (g/day) was given by $F_{bm}/0.871$.

Calculation of child and maternal body composition

The calculated components of the body composition were: total body water, fat mass, and fat-free mass. Total body water (TBW) was calculated as the average of the ^{2}H dilution space

divided by 1.041 and ^{18}O dilution space divided by 1.01 to account for non-aqueous isotope exchange [28]. We calculated the fat-free mass (FFM, kg) of the mothers as TBW (kg)/0.732, assuming that 73.2% of FFM is water [29]. The difference between body weight and FFM gave fat mass. For the children, the FFM was calculated as TBW (kg)/h_{FFM} with h_{FFM} being hydration constant of FFM, which is assumed to be 0.80 for boys and 0.79 for girls of 3 months of age [30].

Calculation of maternal total energy expenditure

Isotope elimination rates (k_O and k_D) were calculated as the gradient of the plot of the natural logarithm of the enrichment in body water versus time since the dose was taken. Rate of carbon dioxide production was calculated using the following formula: rCO_2 (L/day)= 0.455 x TBW(L) x (1.007 k_O – 1.041 k_D) (IAEA, 2009) and total energy expenditure using the modified Weir equation [31]. Total energy expenditure (kcal/day) = rCO_2 (L/day) x (1.1 +3.90/RQ), where RQ was assumed to be 0.85.

Statistical analysis

We compared the mean difference between breastmilk intake based on saliva and urine with paired and independent t-tests. The square root of the mean squared error (MSE) was used to evaluate the goodness of the modelled data fit, which reflects the difference between the measured and model-predicted deuterium enrichments in the mother and child. The p-value was set at 0.05 for each statistical test of significance. To assess the patterns of the differences between breastmilk intakes, the differences between breastmilk intake based on saliva and urine were plotted against their means using a Bland-Altman pair-wise comparison.

Results

Study participants

Table 1 presents the characteristics of the study participants. The mean age and mean body weight of the Rwandan children were slightly higher than those of Dutch children. The body fat mass percentage of the Dutch children (25.7%, SD=2.3) was slightly higher than that of the Rwandan children (23.7%, SD=5.1). The age of mothers ranged from 21 to 38 years with a mean of 30 years. Dutch mothers tended to have a slightly higher mean body weight and fat mass and they were taller on average. Nonetheless, the body mass index of the Dutch mothers (23.2 kg/m^2, SD=4.7) was comparable to that of the Rwandan mothers (23.4 kg/m^2, SD=2.6). In addition, the mothers had a similar fat-free mass (41 kg, SD=3.4).

Isotope kinetic results between saliva and urine samples

The quality parameters of the enrichment data in the current study were in the acceptable ranges (**Table 2**). **Table 3** compares the isotope kinetic results between saliva and urine samples. The deuterium enrichment tended to be higher in saliva samples than in urine samples at each time point. Additionally, the overall mean of deuterium enrichment, and area under the curve were slightly higher for saliva samples (89.3 ppm and 1505 ppm) than for urine samples (87.4 ppm and 1461 ppm). However, enrichments were only statistically different for the area under the curve (p=0.009) (**Table 3**).

The square root of mean square error (MSE) in data from saliva samples (10.4 g/day, SD=6.4) was significantly lower compared to that in data from urine samples (14.6 g/day, SD=6.1), p=0.001). Furthermore, average breastmilk intake based on saliva samples was higher (854.5 g/day, SD=222.3) than that based on urine samples (812.8 g/day, SD=187.1), p=0.029 (**Table 3**).

Table 1: Characteristics of the study participants

Characteristics*	**Dutch**	**Rwandan**	**All**
Number of mother-baby pairs	5	8	13
Children:			
Sex ratio (Male: Female)	1:4	5:3	6:7
Age (months)	3.4, 1.0	3.7, 0.6	3.6, 0.7
Weight at day 0 *(kg)*	6.0, 0.5	6.8, 1.7	6.5, 1.4
Weight at day 14 *(kg)*	6.4, 0.7	7, 1.6	6.8, 1.3
Weight gain *(g/day)*	20.8, 17.3	19.6, 18.5	20.2, 17
Length *(cm)*	61.7, 1.7	60.5, 2.5	61.0, 2.3
Body Mass Index-for-age z-scores	0.14 ,1.3	0.99 ,1.8	0.66 ,1.6
Total body water *(kg)*	3.7, 0.2	3.9, 0.6	3.9, 0.5
Fat-free mass *(kg)*	4.7, 0.3	5.0, 0.7	4.9, 0.6
Fat mass *(kg)*	1.6, 0.3	1.5, 0.4	1.6, 0.3
Fat mass *(%)*	25.7, 2.3	23.8, 5.1	24.5, 5.1
Mothers:			
Age *(years)*	30.8, 0.8	30.5, 6.1	30.6, 4.6
Body weight at day 0 *(kg)*	63.8, 13.2	60.2, 5.3	61.5, 8.8
Height *(cm)*	165.3, 3.3	160.9, 7.2	162.6, 2.2
Body Mass Index *(kg/m^2)*	23.1, 4.7	23.4, 2.6	23.2, 3.4
Total body water *(kg)*	30.2, 2.4	30.0, 2.7	30.0, 2.5
Fat-free mass *(kg)*	41.0, 3.4	41.0, 3.7	40.9, 3.4
Fat mass *(kg)*	22.5, 13	19.3, 3.6	20.5, 8.1
Fat mass *(%)*	33.2, 10.6	31.9, 4.4	32.4, 7

***Note:** * Values are means, standard deviations, except for the number of participants and child sex ratio.*

Moreover, saliva samples resulted in a significantly lower estimated non-breastmilk water intake. At the individual level, for 10 out of 12 mother-child pairs, saliva gave higher breastmilk intake estimates compared to urine, and 1 child was classified as not exclusively breastfed based on non-breastmilk water intake estimated from either saliva or urine (data not presented) (**Table 3**).

Table 2: Kinetic quality parameters for analysis of doubly labelled water.

Participant	**R^2**		**Dilution space**	**Abundance at 14 day**		**Isotope elimination rate**
	^2H	**^{18}O**	**$^2H/^{18}O$**	**^2H**	**^{18}O**	**k_O/k_D**
D1	0.998	0.998	1.037	56.9	29.9	1.2
D2	0.999	0.999	1.012	81.6	21.8	1.3
D3	0.999	0.999	1.005	71.5	22.8	1.2
D4	0.994	0.997	0.997	69.3	22.8	1.2
D5	0.999	0.999	1.006	70.5	22.6	1.2
RW1	0.999	0.999	1.046	41.5	16.8	1.3
RW2	0.998	0.999	1.069	51.8	23.6	1.3
RW3	0.999	0.999	1.047	48.3	22.6	1.3
RW4	0.999	0.999	1.061	48.5	25.3	1.2
RW5	0.999	0.998	1.030	60.6	26.7	1.3
RW6	0.999	0.999	1.014	47.5	22.7	1.2
RW7	0.999	0.994	1.018	42.3	17.0	1.3
RW8	0.998	0.999	1.043	48.9	23.0	1.3
Range	0.994-0.999		0.997-1.069	41.5-81.6	16.8-29.9	1.2-1.3

***Note**: R^2: Coefficient of the determination regression line, ^2H: Deuterium, ^8O; Oxygen-18, k_O: Oxygen-18 elimination rate, k_D: Deuterium elimination rate, ppm: parts per million. D: Dutch, RW: Rwandan.*

The calculated energy intake based on both types of samples showed that saliva-based breastmilk intake provided significantly higher energy intake than urine-based breastmilk intake (2,502 kJ/day vs. 2,377 kJ/day, p=0.029) (**Table 3**).

Mean breastmilk output based on saliva (760 g/day, SD=65.6) was higher but not significantly different from the mean intake based on urine samples (749 g/day, SD=77.1) among Dutch participants (p=0.27). In contrast, saliva samples gave significantly higher mean breastmilk intake (901 g/day, SD=261.2) compared to urine samples (844 g/day, SD=221.2) among Rwandan participants (p=0.045). For both types of body fluid, the mean breastmilk intake of the Dutch children was lower, but not significantly different from that of Rwandan children (p= 0.70 for saliva and p=0.42 for urine) (**Table 3**). However, the variability in differences between breastmilk intakes based on the two types of samples is higher at lower intakes (**Figure 2**).

The mean total daily energy expenditure was significantly higher in the Rwandan mothers than in the Dutch mothers (13,480 kJ, SD=1,966) vs. 10,695 kJ, SD=2,414), p=0.043) (**Table 3**). Maternal total daily energy expenditure did not significantly correlate with breastmilk intake, neither for the total sample (r=0.33, p=0.28) (**Figure 3**) nor for the Dutch and Rwandan group separately (Dutch: r=0.05, p=0.88; Rwandan: r=0.27, p=0.50). Breastmilk intake did not also correlate with mother's age (r=0.006, p=0.98) and mother's body fat (r=0.29, p=0.32), but it did correlate with child's body mass index-for-age (r=0.80, p=0.001), child's fat-free mass (r=0.62, p=0.032), and with the mother's body mass index (r=0.60, p=0.036).

Table 3: Kinetic results based on saliva and urine body fluids

Outcome	**Saliva** (Mean, SD)		**Urine** (Mean, SD)		**Saliva** (Mean, SD)	**Urine** (Mean, SD)	P-value*
	Dutch	Rwandan	Dutch	Rwandan	All	All	
^{2}H enrichment *(ppm)* $T_{1S}=23.6$, $T_{1U}=23.9$	53.2, 11.0	43.6, 11.50	50.0, 9.9	39.7, 10.6	47.4, 11.7	43.8, 11.1	0.049
^{2}H enrichment *(ppm)* $T_{2S}=48.2$, $T_{2U}=48.8$	102.3, 10.6	78.0, 19.24	98.3, 17.3	75.9, 21.4	86.8, 21.1	84.0, 22.2	0.090
^{2}H enrichment *(ppm)* $T_{3S}=71.8$, $T_{3U}=72.6$	131.8, 16.6	95.3, 24.9	130.1, 17.9	88.8, 22.3	109.9, 28.1	105.3, 28.9	0.031
^{2}H enrichment *(ppm)* $T_{4S}=96.0$, $T_{4U}=96.4$	151.0, 14.8	113.9, 19.4	154.2, 15.8	108.4, 17.3	127.4, 25.3	125.1, 28.0	0.371
^{2}H enrichment *(ppm)* $T_{13S}=317.5$, $T_{13U}=317.9$	115.1, 7.5	74.5, 10.2	113.0, 11.0	71.9, 11.8	88.1, 21.2	85.6, 23.0	0.054

Table 3 continues…

^{2}H enrichment *(ppm)* T_{14S}=*342.0,* T_{14U}=*342.8*	106.8, 8.3	67.0, 9.0	106.4, 8.2	66.1, 9.6	80.3, 20.5	79.5, 21.6	0.313
Mean ^{2}H enrichment *(ppm)*	110.0, 10.5	78.6, 14.7	108.6, 11.6	76.8, 14.8	89.3, 20.0	87.4, 20.5	0.106
Area under the curve *(ppm)*	1994, 226.2	1283.8, 202.4	1924.4, 253.3	1230.0, 202.1	1504.1, 382.0	1461, 5, 400.4	0.009
Square root MSE *(mg/kg)*	12.8, 11.0	9.1, 2.9	17.2, 8.1	13.3, 5.0	10.4, 6.4	14.6, 6.1	0.001
Breastmilk output *(g/day)*	760.2, 65.6	901.6, 261.2	748.7, 77.1	844.8, 221.2	854.5, 222.3	812.8, 187.1	0.029
Non-milk oral water intake *(g/day)*	-11.7, 16.2	34.2, 115.8	-7.5, 20.5	70.5, 129.7	18.9, 95.5	44.5, 110.9	0.022
Daily energy intake *(kJ/day)* †					2502.0, 652.7	2380.7, 548.1	0.029

***Note**: SD: Standard Deviation; T: time (hours) of sample collection after dosing; S: Saliva; U; Urine; MSE: Mean Square Error, which is the differences between the measured and model-predicted deuterium enrichment in the mother and child.*

[*] *P-value of the t-test between saliva and urine estimates of each outcome for all participants.*

† Energy intake is estimated based energy density of 0.70 kcal/g according to Dewey et al. (1991) [32].

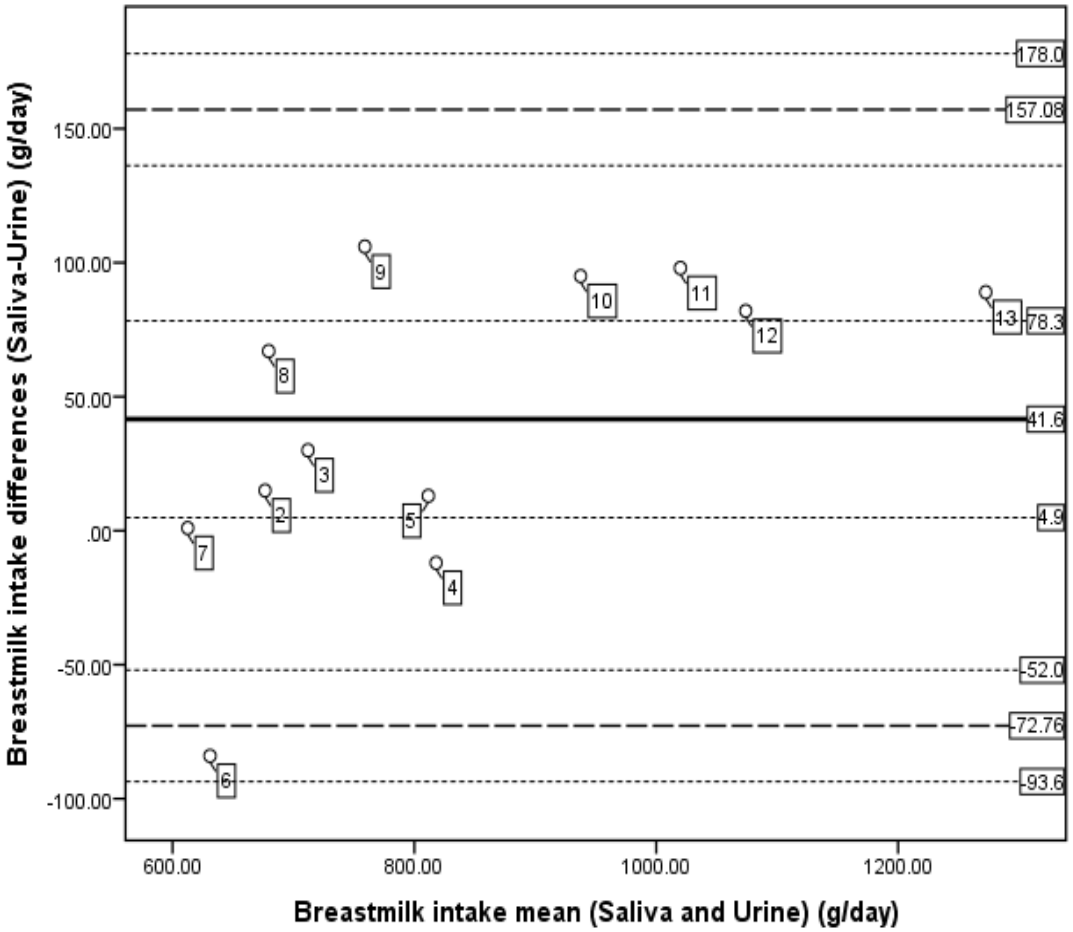

Figure 2: Bland and Altman plot of the differences of breastmilk intakes (Saliva-Urine) plotted against mean intakes (Saliva+urine)/2. ***Note:*** *The central plain line is the mean difference in breastmilk intakes from saliva and urine (41.6 g/day). The lower and upper thick dashed lines indicate the mean difference plus two standard deviations (mean, 2 standard deviations=-72.76, 157.08). The thin dotted lines show the confidence interval for the mean (4.9, 78.3), lower limit (-93.6, -52) and the confidence interval for the upper limit (136.2, 178.0). The numbers in the figure represent the participants per country (2-5 for Dutch and 6-12 for Rwandans).*

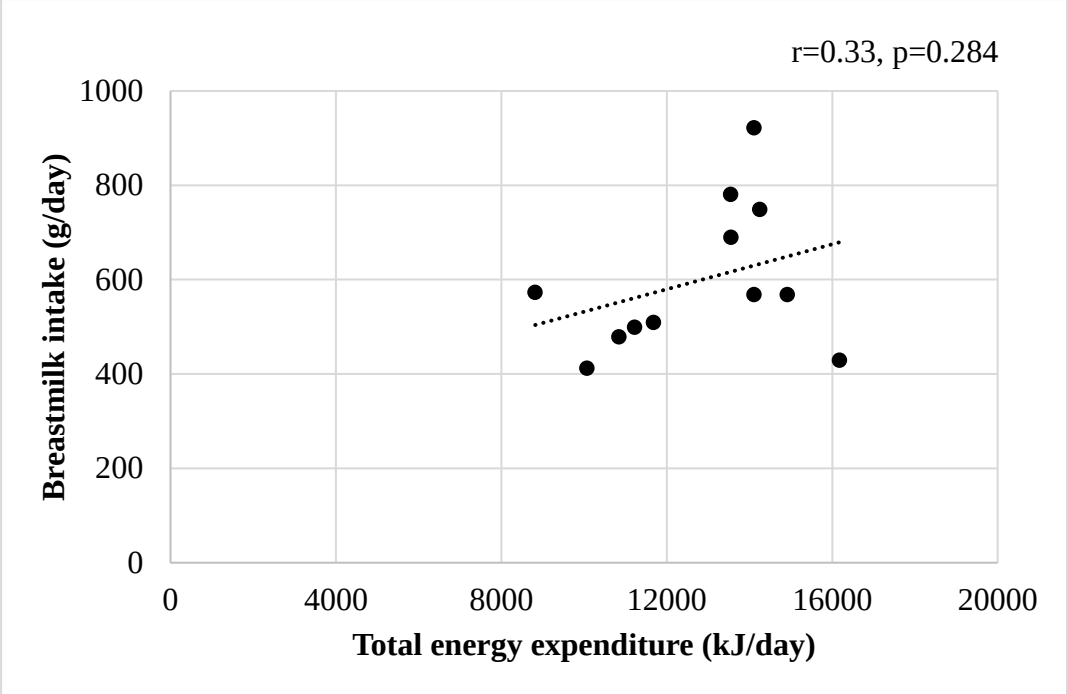

Figure 3: Correlation between maternal energy expenditure and breastmilk intake

Note: *r is the correlation coefficient, and p is the p-value.*

Discussion

The present study showed that saliva samples resulted in ~5% higher estimates of breastmilk intake than urine samples and that maternal energy expenditure did not correlate with breastmilk intake. There are two main reasons that can explain the observed difference between saliva and urine as media for a breastmilk intake assessment. Firstly, it may be due to a higher level of deuterium enrichment in saliva compared to urine and secondarily to a poorer fit of the enrichment data from urine samples.

In our study, saliva samples were slightly more enriched in deuterium than urine samples. This finding is in accordance with earlier observations by Schoeller et al. [15] and Schierbeek et al. (2009) [12] who found a similar difference in enrichment patterns between saliva and urine. This observed difference in deuterium enrichment may be related to fractionation patterns, i.e. the relative abundance of deuterium oxide isotopes in body fluids [13]. Since breastmilk intake is quantified based on deuterium enrichment [8], the different level of deuterium enrichment

between saliva and urine samples is, therefore, likely to influence the magnitude of breastmilk intake estimates. Accordingly, Rickien et al. (2009) attributed a lower estimate of energy expenditure to a slightly lower isotopic enrichment in urine compared to saliva [16].

To quantify breastmilk intake, the deuterium enrichment data are fitted to a model for water turnover in mother and child [8]. With the model, the square root of MSE is calculated to assess the fit of the modelled data. The smaller the square root of MSE, the more precise the data [33]. In our study, the square root of MSE is significantly smaller for saliva samples than for urine samples indicating a poorer fit when urine samples are used. This is probably caused by, on the one hand, the longer time taken by tracers to equilibrate in the contents of the bladder, and on the other hand, the time lag between initial urine production in the kidney and sample collection, particularly in children who are still incontinent at younger age [14, 34]. Therefore, the urine-based data resulted in larger random errors as opposed to saliva-based data. These larger random errors together with a small sample size (12 participants) may have contributed to less reliable measurements based on urine samples. Consequently, this may have resulted in a small but significant difference in estimated breastmilk intake between the two types of sample media. However, based on a cut off of 86.6 g/day for classifying exclusivity of breastfeeding [35], a difference of 5% between two sample media seems to be less important.

Although the mean breastmilk intake based on saliva and urine samples differ, each of these mean intakes exceeds the pooled mean breastmilk intake of 820 g/day reported for exclusively breastfed 3-4 month-old children [36]. Likewise, based on estimated mean energy intakes of 2,502 kJ/day (saliva-based breastmilk) and 2,376 kJ/day (urine-based breastmilk), children in the current study would meet, for example, the energy need of 2,384 kJ/day and 2,184 kJ/day for a 3-month old boy or girl, respectively [37]. In addition, for individual children in this study, a similar proportion of children (50%) would meet their daily energy needs by consuming

breastmilk as estimated based on either saliva or urine. Thus, a mean difference of not more than 5% between saliva and urine samples does not affect estimates of energy adequacy; however, figure 2 shows that the variability in differences between breastmilk intake based on the two types of samples is higher at lower intake.

From a practical perspective, we experienced that collecting urine samples was challenging due to the uncontrollable time of releasing urine by the child, sometimes resulting in a long waiting time and frequent disturbing of the child for checking the wetness of the cotton inlay pad. This waiting time was sometimes lengthened when a child defecated, which required us to change the diaper and inlay pad. For these reasons, urine collection was more cumbersome contrary to saliva. Taken together, saliva, therefore, seems to be more suitable as a medium for studies using the deuterium oxide dose-to-mother technique than urine.

Breastmilk intake was not significantly different between Dutch and Rwandan children. Brown et al. (1998) also reported that breastmilk intake in developing countries does not differ from intake in developed countries [38]. Mean breastmilk intakes estimated in the current study are approximatively within the intake range of 744-925 g/day reported in other studies using the deuterium oxide dose-to-mother technique [10, 17-19, 26, 31, 39]. In addition, despite a small sample size, the breastmilk intake in our study also compares well with the ~700 to 800 g/day reported by Dewey et al. (1981) who used the test weighing method [40].

Since we used doubly labelled water in this study, we measured maternal body energy expenditure in addition to breastmilk intake. The energy expenditure of Rwandese mothers was significantly higher than that of Dutch mothers. This finding agrees well with what Singh et al. (1989) found in the Gambian and English lactating mothers [41]. The rural livelihood conditions for the Rwandan study mothers, dominated by farming activities, are the basis of the observed difference in energy expenditure. Rural residents are generally more active than

urban residents [42], and adult Africans generally participate more in vigorous-intensity physical activity than Europeans [43]. In addition, a study conducted in the Gambia showed that the energy cost of physical activity among mothers during the season of the highest farming activities was up to 2.5 times higher than that of affluent non-farming mothers [41]. These factors explain the difference in energy expenditure between Dutch and Rwandan mothers. Nevertheless, energy expenditure did not correlate to the quantity of breastmilk in either setting. Therefore, it seems that energy expenditure in lactating mothers does not affect breastmilk output in this study, as has also been reported earlier from studies on maternal exercise and lactation performance [44, 45].

The strength of this study is that it used an objective technique to measure breastmilk intake and energy expenditure. In addition, the quality of the enrichment data in the current study was acceptable. This is shown by the accuracy, precision, and other details of the kinetic data based on analysis with gc-pyrolysis-IRMS, which were within an acceptable range and comparable to other studies [24]. Additionally, the study compares the findings between two different settings. However, the small sample size is the major limitation of our study, and therefore results are not representative of the respective source populations.

To conclude**,** saliva samples resulted in higher estimates of breastmilk intake than urine samples. The difference between the two types of body fluid may be attributed to the differences in deuterium enrichment and to larger random errors in urine data indicating a poorer fit. The collection of urine samples is more cumbersome compared to saliva. Therefore, both from a methodological as well as from a practical perspective, saliva sampling is preferable over urine sampling in studies measuring the amount of breastmilk intake with the deuterium dose-to-mother technique. Energy expenditure in lactating mothers does not affect breastmilk output.

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Chapter 6

General Discussion

Background and research questions

Inadequate linear growth and anaemia in children under five years of age have been the major worldwide malnutrition problems since the 1990s. To classify the severity of inadequate linear growth, Waterlow (1972) used the term "stunting" for children whose height-for-age z-scores were below -2 standard deviations [1]. Based on this cut-off, the worldwide prevalence of stunting has substantially declined from 40% in 1990 to 23.2% in 2015 [2]. However, the declining trend differs largely among continents and regions. The slowest decline is observed in the African continent and its Eastern region [2, 3]. Moreover, Africa and particularly East Africa deviate from the desired trajectory for achieving the 2025 targets in stunting reduction [2]. Linear growth deficit starts early in life and Leroy et al. (2014) showed that 62.5% of the accrued deficit at five years of age in the countries of Sub-Saharan Africa was attributable to the deficits accumulated during foetal life and infancy [4]. In Rwanda, 21% of children are already stunted before their first anniversary. Moreover, stunting has been consistently higher among boys compared to girls, and the sex disparity has become wider over time despite the decline in stunting prevalence [5]. Furthermore, it is during infancy when anaemia prevalence hits a peak of 72% among children aged 6-8 months [5].

Based on the UNICEF framework on the causes of malnutrition [6], child linear growth deficit and anaemia arise from the same immediate causes i.e. inadequate dietary intake and illnesses or the interaction between these two. At the next level, inadequate feeding practices, food insecurity, unsanitary home environment, unsafe water, and poor personal hygiene influence inadequate dietary intake and illnesses. At the basis, immediate and distal causes build on poverty and lack or inadequate maternal education.

To effectively accelerate the reduction of these two forms of childhood undernutrition, it is crucial to better understand their aetiology, especially during early life. Therefore, the findings

presented in this thesis are the contributions towards understanding the causes of linear growth deficit, anaemia, and the poor iron status during early life in the Rwandan context.

The research questions of our investigation, as stated in the general introduction of this thesis, and the corresponding key findings, as presented in **chapters 2** to **5**, are summarized in **Table 1**.

Table 1: Research questions and the corresponding main findings

Research questions	**Main findings**
What are the sex-specific factors of stunting among children aged 6-23 months in Rwanda?	- The prevalence of stunting among boys is substantially higher than among girls in Rwanda. - This seems to be most strongly related to the nutritional quality of foods during the complementary feeding period.
What are the patterns and factors determining linear growth in the first year of life (0-12 months of age)?	- Children are born with a length deficit, which gradually deteriorates with the child's age. - Boys developed larger growth deficits over their first life year than girls. - Predictors of poor linear growth were late initiation of breastfeeding, high breastfeeding frequency, early introduction of complementary feeding, and the duration (days) of diarrhoea and malaria diseases. - A strong positive effect of birth weight and birth length on linear growth exists.

Table 1 continues...

How is dietary iron intake related to the occurrence of anaemia, iron deficiency, and iron deficiency anaemia at 12 months of age?	- There is a weakly significant inverse association between dietary iron intake and iron deficiency, but not anaemia and iron deficiency anaemia at 12 months of age. - Iron deficiency contributes 36.5% to anaemia cases at 12 months of age. - Malaria and inflammation are the two most independent predictors of anaemia and IDA at this age.
Is breastmilk intake measured based on saliva samples comparable to breastmilk intake measured based on urine samples?	- Saliva sampling generates slightly higher estimates of breastmilk intake and is more precise as compared to urine.

In the current chapter, we firstly highlight the methodological considerations of the research and then discuss the main findings and give the scope of their public health significance. We conclude this chapter with recommendations for future research.

Methodological considerations of our research

In the next paragraphs, we discuss aspects of the study design, study population, and data collection that may have influenced the interpretation and validity of the findings presented in this thesis.

Study design

To answer our research questions, we used observational data from cross-sectional and longitudinal study designs. To identify sex-specific determinants presented in chapter 2, we analysed data collected from a cross-sectional survey. A major limitation of these data was that we could not show any causal relationships to explain fully why the sex disparity in stunting exists among boys and girls. Nineteen percent (19%) of children had a missing value in the outcome variable and this might have weakened the associations. In addition, with these data, we could not explore if there were any sex-based parental care practices or preferences in favour of girls or to the disadvantage of boys.

The results presented in chapters 3 and 4 were all based on data collected in the longitudinal study design. The longitudinal study design permits to assess the cause-effect relationship between exposures and outcome. In this regard, the design allowed us to measure the length and to collect data on feeding practices repeatedly from the same child.

Study population

Our investigation focused on children under two years of age since children at this age are most vulnerable to different forms of undernutrition notably stunted growth and anaemia in low-income countries [7]. Therefore, assessing factors that may influence undernutrition among infants and young children is relevant and forms an important step towards improving the child's survival by nutritional interventions during the most critical window of opportunity.

In many Sub-Saharan African countries including Rwanda, boys are more affected by stunted linear growth than girls [5, 8, 9]. Thus, it is relevant to try to understand why boys are disproportionately affected by stunting in the context of Rwanda. For this, we analysed data of 1228 children aged 6-23 months of age. Owing to this large sample, data have good internal

validity (**Chapter 2**). However, the study was conducted in only 8 out of 30 districts of Rwanda, and for this reason, the generalisability of our findings might be limited. Healthy infants grow rapidly during the first half of infancy, however, in Rwanda and in some other countries, infants are already stunted at 6 months of age [3, 5, 10]. To contribute to better understanding the aetiology of poor linear growth early in life, we conducted a longitudinal study on a convenience sample of 192 mother-child pairs from birth to birth to 1 year of age (**Chapter 3**).

In our investigation, we also aimed to contribute to the understanding of the objective assessment of exclusive breastfeeding practices by using stable isotopes. In this regard, we conducted a methodological study, which included 13 pairs of mothers and children aged 2-4 months and we assessed if either saliva or urine, as most commonly sampled media for quantification of breastmilk intake, would provide more precise data (**Chapter 5**).

Data collection

Most of the data used in chapters 2, 3, and 4 of this thesis were self-reported. Self-reported data are known to be prone to recall errors and socially desirable responses. Data used in chapter 2 might have been the most prone to recall errors, especially for the feeding practices because of the cross-sectional design, whereas data on the feeding practices used in chapters 3 and 4 were collected in a longitudinal design. The latter are expected to be more reliable since it allowed us to create trust between participants and field assistants, which might have positively influenced the collection of less biased data. In addition to assessing feeding practices, we used dietary data in chapter 4. Reliance on memory might have subjected dietary data to recall errors. Nonetheless, using a multiple pass approach and household utensils to estimate portion sizes minimised errors during 24-hour dietary recall. Finally, a follow-up at 12 months of age may still be early to see the full effects of all early-life determinants on retarded linear growth.

Iron status is infrequently assessed in infants younger than 6 months of age. In one of our studies, we investigated iron status among infants aged 4 and 12 months (**Chapter 4**). For this, we adjusted iron status indicators for inflammatory conditions and this enabled us to estimate the unbiased prevalence of ID. Furthermore, we measured sTfR, which allowed us to estimate body iron stores, although no cut-offs for TfR at this age exist yet.

Discussion of main findings

Patterns of linear growth and irons status indictors

Our studies confirmed the coexistence of stunted linear growth and anaemia in Rwandan children. By analysing cross-sectional data from 1228 children of 6-23 months of age, we observed that boys had more stunted linear growth than girls and this confirms what the two recent Rwandan Demographic and Health Surveys (RDHSs) have consistently reported [5, 11]. In our longitudinal study with 192 children, we observed an increasing linear growth deficit with the child's age and no signs of catch-up growth from birth to 12 months of age. Moreover, a deficit in absolute length at each time point was slightly higher in boys than in girls though it was not statistically different. However, the length deficit between boys and girls started to widen at 6 months of age to the disadvantage of boys, which coincides with the introduction of complementary feeding. This is probably related to slightly higher nutritional requirements for boys, which are not met by the complementary diet. Contrary to stunted growth, anaemia decreased with age from 73% at 4 months of age to 48% at 12 months of age. This downward trend in the prevalence of anaemia with age confirms what the two last RDHSs have reported, although anaemia was assessed from 6 months of age onwards [5, 11]. Despite this decline, the prevalence of anaemia at 12 months of age is of a severe public health problem as acknowledged by WHO [12]. Contrary to a declining anaemia prevalence by 1.5 times from 4 to 12 months of age, the prevalence of iron deficiency almost tripled and it contributed 36.5%

to anaemia cases at 12 months of age. The co-occurrence of stunted growth and anaemia that has been existing in Rwanda over a decade [5, 11] is seen in other countries in the least developed regions of the world at various degrees of magnitude [7]. **Figure 1** depicts the individual significant predictors and their interactions in relation to stunted linear growth and anaemia or iron deficiency based on the findings from our studies.

Complementary feeding practices in relation to linear growth and iron status indicators

The quality of the child's diet is a paramount factor that determines linear growth [15]. Inadequate nutritional quality of the diet is mainly due to low dietary diversity [16-19] or limited consumption of animal foods [20]. Such a diet limits nutrient supply to the body cells and may eventually lead to inadequate utilisation of nutrients to support linear growth [7]. Insufficient diets may worsen the impaired linear growth already arisen during foetal life and the first half of infancy.

By the time children begin with complementary feeding, we found that the majority of children (81%) in Rwanda received cereal-based porridge (**Chapter 2**). Porridge is usually the first and common complementary food throughout childhood in Sub-Saharan Africa [16]. Because it is often of a watery consistency and poor nutritional quality [14, 19], it poses children at increased risk of malnutrition [20, 21]. By considering the first drink given to the child to be indicative of later feeding practices, we contrasted porridge to cow's milk and found that feeding porridge as the first complementary drink predicted poor linear growth particularly among boys (**Chapter 2**).

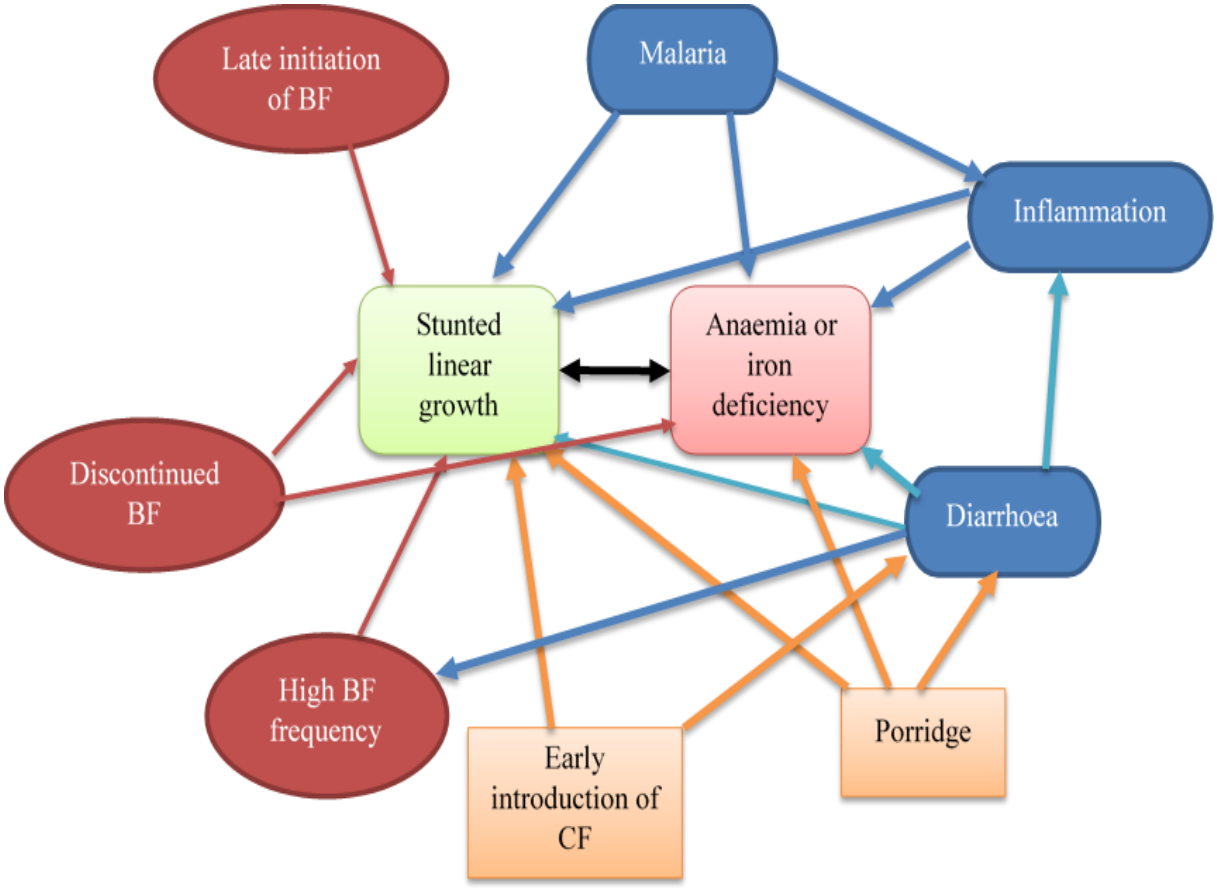

Figure 1: Relationship among predictors in relation to stunted linear growth and anaemia or iron deficiency. ***Note**: **BF**: Breastfeeding. **CF**: Complementary feeding.*

In Rwanda, porridge fed to the children is mainly based on cereals, and a recent study in the northern part of Rwanda confirms that complementary foods provide insufficient nutrients [17]. When poor quality complementary food or drink is introduced before the appropriate age (6 months), linear growth is further compromised. Our longitudinal study shows that almost half (49.5%) of the children were prematurely introduced to complementary foods or drinks and that this feeding practice significantly decelerated the child's linear growth by -0.69 cm from birth to 12 months of age (**Chapter 3**). This early introduction of complementary foods was motivated by a perceived inadequacy of breastmilk production as was reported by 77% lactating mothers. Our findings in chapter 5, however, did not show that this concern is justified since breastmilk intake of Rwandan infants was in the normal range. A larger sample would,

however, be required to study this further. According to the UNICEF report "The state of the world's children 2016", the untimely introduction of complementary foods or drinks is not only practised in Rwanda but is prevalent in many other countries [22]. However, the effect of this poor feeding practice on linear growth is mixed; findings from some studies are consistent with ours [23-25] but others found the contrary [25-27]. This inconsistency may be due to a low sensitivity or specificity of complementary feeding indicators making them a poor measure for the causal relationship between feeding indicators and linear growth [28].

Adding to their poor dietary quality, early-introduced complementary foods may mediate suboptimal linear growth by causing infectious diseases such as diarrhoea. Unsafe diet can be a vehicle of pathogenic microorganisms that, once implanted in intestines, can start using nutrients to their own benefit or can cause nutrient loss by reducing transit time in the intestine or vomiting. In their longitudinal study, Checkley et al. (2008) showed that the risk of stunted linear growth increased with increasing episodes of diarrhoea before two years of age [29]. Other cross-sectional studies also reported the contribution of diarrhoea to a risk of stunting in children under two years of age [30, 31]. In line with this, our study showed that the duration (days) of diarrhoea illness significantly caused linear growth reduction by -0.02 cm per day with the disease in the first half of infancy (**Chapter 3**). In addition to causing stunted growth, intestinal parasitic agents can lead to anaemia and poor iron status [32-34]. In our study, we did not measure the presence of intestinal parasites, but the presence of inflammation, as measured by CRP and AGP concentrations, significantly predicted the risk of anaemia while diarrhoea was not associated with anaemia or iron deficiency at 12 months of age (**Chapter 4**).

Still focusing on infectious diseases, malaria has been linked to both stunted growth [35-38], anaemia and to poor iron status [32, 34]. In our longitudinal study, 29% of the children were reported to have had at least one episode of malaria in entire infancy and the duration (days) that a child had been ill with malaria significantly predicted poor linear growth (**Chapter 3**).

This result contrasts those of Genton et al. (1998) who indicated an increased risk of malaria among children with adequate height-for-age z-scores [39] and of other studies, which reported no association [40, 41]. However, our findings corroborate the findings of earlier longitudinal studies in The Gambia, which found that malaria infection significantly correlated with the risk of linear growth retardation among young children [42, 43]. This effect of malaria on linear growth (**Chapter 3**) confirms what we reported in chapter 2 where stunting prevalence and prevalence ratio were both higher among boys who were affected by malaria than those who were not (61% vs. 42%, and PR=1.22, respectively).

In addition to being a predictor of stunted growth, malaria was significantly related to anaemia (**Chapter 4**). The duration of malaria infection in the previous 30 days at 12 months of age was positively significantly associated with anaemia, iron deficiency, and iron deficiency anaemia at that age. The mechanism linking malaria to the poor haematological status is that malaria causes haemolysis, limits red blood cell formation, and reduces the uptake of dietary iron [33]. The malaria based-reduced iron uptake from diets that, in many rural settings of Africa, are dominated by plant foods known to contain poorly bioavailable iron [16, 17, 44] may accentuate the problem of anaemia and poor iron status in African children. In line with this, most children were found to have eaten plant foods with low intake of iron absorption enhancers (meats or citrus fruits) in the previous 24 hours in our study (**Chapter 4**). Therefore, a combination of both malaria and plant-based diet might explain the anaemia prevalence and iron deficiency observed in the study children at 12 months of age. Even though dietary iron intake was not associated with anaemia, iron deficiency contributed 36.5% to anaemia cases at 12 months of age (**Chapter 4**). In line with our findings, other studies have also shown a lack of a significant association between dietary iron intake and anaemia among study children [45, 46].

In our studies, all indicators of complementary feeding practices including meal frequencies, dietary diversity, or acceptable diet as defined by WHO [47] correlated to neither linear growth **Chapter 2 and 3**) nor to anaemia or iron deficiency (**Chapter 4**). Similarly, the lack of significant associations between indicators of complementary feeding practices and linear growth was also reported by others [48, 49]. However, this is not universal because some other studies did find significant associations between indicators of complementary feeding practices and poor linear growth [13, 25].

Breastfeeding practices in relation to linear growth and iron status indicators

Indicators of breastfeeding practices such as currently breastfeeding, late initiation of breastfeeding, and daily breastfeeding frequencies significantly predicted linear growth in our studies. In chapter 2, with cross-sectional data, we found that discontinued breastfeeding before 24 months of age was a determinant of poor linear growth in girls, whereas for boys this tended to be the opposite. One of the explanations for this may be that the combination of continued breastfeeding with a cereal-based porridge provided too little energy and nutrients to fulfil the higher nutritional demands of boys, whereas this was less of an issue for girls [50]. The lack of a similar association in boys may in part be explained by the reverse causality that triggers mothers to continue breastfeeding children who seem to be smaller or do not thrive well [51, 52]. This may be the case in our study where stunted linear growth significantly affected more boys than girls (43.3% vs. 28%). With our longitudinal data, we could not assess the effect of discontinued breastfeeding practice because almost all children (98.8%) were still breastfed at the end of the follow-up period (12 months of age). Despite this, during infancy, breastmilk is an indispensable source of nourishment to the fast-growing child [53, 54] and therefore influences linear growth. In our longitudinal study, however, frequent breastfeeding episodes were inversely related to the length increments. This may be explained by the fact that children who are frequently breastfed may drink only small amounts, which may be less dense in energy

and nutrients than the richer hindmilk. This breastfeeding behaviour is common in rural areas, because being mostly confined with their children; mothers tend to frequently nurse them regardless if they are hungry or not. Apart from this, the reverse causality, as discussed above, might also have influenced the inverse association between high breastfeeding frequency and length increments.

In surveys, the prevalence of exclusive breastfeeding is usually estimated based on maternal recalls. Estimated from recall data, collected cross-sectionally, the prevalence of exclusive breastfeeding tends to be overestimated. This is confirmed by comparing, for example, the prevalence of exclusive breastfeeding based on our longitudinal data to that established by the Rwandan Demographic and Health Survey. In our longitudinal study, we observed that almost half (49.5%) of the children were not exclusively breastfed at 6 months of age. This prevalence is almost 4 times higher than the 13% reported by RDHS of 2015 [5] and 12.6% reported in chapter 2 of this thesis, both based on cross-sectional data. Moreover, when assessed using maternal recall data, exclusive breastfeeding was found to be overestimated by 40% compared to when assessed using the deuterium oxide dose-to-mother technique [55]. By using the deuterium oxide dose-to-mother technique, we found that saliva sampling was more precise as compared to urine sampling and in addition, this technique showed that one child was not exclusively breastfed, yet at the recruitment, all mothers had reported to breastfeed their children exclusively (**Chapter 5**). Together with the finding in our longitudinal study (**Chapter 3**), this result points to the caution needed while using estimates from maternal recall data.

Stunted linear growth and anaemia: coexistence and interaction

Although stunted linear growth and anaemia are often viewed separately, they may co-exist and a child who is concurrently stunted and anaemic may be at a compounded risk of the adverse health effects. Fast linear growth, which occurs during infancy more than during any other growth stage, demands more nutrients. Particularly at 6 months of age, the demand in iron substantially increases and this partly underpins a higher prevalence of anaemia during the second half of infancy. In addition to anaemia, stunted linear growth continues to accumulate during infancy. The question here is whether linear growth influences the occurrence of anaemia and iron deficiency or the other way around, or whether they interact. In this regard, studies with iron supplementation produced mixed results on linear growth, with some showing a positive effect [56, 57] and others demonstrating no effects [58, 59]. Moreover, findings on the associations between anaemia and linear growth are less consistent; with some studies finding significant associations while others showing the contrary [60]. However, in their trial with oral iron, Ashraf et al. (2009) showed that iron deficiency anaemia impaired linear growth in the first 2 years of age [61]. It is reported that, on the one hand, anaemic children have a higher risk of stunted linear growth than non-anaemic children [34, 60, 62] and on the other hand, being stunted increased the odds of becoming anaemic [32, 60, 63].

Despite that the mechanism on how anaemia or iron deficiency affects linear growth is not yet clearly elucidated, they are both related to a reduced blood capacity to carry oxygen, which subsequently impairs the body's capacity to produce energy [64]. With reduced energy production, the body compensates by reducing energy spent on growth and other body functions. Furthermore, iron deficiency may mediate poor growth by negatively impacting DNA synthesis [65, 66]. Moreover, iron metabolism and protoporphyrin synthesis are both related to insulin growth like factor I (IGF-I); therefore, iron deficiency may decrease the concentration of IGF-I, which affects the influence of IGF-I on erythropoiesis. Decreased IGF-

I concentration interferes with its contribution to the formation of the long bones of the human body [67], thereby leading to short stature. Even if in our study, the length increment at 12 months of age did not differ between anaemic and non-anaemic children, iron deficient and non-iron deficient and iron-deficiency anaemic, and non-iron-deficient anaemic children, we cannot rule out the effect of anaemia and iron deficiency on the observed linear growth deficit. Finally, their coexistence in the same individual or population can also be explained by the fact that both stunted linear growth and anaemia commonly share the same causes [67].

Conclusion

Based on the study research questions, we can conclude that the prevalence of stunting among boys is substantially higher than among girls in Rwanda. This seems to be most strongly related to the nutritional quality of foods during the complementary feeding period. However, children in Rwanda are already born with a length deficit, which deteriorates with the age and shows no signs of catch-up growth during infancy. Late initiation of breastfeeding, high breastfeeding frequency, and early introduction to complementary feeding are significant correlates of decelerated linear growth. In addition, the duration of diarrhoea illness strongly predicts linear growth in the first half of infancy and from birth to 12 months of age while the duration of malaria infection strongly predicts linear growth in the second half of infancy and anaemia, iron deficiency, and iron deficiency anaemia at 12 months of age. In addition to malaria, the presence of inflammation is associated with anaemia occurrence. Moreover, high birth weight and birth length significantly predict the child's linear growth during infancy. Dietary iron intake is low and weakly associated with iron deficiency, but not anaemia or iron deficiency anaemia.

Considering these findings, prenatal interventions seem to be crucial to ensure that children are born with adequate body dimensions and iron reserves, which should provide a strong

foundation to sustain postnatal growth and iron status. Moreover, improving the quality of complementary foods is central to prevent any deterioration of their nutritional status from 6 months of age onwards. However, to make dietary interventions effective, infectious diseases must be controlled.

Public health implications of findings and future research

The public health significance of our findings relies on the fact that we studied the most prevalent forms of undernutrition in Rwanda. As Rwanda has been multiplying efforts to curb the magnitude of stunted growth and anaemia, our findings contribute to better understanding the causes of these two forms of childhood malnutrition. Moreover, we have shown that anaemia already exists in children under 6 months of age. Therefore, early screening for anaemia and iron deficiency among children under 6 months of age would be warranted so that if judged necessary, interventions can be undertaken to improve iron status at a younger age. This with the aim of preparing children to cope better with the considerable physiological iron needs experienced from 6 months of age onwards. Finally, we showed that the current estimates of exclusivity of breastfeeding might be overestimated and therefore caution is required on what mothers report in cross-sectional surveys.

More in-depth research is required to compare feeding habits and dietary intake in relation to nutritional requirements of Rwandan boys and girls during the vulnerable second stage of the first 1000 days of their lives. Additional studies on iron status among infants at birth and in the first half of their infancy are also required. Further investigations should explore the reasons why mothers decide to introduce complementary feeding earlier than recommended. However, we will continue this work by assessing breastmilk quality and quantity at 4 and 9 months of age in the longitudinal cohort.

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Summary

Stunted linear growth and anaemia are major public health concerns in low-income countries, with a disproportionate global burden affecting Sub-Saharan Africa. In Rwanda, stunting among children under five years of age remains high (38%) and it affects more boys compared to girls (43% vs. 33%). However, no studies have attempted to explain the causes of this sex disparity so far. Furthermore, anaemia prevalence hits its peak at infancy, with 72% and 61% of children being anaemic at 6-8 and 9-11 months of age, respectively. Little is known about the causes of anaemia including the contribution of iron deficiency. Therefore, understanding the context-specific factors of poor linear growth and anaemia is key to targeting evidence-based interventions for accelerating the reduction in the magnitude of these two forms of undernutrition. Our research aimed to explore the aetiology of stunted linear growth and anaemia.

To understand the causes of the sex disparity in stunting between boys and girls in Rwanda, we analysed cross-sectional data of 1228 children of 6 to 23 months of age collected from eight districts namely: Gatsibo, Gicumbi, Gisagara, Kamonyi, Karongi, Muhanga, Nyamasheke, and Nyaruguru in the framework of a baseline survey to a nutrition program of the Embassy of Kingdom of the Netherlands in Rwanda. The findings confirmed that stunting significantly affected more boys than girls, with a prevalence of 43.3% vs. 28.0%, respectively. Our analysis revealed that for exposure to any risk factor, boys have a higher prevalence of stunting compared with girls. The multiple regression analyses showed that being fed porridge as first weaning food as opposed to cow's milk was a significant factor for stunting in boys solely (PR=1.44, 95% CI=1.07-1.94, p-interaction=0.048) while discontinued breastfeeding was a significant factor in girls only (PR=1.49, 95% CI=1.05-2.11, p-interaction=0.017) (**Chapter 2**).

To further explore the patterns of early linear growth and to assess the influence of feeding practices in this process among Rwandan children from birth to 1 year of age, we conducted a longitudinal study on 192 mother-child pairs living in a rural area in Muhanga District, one of the eight districts of the cross-sectional study (**chapter 3**). The results showed that children were already born with length deficits (mean length deficit of -1.4 cm), which gradually deteriorated with the child's age (mean length deficit of -2.7 cm at 12 months of age). Moreover, the trend of linear growth deceleration showed no signs of catch-up during infancy. Significant predictors of decelerated linear growth that emerged from multivariate analysis were late initiation of breastfeeding (-0.73 cm, 95% CI: -1.45, -0.01) in the age range of 6-12 months, high breastfeeding frequency (-0.01 cm, 95% CI: -0.02, -0.00) in the age ranges of 0-5 and 0-12 months, and early introduction of complementary feeding (-0.69 cm, 95% CI: -0.90, -0.49) in the age range of 0-12 months. Moreover, the duration (days) of diarrhoea and malaria illnesses did significantly predict decreased liner growth depending on the age range. Meal frequency, dietary diversity, and acceptable diet did not significantly predict linear growth. Conversely, the study confirmed a strong positive effect of birth weight and birth length on postnatal linear growth (**chapter 3**).

Besides exploring linear growth, we also assessed iron status among infants and their mothers at 4 and 12 months postpartum and examined if dietary iron intake among children predicted the occurrence of anaemia, iron deficiency (ID), and iron-deficiency anaemia (IDA) at 12 months of age (**Chapter 4**). In infants, we observed that at 4 months of age, anaemia, ID, and IDA occurred in 73%, 10%, and 8%, respectively while at 12 months of age, anaemia reduced to 48% while ID and IDA increased to 28% and 18%, respectively. At 4 and 12 months of age, ID contributed 10% and 36.5% to anaemia cases, respectively. In their mothers, anaemia, ID, and IDA occurred in 12%, 3%, and 1% at both 4 and 12 months postpartum. Dietary iron intake tended to significantly predict iron deficiency (PR=0.79, 95% CI: 0.62, 1.03), but not anaemia

or iron-deficiency anaemia at 12 months of age. However, the duration of being ill with malaria was significantly associated with anaemia (PR=1.05, 95% CI: 1.00-1.10), ID (PR=1.09, 95% CI: 1.04-1.15), and as well as with IDA (PR=1.15, 95% CI: 1.06-1.25). In addition, the presence of inflammation predicted anaemia (PR=1.01, 95% CI: 1.01-1.10). The iron deficiency at 12 months of age was lower when children had higher body iron reserves at 4 months of age (PR=0.73, 95% CI: 0.59, 0.89).

During infancy, breastmilk is the most important source of energy and nutrients to enhance growth and development, but accurate data on quantity and quality of breastmilk intake in low-income settings are scarce. In the last study presented here, we aimed to assess how the estimated amount of breastmilk intake, determined by the deuterium oxide dose-to-mother technique, differed when it is based on saliva or urine samples (**Chapter 5**). The findings from this small methodological study (n=13 mother-child pairs) showed that the mean breastmilk intake based on saliva samples was significantly higher than that based on urine samples (854.5 g/day vs. 812.8 g/day, p=0.029). This was attributed to slightly higher isotope enrichments in saliva and to a poorer model fit for urine samples as indicated by its square root of the mean square error, which is higher than that for saliva samples (14.6 mg/kg vs. 10.4 mg/kg, p=0.001).

Based on these research findings, we can conclude that stunted linear growth among boys is substantially higher than among girls in Rwanda and this seems to be most strongly related to the nutritional quality of foods during the complementary feeding period. However, children in Rwanda are already born with a length deficit, which gradually deteriorates with the child's age without any signs of catch-up growth during infancy. Late initiation of breastfeeding, high breastfeeding frequency, and early introduction to complementary feeding are significant correlates of decelerated linear growth. However, none of the indicators of complementary feeding practices is significantly related to linear growth. The duration of diarrhoea and malaria

illnesses strongly predicts decelerated linear growth depending on the age ranges. The duration of malaria infection at 12 months of age significantly predicts anaemia, iron deficiency, and iron deficiency anaemia at that same age. In addition to malaria, the presence of inflammation is significantly associated with anaemia prevalence. Therefore, infectious diseases are important determinants of anaemia and iron deficiency among children during infancy. Dietary iron intake is weakly associated with iron deficiency, but not with anaemia or iron deficiency anaemia.

In view of these findings, prenatal interventions seem to be crucial to ensure that children are born with adequate body dimensions and iron reserves, which should provide a strong foundation to sustain postnatal growth and iron status. Moreover, improving the quality of complementary foods is central to prevent any deterioration of their nutritional status from 6 months of age onwards. However, to make dietary interventions effective, infectious diseases must be controlled.

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About the author

Eric Matsiko was on 1st September 1979 in Ruhango District, Rwanda. In 2005, he graduated from the Department of Food Science and Technology, former Kigali Institute of Science and Technology (KIST) now College of Science and Technology at the University of Rwanda. He worked for Conseil Consultatif des femmes, a non-governmental organisation, as manager of its food-processing unit.

In 2009, Belgian Technical Cooperation, Rwanda's office, offered him a scholarship to study a master's program of Nutrition and Rural Development: Main Subject Human Nutrition at Ghent University, Belgium. In 2011, he graduated with a Master's degree in Science in Nutrition and Rural Development**:** Main Subject Human Nutrition. Thereafter, he joined academia as an assistant lecturer of nutrition-related courses at the Catholic University of Rwanda. Later, he joined the University of Rwanda, College of Medicine and Health Sciences, School of Public Health, Department of Human Nutrition and Dietetics where he is still working.

End of 2013, The Netherlands initiative for capacity development in higher education granted him a scholarship for a PhD program in the division of Human Nutrition and Health, Wageningen University and Research. In his thesis, Eric Matsiko focused on "Exploring linear growth retardation in Rwandan children: Ecological and Biological factors". The results of his PhD research work are described in this thesis. During his PhD period, Eric Matsiko attended courses and conferences relevant to his career.

Matsiko looks forwarding to a continued contribution to improving the nutrition of children.

Eric Matsiko can be contacted via his email: ematsiko79@gmail.com

Publications

Publications submitted or to be submitted:

1. Eric Matsiko, Paul Hulshof, Laura Van der Velde, Marlou Floor Kenkhuis, Lisine, Tuyisenge, Alida Melse-Boonstra. **Comparing saliva and urine samples for measuring breastmilk intake with the deuterium oxide dose-to-mother technique among children 2-4 months old, 2019.**
2. Eric Matsiko, Alida Melse-Boonstra, Jeanine Ahishakiye, Kristine Dandanell Garn, Damien Iyakaremye, Lisine Tuyisenge, Edith J. M. Feskens. **Predictors of sex disparity in stunting prevalence between boys and girls aged 6-23 months: a cross-sectional survey in eight districts of Rwanda.** 2019.
3. Eric Matsiko, Edith J.M. Feskens, Lisine Tuyisenge, Alida Melse-Boonstra**. Dietary iron intake tends to predict anaemia, iron deficiency or iron deficiency anaemia among 12-month old Rwandan children. 2019.**
4. Eric Matsiko, Edith J.M. Feskens, Jeanine Ahishakiye, Alida Melse-Boonstra. **Exploring patterns and predictors of linear growth from birth to 12 months of age in Rwanda: A longitudinal investigation**. 2019

Co-authored publications in peer-reviewed journals:

Publications:

1. Megan Parker, Zhen Han, Elizabeth Abu-Haydar, Eric Matsiko, Damien Iyakaremye, Lisine Tuyisenge, malia Magaret, Alexandre Lyambabaje**: An evaluation of hemoglobin measurement tools and their accuracy and reliability when screening for child anemia in Rwanda: A randomized study**. *PLoS One* 2018, **1**:e0187663.
2. Raquel Medeiros Vinci, Liesbeth Jacxsens, Joris Van Loco, Eric Matsiko, Carl Lachat, Thibaultde Schaetzen, Patrick Kolsteren, Michael Canfyn, IlseVan Overmeire,

BrunoDe Meulenaer: **Assessment of human exposure to benzene through foods from the Belgian market**. *Chemosphere* 2012, **8**:1001-1007

Conference abstracts:

1. Eric Matsiko, Paul Hulshof, Laura Van der Velde, Marlou Floor Kenkhuis, Lisine, Tuyisenge, Alida Melse-Boonstra. **Infant breastmilk intakes and mothers body composition and energy expenditure: a comparative study. IUNS 21st ICN International Congress of Nutrition in 2017.** (Oral presentation).
2. Eric Matsiko, Alida Melse-Boonstra, Jeanine Ahishakiye, Kristine Dandanell Garn, Damien Iyakaremye, Lisine Tuyisenge, Edith J. M. Feskens. **Gender-specific determinants of stunting among Rwandan children 6-23 months old. IUNS 21st ICN International Congress of Nutrition in 2017** (Oral presentation).

Overview of completed training activities

Disciple specific courses

Hidden Hunger: Micronutrient Deficiencies in Developing Countries	WUR, Wageningen, NL	2014
Exposure Assessment in Nutrition Research	VLAG, Wageningen, NL	2016
Public Health Research in Practice: Public Health Intervention in real-life settings: Evaluation	VLAG and AGORA, Wageningen, NL	2016
Stable Isotope Methods in Nutrition Research	VLAG, Wageningen, NL	2019

Disciple specific conferences

Micronutrient Global Conference forum	Adis Abeba, Ethiopia	2016
Dutch Nutritional Science days		2016, 2018
International Congress of Nutrition	IUNS, Argentina	2017
SDG Conference: Towards Zero hunger-partnerships for impact	WUR, Wageningen, NL	2018

General courses

Information Literacy including EndNote Introduction	WGS, Wageningen, NL	2013
Data Management	WGS, Wageningen, NL	2014
VLAG PhD week	VLAG, Wageningen, NL	2014
Presenting with Impact	WGS, Wageningen, NL	2018
Scientific writing	WGS, Wageningen, NL	2018
Scientific integrity, WGS, NL, 2018	WGS, Wageningen, NL	2018
Career assessment, WGS, 2018	WGS, Wageningen, NL	2018
Cohort studies, Mixed models for longitudinal and survival data analysis	Erasmus MC, Rotterdam, NL	2018

Optional courses and activities

Research proposal writing	WUR, Wageningen, NL	2014
Scientific meetings, seminars, colloquia organized within a department/research group	WUR, Wageningen, NL	2013-2018

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