Evaluation of growth faltering in rural Gambian children

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Research group affiliation: MRC International Nutrition Group

To my husband, Mr Tigwende Serge Soubeiga, our sons Tegwende and Wendpanga Soubeiga and the rest of the Soubeiga, Nabwera and Tumwa families.

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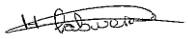
Signed: 

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I would like to sincerely thank my main supervisor Professor Andrew Prentice for seeing the potential in me, and for his invaluable guidance and support throughout this PhD. Thank you too for sharing the gems of what it takes to be a successful, influential and very caring scientist in global health and nutrition. My sincere gratitude goes out to the UK Medical Research Council for funding my fellowship. Many thanks to my second supervisor, Dr Ken Ong for his guidance particularly during the initial stages of developing my ideas and proposals and for his continued insights throughout my field work and analysis. I would also like to thank Dr Sophie Moore, Professor Jay Berkley and Dr Robin Bernstein for their guidance and feedback at important stages of this PhD, as part of my advisory committee. Many thanks also go out to Dr Tony Fulford and Mr Schadrac Agbla for their patience and guidance in the statistical aspects of this work, and for their encouragement throughout my PhD. I would like to thank all the staff at MRC Keneba research field station, where my family and I lived for over 2 years. My family and I will forever remember your warmth and friendship. I am very grateful for all the support that you gave me is ensuring that my research proposals and field work were rigorous whilst also sensitive to the community's cultural practices and expectations. Many thanks also go out to the members of the MRC International Nutrition group (in particular, Dr Branwen Hennig, Dr Modou Jobe, Mr Amat Bah, Mr Phil James, Miss Jenny Howard, Miss Joanne Tapper), MRC Fajara research and clinical teams and The Gambia National Nutrition Agency (in particular Mr Modou Phall and Mr Bakary Jallow), for their regular feedback on my work, sharing their ideas and for their friendship. To Ms Jenny Fleming at The London School of Hygiene and Tropical medicine, thank you for your warmth and guidance on the administrative as aspects of my PhD.

To the participants and their families, I would like to say a big thank you. This work would not have been possible without your participation. I hope that this work will help improve the health and well-being for your community both now and in the future.

Finally, I would like to sincerely thank my husband Mr Tigwende Serge Soubeiga for the sacrifices that he has made to support me in pursuing my career ambitions. To my sons Tegwende and Wendpanga Soubeiga- "thank you for allowing mummy to have time away from you to work." Immense gratitude to my parents, parents in law and siblings for their continued prayers and encouragement.

Abstract

Growth faltering associated with undernutrition in early childhood is endemic in sub-Saharan Africa. Worldwide, over 3 million child deaths annually are attributed to foetal growth restriction, underweight, stunting, wasting, suboptimal breastfeeding and micronutrient deficiencies. Survivors suffer adverse health and socio-economic outcomes. Although rates of stunting have halved worldwide, progress in sub-Saharan Africa has been slow. The prevalence of wasting has not shifted. This work aimed to describe secular trends of growth faltering in early childhood and the hormone correlates of malnourished children during nutritional rehabilitation in rural Gambia. Also, to explore factors associated with severe wasting in infancy.

Firstly, secular trends of growth faltering among under 2's from three rural Gambian villages were described using routinely collected clinic anthropometry data. Over the past four decades, rates of stunting and underweight halved, but significant growth faltering persisted. Secondly, changes in energy regulating hormones during the nutritional rehabilitation of children aged 6-24 months were evaluated. The variations in growth amongst the malnourished children during nutritional rehabilitation were not explained by differences in energy regulating hormones. Baseline C-peptide was the only predictor of future response to nutritional rehabilitation, but would not be a useful clinical marker in isolation. Thirdly, risk factors for severe wasting in infants were explored. Adverse maternal psychosocial circumstances and infant feeding difficulties constrained mothers from practicing the recommended infant feeding practices.

The conclusion from these findings is that current nutrition and health interventions are inadequate in mitigating growth faltering in early childhood in rural Gambia, in the face of poor living conditions and adverse maternal psychosocial circumstances. In addition, the missing contributors of variable growth during outpatient nutritional rehabilitation remain unknown. Further research into the development and upscaling of the nutrition-sensitive interventions is required to address growth faltering in childhood in low and middle income settings.

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List of abbreviations

BHCZ	Birth head circumference z-score
BLENZ	Birth length z-score
BWTZ	Birth weight z-score
CI	Confidence interval
CMAM	Community management of acute malnutrition
CMD	Common mental health disorders
COHORTS	Consortium on Health-Orientated Research in Transitional Societies
EDS	Edinburgh Depression Scale
EED	Environmental enteric dysfunction
ELISA	Enzyme-linked immunosorbent assay
FGD	Focus group discussion
GEMS	Global Enteric Multicenter Study
HAZ	Height-for-age z-score
HCW	Health care worker
HCZ	Head circumference z-score
ICD	Informed consent documents
IDI	In-depth interview
IFA	Iron and folate
IGF-1	Insulin like growth factor 1
IGFBP-3	Insulin like binding protein 3
IQR	Interquartile range
KEMReS	Keneba Electronic Medical Records System
LAZ	Length for age z-score
LNS	Lipid-nutrient supplement
LMICs	Low and middle income countries
LSHTM	London School of Hygiene and Tropical Medicine
MAM	Moderate Acute Malnutrition
MAL-ED	The Malnutrition and Enteric Disease Study

MDG	Millennium Development Goals
MRC	Medical Research Council
MUACZ	Mid upper arm circumference z-score
MMN	Multiple micronutrients
NaNA	The Gambia National Nutrition Agency
PCA	Principal Component Analysis
PCR	Polymerase chain reaction
PhD	Doctor of Philosophy
RF	Responsive feeding
SAM	Severe Acute Malnutrition
SD	Standard deviation
SDG	Sustainable Development Goal
SHINE	Sanitation, Hygiene, Infant Nutrition Efficacy Project
sOB-R	Soluble leptin binding protein
SSA	sub-Saharan Africa
UNICEF	United Nations Children's Fund
WASH	Water sanitation and hygiene
WAZ	Weight-for-age z-score
WHO	World Health Organisation
WHZ	Weight-for-height z-score
WLZ	Weight for length z-score

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Chapter 1: General Introduction

1.1 Preface

Growth faltering due to undernutrition that includes wasting, stunting and micronutrient deficiencies in early childhood is a significant public health problem in many low income and middle income countries (LMICs), including many in sub-Saharan Africa (SSA). It is an attributable factor for 3 million deaths in under 5's worldwide, which is equivalent to 45% of under 5 mortality. In addition, the survivors often have adverse long term health, developmental and socioeconomic outcomes. Our knowledge of evidence based nutrition-specific and nutrition-sensitive interventions has failed to translate into significant reductions in growth faltering in children in SSA. This is partly due to the fact that both the coverage and uptake of these interventions are often poor in resource limited settings. There has also been a greater emphasis on implementation of nutrition-specific interventions, which on their own have limited impact in the context of poverty and poor living conditions. In addition, over the past 2 decades there have been significant improvements in the management and survival of children with severe acute malnutrition (SAM), following the implementation of standardised World Health Organisation (WHO) guidelines in hospital and in the community. However, in LMICs children show variable responses to nutritional rehabilitation and there is limited knowledge about the physiological mechanisms that govern nutritional recovery in these settings. This therefore, limits the implementation of targeted interventions.

1.2 Scope of the thesis

This Doctor of Philosophy (PhD) thesis is a 'mixed model' where the research papers are incorporated into the thesis chapters. Three stand-alone documents with myself as first author have been included. These are from the three research studies that I undertook to investigate different aspects of growth faltering in early childhood in rural Gambia, in order to identify targets to inform the development of future interventions.

1.3 Composition of the thesis

The first research study evaluated the secular trends of growth faltering in children under 2 years in three rural Gambian villages over a period of 4 decades. During this period, the population received unprecedented and sustained levels of health, nutrition-sensitive and nutrition-specific interventions, mainly from the UK Medical Research Council. However, this study showed that although the rates of stunting and underweight had halved over the 4 decades and the seasonal effect on growth faltering had diminished in the most recent decades, the general postnatal patterns of growth faltering after 3 months of age in the under 2's in this population had remained unchanged. This component was important as it highlighted the inadequacy of the current interventions in preventing growth faltering in early childhood in this population. There was therefore an urgent need to explore in greater depth the missing contributors of growth faltering in this population, in order to guide the development of new interventions for rural populations such as this one in The Gambia.

The second study was an exploratory study that evaluated the hormonal changes that occurred during the nutritional rehabilitation of rural Gambian children with moderate or severe acute malnutrition. This study was important as it facilitated the exploration of the dynamics of leptin, its soluble binding receptor (sOBR) and other key energy regulating hormones during nutritional rehabilitation of children not requiring intense inpatient interventions. It demonstrated that variations in growth were not explained by differences in energy regulating hormones. Baseline C-peptide was the only predictor of response to the 28 days of nutritional rehabilitation, but would not be a useful clinical marker in isolation. In addition, there was no evidence of an immediate effect of feeding on the leptin levels during nutritional rehabilitation as had been hypothesised based on findings from a previous unpublished observational study in this population. This provides information for potential the role of C-peptide as a biomarker in combination with others that can be evaluated as tools to facilitate risk stratification and more targeted interventions for malnourished children during outpatient nutritional rehabilitation in order to achieve better health and nutritional outcomes.

The third study used a mixed methods study design to explore maternal and infant factors associated with severe wasting in infants in rural Gambia who had been enrolled into a

micronutrient supplementation randomized trial. As part of the trial, these infants had intensive growth monitoring with regular free access to preventative and treatment health interventions. This study found that increased frequency of complementary feeding (sometimes up to 8 times a day) was associated with severe wasting in these infants. In addition, adverse maternal psychosocial status, inadequate maternal social support networks and infant feeding difficulties constrained mothers from practising the recommended infant feeding and rearing practices. This study highlighted potential targets for intervention that merit further investigation.

1.4 Publications from this thesis

Nabwera HM, Fulford AJ, Moore SE, Prentice AM Growth faltering persists in rural Gambian children despite four decades of interventions. Lancet Glob Health. 2017 Feb;5(2): e208-e216

(Chapter 4)

Nabwera HM, Bernstein RM, Agbla SC, Moore SE, Darboe MK, Colley M, Jallow AT, Bradbory R, Karaffin J, Fulford AJ, Prentice AM Hormonal Correlates and Predictors of Nutritional Recovery in Malnourished African Children. (Submitted to Journal of Tropical Pediatrics 22nd April 2017)

(Chapter 5)

Nabwera HM, Moore SE, Mwangome MK, Molyneux CS, Darboe MK, Camara-Trawally N, Sonko B, Darboe A, Singhateh S, Fulford AJ, Prentice AM Maternal psychosocial stressors and severe wasting in rural Gambian infants: a mixed methods approach. (Submitted to BMC Public Health 25th April 2017)

(Chapter 6)

1.5 Candidate's involvement

I undertook this work during my Medical Research Council (MRC) Career Development Fellowship from March 2012- August 2015. I was based at the MRC Keneba field station in The Gambia. I defined the topics and research questions for this thesis, working closely with my supervisors Prof Andrew Prentice and Dr Ken Ong, with additional support from Dr Sophie Moore. I was responsible for the development of research proposals, ethics

application, research protocols, field work, data and sample collection. This involved supervising, training and development of clinical and field staff. I also coordinated the shipment of biological samples to our US collaborator (Dr Robin Bernstein). All the data cleaning and statistical analysis in this thesis was my responsibility with statistical input from Dr Tony Fulford and Mr Schadrac Agbla. This thesis was written by me and the papers that I have included have the comments of the co-authors incorporated in them.

1.6 Collaborating institutions

As the laboratory in the MRC Keneba did not have the necessary assays, equipment and capacity for running the hormone assays, the samples were sent to our collaborator whose details are below. I familiarised myself with the techniques by visiting the MRC Unit The Gambia laboratories in Fajara, where enzyme-linked immunosorbent assay (ELISA) was used to run immunology assays. Below are the details of the collaborators and their institutions:

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1.7 Funding

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1.8 Study timeline

The time frame for the study activities including the field work and the analysis is shown in Appendix I.

1.9 Definitions

Growth faltering

It is defined as a growth rate below that appropriate for a child's age and sex and is primarily detected through growth monitoring [1, 2].

Undernutrition

This is a *"form of malnutrition resulting from a reduced supply of food or from inability to digest, assimilate, and use the necessary nutrients"* [3].

Wasting

This *"refers to a child who is too thin for his/her height. Wasting is the result of sudden or acute malnutrition, where the child is not getting enough calories from food and faces an immediate risk of death"* [4]. It is defined as a low weight for length/height Z-score that is <-2 SD from the median of the international reference population [1].

Moderate Acute Malnutrition

This is the moderate form of wasting where the weight for length/height Z-score is <-2 SD but ≥ -3 SD from the median of the WHO growth reference standards for all children up to

the age of 5 years; and/or a mid-upper arm circumference (MUAC) of less than 125mm but more than 115mm for children 6 to 59 months of age, without visible oedema [5].

Severe acute malnutrition

This is the severe form of wasting where the weight for length/height Z-score that is <-3 SD from the median of the WHO growth reference standards; and/or or a mid-upper arm circumference (MUAC) of less than 115mm for children 6 to 60 months of age; and/ or the presence of bilateral oedema [6]. In this thesis, this term will be used interchangeably with severe wasting.

Stunting

It is defined as a low length/height for age Z-score that is <-2 SD from the median of the WHO growth standards in children up to 5 years of age [1]. This "*refers to a child who is too short for his/her age. Stunting is the failure to grow both physically and cognitively and is the result of chronic or recurrent malnutrition. Its effects often last a lifetime*"[7].

Underweight

It is defined as a low weight for age Z-score that is <-2 SD from the median of the WHO growth standards in children up to 5 years of age [1]. It refers to a child whose weight is below that expected for their age and may be due to weight loss (e.g. following an acute illness) or failure to gain weight (e.g. due to inadequate feed intake) [8].

Infant

The Oxford Medical Dictionary defines an infant as *"a child incapable of any form of independence from its mother"* [9]. The term is usually used to refer to the period from birth to twelve months of life, which will be the definition in this thesis [10].

Chapter 2: Background and literature review

Health is *"a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity"* according to the World Health Organization (WHO) [11]. Healthy growth and nutrition underpins the survival and well-being of children worldwide and is identified as a global health priority in the Sustainable Development Goals (SDGs) [12]. This requires optimal physiological (including hormonal regulation) and environmental (including adequate and appropriate nutrition, access to clean water and good sanitation, low burden of infections, appropriate stimulation) conditions to enable a child to achieve their genetic growth potential. Unfortunately, many children worldwide live under sub-optimal environmental conditions, which in turn alter their inherent physiology to adapt to these adverse conditions. Thus, they either growth falter (due to undernutrition) or develop obesity (due to overnutrition), both of which are significant public health problems in children worldwide. This PhD focuses on growth faltering in childhood that is currently a greater global health burden than that of obesity in LMICs.

2.1 Global burden of growth faltering

Growth faltering in early childhood (i.e. under 5 years of age) is common in LMICs, including many in SSA [13]. Onset is often in the antenatal period followed by initial postnatal catch up growth up to 3 months of age, when profound growth faltering occurs with evidence of varied degrees of catch up after 18 months of age [13-15]. It results in small for gestational age (SGA) associated with intrauterine growth restriction (IUGR), poor organ development, underweight, stunting and wasting that are associated with significant short and long-term morbidity, and mortality [13, 16, 17]. Reductions in weight for height are generally seen as a short-term response to inadequate dietary intake or utilization, and are thought to precede decreases in height for age [18]. Children who suffer with recurrent episodes of wasting in infancy are more likely to become stunted [18]. Annually 3.1 million under-fives die as a result of undernutrition i.e. IUGR, suboptimal breastfeeding, stunting, wasting and micronutrient deficiencies of zinc and vitamin A [17]. Severe acute malnutrition (SAM) carries an immediate risk of death particularly from infectious disease with mortality rates

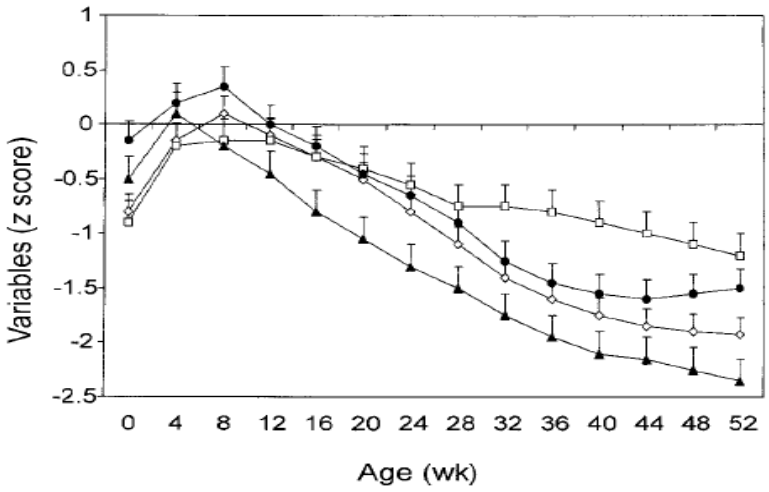
of up to 50% in children undergoing facility-based management [19]. In recent years, significant strides have been made in reducing the mortality due SAM through improved implementation of standardised WHO in-patient and community treatment protocols [20, 21]. The management of SAM according to the in-patient protocol that emphasises the early management of hypoglycaemia and sepsis reduced the case fatality rate by 55% (risk ratio 0.45, 0.32-0.62) [21]. However, the implementation of the in-patient protocol has been challenging in rural African health care facilities due to inadequate infrastructure, poor staffing levels with high staff turnover, limited knowledge of the WHO recommendations for in-patient management of SAM by staff and low staff morale [22, 23]. Communication with carers, play and stimulation of the SAM children is poorly implemented [22]. The community management of acute malnutrition (CMAM) therefore, provides a less resource intensive and more viable model for rural African communities [20, 24, 25]. For the survivors of SAM, there is growing evidence that their risks of relapse and death remain high in the immediate 12 months post-discharge (up to a quarter of overall deaths) particularly in infants and HIV infected children [26, 27]. In addition, they remain stunted [height for age Z-score, HAZ, mean −2.97 (SD 1.3)] and have poor education achievements compared to their peers [27]. Stunting that results from chronic undernutrition is also associated with adverse long-term consequences including impaired cognitive development, poor school achievement, reduced economic productivity in adulthood, adverse maternal reproductive outcomes and non-communicable diseases in adulthood [28-30]. Chronic diseases are especially common in undernourished children who experience rapid weight gain after infancy [28]. In animal models, an adverse prenatal environment may induce long-term metabolic consequences, in particular obesity, insulin resistance, and type 2 diabetes [31]. In addition, maternal undernutrition leads to vascular alterations that are as detrimental to the offspring exposed to a long-term high-fat diet [32]. About 200 million children under 5 years of age fail to reach their potential in cognitive development because of a combination of risk factors such as poverty, poor health and nutrition, and inadequate caring practices. These conditions play an important part in the intergenerational transmission of poverty [33]. In addition, the association between maternal undernutrition, IUGR, and poor growth in infancy and non-communicable diseases (including coronary heart disease) in adulthood has been well described [34].

2.2 Trends and timing of growth faltering

In 2016, the joint UNICEF, WHO, World Bank global trends of undernutrition in the under-fives from 2000-2015 reported a 21% decline in the prevalence of stunting (from 198 [32.7%] to 156 million [23.2%]), 37% of whom are in SSA [7]. However, SSA lagged behind other LMICs in reducing stunting rates by only 17% during this interval compared to 36% and 39% reductions in Asia and Latin America & the Caribbean respectively [7]. In addition, the absolute number of stunted children in SSA increased during this interval from 50 to 88.5 million with the bulk of the burden being in West Africa [7]. From 2012-2015, the prevalence of wasting showed a very slight decline (from 51 [8%] to 50 [7.4%]), 28% of whom are in SSA [7, 35]. Although very valuable in monitoring progress of global under 5 undernutrition targets, these estimates rely on national household surveys and only give a single point prevalence of growth outcomes, which will not take into account the changes with season or socioeconomic circumstances that would affect the growth of these children over time. They also do not provide information on the timing of growth faltering that would inform the timing of interventions.

There is a paucity of longitudinal data describing the timing of growth faltering in children in SSA. ([36-44]. Gray *et al* described growth patterns in a cohort of 123 immunized children in a pastoralist community in northern Uganda over a 6-year period and found that relative to international standards, their weight for age status was optimal only in the first 3 months. Thereafter the weight velocity declined with static weight gain after 24 months [41].

Collinson *et al's* cohort study monitored the growth of 138 infants in rural Gambia from birth to 12 months and plotted their growth trajectories against UK 1990 Growth Charts as shown in **Figure 2.1** [45]. They showed that the infants were born small compared to UK standards, but showed marked catch-up in the 3 months of postnatal life and then, showed significant growth faltering for the remainder of infancy [45]. From 3-12 months, the head circumference for age Z-scores (HCZ) declined by about 2.5 standard deviations (SD), weight for age Z-scores (WAZ) declined by 2 SD, length for age Z-scores (LAZ) by 0.75 SD and body mass index Z-scores (BMI) by 1.5 SD [45].



Mean (±SEM) weight (◇), length (□), head circumference (▲), and BMI *z* scores (●) from birth to age 52 wk in boys (n = 75) and girls (n = 63) combined.

Figure 2.1: Mean anthropometric Z-scores according to age for rural Gambian infants, relative to UK 1990 standards (0 to 12 months) [45]

More recently, Victora *et al* used cross sectional anthropometric data from fifty four LMICs relative to WHO 2006 Growth Reference Standards (**Figure 2.2**) [13].

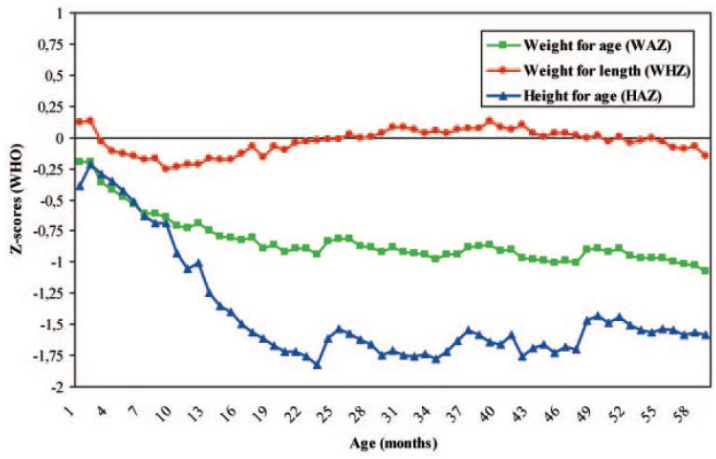


Figure 2.2: Mean anthropometric z scores according to age for all 54 studies, relative to the WHO standard (1 to 59 months) [13]

They showed that the WAZ started close to the median and declined moderately until reaching approximately -1 Z-score at 24 months and remaining reasonably stable after that

[13]. The HAZ also started close to the median and declined dramatically to -1.75z at 24 months, increasing slightly after that [13]. The weight for length/height (WHZ) started just above the median in children aged 1 to 2 months and declined slightly until 9 months of age, picking up after that age and remaining close to the standard thereafter, suggesting that they were able to preserve body composition [13]. The findings of this study formed the basis for the development of the paradigm that the first 1000 days (spanning from conception to a child's second birthday) is the critical window of opportunity for interventions for optimal health, growth and neurodevelopmental outcomes [15, 21, 46]. However, using longitudinal data from rural Gambia Prentice *et al* showed that between 2 and 5 years of life, children demonstrated a very significant recovery of about 0.75 Z- scores in HAZ with an overall increase in height of around 1.5 Z- scores between the 2 years and adulthood in the absence of nutritional interventions, due to the extended pubertal growth [15]. In addition, Stein *et al* who used growth data from the Consortium on Health-Orientated Research in Transitional Societies (COHORTS) that consists of birth cohort data from five LMICs including South Africa, found that in all the cohorts there was a large decrease in HAZ in the first 12 months, persisting to a lesser degree in the second year to a nadir at 24 months (mean heights 2-10 cm below the WHO reference range, HAZ [-1.37± 1.7 vs -1.94±1.15] South Africa vs India) [42]. From 24 months to mid childhood, HAZ increased in the South African but not the Indian cohort HAZ [+0.81 vs -0.01] **Figure 2.3** [42].

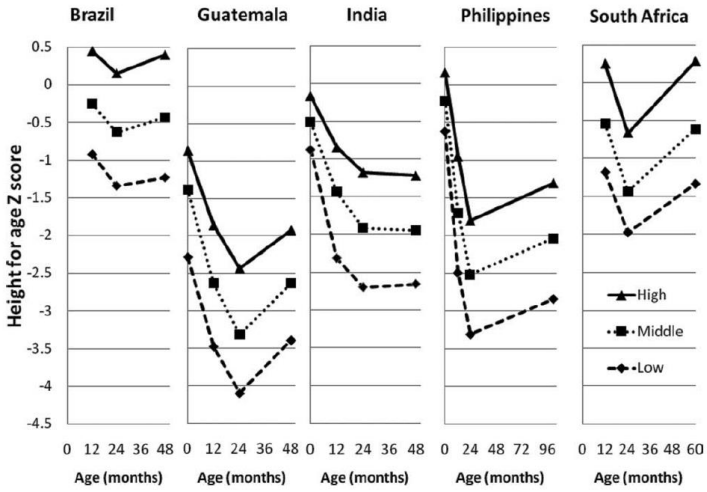


Figure 2.3: Height for age Z-scores at birth, 12 months, 24 months, and mid-childhood of participants in five birth cohort studies, stratified by thirds of attained height [42]

However, despite this catch-up growth, growth failure prior to 12 months was strongly associated with adult stature [42]. These studies highlight the importance of targeted interventions in the first 1000 days, which is the time when profound growth faltering occurs with long term consequences in adulthood. However, they also argue for a greater emphasis on the period after 12 months, as there is still potential for catch-up growth, and improvements in cognitive outcomes, particularly in the adolescent period [15, 47].

2.3 Causes of growth faltering

Attributable factors of growth faltering include poor maternal nutrition resulting in IUGR; inadequate nutrition postnatally; and the high burden of infections during early life (including HIV) with exposure to poor sanitation and hygiene [32, 33]. In addition, the absence or ill health of a mother or a maternal grandmother has been shown to have adverse effects on the growth of children [33, 34].

2.3.1 Infections and environmental enteric dysfunction

Many children in LMICs live in unsanitary conditions due to poverty and an ever widening inequality gap even in countries that have experienced recent economic growth ([48, 49]. As a result, from early in life children become infected with multiple pathogens, particularly via the faeco-oral route [50, 51]. Recurrent and chronic infections in early childhood lead to a proinflammatory state, even in the absence of clinical signs, which inhibit anabolic metabolism that is believed to be an important cause of growth failure in children in LMICs [52-54]. These infections can also result in suppression of appetite, impaired absorption or increased losses of nutrients [55]. Two longitudinal studies in early childhood in rural Gambia showed that even at 2 months of age, children have damaged small intestinal mucosa as indicated by increased gut permeability, and that the gut damage predicts a failure in linear growth [54, 56]. In addition, they also mount an acute systemic inflammatory response (raised C-reactive protein, total free-circulating endotoxin and IgG endotoxin core antibody), that is hypothesized to be due to the translocation of immunogenic molecules across the damaged gut mucosa and is also negatively correlated with growth [54].

Diarrhoea

Although diarrhoea contributes to the burden of malnutrition [57, 58] (and malnourished children are more likely to have moderate to severe diarrhoea with an increased risk of death from it [59, 60]), the majority of undernourished children in LMICs do not present with symptoms of diarrhea [50]. Indeed in rural Gambia the 75% decline in diarrhoeal disease associated with increasing public health interventions between the 1970's and 1990's, was not matched by a similar trend in WAZ, which remained relatively static during that interval (1979, -2.0; 1993, -1.9 at 12 months and 1979, -2.0; 1993, -2.1 at 24 months) (**Figure 2.4a, Figure 2.4b**) [61].

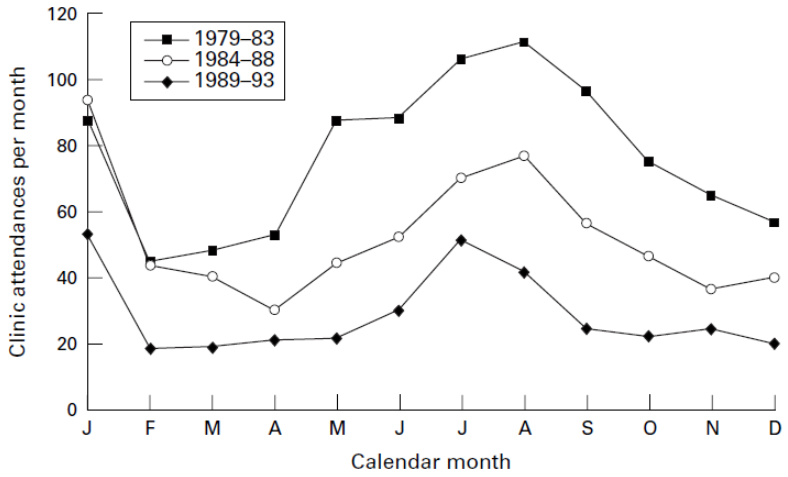


Figure 2.4a: Five year averages of clinic attendance with diarrhea in children under 2 years of age for the periods 1979-83, 1984-88, 1989-93 [61]

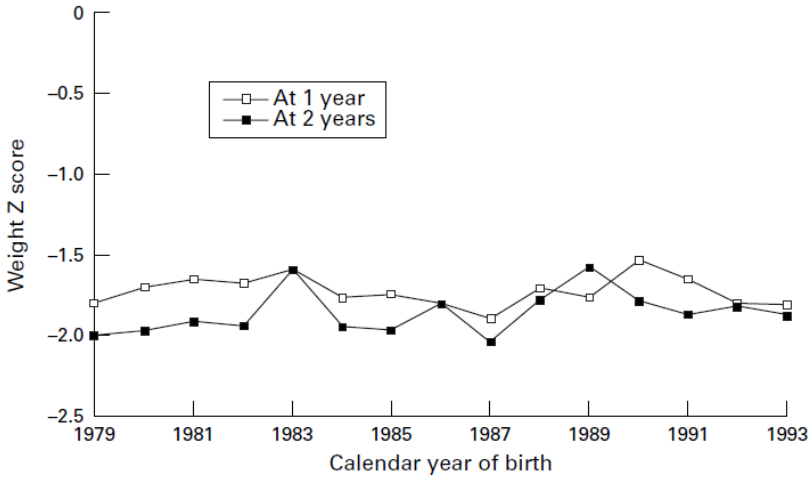


Figure 2.4b: Mean weight Z score for children at 1 year and 2 years of age, 1979-93 [61]

The Global Enteric Multicenter Study (GEMS), a recently concluded multicentre study that had a study site in The Gambia found that *Shigella sp,* which was the second most common causative agent of moderate to severe diarrhoea after rotavirus (most prevalent in children between 12-23 months) was also a risk factor for undernutrition (Kwambana *et al*, personal communication). Observational studies in rural Gambian children found that gastrointestinal infections are acquired from as early as 3 months of age and that *Helicobacter sp,* but not *Giardia sp* infections in infancy were associated with growth faltering (deficits in weight and length) in early childhood [62, 63].

The association between diarrhoea and linear growth has been controversial due to inconsistent findings between studies, which have not been conclusive about its temporal relationship with growth failure (i.e. short term vs long-term) [58, 64-66]. In a study that analysed longitudinal data of under 2's from LMICs in SSA, Asia and South America, Richard *et al* found no association between diarrhoea and short-term linear growth [64]. However, there was an overall negative association between diarrhoea and length at 24 months of age −0.38 cm [95% confidence interval (CI): −0.59, −0.17] using an estimated burden of 23 diarrhoea days per year, that declined with reduced diarrhoea burden estimates [64]. Checkley *et al* in a pooled analysis of longitudinal data from five LMICs showed that the proportion of stunting attributed to ≥5 episodes of diarrhoea before 24 months was 25% [95% CI: 8%, 38%] [65]. What appears evident is that the adequacy of nutritional supplementation between episodes of diarrhoea as well as prolonged diarrhoea-free intervals, facilitate catch up growth, therefore mitigating the negative effects of diarrhoea on linear growth [67, 68]. It has been hypothesised that *"over 90% of normal development in infancy is growth-free and that length accretion is a distinctly saltatory process of incremental bursts punctuating background stasis"* [69]. This is backed by evidence of timed cellular expansion at the endochondral growth plate that can be influenced by favourable or adverse environmental conditions, the mechanisms of which remain to be explored [70]. During recovery from infection-related growth stasis, appetite can transiently be above normal during the period of "catch-up", when high quality nutrient dense foods are required to enhance the anabolic drive [71]. Interestingly, in a cohort of forty infants, Lampl *et al* also found that head circumference growth during infancy is saltatory with median increments of 0.20 cm [95% CI: 0.10, 0.30 cm] in 24 hours, followed by intervals of no

growth from 1 to 21 days that were concordant with linear growth (82% (SD 0.13 of head growth, p≤0.006) [72]. Although this model of saltation has been disputed [73], in LMICs where diets are poor, further exploration of this concept may enable us to provide targeted nutritional interventions (eg nutrient mix) at periods preceding the growth spurts.

Other infections

Undernourished children are also vulnerable to other infectious diseases such as pneumonia where the risk of mortality is increased 15-fold, and malaria where the odds of severe falciparum malaria is increased 4-fold [74, 75]. Although not yet well-defined, this vulnerability is believed to be due to altered immune function (often cellular) and this is an important research priority [74].

Environmental enteric dysfunction

Environmental enteric dysfunction (EED) (formerly environmental enteropathy/tropical enteropathy) is endemic from as early as 3 months of age in LMICs and has been estimate in one study to explain 43% of growth faltering under 15 months of age in rural Gambia [54, 76, 77]. Ingestion of contaminated food or material such as faeces from early childhood results in an altered small intestinal structure and function due to chronic gut inflammation i.e. EED [51, 78]. The structural changes in the small intestine (in particular the jejunum) include partial villus atrophy with crypt hyperplasia and inflammatory cell infiltration mediated by a T-cell immune response [79]. These result in impaired digestion/absorption of nutrients and increased permeability, resulting in bacterial translocation, impaired enteric immune response and systemic inflammation [51, 56, 78]. The specific aetiology is not well defined as there is no clear association with enteric pathogens or diarrhea [80, 81]. This partially explains why nutrition-specific interventions in LMICs often have had limited yield [82-87]. Improving water, hygiene and sanitation (WASH) in LMICs, particularly in poor communities has been assessed as a possible intervention [88]. The use of probiotics or prebiotics, anti-inflammatory agents and antibiotics in children with SAM are some of the other interventions that have been proposed [88]. The Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) study sought to evaluate the association between enteric

pathogens, malnutrition, gut physiology, growth, cognitive development and immune responses in the first 2 years of life in LMICs (Peru, Brazil, Bangladesh, India, Pakistan, Tanzania, South Africa and Nepal) [89]. They analysed non-diarrhoeal stool samples from over 1200 children for enteric pathogens (using molecular biology techniques), enteric inflammation and intestinal permeability, and found that in the first 24 months of life, these children had frequent enteric infections with high levels of both intestinal and systemic inflammation [90]. Higher burdens of enteropathogens, especially those categorized as being enteroinvasive (e.g. Campylobacter) or causing mucosal disruption, were associated with elevated biomarker concentrations for gut immunity (neopterin), inflammation (myeloperoxidase) and systemic inflammation and, indirectly associated with both reduced linear and ponderal growth [90]. Giardia was associated with reduced growth [90]. These findings and future evaluation of the aetiology and clinical diagnostics tools to enable health care workers to identify functional bowel deficits, will enhance the development of more effective interventions to address EED [91].

Water sanitation and hygiene

Inadequate sanitation, including sharing sanitation facilities with 1-2 other households increased the risk of moderate to severe diarrhoea in the GEMS study: Kenya (adjusted OR = 1.41 [95% CI: 1.11, 1.79]), Mali (adjusted OR = 1.23 [95% CI: 1.02, 1.48]), and Pakistan (adjusted OR = 1.58 [95% CI: 1.19, 2.09]) sites ([92]. In Mali, they also found that primary use of piped water (adjusted OR = 0.45 [95%: 0.34, 0.62]), continuous water access (adjusted OR = 0.30 [95%: 0.20, 0.43]), fetching water daily (adjusted OR = 0.77 [95%: 0.63, 0.96]), and breastfeeding (adjusted OR = 0.65 [95%: 0.49-0.88]) significantly reduced the likelihood of moderate to severe diarrhea in under 5's [59]. Water, sanitation and hygiene (WASH) improvements reduce rates of diarrhoeal disease but may also have the potential to improve growth in children by preventing exposure to infectious pathogens [50, 93]. A meta-analysis by Fewtrell *et al* found that hygiene interventions, specifically handwashing and education messages aimed at mothers, reduced the risk of childhood diarrhoea (RR 0·56 [95% CI: 0·33, 0·93] and RR 0·72 [95% CI: 0·63, 0·83]) respectively [93]. Other WASH interventions that reduced childhood diarrhoeal illness were sanitation interventions (pooled RR 0.68 [95% CI: 0.53, 0.87]); overall water supply interventions (RR 0·75 [95% CI: 0·62, 0·91]) with no extra benefit noted standpipe vs household connection; overall water

treatment (RR 0·69 [95% CI: 0·53, 0·89]) and combining WASH interventions (RR 0·67 [95% CI: 0·59, 0·76]) [93].

In related meta-analysis, Dangour *et al* sought to evaluate the effect of WASH interventions over a period of 9-12 months, on the nutritional status of children under 18 years with data from over 4600 children [94]. They found that WASH interventions had no effect on WAZ (mean difference 0.05 [95% CI: -0.01, 0.12]) and WHZ (mean difference 0.02 [95% CI: 0.07, 0.11]), but had a borderline effect on HAZ (mean difference 0.08 [95% CI: 0.00, 0.16]) [94].

Both meta-analyses noted that the quality of studies available to include in their analyses were not of high quality. Following these meta-analyses two intervention trials have been undertaken to further explore the effectiveness of the WASH and nutrition interventions in preventing growth faltering in LMICs. The first is the WASH Benefits study that consisted of two cluster randomized trials that aimed to assess the effect of combined WASH interventions with daily nutrition supplementation from the antenatal period over 24 months on the growth and development of infants and young children in rural Kenya and Bangladesh [95]. The first results from the WASH Benefits trials were presented at EB2017 in Chicago in April 2017. Disappointingly, the WASH interventions had no effect on growth. The nutrition interventions had small effects on growth, in the order of only 0.2 Z-scores. The second is the Sanitation Hygiene Infant Nutrition Efficacy (SHINE) trial that seeks to assess the effect of WASH and nutrition interventions on growth and anaemia incidence in early childhood in Zimbabwe [96]. The data from this trial is keenly awaited and should provide very useful insights into the role of combined WASH with nutrition interventions in promoting child growth and development in poor communities in LMICs. It is likely that these interventions in poor communities may not meet the high socioeconomic and housing thresholds that are required to make the significant differences to growth faltering that have been seen in countries that have experienced economic transition [97].

2.3.2 Infant feeding

Breastfeeding in the first 12 months has a significant impact on child survival worldwide [98, 99]. The WHO recommends that infants under six months are exclusively breastfed and thereafter should receive complementary foods that are safe and nutritionally adequate,

while breastfeeding continues for up to two years or beyond [100]. Breast milk provides all the nutritional requirements for most infants under 6 months and is an important source of nutrients in LMICs, where complementary foods are often nutritionally inadequate [100]. In addition, it is associated with a reduction in childhood infections (particularly gastrointestinal and respiratory), better growth and cognitive developmental outcomes, as well as improved maternal health by enabling child spacing and reducing the risk of breast cancer [98, 101, 102]. A meta-analysis of thirty four trials by Britton *et al* that included over 29,000 mother-infant pairs (healthy mothers and term infants) from fourteen countries found that all forms of extra breastfeeding support to the mothers reduced the risk of stopping breastfeeding before 6 months (RR 0.91 [95% CI: 0.86, 0.96), with a greater effect on exclusive breastfeeding (RR 0.81 [95% CI: 0.74, 0.89]) [103]. However, it is estimated that only 37% of children under 6 months are exclusively breastfeeding in LMICs [101]. Some of the barriers to exclusive breastfeeding include the insufficiency of breast milk alone in meeting the nutritional needs of the infant, family and societal pressures to introduce other feeds as exclusive breastfeeding is not the cultural norm in many communities in LMICs and short maternity leave for mothers in formal employment [104].

Suboptimal complementary infant feeding practices in LMICs are common and associated with stunting and wasting [105, 106]. However, nutrition-specific interventions aimed at improving growth outcomes have had limited yield. A meta-analysis by Dewey and Adu-Afarwuah found that education interventions alone had a small effect on weight (mean effect size 0.28 [range -0.06 to 0.96]) and linear growth (mean effect size 0.20 [range 0.04 to 0.64]) [107]. The provision of complementary food alone also had a moderate effect on weight (mean effect size 0.60 [range -0.02 to 2.99]) and linear growth (mean effect size 0.47 [range -0.04 to 1.81]), but on excluding one trial that appeared to be an outlier from SSA [108], the effect was small for weight (mean effect size 0.26 [range -0.02 to 0.57]) and for length (mean effect size 0.28 [range -0.04 to 0.69]) [107]. Combining the provision of complementary foods with education or another strategy still resulted in modest effects on child growth (mean effect size for weight 0.35 [range 0.18 to 0.66] and mean effect size for length 0.17 [range 0 to 0.32]). When the energy density of complementary foods was increased the effect on growth remained small (mean effect size for weight 0.35 [range -0.13 to 1.37] and mean effect size for length 0.23 [range -0.25 to 0.71]), although this may

be partly attributed to the lack of increase in the energy intake in one of the trials [107]. However, they concluded that trials from food insecure regions such as SSA and South Asia appeared to report greater effects on growth highlighting the need for complementary feeding interventions to be tailored to the needs of the recipient population [107].

Optimal feeding does not only depend on what a child is being fed on but also how, when and by whom the child is fed, and their responsiveness to the child's cues of hunger and satiety [106]. Mothers with large families where there are competing demands on maternal time, have limited time to prepare and adequately monitor and supervise their infants' feeding [109]. Assistance from an older sibling who is inexperienced or a very elderly family member who is be unable to supervise the feeding adequately compounds the problem. Responsive feeding (RF) emphasises the psychosocial aspects of care during complementary feeding i.e. *"being sensitive to hunger and satiety, feed slowly and patiently, and encourage children to eat but not force-feed them, talk to the children and give eye contact" [110].* There is conflicting evidence on the protective effect of responsive feeding on undernutrition in children in LMICs [111]. In an observational study in Ghana, responsive feeding in the context of good care practices (i.e. child feeding practices and use of preventative health services) in mothers with low literacy levels in Accra, RF was found to account for a 0.5 z score improvement in height-for-age (HAZ) and weight-for-age (WAZ), but had no effect on wasting [112]. However, in a randomised trial in Haiti, RF was associated with mean Z-score increases of 0.14 ($p=0.07$) for HAZ, and 0.24 for WAZ and WHZ ($p<0.0001$) [113]. In Bangladesh RF was associated with improved weight gain in under 2's, whilst in India there was a small but significant difference of 0.32 cm [95% CI: 0.03, 0.61] in height but not in weight. However, they found that positive verbalisation by mothers during feeds was increased the acceptance of food [111]. Despite these inconclusive findings, RF is central to the development of healthy eating habits [114]. Unfortunately, force-feeding is common in SSA and is associated with poor feeding habits and nutritional outcomes [111].

2.3.3 Maternal health

Undernutrition

Maternal undernutrition that includes micronutrient deficiencies is common in LMICs and results if adverse health and nutritional consequences for the mothers and their offspring [115]. The direct causes are often food insecurity, multiple infections and poor access to health care on a background of maternal poverty, illiteracy and disempowerment [116]. Growth faltering in early childhood often has its origins in the antenatal period [116]. Both micronutrient and macronutrient interventions have been shown to be effective in improving both maternal and infant nutritional and health outcomes [21, 115].

Macronutrient supplementation

A Cochrane review by Ota *et al* on the effect of education and supplementation in increasing maternal intake of energy and protein found that this can potentially reduce the risk of low birth weight (risk ratio 0.04 [95% CI: 0.01, 0.14]) (one trial in Bangladesh, 300 women) even in LMICs [117, 118]. The effects on birth outcomes were more significant when undernourished mothers were supplemented, resulting in a reduced risk of still births (risk ratio 0.60 [95% CI: 0.39, 0.94] and small for gestational age SGA (risk ratio 0.79 [95% CI: 0.69, 0.90]) [117].

In Ghana, a randomized trial by Adu-Afarwuah *et al* found that antenatal lipid-based nutrient supplement (LNS) improved the LAZ of their children at 18 months when compared to iron and folic acid (IFA) and MMN (-0.69 ± SD 1.01 vs -0.87 ± 0.99, p = 0.006; -0.69 ± SD 1.01 vs 0.91 ± 1.01, p = 0.009 respectively) [119].

Micronutrient supplementation

A recent meta-analysis by Haider and Bhutta involving 137,791 women mainly from LMICs, assessing the effect of multiple micronutrient (MMN) supplements given to pregnant women, compared with iron with or without folic acid, found that MMN reduced the risk of SGA at birth (risk ratio 0.91 [95% CI: 0.84, 0.97]), low birthweight (risk ratio 0.88 [95% CI: 0.85, 0.90]), and stillbirths (risk ratio 0.92 [95% CI: 0.86, 0.99]) [120].

Mental health

Maternal depression and anxiety that affect 15-28% of mothers in SSA and Asia, impair parenting skills and are associated with poorer infant health, growth and developmental outcomes [121, 122]. Risk factors include marital discord, young age of the mother, birth of a child of the non-preferred sex, HIV, violence, conflict and migration [121]. Until recently, maternal mental health was often given a low priority in the development and implementation of maternal and child health interventions to address growth faltering. There has been conflicting evidence on the association between maternal depression and child undernutrition in LMICs, with an overall stronger association in South Asia than Africa [122]. In their multi-country community based cross sectional survey, Harpham *et al* found increased adjusted odds for stunting and underweight associated with maternal common mental disorders (CMD) in India (1.4 [95%CI: 1.2, 1.6], 1.1 [95%: 0.9, 1.4]) and Vietnam (1.3 [95%: 0.9, 1.7], 1.4 [95%: 1.1, 1.8]), but not in Peru (1.1 [95%: 0.9, 1.4], 0.9 [95%: 0.6, 1.2]) or Ethiopia (0.9 [95%: 0.7, 1.2], 1.1 [95%: 0.9, 1.4]) [123]. A prospective case control study in Nigeria found that infants of mothers who suffered with postnatal depression had poorer growth at the third month (weight OR 3.41 [95% CI: 1.30, 8.52]; length OR 3.28 [95% CI: 1.03, 10.47]) and the sixth month postpartum (weight OR 4.21 [95% CI: 1.36, 13.20]; length OR 3.34 [95% CI: 1.18, 9.52]) [124]. They also found that depressed mothers were more likely to stop breastfeeding earlier and their infants more likely to have episodes of diarrhoea and other infectious illnesses [124]. A meta-analysis of mainly retrospective case control and cross-sectional studies done in LMICs showed that children of mothers with depression or depressive symptoms were more likely to be underweight (OR: 1.5 [95% CI: 1.2, 1.8) or stunted (OR: 1.4; 95% CI: 1.2, 1.7) [125]. Sub analysis of three longitudinal studies showed a stronger effect: the OR for underweight was 2.2 (95% CI: 1.5–3.2) and for stunting, 2.0 (95% CI: 1.0–3.9) [125]. Most recently, Ashaba *et al* found that maternal depression was significantly associated with severe wasting with an adjusted OR 2.4 [95% CI: 1.11, 5.18], in their inpatient nutrition rehabilitation unit in southwest Uganda [126].

2.3.4 Socioeconomic circumstances

Maternal education is associated with reduced child morbidity and mortality [21]. It is also associated with a lower risk of severe wasting [127, 128]. Mothers who have accessed

schooling demonstrate appropriate child care practices including infant feeding, preventative health seeking and hygiene behavior [128]. In addition, maternal schooling also has implications for gender empowerment and therefore improved diversity, quality and quantity of complementary feeds and resources to implement the appropriate hygienic practices [129, 130].

Mothers in poor communities in LMICs often face food insecurity and are therefore unable to diversify infant diets according to the complementary feeding recommendations, resulting in infant growth faltering. Under these circumstances, maternal resilience, achieved through peer support groups within communities, has been shown to result in sustained improvements in infant feeding practices [131]. In addition, in rural Gambia, the involvement of maternal grandmothers in infant care was associated with improved child growth and survival but the presence of paternal grandmothers seemed to have no benefit [132]. Interventions that promote maternal economic status and living standards also have a positive impact on the growth in childhood in LMICs [130].

2.4 Energy and growth regulating hormones in the context of undernutrition

Healthy growth during childhood and adolescence is regulated by the growth hormone/insulin like growth factor-1 (GH-IGF-1) axis (**Figure 2.5**) [133].

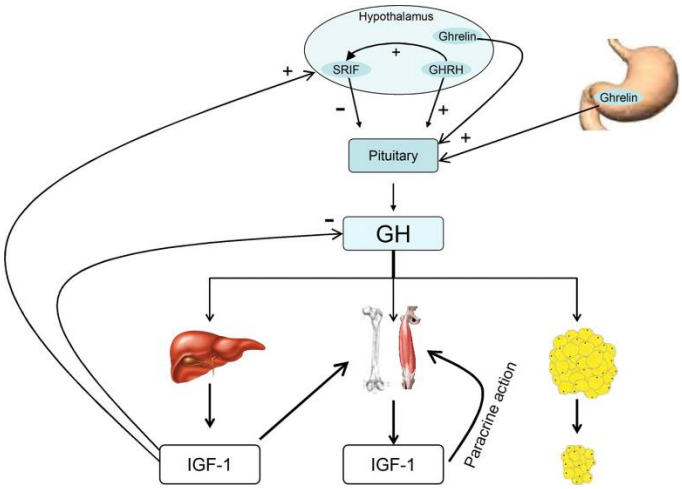


Figure 2.5: Simplified diagram of GH/IGF-1 axis involving hypophysiotropic hormones controlling pituitary GH release, IGF-1 production in the liver and elsewhere, and tissue responsiveness to GH and IGF-1. GH increases fat mobilization, decreases body fat and decreases adipocyte size and lipid content. Arrows denote stimulation (+) or inhibition (–). SRIF, somatotropin release-inhibiting factor; GHRH, GH-releasing hormone [133]

The pulsatile secretion of GH from the anterior pituitary gland is promoted by growth hormone release hormone (GHRH) and ghrelin (a neuropeptide hormone and gastric orexigenic hormone); and inhibited by somatotropin release-inhibiting factor (SRIF, somatostatin) and IGF-1 [133, 134]. These are themselves modulated by peripheral metabolic signals and other neuropeptides in the hypothalamus [133].

IGF-1 is a GH-dependent peptide that is mainly synthesized by the liver [133]. The half-life of circulating IGF-1 is prolonged and its signaling is either enhanced or reduced when bound to insulin like growth factor binding proteins (IGFBP), of which IGFBP-3 is the most abundant, accounting for 80% of all IGFBPs [133]. IGF-1 levels are significantly reduced during periods of fasting and increase with re-feeding, correlating well with the changes in nitrogen balance [135]. It is an important link between nutrient intake and cellular anabolic

responses [135]. Cortisol and inflammatory cytokines reduce the GH stimulation of IGF-1 [133].

Ghrelin, leptin and peptide YY are three important hormones involved in appetite regulation and therefore signaling in energy homeostasis. Ghrelin stimulates food intake; leptin and peptide YY suppress food intake [55, 136]. Leptin was initially discovered in 1994 as an adipocyte derived hormone, whose absence resulted in morbid obesity in the ob/ob mouse [137]. In subsequent years, the role of leptin in states of energy deprivation has gained significance [138, 139]. It also plays a key role in immune regulation, fertility and modulation of intestinal barrier function [139, 140]. Low leptin levels in cord blood closely reflect decreased adiposity at birth and strongly predict high rates of weight gain in infancy and catch-up growth [141]. Soluble leptin receptor (sOB-R) is the primary leptin-binding protein in human blood [142]. In the first years of life, sOB-R is detectable in remarkably high concentrations and levels decline continuously thereafter [141].

Nutrients drive intrauterine growth by providing substrate for tissue accretion, whereas hormones regulate nutrient distribution [143]. *"The main hormonal drivers of intrauterine growth are insulin, insulin-like growth factors and thyroid hormones. Together with leptin and cortisol, these hormones control cellular nutrient uptake and the balance between accretion and differentiation in regulating tissue growth" [143].* The infancy phase of growth in healthy Western populations lasts for the first 9 months of postnatal life and is regulated by insulin and the IGF axis [144, 145]. Subsequently, GH becomes responsible during the childhood growth phase and plays an important role in regulating longitudinal growth, body weight, and body composition [144]. The GH-IGF-1 axis regulates postnatal skeletal muscle hypertrophy and bone growth [146]. GH exerts anabolic actions in skeletal muscle by both promoting muscle development and facilitating nutrient uptake and utilization in the muscle, thereby coordinating global energy expenditure and body composition mediated through IGF-1 [146]. However, GH also functions independently of IGF-1 receptor signaling, to facilitate normal insulin action in skeletal muscle, which ultimately impacts upon global nutrient metabolism [146, 147].

Surprisingly little is known about the physiological mechanisms by which the different environmental factors inhibit growth in children in LMICs. Periods of inadequate energy and

nutrient intake, inadequate absorption due to infectious episodes and increased metabolism due to infections, are likely to alter the GH-IGF-1 axis and other key hormonal axes involved in growth and energy regulation, which will alter the timing and duration of the various phases of growth [148].

In a zinc supplementation trial, Doherty *et al* found that insulin and IGF-1 respond to dietary changes in carbohydrates and proteins and that increments in IGF-1, IGFBP-3 and markers of bone and collagen formation occurred during nutritional rehabilitation of children with SAM [147].

Observational studies in LMICs comparing hormone levels in pre-pubertal children with SAM undergoing intensive in-patient nutritional rehabilitation found that at baseline, children with SAM had significantly lower leptin, insulin, IGF-1 and IGF-binding protein 3 (IGFBP-3); and significantly higher basal cortisol, GH, soluble leptin binding receptor (sOB-R) and IGFBP-1 compared to their well-nourished counterparts [147, 149, 150]. These normalize to the same levels as in well-nourished children within 2 weeks of intensive nutritional rehabilitation with associated weight gain [147, 149, 150]. In addition, at the end of nutritional rehabilitation, leptin increased significantly in the undernourished children, sometimes reaching 166% of levels observed in well-nourished children, even with modest weight gain [147, 149, 150]. Conversely, Stein *et al* also found that sOB-R decreased significantly below the levels observed in well-nourished children and the molar excess of sOB-R over leptin was found to be negatively correlated to leptin, IGFBP-3, body mass index (BMI) and mid upper arm circumference (MUAC), and hypothesized that sOB-R may a modulatory effect on leptin in children with SAM during nutritional recovery [147, 149, 150]. This supports the notion that prior to intervention, children with SAM are in a pro-inflammatory and catabolic state. With treatment that includes antibiotics, the inflammation is reduced therefore promoting anabolism, which in the presence of intensive protein and energy therapeutic feeding, results in rapid growth (weight and potentially linear) regulated by the GH/IGF-1 axis. The hormone responses during CMAM where the nutrient provision is less intensive are largely unknown. This knowledge would help to improve the timing and intensity of interventions during CMAM.

2.5 Summary

Growth faltering due to undernutrition in early childhood is a significant public health problem in LMICs. Despite the implementation of several nutrition-specific and nutrition-sensitive interventions, progress has been limited, particularly in SSA where the number of stunted children is increasing. Possible explanations for this are that the coverage has been inadequate due to resources limitations in many of these countries. However, it is evident that many gaps remain in our knowledge of the aetiological factors (that may differ between regions) of growth faltering; the timing and duration of growth faltering; the physiological mechanisms by which adverse environmental factors alter the growth trajectories of children; and the influence of maternal psychosocial status and family dynamics on growth faltering. Exploring and building evidence in these areas would enhance the development of new interventions, but also help strengthen the existing interventions and therefore contribute to the development of better policies.

Chapter 3: Study description

3.1 Statement of the problem

Growth faltering due to undernutrition in early childhood is a persisting public health problem in many sub-Saharan African countries, including The Gambia. Current evidence-based interventions have not succeeded in making a significant and sustained impact on the growth of children in these settings. The increased risk of morbidity and mortality associated with undernutrition has also hampered progress in reducing mortality and improving the health and developmental outcomes in under 5's. In addition, malnourished children have variable responses to nutritional rehabilitation. Knowledge of the physiological mechanisms that govern these responses is limited. There is therefore an urgent need to understand the timing and missing contributors of growth faltering in this age group in SSA and identify the predictors of nutritional recovery in order to accelerate the development of new targeted and innovative interventions.

This study was designed to describe the secular trends of growth in early childhood over 4 decades, in a rural Gambian community. In addition, the study described the energy regulating hormone correlates in malnourished children during nutritional rehabilitation and explored factors associated with severe wasting in infants in this setting.

3.2 Study site

This work was undertaken in the West Kiang district of The Gambia. The West Kiang district is the largest but least populated of the 6 districts of the Lower River Division of the Gambia. It consists of 36 villages of varying sizes with a total stable population of almost 15,000, of whom 2,300 (15%) are children under 5 years of age [151]. It falls under the local government area of Mansa Konko [152].

The main income generating activity is subsistence farming, but over the past decade this district has been prone to food insecurity due to erratic rainfall patterns, necessitating emergency relief food supplies [153]. The climate has a long dry 'harvest' season

(November- May) and a short wet 'hungry' season (late June to mid-October), when agricultural work, depletion of food supply and infectious diseases peak [154]. Breastfeeding from birth to 2 years is the norm and complementary feeds are often nutritionally inadequate [155]. Mandinka are the predominant ethnic group, but there are also other ethnic groups in the district including Fula and Jola [151]. The majority of the population are Muslims but traditional African beliefs are an important part of their spiritual lives. Polygamy is a popular and acceptable practice and isolated nuclear family units are rare, as most compounds will have extended family relations including grandparents living within the family unit. In rural Gambia, the value and status of women is often based on their reproductive capacity, therefore fertility rates have remained high and the uptake of contraception low [156]. The majority of married women live in a compound belonging to their husband or his family [157]. The bulk of domestic duties, agricultural work and rearing of children is undertaken by the women, who are also often the recipients of domestic violence [152]. An increasing number of children including girls are accessing primary school education, but countrywide only 30% transition to secondary school [158]. The literacy levels particularly amongst women in rural areas remain low and a large proportion of the population live below the moderate poverty line of living on less than US$ 2/ day [152, 159].

The UK Medical Research Council (MRC) has been providing free comprehensive primary health care services including antenatal and child health clinics, for over 40 years to three rural villages in the West Kiang district- Keneba, Manduar and Kantong Kunda [151, 154]. This has contributed to over 80% declines in both the infant and under 5 mortality in these three villages over the past 4 decades [154], well ahead of the Gambian national estimates [152]. Unfortunately, the rates of undernutrition in children under 5 in The Gambia remain high, with the 2010 multiple indicator clusters surveys showing that 17% are underweight, 23% are stunted and 10% are wasted [152]. In recent years, the West Kiang demographic surveillance system (DSS) has been established that includes all the 36 villages (**Figure 3.1a and Figure 3.1b**). The inhabitants in all these villages can access free health care services at the MRC Keneba clinic, but continue to have their routine antenatal and child health checks at their local health care facilities run by The Gambian Ministry of Health [151, 152].

Figure 3.1a: Map of The Gambia depicting the West Kiang

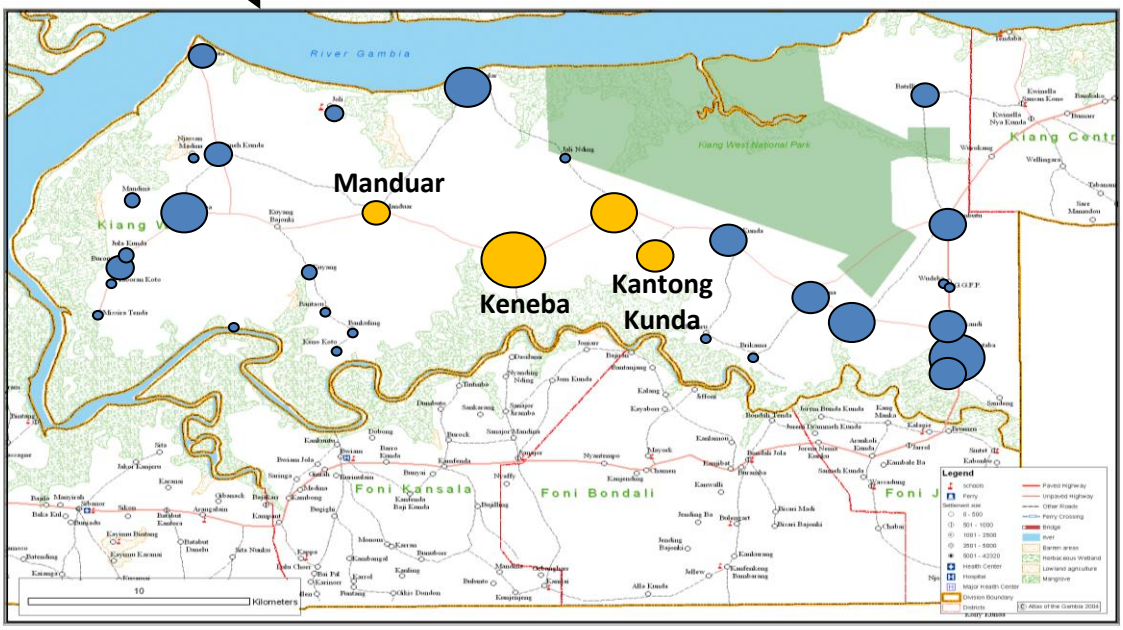


Figure 3.1b: Map of the study area clearly marking the West Kiang Demographic Surveillance Survey area

3.3 Study populations

This work included children under 2 years of age both in the community and health care facilities within the West Kiang district, including the MRC Keneba clinic and the surrounding government health centres. Further details of the study populations within each research study are presented in Chapters Four, Five and Six. The secondary analysis of anthropometric MRC Keneba clinic data, utilised data that was collected among infants from

May 1976 to February 2012. The other two studies were conducted between June 2013 to August 2015.

3.4 Objectives

3.4.1 General objective

To evaluate the patterns and timing of growth faltering in rural Gambian children using postnatal anthropometric measurements, and to evaluate the predictors of growth faltering and the dynamics of growth and energy mediating hormones during nutritional rehabilitation.

3.4.2 Specific objectives

1. To describe the growth patterns of rural Gambian children under 2 years of age over the past 4 decades.
2. To evaluate the effect of seasonality, infections and maternal height on postnatal growth.
3. To investigate whether the high degree of variability in children's responses to nutritional rehabilitation in malnourished children could be explained and predicted by differences in energy and growth regulating hormones.
4. To investigate the acute response of leptin to feeding in malnourished and non-malnourished controls.
5. To explore the maternal and infant health, maternal psychosocial and environmental factors that are associated with severe wasting in rural Gambian infants.

3.5 Sampling and sample size calculations

In the three research studies, children were sampled from the following sites: the MRC Keneba DSS that incorporates the community of West Kiang district of The Gambia, the MRC Keneba clinic and the government health care facilities within the West Kiang district. The sample sizes for the research studies were calculated separately as they were based on their respective primary objectives and will be discussed in detail in Chapters Four to Six.

3.6 Ethical considerations

The proposals for the research studies that are presented in this thesis were approved by the Joint Gambian Government/MRC Unit The Gambia Ethics Committee (Appendix IX). Approval for research Studies II and III was also sought and granted by the London School of Hygiene and Tropical Medicine (Appendix IX). For research Studies II and III, informed consent both written and verbal was obtained from individual participants (for adults) or carers (for children) (Appendix VII). For the retrospective secondary cohort analysis, the ethics approval was based on the approval for demographic surveillance that was granted by the Joint Gambian Government/MRC Gambia Ethics Committee in 1981. In addition, some of the data collection pre-dated the establishment of a formal ethics process in The Gambia. Further details on the consenting process for research Studies II and III will be presented in Chapters Five and Six.

3.7 Study designs

To address the five objectives of this work, three main study designs were employed. These are summarised and discussed in this sub-section.

3.7.1 Retrospective cohort study

This study design utilises data that has already been collected and often for other purposes other than the objectives of the relevant study [160]. This provides valuable information on the timing of the outcome of interest that is useful in exploring the aetiology of disease.

This study design was used for Study I, to describe the secular trends of growth in under 2's in a rural Gambian community spanning four decades. This design was advantageous as all the relevant data on exposures of interest (e.g. seasonality, infectious diseases) and timing of growth faltering in early childhood (outcomes) had already been collected therefore saving on the resources required for collecting the data. In addition, as no additional variables were required for this analysis, the challenge of recall bias when using retrospective cohorts was avoided. Also, as there was very limited migration in the study population, the bias introduced by loss to follow-up was limited. The large volume of anthropometric data at different time points in the first 2 years of life in this community

allowed comparisons of effect size estimates over time in body size and an estimation of the effect of seasonality on growth faltering between decades. It provided background information on growth faltering in children over different time periods in this population that led to the development of hypotheses that were tested in a subsequent study.

A major limitation of this design was that the data were not collected to address the study objectives, therefore, it was not possible to control for bias, confounders or random errors that may have occurred during data collection. It was also not possible to minimise missing data as some of the data had been collected four decades before. In addition, there were variations in the accuracy of measurements between decades particularly in the birth length, which I had to omit from the analysis.

3.7.2 Cross-sectional study at baseline (cases vs controls) and longitudinal for cases (Quasi experimental design)

This study design was employed Study II, as this was an exploratory study aimed at providing data and generating hypotheses for future interventional studies in malnourished children. The cross-sectional design at baseline facilitated the estimation of differences in energy regulating hormones between malnourished (case) and non-malnourished (control) children who were not matched, whilst also assessing the acute changes in these hormones with feeding. This was advantageous as multiple outcomes (i.e. energy regulating hormone levels and C-reactive protein) were assessed whilst enabling comparisons to be made between the cases and controls therefore, limiting the costs involved [160]. The longitudinal aspect of the study was advantageous as it enabled the evaluation of hormone changes over time in malnourished children, hence providing key insights into the timing and energy regulating hormone responses during nutritional rehabilitation. It has also helped generate hypotheses that can be tested in adequately powered intervention studies.

A major limitation of this study design was the problem of confounding that may have affected the comparison of hormonal levels between the three groups and between pre-and post-test meal. Such factors could include age, sex, season, circadian rhythms and severe illness in the severely malnourished children. In addition, there would also have been recall bias in the responses to the brief infant feeding and socioeconomic questions that were

administered to carers at recruitment, with carers of malnourished children either overreporting or underreporting what they consider to be risk factors for malnutrition [160].

3.7.3 Case control and mixed methods study

Study III was a retrospective study design nested within a randomised micronutrient supplementation trial with a different study objective from the ones of interest for this work. The study population therefore had a number of variables of interest (i.e. potential risk factors for severe wasting in infants) that had already been collected during the trial therefore limiting the problem of recall bias. In addition, both the cases (children who had at least one episode of severe wasting in infancy) and controls (children who had not become severely wasted in infancy and were matched to the cases by age, gender, distance from the MRC Keneba clinic and village size) were recruited from the community, hence limiting the effect of selection bias. This design also facilitated the exploration of a large number of variables of interest (exposures) with fewer resources (i.e. money and time), than would have been required for a cohort study [160].

The mixed methods research approach is a form of scientific inquiry in which the investigator collects and analyses data, integrates the findings, and draws inferences using both qualitative and quantitative approaches or methods in a single study or a program of inquiry [161]. A key strength of this mixed methods study approach is that it enables the researcher to explore the meaning of a phenomenon through description [162]. For this study, the use of standard questionnaires in the formative phase, followed by informal observations and in-depth interviews (IDIs) with a diverse group of independent respondents, facilitated triangulation of the findings that enhanced the comprehensiveness and credibility of the study [163, 164]. Another strength of this work was the iterative study design. The homes of some of the participants were visited 2-3 times from the formative phase to the interview phase. This helped the research team and I to build a rapport with the mothers and members of their households, which facilitated the sharing of sensitive information by carers during the research process. Prolonged engagement with participants also enhances the credibility of findings in qualitative studies [163].

One major limitation of the study design, that is that the data on many of the exposures was collected retrospectively, and often over 12 months after the child had growth faltered with the associated challenges of recall bias. These influences have been mitigated by using some of the prospectively collected data to either (i) validate some of the retrospective data (breastfeeding and complementary feeding) or (ii) use as actual exposure variables (infant feeding under 6 months, maternal education and access to water). Nevertheless, this study has generated a number of hypotheses that can be used to design future intervention studies.

3.8 Data validation and storage

Data from the Studies II and III were double entered into the MRC Keneba secure SQL server study databases that were routinely backed up. All the databases were password protected with restricted access to the research team members. The dataset for the retrospective study was extracted from the MRC Keneba clinic databases (including the Keneba Electronic Medical Records System) [151]. The datasets for all the studies were received in Excel spreadsheet format and imported into STATA (version 12.0, StataCorp, College Station, TX) for data cleaning and analysis. Values for weight, length, head circumference and mid upper arm circumference (MUAC) were excluded from the analysis if they were not biologically plausible for the child's age. WHO macros were used to transform anthropometric data to WHO (2006) Z scores using the 2006 growth reference standards [1] .

Audio-recordings of all the interviews were downloaded into a password protected study folder on the MRC Keneba secure server that was only accessible to the research team. The raw data was stored in its original format. Audio-recordings from all the interviews that were conducted in Mandinka and Fula were translated into English and transcribed verbatim using standardised transcription principles [165]. Data analysis took place alongside data collection in order to allow questions to be refined and new avenues of inquiry to develop [166]. NVivo 10 software (QSR International Pty Ltd 2012) was used to aid in further data management and analysis using codes.

3.9 Statistical methods

This provides a summary of the statistical methods that were used. A more detailed discussion of their application to the data of three research studies will be presented in Chapters Four to Six.

3.9.1 Measures of effect size and evidence of association

The mixed effects or random effects regression models are regression models that incorporate both fixed effects (i.e. *"levels of primary interest"*) and random effects (i.e. *"levels not of primary interest e.g. subject effects"*) and is used when analysing correlated data such as repeated measurements on individual participants over time [167]. They are used to estimate the effect size changes over time for specific characteristics with repeated measurements in individual participants, within longitudinal studies [168]. These datasets are often unbalanced due to variations in measurements over time making it difficult to apply a multivariate model for analysis [168]. These models combine two stages of statistical modelling i.e. assumption of linear regression modelling for individual participants' parameters that have a bivariate normal distribution in the population (random effects) [168]. These models also allows for analysis of between and within individual variation [168].

Random-intercept linear regression model

This model is used to estimate the effect of individual participants' characteristics on a continuous outcome in longitudinal or clustered studies and allows for accounting for the between and within individual variations [169]. Let Y_{ij} be a continuous outcome for time point i and participant j, $(X_{1ij}, \dots, X_{pij})$ p participants' characteristics. The model specification is generally as model [169]:

$$Y_{ij} = \beta_0 + \beta_1 X_{1ij} + \cdots + \beta_p X_{pij} + \gamma_j + \varepsilon_{ij}$$

where γ_j and ε_{ij} are the random-intercept and individual participant's residual at time point i respectively and are both normally distributed with mean 0. γ_j represents the combined effects of omitted or unobserved individual participant's characteristics and captures the

within participant variability. $\beta_0 + \gamma_j$ is a participant-specific intercept[169]. The coefficients β_1 to β_p are assumed to be the same for all individual participants.

In Research Paper I, this was used to estimate the effect size changes in growth parameters over the four decades in under 2's within the retrospective cohort analysis.

Random-intercept logistic regression model

This model is used to estimate the effect of individual participant's characteristics on a binary outcome in longitudinal or clustered studies and allows for accounting for the between and within individual variations [170]. Let Y_{ij} be a binary outcome with value 1 if the event occurs and 0 otherwise. The model is specified as follows:

$$logit[p(Y_{ij}=1)] = \beta_0 + \beta_1 X_{1ij} + \ldots + \beta_p X_{pij} + \gamma_j$$

γ_j captures the within-participant variability on the log-odds scale [170].

In Research Paper I, to describe secular changes in rates of stunting, wasting and underweight a random effects logistic regression of the binary variable was fitted on the first four orthogonal polynomials in age and the first pair of Fourier terms for season. Effects of age and season on repeated growth parameters were fitted using random effects models. Models for males and females and each decade were fitted separately.

Random-slope linear regression

This model is an extension of the random-intercept linear r egressionmodel where the coefficients β_1 to β_p are allowed to vary across individual participants [169]. It is a useful means for controlling for confounding and reducing the standard error of regression coefficients of exposure variables as exposure variable with strong associations with outcome variables are included [171]. The random-slope captures the variability of the effect across individual participants [169]. The model specification is as follows [169]:

$$Y_{ij} = \beta_0 + (\beta_1 + u_{1j}) X_{1ij} + \ldots + \beta_p X_{pij} + \gamma_j + \varepsilon_{ij}$$

$\beta_1 + u_{1j}$ is the participant-specific effect of X_{1ij} on Y_{ij}. u_{1j} is also assumed to be normally distributed with mean 0 and uncorrelated with γ_j [169]

In Research Paper II, random-slope linear regression was used to assess the change in weight, MUAC, knee heel length, WHZ, WAZ and HAZ over time and allow for variation in growth rate between individual children.

The differences in hormone levels between nutritional groups at baseline was assessed using random-intercept and random-slope linear regression models that accounted for repeated measurements in each individual child i.e. pre- and post-prandial levels and over time. These models were also used to evaluate the hormone and anthropometric changes over time, allowing for interaction between nutritional groups and time points, adjusted for age and gender.

Fourier regression

Fourier regression is a form of regression analysis that is a very useful tool for analysing periodic or cyclic data such as seasonality. It facilitates the measurement of association and effect size estimates of the complex patterns seen in these analyses [172]. *"It fits a linear regression model in which the cyclic component, c(ϑi), is represented by a truncated Fourier series"* [172]. Fourier regression represents the seasonal pattern as a Fourier series whose higher order terms are regarded as high-frequency noise and discarded. The resulting truncated series is a linear combination of trigonometric functions of θ, the angle representing the phase of the year when the measurement was made, and whose coefficients are readily estimated as part of the regression model [172].

In Research Paper I, the first three pairs of Fourier terms were fitted whilst controlling for age by including the first three orthogonal polynomials in age (age1, age2, age3) in the model. Thus the *j*th observation for the *i*th individual is given by:[172]

$$Y_{ij} = \beta_0 + \beta_1 age1_{ij} + \beta_2 age2_{ij} + \beta_3 age3_{ij} + \sum_{k=1}^{3} \left[\alpha_k \, sin\left(k\theta_{ij}\right) + \beta_k \, cos\left(k\theta_{ij}\right)\right] + \tau_i + \varepsilon_{ij}$$

where the α_k and γ_k are the coefficients for the Fourier terms and the βs the remaining regression coefficients; τ_i is the random effect due to the i^{th} individual and ε_{ij} is the error term.

In Research Paper I, Fourier regression was used to estimate the effect of season on body size. The seasonal estimated patterns were averaged across all age groups as seasonal patterns themselves may have varied with age.

To quantify the children's susceptibility to seasonal changes (as plotted in Figure 4.2 of Research Paper I) the amplitude of the seasonal pattern was estimated, which was defined as the square root of half the sum of the squared Fourier coefficients:

$amplitude = \sqrt{\sum_k [\alpha_k^2 + \gamma_k^2] / 2}$.[172]

The delta method was used to estimate the standard error of these estimates (employing Stata's post-estimation command *nlcom*).

Conditional logistic regression

Logistic regression is commonly used to analyse binary outcome variables and to evaluate the effect of a single or multiple exposure variables, hence enabling the estimation of effects using odds ratios [171].

Conditional logistic regression is a type of logistic regression used for analysing matched case-control studies, in which cases are only compared to controls in the same matched set [171]. This helps to further control for the known confounders [171]. The matched set is used as a covariate in the conditional logistic regression.

logit[p(Yij=1)] = βset_1 + …. + βset_m + β1X1 + … + βpXp [171]

The intercept in the ordinary logistic regression is substituted by the matched set effects (βset k (k=1, …,m) is the kth matched set effect) [171].

In multiple logistic regression models with three or more variables, the assumption is that there is no interaction between the variables. Therefore, the effect of each is estimated based on controlling for the effect of all the other exposure variables [171].

In Research Paper III, conditional logistic regression was used to assess the association of severe wasting with the multiple exposure variables that were hypothesised to be possible risk factors (carer, infant and environmental).

Kruskal-Wallis test

This is a non-parametric hypothesis test that examines the difference in medians between more than two independent groups [171].

In Research Paper II, this was used to assess for the difference in baseline non-normally distributed numerical characteristics between the 3 nutritional groups.

Two-sample student t-test

This is a statistical hypothesis test that can be used to assess the difference in mean between 2 independent groups of participants where the sample population is assumed to be normally distributed [171].

This uses the t-statistic with the null hypothesis being that there is no difference in means between the groups.

In Research Paper III, the student t-test was used to assess the differences in means of the baseline characteristics between cases (children who became severely wasted in infancy) and controls.

Wilcoxon-Mann-Whitney test

This is a non-parametric test hypothesis test that is used to assess difference in the medians between 2 independent groups of participants [171].

The equivalent test for differences in means between two normally distributed sample populations is the two-sample t-test [171].

In Research Paper III, this was used to test for the difference in medians of baseline characteristics between cases (children who became severely wasted in infancy) and controls.

Repeated measures Analysis of Variance (ANOVA)

This is a hypothesis test that is used to assess the difference in means in more than 2 groups of participants evaluating multiple measures at different time points [171].

The assumptions are that the measures within the groups are normally distributed, the expected values of the errors are zero, the variances of all errors are equal to each other and that the errors are independent [171].

In Research Paper II, the repeated measure ANOVA was used to assess which biochemical indices were good predictors of nutritional recovery.

Wilcoxon signed-rank test

This is a non-parametric hypothesis test that assesses for the difference in medians between paired observations [171].

It is the equivalent to the paired t-test in a normally distributed sample population comparing differences in means [171].

In Research Paper II, this was used to compare the median hormone levels in the fed and fasted state within the three nutritional groups (severe acute malnutrition, moderate acute malnutrition and controls) at baseline.

3.9.2 Significance test in regression models

Wald test

This is an approximation of the likelihood ratio test and is a widely used hypothesis testing approach [171]. This helps in the decision of which exposure variables improve the model fit and which ones do not [171].

In Research Paper II, the Wald test was used to test for interaction between time (Day 0 versus Day 14 and Day 0 versus Day 28) and nutritional group (severe acute malnutrition versus moderate acute malnutrition). Interaction terms, age at recruitment and sex were not included in the final model if there was no evidence at 5% level of significance.

3.9.3 Other statistical methods

Principal component analysis

Principal component analysis (PCA) is a multivariable statistical method that uses an orthogonal transformation to convert a set of possible correlated quantitative variables into linearly uncorrelated variables i.e. principal components [173]. The quality of principal components can be evaluated using techniques such as the Kaiser-Meyer-Olkin (KMO) measure described below [174]. KMO values range from 0 to 1 with values closer to 1 indicative of sampling adequacy and values close to zero indicative of widespread correlations [174].

In Research Paper III, the PCA was used to determine household socioeconomic status using an asset based index in which I included the following six socioeconomic indicators: ownership of television, car, electricity, motorcycle, bicycle, or animal cart (yes/no). Variables with a KMO value of <0.50 or missing values were dropped from the model.

Chi-squared test

This is used to test the association between the categorical exposure variables and the outcome variables (often binary) ([171]. It tests the null hypothesis that there is no association between the exposure and outcome variables.

In Research Paper III, this was used to evaluate possible differences in the baseline characteristics of the infants/their carers and the environment between the cases (children who became severely wasted in infancy) and controls.

Fisher's exact

This was used as a measure of association between categorical exposure variables and the outcome (often binary) when the sample size is too small therefore, rendering the chi-squared test invalid as the expected numbers within each cell are very small [171].

In Research Paper II this test was used to evaluate the association between baseline characteristics of the children/carers and their nutritional status.

Chapter 4

Research Paper I

Article cover sheet

Title: **Growth faltering in rural Gambian children after four decades of interventions: a retrospective cohort study**

1. For a 'research paper' already published

   1.1 Where was the work published? Lancet Global Health

   1.2 When was the work published? February 2017

      1.2.1 If the work was published prior to registration for your research degree, give a brief rationale for its inclusion N/A

      1.2.2 Was the work subject to academic peer review? Yes

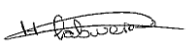
      1.2.3 Have you retained the copyright for the work? Yes

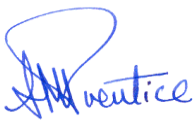
      If yes, attach evidence of retention: CC BY licence has been applied.

      https://doi.org/10.1016/S2214-109X%2816%2930355-2

2. For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper.

   As part of my PhD, I conceived, designed and undertook the data analysis for the study in collaboration with the all the authors (Prentice AM, Fulford AJ, Moore SE). I wrote the manuscript and my co-authors edited and provided comments on the drafts that were incorporated into the submitted version of the manuscript. I also incorporated the peer reviewer's comments in the final version of the manuscript.

Candidate's signature: 

Supervisor or senior author's signature to confirm role as stated in (2): 

Growth faltering in rural Gambian children after four decades of interventions: a retrospective cohort study

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4.1 Summary

Background Growth faltering remains common in children in sub-Saharan Africa and is associated with substantial morbidity and mortality. Due to a very slow decline in the prevalence of stunting, the total number of children with stunting continues to rise in sub-Saharan Africa. Identifi cation of effective interventions remains a challenge.

Methods We analysed the eff ect of 36 years of intensive health interventions on growth in infants and young children from three rural Gambian villages. Routine growth data from birth to age 2 years were available for 3659 children between 1976 and 2012. *Z* scores for weight-for-age, length-for-age, weight-for-length, mid-upper-arm circumference, and head circumference were calculated using the WHO 2006 growth standards. Seasonal patterns of mean *Z* scores were obtained by Fourier regression. We additionally defined growth faltering as fall in *Z* score between 3 months and 21 months of age.

Findings We noted secular improvements in all postnatal growth parameters (except weight-for-length), accompanied by declines over time in seasonal variability. The proportion of children with underweight or stunting at 2 years of age halved during four decades of the study period, from 38·7% (95% CI 33·5–44·0) for underweight and 57·1% (51·9–62·4) for stunting. However, despite unprecedented levels of intervention, postnatal growth faltering persisted, leading to poor nutritional status at 24 months (length-for-age *Z* score –1·36, 95% CI –1·44 to –1·27, weight-for-age *Z* score –1·20, –1·28 to –1·11, and head circumference *Z* score –0·51, –0·59 to –0·43). The prevalence of stunting and underweight remained unacceptably high (30·0%, 95% CI 27·0–33·0, for stunting and 22·1%, 19·4 to 24·8, for underweight).

Interpretation A combination of nutrition-sensitive and nutrition-specifi c interventions has achieved a halving of undernutrition rates, but despite these intensive interventions substantial growth faltering remains. We need to understand the missing contributors to growth faltering to guide development of new interventions.

Funding UK Medical Research Council, UK Department for International Development.

4.2 Introduction

The combination of fetal growth restriction, underweight, stunting, and wasting in later childhood, suboptimal breastfeeding, and micronutrient deficiencies have been estimated to cause more than 3 million child deaths annually, equivalent to 45% of the global total.[1] Among these factors, the association between undernutrition and mortality is confounded by the eff ects of deprivation but is probably at least partly causal as evidenced by the greatly elevated hospital case fatality rates of undernourished children compared with better nourished children.[2]

The Millennium Development Goals (MDGs) adopted underweight as a key indicator for MDG1, but stunting has since been adopted as the preferred indicator because it off ers a more stable index of long-term malnutrition. Latest estimates suggest that rates of stunting have been declining in most regions, but there remain 159 million children with stunting worldwide.[3] The prevalence of stunting has declined most slowly in sub-Saharan Africa, and as a consequence of population growth the absolute number of children with stunting has increased.[3]

Stunting rates fall rapidly as countries pass through the economic transition, but the key elements of progress that alleviate growth faltering are poorly understood, thus limiting the design of interventions and the targeting of health and development inputs in populations that remain impoverished. In this study, we analyse a longitudinal dataset spanning almost four decades of growth monitoring in three rural African villages that have received an unprecedented level of health-orientated interventions. A meta-analysis[4] of previous interventions for water, sanitation, and hygiene (WASH) has not yielded strong grounds for optimism regarding the likely efficacy of such investments at the levels currently offered. The analysis of randomised trials included more than 4600 children studied over 9–12 months of intervention, and its findings showed no evidence of any beneficial effect on weight-for-age or weight-for-height and only a marginally significant effect on height-for-age of less than a tenth of a standard deviation (0·08 *Z* score, 95% CI 0·00–0·16). Additionally, the *Lancet* Series on Maternal and Child Nutrition[5] reinforced the conclusion that nutrition interventions

4.3 Research in context

Evidence before this study

We searched PubMed and subsequent reference lists of relevant articles, with combinations of the terms "secular trends in growth", "growth faltering", "undernutrition", "wasting", "stunting", "underweight", "African children", "rural", "underfive", and "infants" between June 1, 2012, and Feb 28, 2015. All studies published between Jan 1, 1980, and Feb 28, 2015, that had the relevant search terms (irrespective of language) were included. The quality of the evidence was inadequate for the research questions that we posed, including what the secular trends were in growth in rural African children younger than 2 years during the past four decades, and how the effect of seasonality on the growth of these children has changed during the past four decades. A small number of longitudinal studies from east and central sub-Saharan Africa described the growth patterns in cohorts of young African children over a period of less than a decade, assessing the effect of seasonality, immunisation uptake, and maternal health factors on the patterns of growth faltering. These findings showed that weight declined after the first 3 months in infants and that improved growth in infancy was associated with immunisation status and indices of adequate maternal nutritional status, whereas the rainy season was associated with reduced growth velocity. However, none of these studies described secular trends in these growth patterns. Additionally, the multicountry analyses used cross-sectional data, making interpretation of trends in growth faltering over time within individual populations difficult. Several studies from southern Africa assessed the secular trends in growth in children of school age and older children (older than 8 years), whereas other researchers combined different cohorts in their analyses, making it diffi cult to contextualise the associated trends in the social environmental and health interventions within the respective populations.

Added value of this study

To our knowledge, this study is the first to describe in fine detail the secular trends in longitudinal and seasonal growth patterns of children in a rural sub-Saharan African community with a constant sampling frame. We have documented the introduction of a series of nutrition-specific and nutrition-sensitive interventions resulting in an unprecedented level of health care in these villages. Simultaneous socioeconomic transitions have occurred with increased access to formal education, employment, and income through remittances from family members overseas. Families have become much less reliant on subsistence farming for their income and nutritional needs. These changes have resulted in reduction of mortality to a tenth of its former level in children younger than 5 years, and major reductions in diarrhoeal and other morbidity. Growth has improved but, despite these profound health and socioeconomic changes, the patterns of childhood growth faltering persist with stunting prevalence remaining at 30%. Our findings indicate that communities must exceed a very high threshold for health and environmental change before growth faltering will be eliminated.

Implications of all the available evidence

Children in resource limited settings, particularly in sub-Saharan Africa, continue to have suboptimal growth patterns despite access to public health interventions such as immunisation, clean water, and sanitation. Our analysis suggests that mitigation of growth faltering will need these public health interventions to be combined with many other improvements in children's environments, perhaps including improved housing with the provision of piped water directly into the home. Evidence from countries that have passed through the economic transition suggests that poverty reduction promotes such improvements and is accompanied by rapid declines in stunting.

alone will have little effect on childhood undernutrition and estimated that, even if scaled up to 90% coverage, the implementation of all of the currently identified evidence-based interventions relating to nutrition would eliminate only about 20% of stunting globally. The results of ongoing trials to test the effect on growth of WASH interventions are keenly awaited.[6,7]

The implication therefore is that there is a very high threshold for improvements in living conditions, disease elimination, dietary sufficiency, and access to health care that must be exceeded to eliminate malnutrition. On this basis, we predict that current WASH interventions might not be sufficiently intensive to yield a substantial improvement in child growth, and that greater efforts will be required to meet the new UN Sustainable Development Goals (SDGs).[8] In this study we assessed the aggregate improvements in child growth associated with progressive improvements in a wide range of nutrition-specific and nutrition-sensitive interventions in three rural Gambian villages that have been under continuous growth monitoring for almost 4 decades.

4.4 Methods

4.4.1 Study design and participants

We did a retrospective cohort study using routine growth monitoring data for all children whose date of birth had been recorded to assess trends in growth faltering in children younger than 2 years in the West Kiang region of The Gambia during the past four decades.

Three rural villages in this region (Keneba, Manduar, and Kantong Kunda) have benefi ted from free health care provided by the UK Medical Research Council for the past 40 years. Since the 1970s there have been increasing levels of support and interventions (panel) such that these villages have benefited from unprecedented levels of nutrition-specific and nutrition-sensitive interventions compared with other such communities in rural low-income settings.

Growth monitoring was done on a monthly basis in the 1970s but from 1983 onward, measurements were made at birth, 6 weeks, 3 months and then every 3 months thereafter. Diseases were recorded both at regular child 'well baby' clinics and when mothers presented with a sick child, and here we focus on clinical diagnoses for pneumonia, chest infections, diarrhoea, and malaria. Malaria diagnoses were based on positive blood fi lms and, since 2007, on rapid diagnostic tests.[9]

As described elsewhere,[9] the climate in the intervention area has a long, dry harvest season (November to May) and a wet so-called 'hungry' season (late June to mid-October) when agricultural work, depletion of food supply, and infectious diseases are at their peak.

Ethics approval for the demographic surveillance of the three villages was granted by the Joint Gambian Government/Medical Research Council Unit The Gambia Ethics Committee.

4.4.2 Procedures

Standard anthropometric measurements were done in the clinic by trained clinic staff and *Z* scores were calculated against the WHO 2006 growth standards.[10] We defined stunting, wasting, and underweight as height-for-age, weight-for-length, and weight-for-age of less than 2 SDs (*Z* scores) below the WHO reference median. Further details are provided in the appendix.

See Online for appendix

4.4.3 Statistical analysis

We fitted the effects of age and season on repeated growth parameters using random effects models. Models for boys and girls and each decade were fitted separately. To describe secular changes in rates of stunting, wasting, and underweight, we fitted random effects logistic regression of the binary variable on the first four orthogonal polynomials in age and the first pair of Fourier terms for season (appendix). To describe the effect of season on growth, we obtained seasonal patterns of body size by Fourier regression, as described in the appendix.[11] To describe the changes in body size with age, we produced plots of mean *Z* score versus age by fitting age with ten-knot cubic regression splines and controlling for season by including the first pair of Fourier terms. We quantified growth faltering as the drop in *Z* score during the 18 month interval between 3 months and 21 months of age. These estimates are all simple linear combinations of the regression coefficients and their standard errors calculated using the variance–covariance matrix for the regression coeffi cients (ie, the Fisher information matrix), using Stata's post-estimation command *lincom*. We did not do any formal statistical hypothesis tests. With such large volumes of observational data almost any difference examined would

Panel: **Timeline of interventions introduced for three rural Gambian villages**

1976–79
- Lower basic primary education (grades 1–4)
- Routine daily food supplementation for all infants aged 3–12 months
- Routine child health clinic
- Infant vaccines (BCG, measles, yellow fever, oral polio, combined diptheria, tetanus, pertussis), maternal vaccine (tetanus toxoid)
- Antenatal clinic
- Free availability of oral rehydration solution
- Health and nutrition education

1980–89
- International Trypanotolerance Centre provided hand pumps and tap water
- Pit latrines in compounds
- Medical Research Council Gate clinic opened
- Mobile midwifery service
- Infant vaccine (hepatitis B)

1990–99
- Upper basic primary education
- Emigration of young people (education and remittance)
- Vitamin A supplementation in infants
- Infant vaccine (*Haemophilus influenzae* type B)

2000–12
- Infant vaccines (pneumococcal and rotavirus)

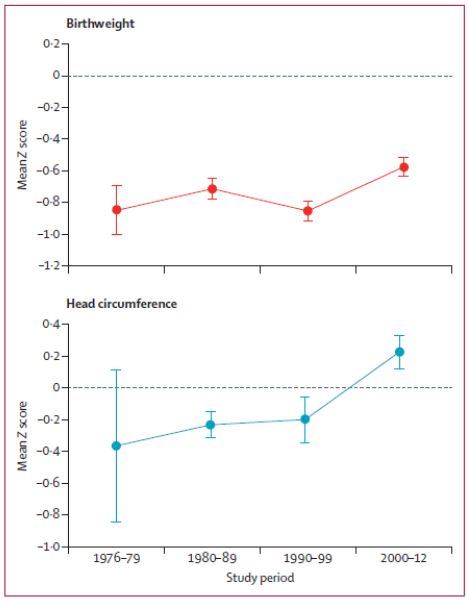

Figure 4.1: **Secular changes in weight and head circumference at birth**
Figures shows mean (SE) *Z* scores for both sexes combined, calculated using the WHO 2006 growth reference standards.

	1976–79 (n=560)	1980–89 (n=920)	1990–99 (n=879)	2000–12 (n=1300)	Overall (n=3659)
Sex					
Boys	267	453	455	665	1840
Girls	293	467	424	635	1819
Mean (SD) birthweight, kg (n=2728)					
Boys	3·1 (0·3)	3·1 (0·4)	3·0 (0·4)	3·1 (0·4)	3·0 (0·4)
Girls	2·8 (0·5)	2·9 (0·4)	2·8 (0·4)	3·0 (0·4)	2·9 (0·4)
Premature	15/76 (20%)	58/714 (8%)	63/721 (9%)	84/687 (12%)	220/2198 (10%)
Primiparity	55/478 (12%)	94/867 (11%)	79/788 (10%)	175/1176 (15%)	403/3300 (12%)
Mean (95% CI) maternal height, cm	158·1 (157·4–158·9)	158·4 (157·7–159·1)	159·4 (158·8–160·0)	160·9 (160·3–161·6)	159·8 (150·6–170·4)

Data are mean (SD), n/N (%), or mean (95% CI).

Table 4.1: **Maternal and infant baseline data**

	1970s	2000s	Change between 1970s and 2000s
Birth (mean WHO Z scores)			
Birthweight	−0·85 (−1·00 to −0·70)	−0·59 (−0·65 to −0·53)	0·26 (0·18 to 0·34)
Head circumference	−0·36 (−0·84 to 0·12)	0·22 (0·11 to 0·33)	0·58 (0·33 to 0·83)
Sex difference at birth (male minus female mean WHO Z scores)			
Birthweight	0·47 (0·18 to 0·76)	−0·03 (−0·15 to 0·09)	−0·50 (−0·81 to −0·19)
Head circumference	0·82 (−0·20 to 1·84)	−0·05 (−0·26 to 0·16)	−0·87 (−1·91 to 0·17)
Body size at 2 years (mean WHO Z scores)			
Length-for-age	−2·10 (−2·22 to −1·98)	−1·36 (−1·44 to −1·27)	0·74 (0·59 to 0·89)
Weight-for-age	−1·64 (−1·77 to −1·51)	−1·20 (−1·28 to −1·11)	0·44 (0·29 to 0·59)
Weight-for-length	−0·71 (−0·82 to −0·59)	−0·68 (−0·76 to −0·60)	0·03 (−0·11 to 0·17)
Mid-upper-arm circumference	−1·02 (−1·14 to −0·89)	−0·74 (−0·82 to −0·66)	0·28 (0·13 to 0·43)
Head circumference	−1·28 (−1·48 to −1·08)	−0·51 (−0·59 to −0·43)	0·77 (0·55 to 0·99)
Prevalence of stunting, underweight, and wasting at 2 years			
Stunting	57·1% (51·9 to 62·4)	30·0% (27·0 to 33·0)	−27·1% (−33·2 to −31·5)
Underweight	38·7% (33·5 to 44·0)	22·1% (19·4 to 24·8)	−16·6% (−22·5 −21·1)
Wasting	11·3% (7·9 to 14·8)	10·8% (8·7 to 12·8)	−0·5% (−4·5 to −3·2)
Growth faltering*			
Length-for-age	−0·87 (−0·92 to −0·82)	−0·70 (−0·73 to −0·67)	0·17 (0·11 to 0·23)
Weight-for-age	−0·80 (−0·86 to −0·74)	−0·76 (−0·78 to −0·74)	0·04 (−0·03 to 0·11)
Weight-for-length	−0·95 (−1·02 to −0·88)	−0·86 (−0·89 to −0·82)	0·09 (0·01 to 0·17)
Mid-upper-arm circumference	−0·45 (−0·53 to −0·38)	−0·40 (−0·44 to −0·37)	0·05 (−0·04 to 0·14)
Head circumference	−0·79 (−0·88 to −0·70)	−0·51 (−0·54 to −0·47)	0·28 (0·18 to 0·38)
Seasonality†			
Length-for-age	0·13 (0·12 to 0·14)	0·04 (0·03 to 0·05)	−0·09 (−0·10 to −0·08)
Weight-for-age	0·27 (0·26 to 0·28)	0·12 (0·12 to 0·13)	−0·15 (−0·16 to −0·14)
Weight-for-length	0·32 (0·30 to 0·34)	0·16 (0·15 to 0·17)	−0·16 (−0·18 to −0·14)
Mid-upper-arm circumference	0·24 (0·22 to 0·26)	0·11 (0·10 to 0·12)	−0·13 (−0·15 to −0·11)
Head circumference	0·12 (0·10 to 0·13)	0·03 (0·02 to 0·04)	−0·09 (−0·11 to −0·07)
Annual incidence of diarrhoeal infection between birth and age 2 years‡	3·13 (2·89 to 3·37)	0·84 (0·76 to 0·91)	−2·29 (−2·54 to −2·04)

Data are effect size (95% CI). *Difference in mean Z score between 3 months and 21 months. †Amplitude of seasonal Z score fluctuation between birth and age 2 years. ‡ Diarrhoea is the infection usually associated with growth faltering.

Table 4.2: **Effect size estimates for changes in body size by decade**

be significant, so statistical significances poorly discriminate between important and trivial patterns in the data. Instead, we focused on estimation of effect sizes and their confidence intervals. All analyses were done with Stata 12.

4.5 Role of the funding source

The UK Medical Research Council has provided sustained support for our unique cohort over many years and approved our general research plans (including longitudinal data collection) every 5 years. MRC played no other role in interpretation of the data or preparation of the manuscript. The corresponding author had access to all of the data and had final responsibility for the decision to submit for publication.

4.6 Results

From May 1, 1976, to Feb 29, 2012, 4474 children younger than 2 years from these villages were seen at the child clinics in Keneba. Children were included in this analysis if their date of birth was known accurately and they visited the clinic on six or more occasions, giving a total of 3659 children eligible for the study. Those ineligible included 24 with unknown date of birth and 791 visitors who attended the clinic on five or fewer occasions. The median number of visits per child was 16 (IQR 13–26), resulting in a total of 59 371 visits at which anthropometric measurements were made. Most deliveries occurred at home in the presence of a traditional birth attendant but a trained midwife completed a baby check including anthropometric measurements within 5 days of delivery (mostly within 72 h).

We analysed secular trends in birth size, because it is an important determinant of postnatal growth and attained size. Data about birth size were available for 2728 (75%) babies (figure 4.1, table 4.1). We excluded length data because there were more missing data than for weight and head circumference and because birth lengths measured with a length mat in the babies' homes are inherently less reliable than the other measurements. During the four decades of the study period, birthweight Z score increased by 0·26 (95% CI 0·18 to 0·34) from a

starting point of –0·85 (figure 1.1, table 2.1). Head circumference at birth Z score increased by 0·58 (95% CI 0·33 to 0·83) from –0·36, thus ending up slightly above the WHO standards at 0·22 (95% CI 0·11 to 0·33). A small part of this increase might be attributable to a steady increase in maternal height totalling 28 mm (95% CI 18 to 38; table 1.1).

Figure 2 captures the characteristic growth patterns of these rural infants. They are born small and continue to fall away from the WHO standard length centiles throughout the first 2 years of life. Their weight shows early catchup while the infants are still fully breastfed and largely protected from infections; this trend is magnifi ed in their weight-for-length due to the simultaneous decline in length. Mid-upper-arm and head circumferences show a similar resilience in very early infancy.

The figure also illustrates the secular trends in growth during the four decades. Length shows a consistent, but limited, improvement. At 2 years, length-for-age *Z* score had improved by 0·74 (95% CI 0·59 to 0·89) from a starting point of –2·10 (table 2.1). Weight and head circumference showed an initial improvement by the second decade but little further gain. Weight-for-length showed absolutely no change in the second year of life. Mid-upper-arm circumference increased by a quarter of a *Z* score (table 2.1).

The prevalence of stunting at 2 years almost halved from 57% to 30% and the prevalence of underweight decreased from 39% to 22% (table 2, figure 3.1). There was no change in the prevalence of wasting.

Growth failure is markedly seasonal in this environment (figure 2.1), with greater defi cits occurring in the rainy season (July to November) when infections are more common and maternal care declines due to the pressures of farming. Figure 4.1 shows that there has been a substantial attenuation of the seasonality of growth during the four decades studied. When assessed as the amplitude of *Z* score fluctuation, this measure was signifi cant for all indices (table 2) in the order of a tenth of a *Z* score.

We defined growth faltering on the basis of the diff erences in *Z* score between 3 months and 21 months post partum. In the 1970s, *Z* scores for length-for-age, weight-for-age, weight-for-length, and head circumference all fell by between 0·79 and 0·95 (table 2). Over time, this fall was slightly attenuated for length-for-age (*Z* score 0·17, 95% CI 0·11–0·23) and weight-for-length (0·09, 0·01–0·17), and more markedly attenuated for head circumference (0·28, 0·18–0·38; figure 4.5). The decline in *Z* score for weight-for-age and mid-upper-arm circumference did not change during the period studied.

The incidence of diarrhoea, malaria, and bronchiolitis in the children younger than 12 months fell by 80% during the four decades studied. Conversely, the incidence of pneumonia seemed to increase during the four decades (figure 4.6).

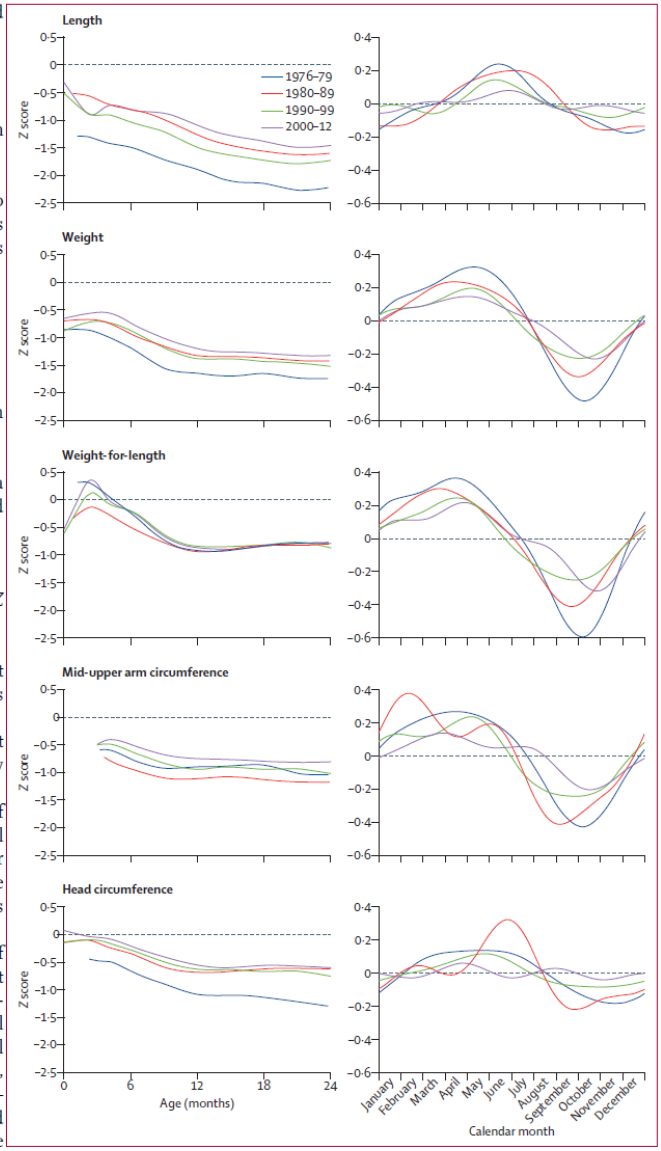

Figure 4.2: **Secular and seasonal trends in child growth**
Figure shows mean age and Z scores for sexes combined, calculated by comparison with the WHO 2006 growth reference standards. Length refers to length-for-age; weight refers to weight-for-age.

4.7 Discussion

Goal 2 of the SDGs, "to end hunger, achieve food security and improved nutrition, and promote sustainable agriculture", is accompanied by the target to achieve the internationally agreed goals for stunting and

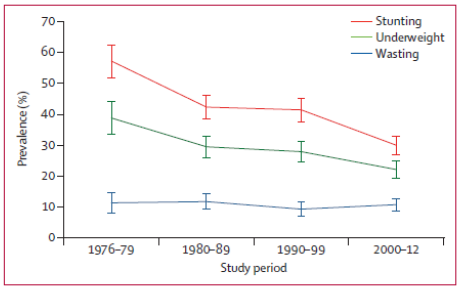

Figure 4.3: **Secular trends in stunting, underweight, and wasting at 2 years of age** Stunting, underweight, and wasting are defined as proportion below −2 Z scores against WHO 2006 growth reference standards.

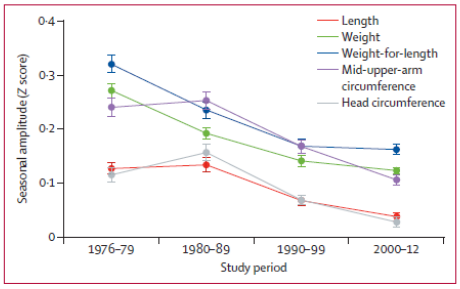

Figure 4.4: **Amplitude of the seasonality by decade**
Figure shows seasonal Z score amplitude for sexes combined, calculated by comparison with the WHO 2006 growth standards.

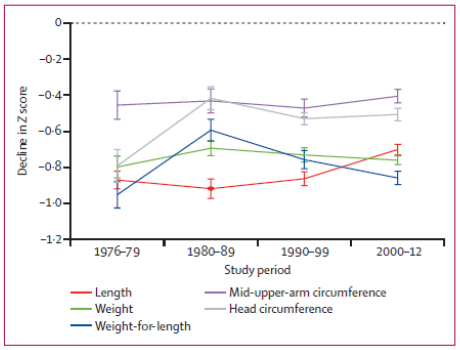

Figure 4.5: **Fall in Z scores between 3 and 21 months of age for each decade**
Figure shows fall in Z scores for sexes combined, calculated by comparison with the WHO 2006 growth standards.

wasting in children younger than 5 years by 2025. For stunting, this goal would require a 40% reduction from the current estimate of 159 million stunted children to reach the target of less than 100 million. In Africa there has been a disappointing decline in the prevalence of stunting from 42% in 1990 to 32% in 2015[12] and, because of population growth, the absolute numbers of children with stunting actually increased from 47 million to 58 million during this period. The prevalence of stunting is now predicted to stabilise at that level because continued population growth off sets a slower-than-required decline in prevalence. By comparison, during the same period the prevalence of stunting in Asia decreased from 48% to 25% and the total number of children with stunting declined from 189 million to 84 million.[12]

Elimination of stunting creates a complex and paradoxical challenge, which suggests that one or more key causative factors remain unknown. On the one hand, nutrition-specifi c interventions have repeatedly shown very limited effi cacy even when implemented under the optimal conditions of randomised trials,[2,13–17] whereas on the other hand, stunting resolves rapidly as wealth and living conditions improve in countries passing through the economic transition.[11,18]

The longitudinal data presented in this study add to this challenge. During almost four decades the Medical Research Council has made sustained investments in health care and nutrition-related infrastructure within our core study villages; these inputs are unparalleled across rural Africa and would be prohibitively expensive for governments of low-income countries to roll out nationwide. These villages have access to antenatal and postnatal care, and round-the-clock access to clinicians and nurses in a well equipped and efficient primary health-care clinic. All health services are free of charge. All children are fully vaccinated, receive vitamin A, mebendazole, and other health interventions as per WHO protocols. Breastfeeding rates are among the very best worldwide and are further supported by Baby Friendly Community Initiatives accompanied by regular messaging in support of exclusive breastfeeding for 6 months. Open defecation and water obtained from contaminated open wells have been universally replaced by latrines in all compounds and tube well water supplied through clean pipes to standpipes around the villages. These interventions have had a profound effect on mortality in children younger than 5 years[9] and the incidence of most diseases, especially diarrhoea (which has been previously implicated as a major cause of growth failure; figure 6).[19] Further, children attend regular well-baby checks with growth monitoring and we provide a dedicated treatment centre for severely malnourished children to treat those who do become malnourished. The remittance economy from village members who have migrated overseas, together with incomes from employment at the Medical Research Council, have greatly improved food security and attenuated the stress of the so-called hungry season as refl ected in the reduction in the amplitude of seasonal growth faltering in fi gure 4. This increased wealth has also improved housing conditions and dispersed families over a wider area, reducing overcrowding. Child

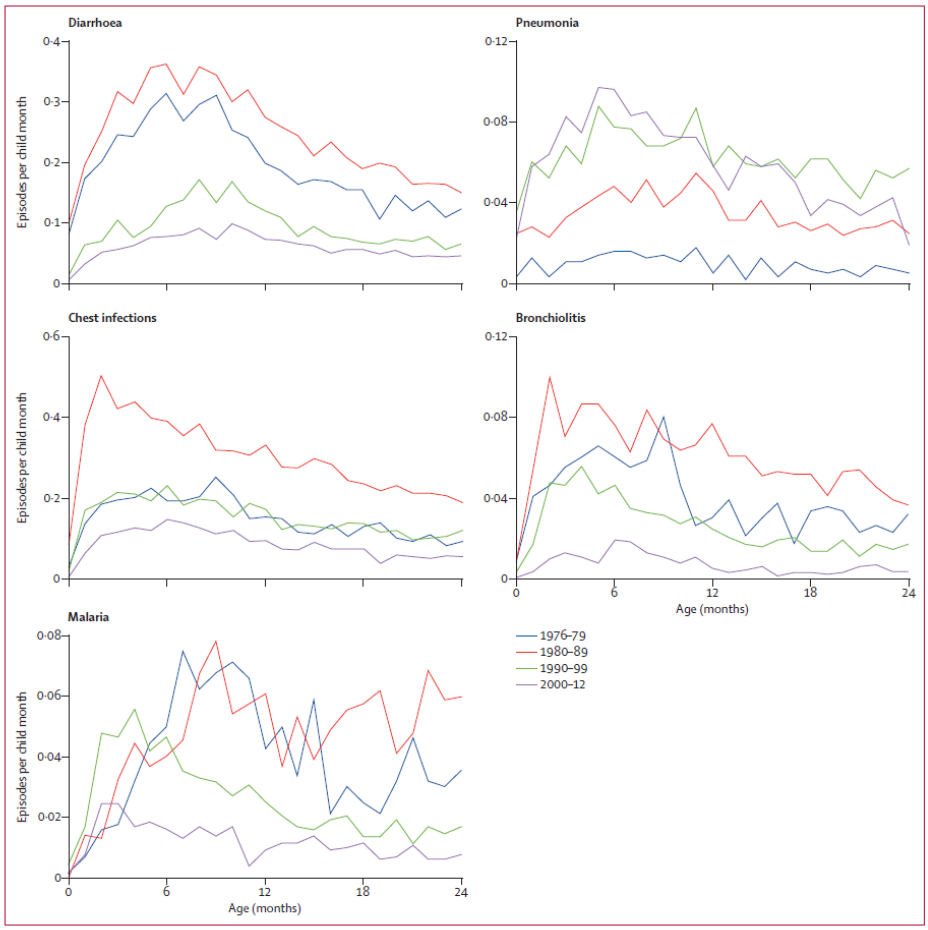

Figure 4.6: **Disease episodes for each decade**

Figure shows episodes per child per month between 0 months and 24 months of age. The apparent contrary increase in the incidence of pneumonia probably results from the changing clinical definitions of pneumonia among the series of doctors who have worked in the Medical Research Council Keneba clinic during the past four decades, especially with the introduction of WHO Integrated Management of Childhood Illness guidelines in 1997.[20] It is also likely that, with the more stringent WHO definitions for malaria, pneumonia was frequently stated as the diagnosis. This phenomenon has been reported in other settings in the tropics.[21]

mortality has fallen, birth spacing has increased, and family size has decreased. There is now free universal primary education with enrolment of about 97%, although this figure drops for secondary education particularly for girls to 30%.[22] Furthermore we have, over the years, conducted and published a series of randomised trials of nutritional interventions targeted at pregnant and lactating mothers, infants, and children, with the main aim to improve growth (appendix). Our findings have shown at most modest improvements in infant growth, consistent with results from systematic reviews and meta-analyses.[2,13,23]

The modest increase of 2·8 cm in the mean maternal height is indicative of a small degree of improvement in maternal nutrition during the four decades. Meta-analysis of the relationship between maternal height and birthweight[24] yielded an expected effect of 8 g more birthweight per cm of maternal height. The COHORTS group reported a similar value of 0·024 *Z* scores per cm.[25] Therefore the increase in maternal height probably contributed only about 20–30 g of the observed 120 g increase in birthweight.

A limitation for our data was the difficulty in deriving a consistent sampling frame for the population under

study in an area undergoing rapid change, particularly in the later decades. Changes in the population structure during the past 40 years might have influenced the trends we have reported. We attempted to control for this factor by excluding the children who attended our clinic fewer than six times as likely visitors. However, exclusion of these children might have created a sampling bias, because infrequent attenders might represent resident children who engaged poorly with health care. This potential bias would only affect the trends displayed if the population prevalence of poor attenders changed during the period studied.

Another limitation was missing data from the 1970s, particularly birth data, limiting our ability to evaluate the trends in these parameters. Additionally, we omitted birth length data because of poor reliability in the measurements and the small number of measurements that were available in all the four decades. Comparison of the trends noted in our core study villages with those in neighbouring villages receiving less intensive intervention would have been desirable, but such data were not available.

Growth has improved during these four decades but, despite the unprecedented levels of investment, the prevalence of low birthweight (12%), childhood stunting (30%), and underweight (22%) remains high. The prevalence of wasting has not changed, and growth faltering between 3 months and 21 months has been only marginally attenuated. These data suggest that the refractory stunting must be caused by factors (beyond the improvements and interventions provided in the study villages) that are corrected as nations pass through the economic transition and advance from low-income and lower-middle-income status. Environmental enteropathy affecting almost all children in low-income settings has been proposed as the mechanism linking growth failure with WASH deficits.[26] Our results, together with a previous analysis[27] of associations between poor child growth and a range of indicators of socioeconomic status and living conditions in this same community, suggest that there is a very high threshold for WASH improvements that must be achieved before growth faltering can be eliminated. Improved housing conditions, possibly including the provision of piped water directly into the home, might be a necessary step in the global challenge to eliminate childhood malnutrition.

Our study villages of Keneba, Kantong Kunda, and Manduar are highly unusual (and possibly unique) in having the combination of intensive interventions over a protracted period accompanied by systematic growth monitoring; our results might therefore not be generalisable. However, before Medical Research Council inputs and in all other respects such as environment and farming practices they share many characteristics with countless other rural villages in sub-Saharan African in areas of low malaria endemicity. Therefore, we believe that our findings and suggestions for future interventions are likely to be applicable to other similar settings in rural Africa.

4.8 Contributors

AMP, AJF, SEM, and HMN designed the study; AJF and HMN analysed the data. HMN, AJF, SEM, and AMP prepared the manuscript and are responsible for the final content. All authors read and approved the final manuscript.

4.9 Declaration of interests

We declare no competing interests.

4.10 Acknowledgments

We thank the staff at MRC Keneba that have served the community in the Keneba, Manduar and Kantong Kunda villages during the four decades, the children and their parents/carers. The MRC International Nutrition Group is supported by the UK Medical Research Council (grant no MC-A760-5QX00) and the UK Department for International Development under the MRC–DFID Concordat agreement.

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4.8 Authors' information

HMN is a fourth year PhD student in the Department of Population Health at the London School of Hygiene and Tropical Medicine, London. This paper forms part of her PhD thesis on the evaluation of growth faltering in rural Gambian children. AJF was the lead statistician for the MRC International Nutrition Group with a great deal of experience in nutrition epidemiology. SEM is a principal investigator and UK MRC grant holder currently based at the Division of Women's Health, King's College London, London, UK. AMP supervised HMN in all aspects of her work and is Head of the MRC International Nutrition Group at LSHTM and the Nutrition Theme Leader at the MRC The Gambia Unit and has undertaken a vast amount of research in maternal and child nutrition in sub-Saharan Africa.

Chapter 5

Research Paper II

Article cover sheet

Title: **Hormonal Correlates and Predictors of Nutritional Recovery in Malnourished African Children**

1. For a 'research paper' prepared for publication but not yet published

   1.1 Where is the work intended to be published?

   This paper has been submitted to the Journal of Tropical Pediatrics.

   1.2 List the paper's authors in the intended authorship order

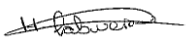
   Helen M Nabwera, Robin M Bernstein, Schadrac C Agbla, Sophie E Moore, Momodou K Darboe, Mariama Colley, Amadou T Jallow, Richard Bradbury, Jennifer Karafin, Anthony J Fulford and Andrew M Prentice

   1.3 Stage of publication: Not yet submitted/**Submitted**/Undergoing revision from peer reviewer's comments/ In press Submission

   22nd April 2017

2. For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper.

   As part of my PhD, I conceived, designed and undertook the data collection and analysis for the study in collaboration with the all the authors (Bernstein RM, Agbla SC, Moore SE, Darboe MK, Colley M, Jallow AT, Bradbury R, Karafin J, Fulford AJ, Prentice AM). I wrote the manuscript and my co-authors edited and provided comments on the drafts that were incorporated into the submitted version of the manuscript.

Candidate's signature: 

Supervisor or senior author's signature to confirm role as stated in (2): 

Hormonal Correlates and Predictors of Nutritional Recovery in Malnourished African Children

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5.1 Disclosure statement: The authors have nothing to disclose

5.2 Abstract

Background: Malnourished children show variable growth responses to nutritional rehabilitation. We aimed to investigate whether these differences could be explained by variations in growth and energy regulating hormones.

Methods: Quasi-experimental study. Sixty children aged 6-24 months who presented to the Medical Research Council, Keneba outpatient clinic in rural Gambia were recruited to controls if weight-for-height Z-score (WHZ) >-2 (n=22), moderate acute malnutrition if WHZ <-2&>-3 (n=18) or severe acute malnutrition if WHZ <-3 (n=20). These children would be representative of children presenting to a rural Gambian health facility, but would not be representative of the children presenting to an urban health care facility. Pre- and post-prandial plasma hormone (leptin, soluble leptin receptor, ghrelin, IGF-1, IGFBP3, cortisol, C-peptide, insulin) and salivary CRP levels were measured at baseline and during nutritional rehabilitation over a four-week period using ELISA. All malnourished children received a ready-to-use therapeutic food (RUTF) and those with severe acute malnutrition (SAM) also received milk-based formulas.

Results: In univariable analyses, increases in weight-for-age Z-score (WAZ) in malnourished children were positively correlated with insulin (F-ratio 7.8, p=0.006), C-peptide (F-ratio 12.2, p<0.001) and cortisol (F-ratio 5.0, p=0.03). In multivariable analysis, only baseline C-peptide (F-ratio 7.6, p=0.009) predicted the changes in WAZ over 28 days of interventions.

Conclusion: Although it cannot be used in isolation, baseline C-peptide was a predictor of future response to rehabilitation in rural Gambian, malnourished children.

***Key words*:** hormones, malnutrition, nutritional rehabilitation, Gambian children

5.3 Background

Growth faltering is endemic in under 2's in sub-Saharan Africa and is associated with high rates of morbidity and mortality [17]. A proportion of these children develop life-threatening severe acute malnutrition (SAM) that requires urgent and intensive health service investment. The causes of the wide variability in the response of children suffering from SAM to nutritional rehabilitation programmes are unknown [175], (excepting for HIV infection) even when such programmes are rigorously implemented according to latest international guidelines [20, 175]. The identification of simple prognostic indicators, measurable at initial diagnosis, would greatly assist in triaging malnourished children between those who require high-cost, labour-intensive tertiary level care and those suitable for community management of acute malnutrition (CMAM).

Healthy growth is regulated by hormonal pathways that are sensitive to nutritional and infectious stressors [145]. Periods of inadequate energy and nutrient intake or increased metabolism due to infections, alter the hormonal regulation of growth by the growth hormone-insulin-like growth factor (GH-IGF) axis that can shift the timing and duration of the various phases of child growth [71, 148]. Linear growth is regulated by growth hormone, mediated by insulin-like growth factor-1 (IGF-1) and the predominant binding protein: insulin-like binding protein-3 (IGBP3) [176]. Increase in the molar ratio of IGF1: IGFBP3 is associated with high growth velocity suggesting increased levels of bioactive IGF-1 [177]. Previous studies have evaluated the energy regulating hormonal changes in children with SAM undergoing intensive in-patient nutritional rehabilitation [149, 150, 178-182].

The primary aim of this study was to investigate whether the high degree of variability in children's responses to nutritional rehabilitation could be explained and predicted by differences in energy and growth regulating hormones.

5.4 Methodology

5.4.1 Study population

The study was conducted at the Medical Research Council Unit The Gambia's rural field station in Keneba. The study participants were children aged 6-24 months who presented to the outpatient clinic. They were assigned to one of the 3 nutritional groups, according to

the World Health Organization (WHO) classifications using weight-for-height Z-scores (WHZ), mid upper arm circumference (MUAC) and clinical assessment [1, 6]. Controls had a WHZ above -2. Children were excluded if they had significant medical complications requiring resuscitation, were HIV infected, or had congenital or chronic medical conditions.

5.4.2 Study design and interventions

A quasi-experimental study design. At baseline, all the children received 20ml/kg of Formula 75 (Nutriset) as a test meal. Children with MAM and SAM had further test meals on Days 14 and 28. All malnourished children were managed according to WHO and national malnutrition guidelines, supervised by the clinic staff [6, 183].

Anthropometric measurements including weight, height, head circumference, mid-upper arm circumference (MUAC) and knee-heel length were taken by trained field workers.

5.4.3 Biological sampling and analysis

At enrolment, pre- and post- test meal venous blood, saliva and urine samples were collected from all the children. Subsequent samples were collected from MAM and SAM children on Day 14 and 28. The hormone and salivary CRP analyses were performed using ELISA on plasma and saliva respectively (R&D Systems, Minneapolis, USA; ALPCO, New Hampshire, USA; Merck Millipore, Darmstadt, Germany; Salimetrics, Pennsylvania, USA).

5.4.4 Statistical analysis

The study sample size was derived using the reported variability in energy regulating hormones in malnourished under 5's over time and between malnourished children and controls, from previous work in this setting (Nweneka, Prentice *et al*, unpublished) and Stein *et al*'*s* data [149]. Baseline characteristics across nutritional groups were compared using the Fisher's exact and Kruskal-Wallis tests where applicable. Molar ratio IGF1: IGFBP3 was calculated using a conversion of 1 ng/ml IGF-1 = 0.13 nM IGF-1 and 1 ng/ml IGFBP-3 = 0.036 nM IGFBP-3, which results in multiplying the IGF-1/IGFBP-3 ratio by a constant of 3.61 [184]. Wilcoxon signed rank test was used to compare pre- and post-prandial hormonal levels within each nutritional group at baseline. A mixed effects model was used to assess differences in hormone levels between nutritional groups at baseline and over time. A

piecewise linear random slope model was used to assess the change in anthropometry over time using three time intervals: 0-14 days, 14-28 days and 29-180 days. The Wald test was used to test for interaction between time and nutritional group. Repeated measures ANOVA was used to assess which biochemical indices were good predictors of nutritional recovery. Analyses were conducted using Stata 12.1 (Stata Corp) and DataDesk 7.0.2 (Data Description Inc, Ithaca, NY).

(Further details in **Supplementary Material**).

5.4.5 Ethical considerations

The study was approved by the Gambia Government/MRC Unit The Gambia Joint ethics committee, SCC 1306 and the London School of Hygiene and Tropical Medicine ethics committee. All guardians had the study explained to them in detail by the field staff in their local language and signed a consent form in English.

5.5 Results

Study population

Sixty children were recruited into the study and completed follow up from June 2013 to October 2014. None of the children with SAM had pedal oedema at presentation and no children died during the study. Diarrhoea was a more common presenting symptom in children with SAM than in the other groups (58% vs 29% (MAM) and 11% (Controls)); p=0.01 (**Table 5.1**).

Anthropometric changes over time

The change in anthropometric measurements was variable across all nutritional groups. The children with MAM and SAM showed significant catch-up growth between 0-14 days in all the anthropometric parameters (**Table 5.2**).

A key feature of the nutritional recovery in both the MAM and SAM groups was the very high degree of variability (**Figure 5.1)**. This variability in WAZ recovery was not predicted by baseline age, anthropometry, breastfeeding status, salivary CRP, amount consumed at the test meal, presence of diarrhoea or a urinary tract infection.

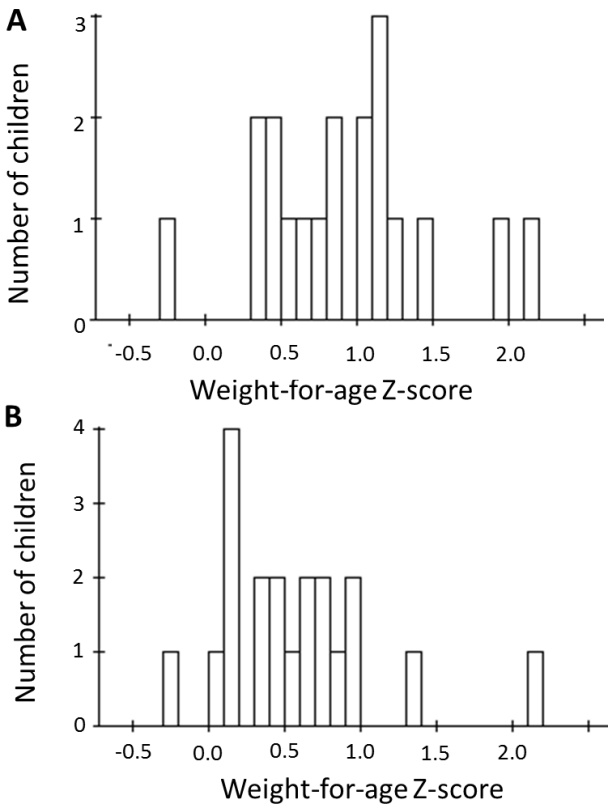

Figure 5.1: Weight-for-age z-score gain

(A. Children with moderate acute malnutrition; B. Children with severe acute malnutrition)

Hormone status at baseline

There was no evidence of a difference between the pre- and post-prandial levels of any of the hormones including leptin, in any of the nutritional groups (**Figure 5.2**). There was a very strong correlation (r <0.9 in all cases) between the pre- and post-prandial values with no significant deviation from the Y=X line, indicative of a lack of difference between the pre- and post- prandial levels (see Bland-Altman plots in **Supplementary Figure 5.1**). The strength of these correlations validates the precision of the assays and shows high discrimination ratios indicating that each of the indices has the potential to be good predictors of response. The pre- and post-prandial levels were therefore averaged for subsequent analysis (Further details in the Supplementary material).

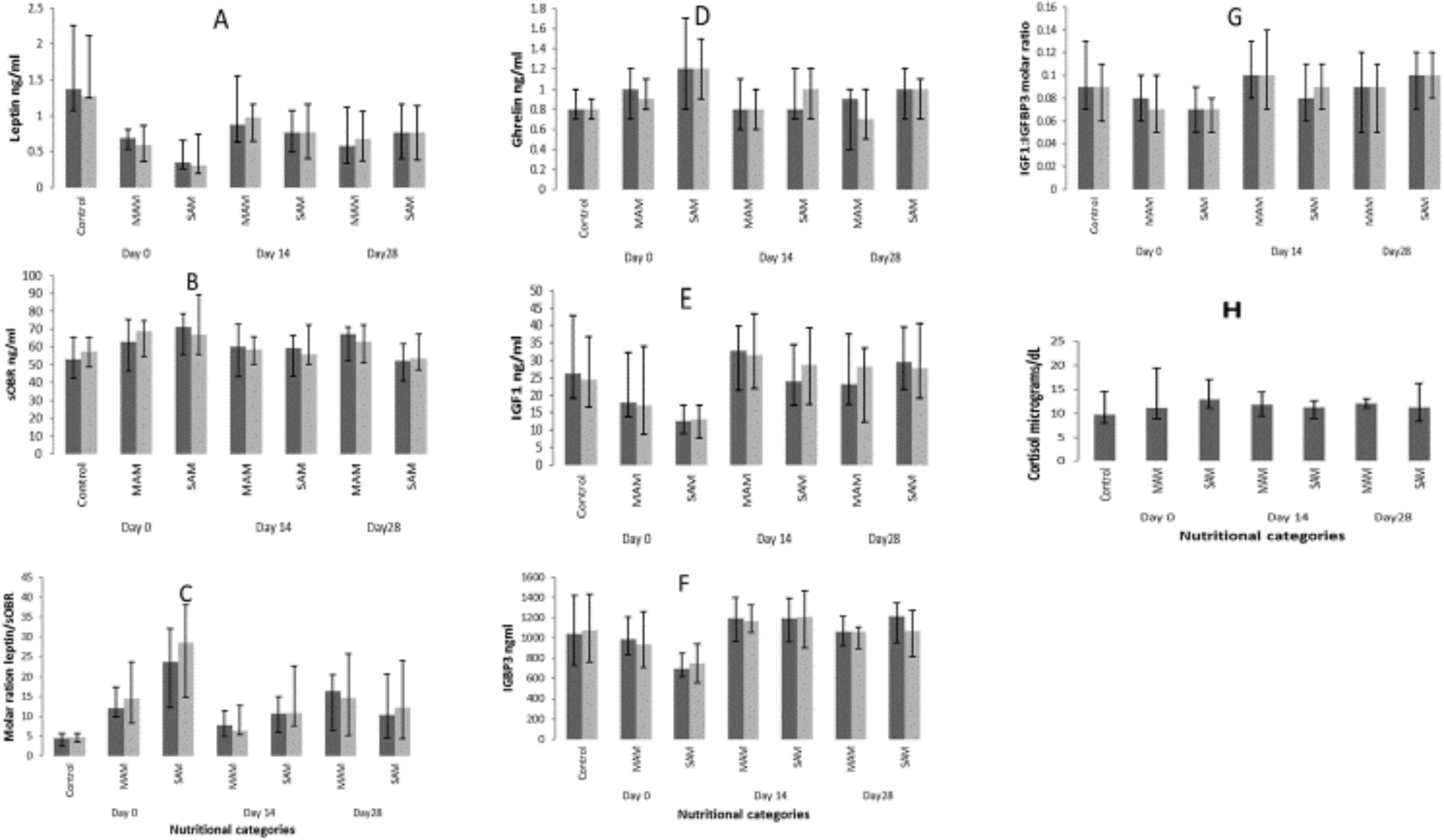

Figure 5.2: Changes in hormone and receptor levels over time by nutritional category

In **Table 5.3,** the geometric mean ratios are equal to the log of the mean concentrations of the hormones or their binding proteins for children with MAM or SAM compared to controls. Compared to controls the geometric mean ratio of leptin were significantly lower in both MAM (0.4 [95% CI: 0.3, 0.6], p<0.05) and SAM (0.3 [95% CI: 0.2, 0.5], p<0.05). IGF-1 and IGF-binding protein 3 (IGFBP3) were significantly lower in only SAM (0.5 [95% CI: 0.3, 0.7], p<0.05) and 0.7 [95% CI: 0.5, 0.9], p<0.05) respectively. Compared to controls the molar excess of sOBR was significantly higher in both MAM (3.4 [95% CI: 1.9, 6.1], p<0.05) and SAM (4.9 [95% CI: 2.8, 8.6], p<0.05). The was no evidence of a difference in geometric mean ration of cortisol, insulin or C-peptide in the MAM/SAM groups compared to controls (**Table 5.3**).

Hormone changes over time

The lack of difference in hormone levels between the pre- and post-prandial states persisted at the subsequent time points in the MAM and SAM groups (**Figure 5.2**). In the multivariable analysis shown in **Table 5.4**, the geometric mean ratios are equal to the log of the mean concentrations of the hormones or their binding proteins at Day 14 or 28 compared to baseline (Day 0). From Day 0-14, significant increases in the geometric mean ratios in both MAM and SAM were found for the following: total leptin (1.6 [95%CI: 1.1, 2.4], p=0.002 and 1.8 [95%CI: 1.3, 2.6], p<0.001); IGF-1 (1.6 [95%CI: 1.3, 2.1], p<0.001 and 2.1 [95%CI: 1.7, 2.8], p<0.001); IGFBP3 (1.3 [95%CI: 1.1, 1.5], p=0.002 and 1.7 [95%CI: 1.5, 2.0], p<0.001) respectively. There were significant decreases in the molar excess of sOBR: total leptin in both the MAM and SAM groups (0.5 [95%CI: 0.4, 0.8], p<0.001) and 0.5 [95%CI: 0.3, 0.7], p<0.001) respectively (**Table 5.4**). The was no evidence of a difference in geometric mean ration of insulin or C-peptide over time in either the MAM or SAM groups.

Hormonal correlates of weight gain

There was significant correlation between many of the hormonal measurements over time and this introduced confounding into the multivariable analysis; therefore, univariable analyses are reported. Changes in WAZ among the SAM and MAM groups combined were positively correlated with insulin (F-ratio 7.8, p=0.006) and C-peptide (F-ratio 12.2, p<0.001) but not with any of the other hormones or their binding proteins. Surprisingly the association with leptin did not reach statistical significance (p=0.07).

Hormonal predictors of weight gain

The change in WAZ over the 28 days of active intervention (combined MAM and SAM) was predicted by Day 0 C-peptide (F-ratio 5.4, p=0.03) and cortisol (F-ratio 5.0, p=0.03) (both were positive associations) but not by any of the other hormonal indices nor by salivary CRP. In multivariable analysis with both C-peptide and cortisol, the predictive value of C-peptide strengthens (F-ratio 7.6, p=0.009) and it predicted 13.9% of the variance in weight recovery.

(Further details in **Supplementary Material**)

5.6 Discussion

Our study shows that in this setting, the significant part of nutritional recovery occurs in the first 2 weeks of nutritional rehabilitation but is very variable between children. Insulin and C-peptide (a polypeptide found in the proinsulin molecule and a marker of endogenous insulin production) were the only hormones that were correlated to changes in WAZ in malnourished children and to a lesser extent cortisol.

Even in the modest samples of MAM and SAM children studied here, we noted a range spanning more than 2 Z-scores in weight (WAZ) in response to the interventions; some children even deteriorated over the 28 days. With an increasing move towards treating uncomplicated cases of SAM in the community, in order to reduce the costs of in-patient treatment and increase coverage [20], it would be very useful to identify predictors of likely response to therapy in order to guide the triaging of patients between in-patient and out-patient care protocols. Out of all the anthropometric, health and biochemical indices tested, only C-peptide and baseline cortisol predicted WAZ gain over 28 days. We interpret the cortisol result as indicating that these children were more acutely sick at baseline and once brought into clinical care made the fastest response. Children had characteristic C-peptide values suggesting the possibility that differences in insulin production represent a constitutive determinant of the propensity to store energy and nutrients when available. Nonetheless C-peptide only predicted 13.9% of the variance in recovery rates and attempts to combine this with other measures yielded no significant improvement in the prediction.

Previous studies have also shown that at the end of nutritional rehabilitation, leptin increases in the undernourished children, despite modest weight gain, sometimes reaching

166% of levels observed in well-nourished children [149, 150, 179, 182]. Somewhat surprisingly we did not find such marked changes in our study possibly because almost all the children were on oral feeds from the onset of their nutritional rehabilitation or supplementation hence resulting in a more natural course of nutritional recovery. We also found that sOBR and the molar excess of sOBR decreased during nutritional rehabilitation as did Stein *et al* [149], however, the levels of sOBR and the molar excess of sOBR did not drop below the levels observed in controls, as they found, again a possible reflection on the difference in energy regulating hormone responses with the different modes of feeding during nutritional rehabilitation (nasogastric versus oral) [20, 149].

A recent pilot study of children aged 18±4m being rehabilitated from SAM in our centre in rural Gambia, with age and sex matched community controls, found that in both groups a significant postprandial rise in leptin levels was found (Nweneka, Prentice *et al*, unpublished). We hypothesised, on the basis of Stein's prior finding of very high sOBR: leptin ratios in malnutrition [24], that this rapid rise of postprandial leptin was due to a circulating reservoir of leptin bound to the soluble binding receptor (sOBR) that is released into the circulation acutely with feeding. As no other studies, have shown acute post-prandial rises in plasma leptin we were concerned that the initial study resulted from a methodological artefact and sought replication in the current study. Our concerns were validated as we failed to replicate an acute leptin response. Although there was no evidence of an immediate effect of feeding on the hormone levels during nutritional rehabilitation, our other findings on the more chronic responses of leptin and sOBR were consistent.

This study had a number of limitations. Our sample size was modest in recognition of our ethical responsibilities in studying young children but had been validated as informative by a prior pilot study. Nonetheless our findings confirm and extend our understanding of the endocrine changes observed in other populations recovering from malnutrition. We also excluded malnourished children who were severely unwell and those who were HIV infected, therefore our findings can only be generalised to malnourished children with few or no complications. This is both a limitation and a strength since the primary intention of this study was to identify possible prognostic indicators that would guide treatment decisions and children with complications necessitate in-patient care so the treatment pathway is already established. None of the children in the study had kwashiorkor and we

were therefore unable to make comparisons of the hormonal changes between children with marasmus and kwashiorkor.

5.7 Conclusion

We conclude that insulin and C-peptide were the variables most strongly associated with WAZ gain and C-peptide was the only variable for which the Day 0 values predicted the response to nutritional rehabilitation. C-peptide would not be a useful prognostic tool in isolation.

5.8 Acknowledgements

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5.9 Authors contributions

The author contributions included the following: Helen M Nabwera, Andrew M Prentice, Sophie E Moore, Anthony J Fulford, Robin M Bernstein, Momodou K Darboe conceptualised and designed the study; Helen M Nabwera, Mariama Colley coordinated the data collection; Amadou T Jallow, Robin M Bernstein, Richard Bradbury, Jennifer Karafin processed the plasma samples and undertook laboratory hormone analyses; Helen M Nabwera, Schadrac C Agbla performed the data analysis and drafted the initial manuscript; all authors reviewed and revised the manuscript.

Tables

Table 5.1: Baseline characteristics

	Nutritional category			*P*
	Controls (N=22)	MAM (N=18)	SAM (N=20)	
Age in months, median (IQR)	12.75 (10.2, 19.3)	16.5 (12.0, 22.0)	12.0 (10.3, 16.5)	0.22[a]
Females, n (%)	11 (50)	8 (44)	10 (50)	0.90[b]
Village in West Kiang, n (%)	17 (77)	17 (94)	18(90)	-
Mandinka, n (%)	15 (68)	17(94)	17 (85)	-
Age of weaning in months, median (IQR)	6.0 (5.0, 6.0)	6.0 (6.0, 6.0)	6.0 (5.5, 6.0)	0.82[a]
WHZ, median (IQR)	-1.2 (-1.8, 0.1)	-2.6 (-2.8, -2.1)	-3.4 (-3.9, -3.2)	<0.001[a]
WAZ, median (IQR)	-1.5 (-1.7, -0.1)	-2.8 (-3.1, -2.1)	-3.2 (-3.4, -2.9)	<0.001[a]
HAZ, median (IQR)	-0.7 (-1.8, 0.03)	-1.7 (-2.5, -1.0)	-1.9 (-2.3, -0.9)	0.08[a]
Salivary CRP, ng/mL**,** median (IQR)	2.9 (2.4, 4.1)	4.9 (2.8, 10.3)	5.6 (4.1, 9.9)	0.04[a]
Urinary tract infections, n (%)	4 (19)	2 (12)	1 (6)	0.47[b]
Diarrhoea, n (%)	2 (11)	4 (29)	11 (58)	0.01[b]
Antibiotics prescribed	9 (41)	11(61)	18 (90)	0.003[b]
Mother had no formal education, n (%)	20 (91)	12 (67)	16 (80)	0.17[b]
Father had no formal education, n (%)	9 (41)	10 (56)	15 (75)	0.09[b]

Abbreviations: MAM Moderate Acute Malnutrition; SAM Severe Acute Malnutrition; WHZ weight-for-height z-score; WAZ weight-for-age z-score; HAZ, Height-for-age z-score.

[a] Kruskal-Wallis test.

[b] Fisher's exact test.

Table 5.2: Change in anthropometric measurements by nutritional group*

	Controls		MAM		SAM		*P*[a]
	Change per day (95% CI) *	*P*	Change per day (95% CI) *	*P*	Change per day (95% CI) *	*P*	
Weight (Kg)							
Within 0-14 days	0.003 (-0.003, 0.01)	0.37	0.03 (0.02, 0.04)	<0.001	0.04 (0.03, 0.04)	<0.001	<0.001
Within 15-28 days	-0.002 (-0.01, 0.005)	0.62	0.002 (-0.005, 0.01)	0.50	0.008 (0.002, 0.01)	0.008	0.08
Within 29-180 days	0.008 (0.006, 0.01)	<0.001	0.005 (0.003, 0.007)	<0.001	0.006 (0.004, 0.007)	<0.001	0.06
MUAC (cm)							
Within 0-14 days	0.008 (-0.003, 0.02)	0.15	0.04 (0.03, 0.05)	<0.001	0.05 (0.04, 0.06)	<0.001	<0.001
Within 15-28 days	-0.002 (-0.01, 0.01)	0.70	0.02 (0.004, 0.03)	0.01	0.01 (0.002, 0.03)	0.02	0.04
Within 29-180 days	0.003 (0.001, 0.004)	<0.001	0.003 (0.001, 0.004)	<0.001	0.003 (0.001, 0.004)	<0.001	0.37
Kneel heel (cm)							
Within 0-14 days	0.004 (-0.01, 0.01)	0.44	0.02 (0.01, 0.03)	<0.001	0.02 (0.008, 0.03)	<0.001	0.02
Within 15-28 days	0.003 (-0.003, 0.01)	0.34	0.003 (-0.003, 0.01)	0.34	0.003 (-0.003, 0.01)	0.34	0.94
Within 29-180 days	0.01 (0.007, 0.01)	<0.001	0.005 (0.002, 0.008)	0.003	0.009 (0.006, 0.01)	<0.001	0.02
WHZ-scores							
Within 0-14 days	-0.02 (-0.03, -0.004)	0.009	0.05 (0.03, 0.06)	<0.001	0.05 (0.04, 0.06)	<0.001	<0.001
Within 15-28 days	-0.004 (-0.02, 0.01)	0.54	-0.004 (-0.02, 0.01)	0.54	-0.004 (-0.02, 0.01)	0.54	0.70
Within 29-180 days	0.004 (0.001, 0.008)	0.03	0.004 (0.001, 0.008)	0.03	0.004 (0.001, 0.008)	0.03	0.24
WAZ-scores							
Within 0-14 days	-0.007 (-0.014, < 0.001)	0.06	0.036 (0.03, 0.04)	<0.001	0.040 (0.033, 0.047)	<0.001	0.39
Within 15-28 days	-0.008 (-0.015, -0.001)	0.03	-0.007 (-0.016, 0.002)	0.11	0.003 (-0.005, 0.010)	0.52	0.16
Within 29-180 days	<0.001(-0.0001, 0.002)	0.09	<0.001 (<-0.001, 0.002)	0.09	<0.001 (<-0.001, 0.002)	0.09	0.004
HAZ-scores							
Within 0-14 days	0.009 (-0.002, 0.020)	0.10	-0.004 (-0.016, 0.009)	0.54	0.015 (0.004, 0.026)	0.01	0.04
Within 15-28 days	-0.009 (-0.019, 0.002)	0.11	-0.006 (-0.019, 0.006)	0.31	-0.005(-0.016, 0.006)	0.36	0.31
Within 29-180 days	-0.001 (-0.002, <-0.001)	0.03	-0.001 (-0.002, <-0.001)	0.03	-0.001 (-0.002, <-0.001)	0.03	0.34

[a]Interaction test between time and nutritional group. *All estimates adjusted for age and gender.

Abbreviations: MUAC mid-upper arm circumference; WHZ weight-for-height z-score; WAZ weight-for-age z-score; HAZ height-for-age z-score.

Table 5.3: Baseline hormone and receptor levels by nutritional group

Hormone	Control [a]			MAM [b]			SAM [c]			Geometric mean ratio *(95% CI)* [b vs a]	Geometric mean ratio *(95% CI)* [c vs a]
	Fasted	Fed	*P* α	Fasted	Fed	*P* α	Fasted	Fed	*P* α		
Leptin, ng/mL, median (IQR)	1.4 (1.1-2.3)	1.3 (1.3-2.1)	0.52	0.7 (0.5-0.8)	0.6 (0.4-0.9)	0.49	0.4 (0.3, 0.7)	0.3 (0.2, 0.7)	0.13	0.4 (0.2, 0.6) **	0.3 (0.2, 0.5) **
sOBR, ng/mL, median (IQR)	53.1 (42.5-65.2)	57.5 (48.8-65.7)	0.88	62.9 (75.3-46.4)	68.6 (54.8-74.8)	0.21	71.2 (55.9-78.6)	67 (55.8-89.1)	0.70	1.2 (0.9, 1.4)	1.2 (1.0, 1.5) **
Molar excess sOBR/Leptin	4.4 (2.4-5.6)	4.7 (3.5-5.6)	0.43	12.1 (9.8-17.3)	14.5 (8.2-23.7)	0.06	23.7 (12.2-32.2)	28.6 (14.7-38.2)	0.07	3.4 (1.9, 6.1) **	4.9 (2.8, 8.6) **
Total ghrelin, ng/mL, median (IQR)	0.8 (0.7-1.0)	0.8 (0.7-0.9)	0.73	1.0 (0.7-1.2)	0.9 (0.8-1.1)	0.97	1.20 (0.81-1.70)	1.2 (0.9-1.5)	0.33	1.3 (0.9, 1.8)	1.6 (1.1, 2.3) **
IGF-1, ng/mL, median (IQR)	26.2 (19.2-42.9)	24.5 (16.5-36.9)	0.49	17.8 (13.8-32.3)	17.2 (8.7-34.0)	0.15	12.5 (9.1-17.0)	13.0 (7.8-17.0)	0.79	0.7 (0.5, 1.1)	0.5 (0.3 ,0.7) **
IGFBP3, ng/mL, median (IQR)	1039.1 (730.1-1420.7)	1073.4 (762.7-1430.6)	0.33	990.5 (834.5-1207.6)	937.1 (709.2-1258.2)	0.68	697.2 (614.0-846.4)	747.6 (559.8-944.2)	0.19	0.9 (0.6, 1.1)	0.7 (0.5, 0.9) **
Molar ratio IGF1:IGFBP3	0.09 (0.07-0.1)	0.09 (0.06-0.1)	0.36	0.08 (0.06-0.1)	0.07 (0.05-0.1)	0.74	0.07 (0.05-0.09)	0.07 (0.05-0.08)	0.35	0.8 (0.6, 1.1)	0.7 (0.6, 0.9) **
Cortisol, µ/dL, median (IQR)	9.7 (7.9-14.6)	-	-	11.1 (8.8-19.5)	-	-	12.9 (11.1-17.1)	-	-	1.2 (0.8, 1.7)	1.4 (0.9, 1.9) *
C-peptide, pM, median (IQR)	240.7 (129.3-309.6)	-	-	212.3 (154.8-294.7)	-	-	218.9 (145.7-464.5)	-	-	1.1 (0.7, 1.8)	1.2 (0.7, 2.0)
Insulin, µIU/mL, median (IQR)	2.1 (1.1-4.4)	-	-	2.2 (0-5.9)	-	-	1.1 (0-3.05)	-	-	1.0 (0.4, 2.3)	0.7 (0.3, 1.7)

Abbreviations: MAM, Moderate Acute Malnutrition; SAM, Severe Acute Malnutrition; sOBR soluble binding receptor; IGF-1 insulin-like growth factor-1; IGFBP3 insulin-like growth factor.
α Wilcoxon signed-rank test comparing fasted and fed hormonal levels. *Bonferroni adjusted P<0.10 ** Bonferroni adjusted P<0.05

Table 5.4: Hormone changes over time by nutritional group

Hormones	MAM *Geometric mean ratio (95% CI)			SAM *Geometric mean ratio (95% CI)			P^b
	Day 14 vs 0	Day 28 vs 0	P^a	Day 14 vs 0	Day 28 vs 0	P^a	
Leptin, ng/mL	1.6 (1.1, 2.4)	1.1 (0.8, 1.6)	0.002	1.8 (1.3, 2.6)	1.6 (1.1, 2.3)	<0.001	0.18
sOBR, ng/mL	0.88 (0.8, 0.9)	1.0 (0.9, 1.1)	0.02	0.8 (0.7, 0.9)	0.8 (0.7, 0.9)	<0.001	0.02
Molar excess sOBR/Leptin	0.5 (0.4, 0.8)	0.8 (0.6, 1.3)	<0.001	0.5 (0.3, 0.7)	0.5 (0.3, 0.7)	<0.001	0.04
Total ghrelin, ng/mL	0.9 (0.7, 1.1)	0.8 (0.6, 0.9)	0.007	0.7 (0.6, 0.9)	0.7 (0.6, 0.9)	<0.001	0.11
IGF-1, ng/mL	1.6 (1.3, 2.1)	1.3 (1.0, 1.7)	<0.001	2.1 (1.7, 2.8)	2.2 (1.7, 2.8)	<0.001	0.001
IGFBP3, ng/mL	1.3 (1.1, 1.5)	1.1 (1.0, 1.3)	0.002	1.7 (1.5, 2.0)	1.5 (1.3, 1.7)	<0.001	<0.001
Molar ratio IGF1:IGFBP3	1.3 (1.1, 1.6)	1.1 (1.0, 1.5)	0.005	1.2 (1.0, 1.5)	1.5 (1.2, 1.8)	<0.001	0.02
C-peptide, pM/L	1.1 (0.7, 1.8)	1.2 (0.7, 2.0)	0.69	0.9 (0.5, 1.5)	0.9 (0.6, 1.6)	0.91	0.70
Insulin, µIU/mL	0.8 (0.3, 2.1)	1.4 (0.5, 3.5)	0.40	1.9 (0.8, 4.7)	1.6 (0.7, 4.0)	0.16	0.23
Cortisol, µ/dL	0.9 (0.6, 1.3)	0.9 (0.6, 1.3)	0.64	0.7 (0.5, 1.0)	0.8 (0.6, 1.1)	0.03	0.47

Abbreviations: MAM Moderate Acute Malnutrition; SAM Severe Acute Malnutrition; sOBR soluble binding receptor; IGF-1 insulin-like growth factor-1; IGFBP3 insulin-like growth factor.

[a] Bonferroni's adjusted test assessing differences over time.

[b] Wald test assessing interaction between nutritional group and time.

5.10 Figure Legend

Figure 1: Weight-for-age z-score gain.

A. Moderate Acute Malnutrition group. Number of children by weight-for-age z-score gain.
B. Severe Acute Malnutrition group. Number of children by weight-for-age z-score gain.

Figure 2: Changes in hormone and receptor levels over time by nutritional category.

A. Leptin levels in nanograms per millilitre.

   Dark grey bars = pre-test meal levels, pale grey bars = post-test meal levels.
B. Soluble leptin receptor (sOBR) levels in nanograms per millilitre.

   Dark grey bars = pre-test meal levels, pale grey bars = post-test meal levels.
C. Molar excess of sOBR: leptin.

   Dark grey bars = pre-test meal levels, pale grey bars = post-test meal levels.
D. Total ghrelin in nanograms per millilitre.

   Dark grey bars = pre-test meal levels, pale grey bars = post-test meal levels.
E. Insulin like growth factor 1 (IGF-1) in nanograms per millilitre.

   Dark grey bars = pre-test meal levels, pale grey bars = post-test meal levels.
F. Insulin like growth factor binding protein 3 (IGFBP3) in nanograms per millilitre.

   Dark grey bars = pre-test meal levels, pale grey bars = post-test meal levels.
G. Molar excess of IGF-1: IGFBP3.

   Dark grey bars = pre-test meal levels, pale grey bars = post-test meal levels.
H. Pre-prandial Cortisol in micrograms per decilitre

Chapter 6

Research Paper III

Article cover sheet

Title: **Maternal psychosocial stressors and severe wasting in rural Gambian infants: a mixed method approach**

1. For a 'research paper' prepared for publication but not yet published

2.1 Where is the work intended to be published?

This paper has been submitted to BMC Public Health.

2.2 List the paper's authors in the intended authorship order

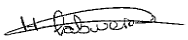
Helen M Nabwera, Sophie E Moore, Martha K Mwangome, Sassy C Molyneux, Momodou K Darboe, Nyima Camara-Trawally, Bakary Sonko, Alhagie Darboe, Seedy Singhateh, Anthony J Fulford, Andrew M Prentice

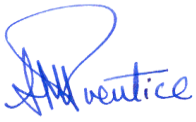
2.3 Stage of publication: Not yet submitted/**Submitted**/Undergoing revision from peer reviewer's comments/ In press Submission

25th April 2017

3. For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper.

As part of my PhD, I conceived, designed and undertook the data collection and analysis for the study in collaboration with the all the authors at the different stages of this work (Moore SE, Mwangome MK, Molyneux SC, Darboe MK, Camara-Trawally N, Sonko B, Darboe A, Singhateh S, Fulford AJ, Prentice AM). I wrote the manuscript and my co-authors edited and provided comments on the drafts that were incorporated into the submitted version of the manuscript.

Candidate's signature: 

Supervisor or senior author's signature to confirm role as stated in (2): 

Maternal psychosocial stressors and severe wasting in rural Gambian infants: a mixed methods approach

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6.1 Abstract

Background: Severe wasting affects 16 million under 5's and carries an immediate risk of death. Prevalence remains unacceptably high in sub-Saharan Africa and early infancy is a high-risk period. We aimed to explore risk factors for severe wasting in rural Gambian infants.

Methods: We undertook a retrospective case-control study from November 2014 to June 2015, in rural Gambia. Participants were recruited from the Early Nutrition and Immune Development (ENID) trial that was conducted from 2010-2015. Cases had WHO standard weight-for-length z-scores (WLZ) <-3 on at least 1 occasion in infancy. Controls with a WLZ >-3 in the same interval, matched on age, gender, village size and distance from the clinic were selected. Standard questionnaires were used to assess maternal socioeconomic status, water sanitation and hygiene and maternal mental health. Conditional logistic regression using a multivariable model was used to determine the risk factors for severe wasting. Qualitative in depth interviews were conducted with mothers and fathers who were purposively sampled. In addition, research staff were also interviewed in order to explore their aid with the validation of the in-depth interviews with the carers. A thematic framework was used to analyse the in-depth interviews that involved extensive exploration of the transcripts to identify emerging themes.

Results: Two hundred and eighty (77 cases and 203 controls) children were recruited. We conducted in-depth interviews with 16 mothers, 3 fathers and 4 research staff members. The mean age of introduction of complementary feeds was not significantly different between cases and controls (5.2 [SD 1.2] vs 5.1 [SD 1.3] months, Student t-test, p=0.72). Increased odds of severe wasting were associated with increased frequency of complementary feeds (range 1-8) [adjusted OR 2.06 (95%: 1.17-3.62), p=0.01]. Maternal adherence to the recommended infant care practices was influenced by her social support networks, most importantly her husband, by infant feeding difficulties and maternal psychosocial stressors.

Conclusion: In rural Gambia, adverse psychosocial circumstances and infant feeding difficulties constrain mothers from practising the recommended child care practices. Interventions that promote maternal resilience through gender empowerment, prioritising

maternal psychosocial support and encouraging the involvement of fathers in infant and child care promotion strategies, would help prevent severe wasting in these infants.
Key words: infant feeding, severe wasting, maternal stressors

6.2 Background

Severe wasting affects 16 million under 5's worldwide and carries an immediate risk of death [7, 17]. Survivors suffer significant short- term and long- term health issues, and psychosocial and economic consequences that are often intergenerational [28, 185-187]. The prevalence of wasting remains unacceptably high in sub Saharan Africa, with rates approaching 1-2% in the West African region [188]. In low income settings, postnatal growth faltering starts in early infancy and children can accumulate up to 80% of their total growth deficit in weight at 3 years in the first 12 months of life [15, 189, 190]. Attributable factors include poor maternal health, nutrition and socio-economic status, and infant factors including inadequate dietary intake and recurrent infections that could indicate sub-optimal child care practices [17, 191, 192]. It has been estimated that, with optimal coverage, a combination of nutrition specific and nutrition sensitive interventions could reduce under 5 deaths by 15% in low income settings [21, 115, 129]. Unfortunately, the delivery platforms for these evidence based interventions are often under resourced, and when combined with low uptake, result in poor coverage with limited impact on maternal and child undernutrition [115, 130]. Scaling up of nutrition sensitive interventions has the potential to enhance progress in childhood undernutrition [130]. The Gambia, where half of the population is rural, was one of the few African countries to meet the fourth Millennium Development Goal of reducing under 5 mortality by two thirds from 1990-2015 [152]. Unfortunately, over this interval the prevalence of wasting in this age group remained unchanged at 10% [152, 193].

This study aimed to explore the maternal and infant health, psychosocial and environmental factors that are associated with severe wasting in rural Gambian infants, in order to identify targets for intervention that would contribute to the evidence base for scalable nutrition interventions.

6.3 Methods

6.3.1 Study design

The study utilised a mixed methods approach involving both quantitative and qualitative methods. In the quantitative methods, we used a retrospective case-control study design among children who had been enrolled into the Early Nutrition and Immune Development (ENID, ISRCTN49285450) randomised trial (details in Supplementary Material) [194]. The quantitative phase aimed to identify and quantify the risk factors associated with severe wasting in infancy in this population. In the qualitative phase, a descriptive-exploratory approach was used to explore constraints, knowledge, attitude and practice of infant rearing and feeding among carers. The use of a mixed method approach evolved out of the need for an interdisciplinary approach to address complex health problems such as severe wasting in infancy, that quantitative or qualitative approaches alone were not able to adequately address [195].

6.3.2 Setting

This study was undertaken in the West Kiang district of rural Gambia that contains 36 villages of varying sizes with a total stable population of almost 15,000, of whom 2,300 (15%) are children under 5 years of age [151]. The main income generating activity is subsistence farming, but over the past decade this district has been prone to food insecurity due to erratic rainfall patterns, necessitating emergency relief food supplies [153, 159]. The climate has a long dry 'harvest' season (*November- May) and a short wet 'hungry' season (late June to mid-October), when agricultural work, depletion of food supply prior to harvest and infectious diseases peak [154].* Breastfeeding from birth to 2 years is the norm and complementary feeds are often nutritionally inadequate [155]. Mandinka are the predominant ethnic group, but there are also other ethnic groups including Fula and Jola [151]. The majority of the population are Muslims but traditional African beliefs are an important part of their spiritual lives. Polygamy is a popular and acceptable practice and isolated nuclear family units are rare as most compounds will have extended family relations including grandparents living within the family unit. In rural Gambia, the value and status of women is often based on their reproductive capacity therefore fertility rates have remained high and the uptake of contraception low [196]. The majority of married women

live in a compound belonging to their husband or his family [157]. An increasing number of children including girls are accessing primary school education, but in the local government area that includes the West Kiang district, only 42% transition to secondary school [152]. The literacy levels, particularly amongst women in rural areas remain low and a large proportion of the population live below the moderate poverty line of less than US$ 2/ day [152, 159].

The UK Medical Research Council (MRC) has been providing free comprehensive primary health care services including antenatal and child health clinics, for over 40 years to 3 rural villages in the West Kiang district: Keneba, Manduar and Kantong Kunda [151, 154]. This has contributed to a greater than 80% decline in both the infant and under 5 mortality rates in these 3 villages over the past 4 decades [154], well ahead of the Gambian national estimates [152]. Unfortunately, the patterns of undernutrition in children in this population have not followed a similar trend, and at 2 years of age 11% are wasted, 22% are underweight and 30% are stunted [193] , in line with the national estimates [152]. In recent years, the West Kiang Demographic Surveillance System (DSS) has been established that includes all the 36 villages (including Keneba, Manduar and Kantong Kunda). The inhabitants in all these villages can access free health care services at the MRC Keneba clinic, but continue to have their routine antenatal and child health checks at their local health care facilities run by the Ministry of Health in The Gambia [151].

6.3.3 Sampling and study population

Quantitative

Sample size

Our sample size calculations were based on analysis of the matching variables (in this case those derived from the questionnaire that include access to clean drinking water, hand washing habits and sanitation; infant feeding practices; socio-economic status, family structure and maternal depression) using the normal approximation to the binomial and assuming a case-control design (Supplementary Table 1). Our power calculations showed that 97 cases and 291 controls would be sufficient to identify, with 90% certainty at the 5% level of significance, factors for which the OR of becoming severely malnourished is 2 or more.

Inclusion and exclusion criteria

All the children who had ever been in the ENID trial were eligible. The weight-for-length z-scores (WLZ) for the infants were calculated using WHO Anthro (version 3.2.2, January 2011) and macros according to the WHO 2006 growth reference standards [1]. Cases were all the children who had a WLZ <-3 i.e. were severely wasted, on at least 1 occasion between 0-12 months of age (excluding the first week of life). Controls were all the appropriately matched children with a WLZ>-3 at the age when the cases had a WLZ<-3. The controls were matched based on age (i.e. similar date of birth (+/- 1 month), gender, village size (large [>750 inhabitants], medium [250 – 750 inhabitants] and small [<250 inhabitants]) and distance from the MRC clinic in Keneba (binary near [<19km]-far [≥19km]) The ENID trial excluded all multiple pregnancies, infants exposed to HIV and those with congenital anomalies [197]. In addition, children who presented to the clinic with severe acute malnutrition (SAM) requiring admission to the nutrition rehabilitation unit were excluded from the ENID trial [194].

Qualitative

Based on the preliminary analysis of the quantitative data, maternal illiteracy, history of a child death and maternal depression were associated with severe wasting in infancy. We therefore used these findings to develop a sampling framework for the in-depth interviews (IDIs) (**Figure 6.1**).

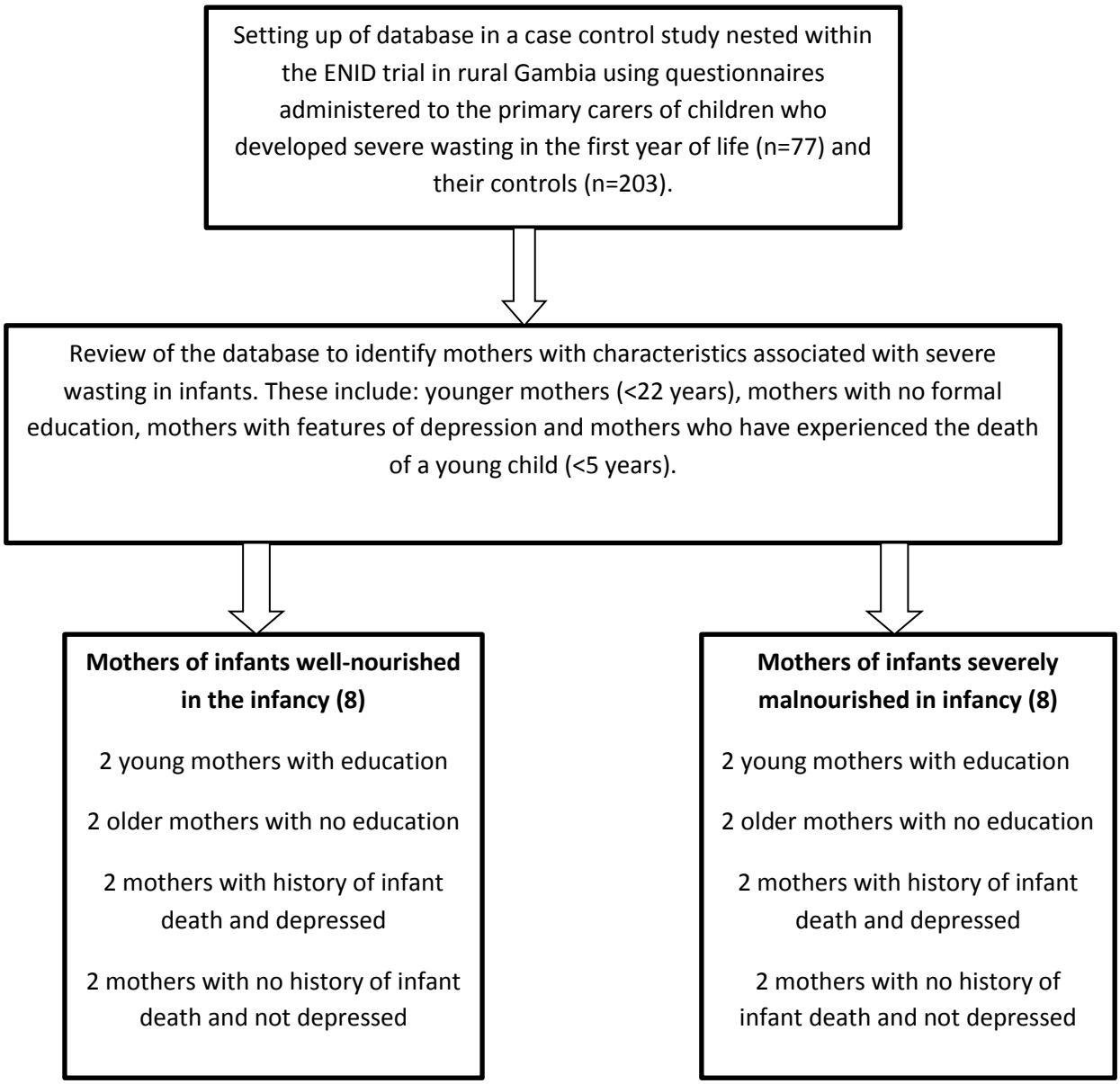


Figure 6.1: Sampling framework for qualitative data

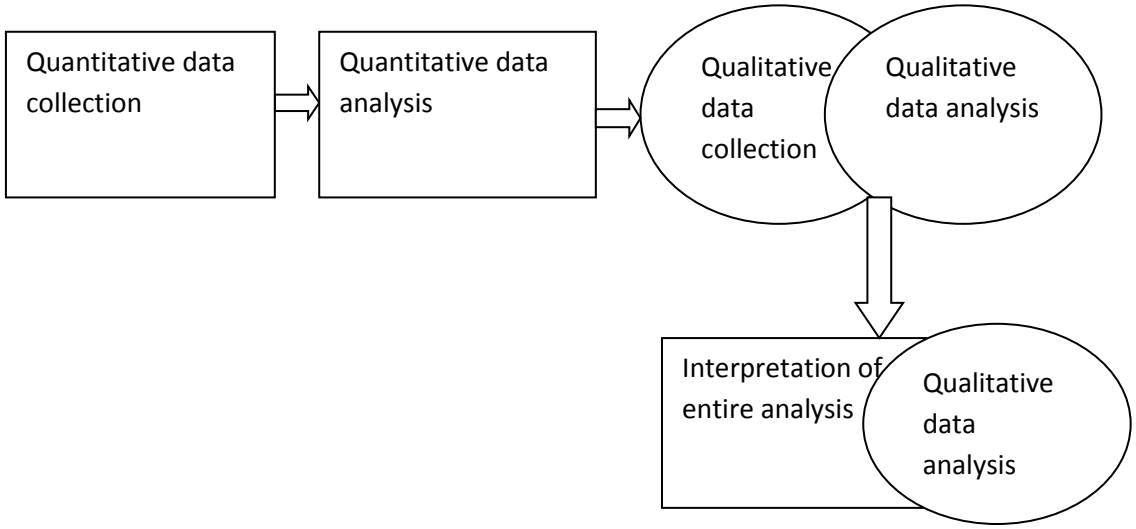
We used stratified purposive sampling where mothers of infants who were in both groups i.e. cases and controls, were selected, in order to capture a diversity of views and experiences about psychosocial, cultural, economic and environmental factors that influenced their infant feeding and rearing practices and their perceptions on how these

impacted on the nutritional status of their children. It also allowed us to look for any major differences across the 2 sets of carers i.e. those with or without severely malnourished children.

Purposive sampling is commonly used in qualitative research to identify a particular group of people who possess certain characteristics or who reside in circumstances pertinent to the phenomenon being studied and are therefore *"information-rich"* [198]. Stratifying the sampling framework also allowed us to identify any major variations in practices or perceptions within each strata [198]. This iterative process led us to undertake IDIs with fathers of some of the cases and controls, and with the research staff. We stopped recruiting participants for IDIs when we got to the point of *"data saturation"* i.e. the point in data collection and analysis when *"new information does not generate any new themes or variability within the themes in the dataset"* [199]. In total, we undertook 19 IDIs with carers (8 with mothers and 2 with fathers of cases, 8 with mothers and 1 with a father of controls) and 4 with the research staff (3 male, 1 female).

6.3.4 Data collection

We used the sequential explanatory strategy for data collection as illustrated in **Figure 6.2** [195]. This involved quantitative data collection and preliminary analysis to guide the development of the qualitative sampling framework, in the first phase followed by qualitative data collection and analysis in the second phase [195].



* Adopted from Creswell JW. Research Design- Qualitative, quantitative and mixed methods. 3rd ed. U.S.A: SAGE; 2009.

*Figure 6.2: Sequential explanatory strategy for data collection model (Creswell, 2009) **

Quantitative

Baseline maternal demographic data including maternal age, parity, residence, marital status, socio-economic status and details of her spouse were collected at recruitment into the ENID trial [197]. In addition, data on the infant anthropometry, feeding and illness episodes were collected prospectively during the trial [194].

Questionnaires

Between November 2014 and March 2015, we administered two questionnaires to the main carers of the infants. The first one addressed questions about the demographic and socioeconomic status; infant feeding; and water, sanitation and hygiene (WASH) practices. The second one was a modified version of the Edinburgh Depression Scale (EDS) that was used to assess for symptoms of depression in the mothers. This has 10 items and is a validated screening tool for depression in postnatal and non-postnatal women in a wide range of settings worldwide, including West Africa [200-202]. This tool was translated into Mandinka according to the principles of the WHO translation protocol [203]. Further details about this tool can be found in the Supplementary Material. One trained male senior field worker administered the modified EDS closely supervised by HMN. Women who had a modified EDS ≥ 12 (total score 30) were classified as being depressed, and were referred to the primary health care services at MRC Keneba clinic for counselling by trained nurse counsellors and/or assessment by the doctors in the clinic, who were experienced in managing depression. During the study one participant who did not respond to these primary care interventions, was referred to the Edward Francis Small Teaching Hospital in Banjul for specialist psychiatric assessment and management.

Anthropometry and infection episodes

The collection of anthropometric data and infant infection episodes for the ENID Trial has been described in detail elsewhere [155, 194]. In summary, the measurements in the infants including weight, height, were performed by trained senior midwives and field workers. Lengths were measured on a Raven Kiddimetre® (Raven Equipment, Great Dunmow, Essex, UK) to the nearest 0.1 cm. Weight was measured on minimally clothed infants and recorded to the nearest 10 grams using electronic Seca 336 scales that were calibrated regularly. Infants had their measurements done at birth (within 72 hours of delivery) and study visits on weeks

1, 8, 12, 16, 24, 40 & 52. All infants had a weekly visit from the trial field workers who recorded all their illness episodes including diarrhoea and chest infections during that interval.

Qualitative

Informal observations

During these sessions, researchers systematically watch people and events to observe everyday behaviours and relationships, in order to understand the perspectives of the study population as well as the physical, social, cultural, and economic context that in which they live [204]. Our female study nurse undertook these informal observations systematically using an observation tool that encouraged assessment of the environement, hygiene practices of the carer, infant and young child feeding practices as well as the dynamics and interactions within the household. These were conducted one week prior to the respective IDIs. During these visits, she systematically observed and documented details of the home environment. These included the daily activities of the mothers, with a focus on their child rearing practices including the preparation of food for the infants or children in the household, hygiene and sanitation practices. She also observed the dynamics within families – non-verbal communication and the levels of interaction between the mothers and other members of the family e.g. the father, the index child, the grandparents and co-wives. These observations provided a rich source of information about the context prior to the IDIs whilst enabling the study nurse to develop a rapport with the mothers that enhanced the conduct of the interviews.

In-depth interviews

In these sessions one interviewer interviews only one person [198]. The IDIs were done between April and July 2015. Our female study nurse conducted the IDIs with carers in Mandinka and Fula. They were all undertaken in the respondents' compounds, which was their preference. HMN conducted the IDIs with the research staff in English. The IDI guide was divided into the following four areas of inquiry: infant feeding; parenting skills; hygiene; and mental health/learning difficulties. Each section consisted of a combination of structured and open ended questions. We asked all respondents the same questions in a similar sequence, and the interviewer probed inductively on relevant responses. The interviews were audio recorded and the interviewers took field notes for all the interviews.

The interviews in Mandinka were transcribed by an independent transcriber, and those in Fula by a field worker from the study team, who had previous experience of transcribing qualitative interviews. This concurrent transcription and translation of these interviews was largely due to the limited time and resources available to undertake a two-stage process of transcribing in the local languages, then translating. In addition, they are not formally written languages which would add to the difficulty. The study nurse reviewed the transcripts for translation accuracy and discussed these with HMN during debriefing sessions. Where the translation was problematic, we compared the transcripts to the original audio recordings and revised them accordingly. In addition, two randomly selected transcripts were translated and transcribed by a field worker, who was a member of the research team and we found that there was agreement with the original transcriptions. This helped to establish both the validity and reliability of the transcripts [205].

6.3.5 Data analysis

Quantitative

Date were analysed using Stata (version 12.0, StataCorp, College Station, TX). We incorporated available prospectively collected data for the following variables: infant feeding data on timing of the introduction of complementary feeds, parity, infant morbidity and wealth indices. Descriptive statistics were computed to report number and percentage for categorical data, and the mean or median and standard deviation (SD) or interquartile range (IQR) for continuous data. To assess the difference in frequencies, Chi-squared or Fisher's exact tests were used and the Two sample Student T-test or Wilcoxon Ranksum test was used to assess the difference in means and medians of the baseline characteristics between the cases and controls. A principal component analysis (PCA) was used to determine household socioeconomic status using an asset based index in which we included the following six socioeconomic indicators: ownership of television, car, electricity, motorcycle, bicycle, or animal cart (yes/no), that were prospectively collected in the ENID trial. The adequacy of these indicators was assessed for inclusion in the PCA using the Kaiser-Meyer-Olkin measure [174], and they explained over 22% of the variability in the combined socioeconomic factor score (Further details in Supplementary Material). The households that the children came from were classified into quintiles based on Filmer and Pritchett's method [206].

The association between the demographic, psychosocial, infant feeding and WASH characteristics and severe wasting in infancy was assessed using conditional logistic regression, where cases were only compared to controls in the same set for crude odds ratios (ORs) and then, adjusted for confounders and collinearity in the multivariable model [171]. Using a backward stepwise model selection criteria where explanatory variables with a sparse amounts of data (e.g. sibling deaths) and those with unreliable estimates where the 95% confidence intervals were ≥ 10, were dropped from the model. In addition, we incorporated the prospectively collected data variables where the retrospectively collected data was either not available or was not robust e.g. age of introduction of complementary feeds and household assets in order to improve the accuracy of our findings. The final conditional regression model had 18 dependent variables [171]. This enabled us to estimate the effect of these exposures on severe wasting in infancy through computing adjusted ORs. We used 95% confidence intervals (CI) and p-value <0.05 to determine statistical significance. Our purpose was exploratory rather than intended as a definitive test of a particular hypothesis.

Qualitative

In qualitative research data analysis is an inductive process that seeks to learn about the perceptions that participants hold about a problem or issue by identifying patterns or themes [207]. HMN used the information from the informal observations, interview transcripts and summary information from the debriefing sessions, to develop summary sheets of caregivers in both groups. She then used headings and sub-headings based on the study questions and emerging themes to categorize information from the summary sheets. These ensured that we could comprehensively describe each group of mothers. This process formed the preliminary stages of data analysis as it involved reading and re-reading of the observation tools, interview transcripts and field notes, and thorough familiarization with the data from each caregiver. We undertook data analysis alongside data collection in order to allow questions to be refined and new avenues of inquiry to develop [208]. We used NVivo 10 software (QSR International Pty Ltd 2012) in subsequent data management and analysis using codes. After extensive familiarization with the data, HMN developed a coding scheme [209]. Two of the authors (HMN, MKM) agreed on the coding scheme who together reviewed the first 10% of respondents' transcripts independently and through multiple iterations came to an eventual

consensus. We revised the coding scheme further during the analysis. These multiple iterations are a key inductive process in the development of data coding schemes [210]. HMN undertook the NVivo coding of all the 23 transcripts. Using a thematic analysis approach, we grouped the data into themes and sub-themes and evaluated key emerging themes and how the themes/subthemes were interconnected [166, 211, 212]. To guide our analysis, we used a conceptual framework (Figure 6.3) that was based on the UNICEF Conceptual framework for undernutrition in children (Supplementary Material) [213].

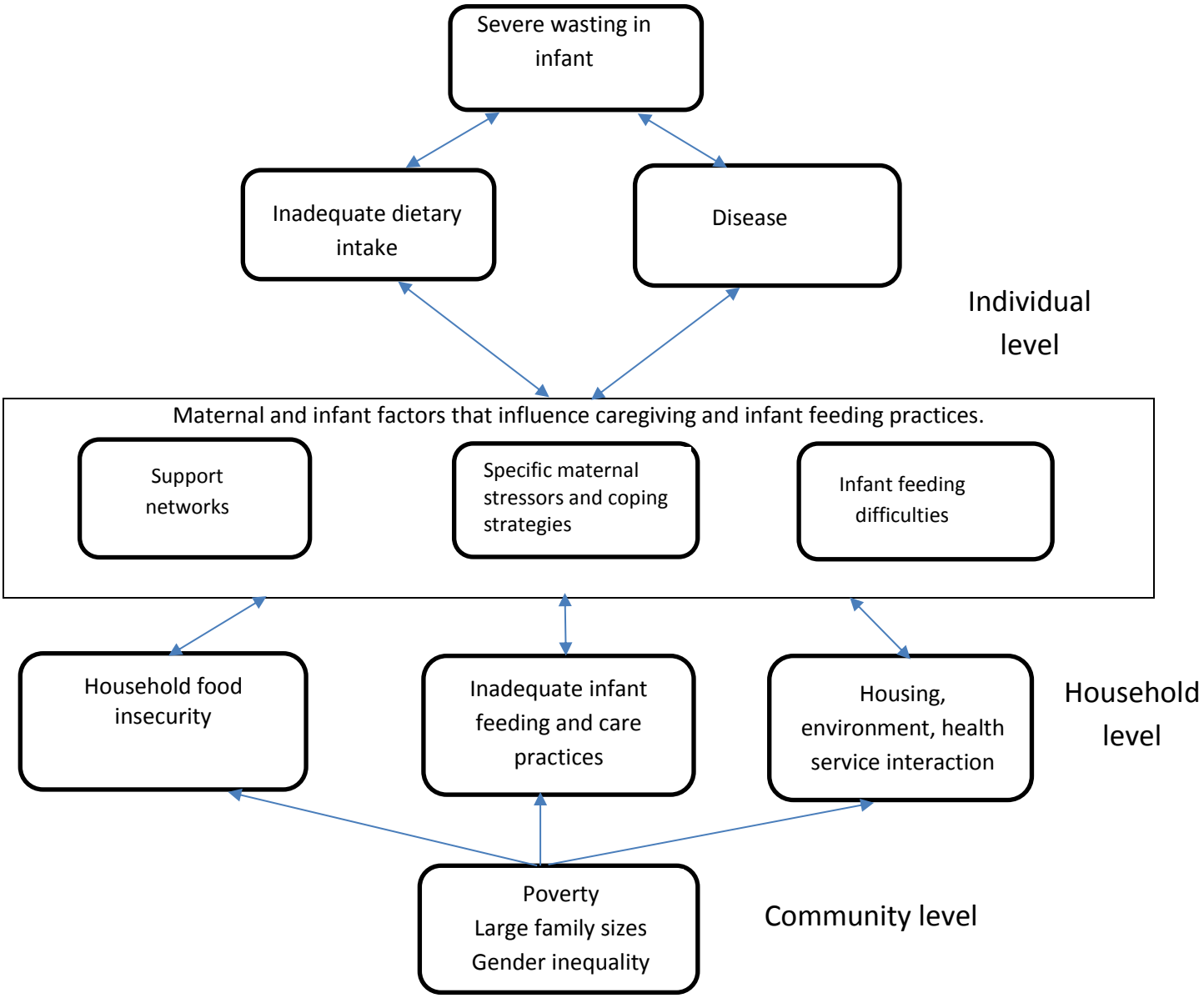


Figure 6.3: Conceptual framework of maternal and infant factors that influence infant nutritional status

6.4 Results

6.4.1 Quantitative

Eighty-nine children were identified as having a WLZ <-3, but we excluded one as their WLZ was clearly an error. Only 77 could be appropriately matched to controls. Sixty-one were matched 1:3 (three controls per case), five were matched 1:2 (two controls per case) and eleven were matched 1:1 (one control per case) giving a total of 280 children (78% of the expected sample size). Seventy-two (94%) of the cases and 200 (99%) of the controls were alive at the time of this follow-up study. The mean age of the cases and controls at the time of the study was 2.9 (SD 0.8) years **(Table 6.1).**

Comparison of characteristics of cases and controls

The mean WLZ for the cases and controls were -3.5 (SD 0.5) and -0.6 (SD 1.1), p<0.005 respectively at the age they were matched. The mean WLZ of the cases and controls at 12 months remained significantly different (-2.1 [SD 1.3] and -0.8 [SD 1.1], p<0.005). There was no difference between the 2 groups regarding age, gender, distance from the MRC clinic and village size. At the start of this follow up study, all the children were over 6 months of age with a mean age of 2.9 years (SD 0.8) for cases and controls. The primary carers during the first 12 months for all the children in the study were their mothers **(Table 6.1)**.

Maternal

The overall prevalence of maternal depression was 13% with no significant difference between the mothers of the cases and of the controls (13% vs 12%, Chi square, p=0.89). Most households had one or more additional children under the age of 5 years. Overall the mothers had the freedom to move around without restrictions. There was no significant difference in maternal level of education between cases and controls (Chi square, p=0.20) and the majority had attended Arabic school (Islamic studies). The wealth indices of the households were ranked from the poorest (Q1) to the wealthiest (Q5). There was no difference in the wealth indices between the mothers of the cases or controls (**Table 6.1**).

Infant feeding

Infant feeding choices were often made by the mothers. Infants were often breastfed up to 24 months, with the introduction of complementary feeds at an average of 5.2 months. The

commonest complementary feed was maize meal ("coos") porridge. Infants were often fed 3 complementary meals a day and mothers in both groups most frequently reported practising scheduled complementary feeding (70% cases vs 72% controls, Chi square, p=0.81). The mothers of the cases reported a higher frequency of complementary feeding (mean 4 vs 3, Student T-test, p=0.002) (Range 1-8). Mothers reported that their infants often fed from their own feeding bowls using a spoon **(Table 6.1)**.

Illness episodes in infancy

Overall children in both groups had a median of 2 (IQR 1, 4) diarrhoea episodes in the first 12 months of life with no significant difference in the number of illness episodes between cases and controls (10 [IQR 6, 14] and 9 [IQR 6,13], p=0.66) **(Table 6.1)**.

Sibling deaths

Overall, a third of mothers had experienced the death of a child other than the index child, with the most recent loss being at a younger age in the controls (median 2 [IQR 1, 5] vs 6 [2, 7] months, Wilcoxon Ranksum test p=0.01). **(Table 6.1)**.

Environment

Overall traditional pit latrines were the commonest form of sanitation within compounds (95% of participants). Overall, the source for clean drinking water was less than 30 minutes away for most mothers (>96%). Only a third of mothers undertook water purification and this was often filtration using a cloth and over 90% reported that they practised hand washing with soap during their daily activities **(Table 6.1)**.

Characteristics of cases in the first 12 months

The mean age of first growth faltering episode when WLZ< -3 was 6.5 (SD 3.5) months and at 12 months their mean WLZ was -2.07 (SD 1.32, n=53). There were more male infants (48 [57.8%]) among the cases. The mean maternal and paternal ages were 34.9 (SD 6.37) and 49.2 (SD 12.4) years respectively. The largest proportion of infants were from a medium and a large sized village that were 5km and 19km from the MRC clinic (**Table 6.1 & 6.2**).

Risk factors for severe wasting

In the univariable conditional logistic regression analysis, the only factor that was associated significantly with severe wasting in the infants was increasing frequency of complementary feeds (OR 1.51 [1.13-2.01], p=0.005) **(Table 6.3)**. In the multivariable conditional logistic regression model, this association remained significant (OR 2.06 (1.17-3.62), p=0.01). The treatment of water showed a trend towards a protective effect but this was not statistically significant (OR 0.31 (0.09-1.04), p=0.06). Maternal depression was not significantly associated with severe wasting in infants in this model (OR 1.37 (0.32-6.00), p=0.67) **(Table 6.4)**.

6.4.2 Qualitative

Mothers in this study were knowledgeable about the infant feeding and rearing recommendations having received intensive and sustained health promotion messages from the ENID trial study team. The mothers who we interviewed were all married and lived in their marital homes with their husbands and parents-in-law. All the mothers experienced poverty and were financially dependent on their husbands with only a minority having accessed formal 'English' education. Therefore, the infant feeding and rearing capacity of mothers was heavily influenced by 3 key factors: i) her social support network, most importantly her husband; ii) infant feeding difficulties; and iii) maternal psychosocial stressors.

Support networks

We observed that within households, mothers were preoccupied with domestic chores (including food preparation) and 'gardening' (farming) in order to provide food for their families. In addition, they also bore the responsibility of caring for their aging parents-in-law. Apart from mothers who were still breastfeeding, they were often unable to supervise feeding or play sessions with infants or young children and relied on a family member, e.g. paternal grandmother, or an older sibling for support with this. When these support networks were not available, these sessions were unsupervised. This lack of supervision sometimes posed a risk to the infants, e.g. being too close to a fire, or sharing a feeding bowl with a domestic animal.

"Yes, because if the mother leaves the child at home with the younger ones who cannot take care of that child, for example...I met the child eating with a dog. You see, and whilst the elder sister was there who could not even take care of that child. When I asked, she said the mother had gone to the garden..." (Field worker, 150211_003)

Under these circumstances, mothers were therefore unable to quantify the amount of food their infants had consumed and often assumed they were full when they stopped crying or feeding.

In this challenging context for mothers, the support of their husbands appeared to be a crucial mitigating factor. Both the male and female respondents perceived the role of the father as being a provider even when he was not permanently based in the home, i.e. bringing in resources to support the mothers' infant feeding and rearing strategies such as purchasing ingredients for enriching complementary feeds (sugar and butter for enriching "pap" (maize meal porridge)) or equipment for maintaining hygiene such as soap, as well as transport to access health care facilities for the infant and other family members.

"Fathers actually have a role to play, because fathers should stand in the care of both the mother and the child. Both the mother and father own the child. … father's role is to buy soap for the mother, in order to launder and if he (infant) starts to eat, you can buy food stuff which will benefit him (infant) and give to the mother to give him. But for today if you want to leave him in the care of the mother only, "baa fele a,amang semboo soto" (the mother is not financially strong)"…. (Mother, control_MAL130J, Bajana)

In addition, mothers also thrived on a supportive marital relationship with their husbands and this seemed to enhance their ability to make decisions in line with the infant feeding and rearing strategies. Mothers were the primary decision makers on matters pertaining to infant feeding and rearing, including accessing preventative or minor illness health care services that were often accessible on foot. This is because, their husbands, apart from those who worked for the MRC or were government health care workers (HCWs), had limited knowledge of the infant feeding and rearing guidance and therefore relied heavily on the mothers to make these choices. However, when their infants were very sick and required admission to the MRC Keneba clinic, mothers sought the financial support of their husbands for transportation.

"Yes, if they involve themselves in, it will give better care to the children. When a mother doesn't have something and you the father have it then you put it there. …it will also add the child care. But if the mother doesn't have it and you (husband) too did not do it, caretaking will not take place. He (infant) will be left out to get sick." (Mother, case_MAL019P, Kuli Kunda)

Although, the regular pastime for men in the West Kiang is to sit in the "bantaba" (a traditional meeting place for the men of the village), when not engaged in agriculture or male-led work, some husbands recognised the domestic pressures that the mothers were under, reflected on this and considered/admitted to offering practical assistance to their wives (mothers) so that the care of the infant was optimised, despite this being culturally alien. For them the aims were to support the mother but also their infants.

"Yes I (husband/father) helped the mother in that because the reason why I helped her, I like myself that is why. I know that she alone cannot do all that… If she is at one place, I can also be at the other place because to leave it with her alone, she is not a slave. We are all marriage partners. … I do not leave my wives. I always help them. And in terms of hygiene, I helped them there too. Because I clear my environment, I tell them "make this place and make the other place". If you want the children's health, you have to take care of your place. If you do not take care of them, there will be no health." (Father, case_MAL008F, Jali)

Mothers also felt that the practical involvement of their husbands in the care of their infants enhanced their nutrition. Mothers mentioned support such as feeding, bathing and taking the infant to the clinic to enabled the mother to complete her tasks and therefore had time to give appropriate attention to the feeding of their infants.

"Yes they also have a role to play. That is because a child is closer to the wife that is why caretaking starts with the wife. But the husband also has a role. … The child's needs should also be the role of the husband. The needs such as make him clean, and should feed him. Like in the case of feeds, whatever your earnings can allow you to afford, you should give to the child because that will improve his health." (Mother, case_MAL015R, Jali)

However, when this support from their husbands was not forthcoming e.g. due to abandonment of mothers and their children because of marital tension or the cultural union

of widows with a close male relative; the mothers relied more heavily on the support of the maternal grandmother/parents or peers. This support was less reliable as often these parties themselves relied on financial support from elsewhere. Under these circumstances, mothers found that this constrained their ability to adhere to the infant feeding recommendations, by limiting their options for enriching the popular complementary food pap. In desperation, some mothers opted to increase the breastmilk content in the diets of their over 6-month-old infants and gave less or no complementary food, which was presumably detrimental to the nutritional status of their infants.

"…I just noticed that when I asked him to do… he did not do it. When I ask from him once, twice and thrice, and he does not do it, I never bother myself to tell him again. When I go to my people if on a particular day they too do not have it, then I leave it. I do not cook for him on that day and keep it for him, I then breastfeed him." (Mother, case_MAL019P, Kuli Kunda)

"You see a man; he may sit without digging toilet at home. He will not buy soap and will not buy anything. You will be responsible of putting everything in your food. You will buy soap and there is no toilet in the compound. That can bring difficulty. (Mother, case_MAL041S, Nyorro Jattaba)

Within the homesteads paternal grandmothers were often elderly and mothers perceived their contribution to the care of infants as being mainly to carry them while the mothers undertook their domestic chores. Their role in infant feeding and rearing therefore appeared to be marginal with mothers opting to use the recommended infant feeding information from health care workers (HCWs) in their decision-making processes.

Mothers also highlighted the importance of peer support in encouraging them to adhere to the recommended infant feeding and rearing strategies. Within villages the mothers met regularly and shared their knowledge and experiences of infant feeding with, among others, peer counsellors who had been trained by the Gambia National Nutrition Agency (NaNA) to promote exclusive breastfeeding under 6 months. Peer support also proved critical when a mother was bereaved and therefore not able to care for her infant and young children. In these situations, her peers would take on the care of the children until she was able to

herself but duration of this support was variable and appeared to depend on a mother's ability to maintain good social interactions with her peers.

"Yes, I spread the information among the women at our "bantabato" (meeting ground) to tell the women that we should all do it that way. ... I myself get up and ask them to give me my child to breastfeed." (Mother, control_MAL256C, Jali)

Infant feeding difficulties

Whenever mothers experienced challenges with infant feeding they tried to find solutions amongst themselves and often did not consider consulting with a HCW. These challenges would only become evident to the HCWs when the infants were admitted to the MRC clinic with an acute illness or severe wasting. Having an infant who persistently cried even after being breastfed was perceived by mothers and the community at large as a sign of a "hungry baby" and mothers therefore opted to introduce pap even in the first week of life, as a breast milk supplement. Both primiparous and multiparous mothers often perceived breastmilk insufficiency. Multiparous mothers reported having adequate amounts of breast milk for the first few children but subsequently inadequate amounts to breastfeed the later infants. In addition, on the rare occasion when they were consulted, HCWs were not able to offer practical solutions to this challenge, but instead reassured carers that this was the norm for some women.

"When he was a baby, N (mother) did not have milk. That disturbs her and it makes her prepare food for him early, which can make him stop crying. …When we started cooking food for him, he stopped crying. When he eats until belly full, then he keeps quiet and sleeps for sometimes lying without disturbing anybody on anything." (Father, case_MAL035Z, Jiffarong)

Some infants refused to take pap and in food insecure households, mothers had no alternatives to offer so opted to increase the amount of breast milk in their diet, including those over 6 months of age. In adequately resourced households, mothers tried alternatives such as ground rice or potato powder, which some infants preferred and were therefore able to receive adequate volumes of complementary feeds. In addition, these infants were "force fed" by mothers who were desperate to ensure that they were adequately fed.

"…Then when I cook and was giving to him, he doesn't agree to drink. No matter how I force him, he spits it out. Now I cook porridge for him and later stop cooking it because he doesn't want to drink it. I only give him breast milk." (Mother, case_MAL065Y, Karantaba)

Maternal psychological stressors

Mothers in this community faced several adverse events that had an impact on their psychological status, hence limiting their ability to appropriately care for their infants and to access health promotion services. Religion was the basis for many of their coping strategies and HCW were also important in this process.

Ill health of child

Having a sickly infant who required several admissions to health care facilities was stressful for mothers. The admissions particularly when the infant was unwell were not conducive for health promotion as HCWs focused on treating and saving the infant's life during which time the mothers were distressed and preoccupied with their infant's illness. By contrast mothers whose infants were healthy could access and assimilate health promotion messages regularly.

Death

The death of a child was a common experience for mothers. They found it more difficult to recover from the death of an older child who they had formed a relationship with, than from the death particularly of a newborn infant. Their experience of miscarriages was secretive and often not shared with their husbands. However, although distressing, mothers showed resilience by rationalising these losses and therefore did not feel that these bereavements affected their parenting capacity and therefore the growth and nutrition of their infants.

"When a human being dies, and now moves from you, it makes you sad. But it is God that created death. And also in terms of Islam, if you want to do it in another way, it diminishes your Islam. I was very used to him (deceased child) truly speaking when he was with me. When he was taken from me, I felt that someone was taken from me but I then held onto this that it is God who gave me and when He was giving to me I was not aware of it.

Therefore, now if He is in need of him and I can take it to be God's property." (Mother, case_ MAL015R_Jali)

In this community, widows had limited autonomy in decision making for themselves or their children. In addition to grieving for the loss of their husband, the cultural practice that required them to remarry a male relative of the deceased (levirate marriage) sometimes exposed them and their children to adverse psychosocial circumstances as they were often remarried as the third or fourth wife to husbands who did not have the resources to support them adequately. This in turn limited a mother's ability to care for her infant optimally.

Lack of autonomy in child spacing

Although the norm in this community was for mothers to breast feed children until 2 years of age, short birth spacing reduced the length of time a mother could feed her index infant as the belief in the population is that pregnant women should not breastfeed. Mothers found this stressful as it constrained their ability to adhere to the societal norms of breastfeeding whilst increasing their work load i.e. increasing number of young children to care for.

"… because you may have a child less than 2 years and you later have another one who is less than 1 year you then conceive another one. Now if you want to take care of them, one is just removed from breastfeeding, the other is lactating and pregnant with another. That caring becomes difficult." (Mother, control_MAL138F, Jiffarong)

6.5 Discussion

Our study showed that an inappropriately high frequency of complementary feeding reported by mothers was associated with an increased the odds of severe wasting in infants and that this, in addition to other poor infant feeding and rearing practices in this community were influenced by a mother's lack of social support networks; inability to cope with psychosocial stressors and her experience of infant feeding difficulties in the context of a heavy burden of domestic chores. We also found some weak evidence of a protective effect of household water treatment on severe wasting which is an indication of mothers' abilities to appropriately prioritise domestic tasks within a supportive household of

community. However, we found no evidence of the association between maternal depressive symptoms and severe wasting in infants in this population.

Our finding that suboptimal infant feeding practices were associated with severe wasting was consistent with previous findings in this population from over two decades ago [105]. The increased odds of severe wasting in infants with increased frequency of complementary feeds with the reported practice of unsupervised infant feeding sessions both highlight the challenges that mothers experienced with adhering to complementary feeding recommendations when their support networks are inadequate. In the absence of adequate supervision during feeds, infants were likely to have ingested inadequate quantities of food necessitating more regular feeds. These findings are consistent with a report of time allocation for rural African women that estimates they spend 3 hours a day on meal preparation and only one hour for child care [214]. However, as drawing causal inference from a case control study can be problematic, the increased odds of wasting associated with the increased frequency of feeds in infants may also be explained by the action that mothers may have taken when their infants became severely wasted in an attempt to improve their nutritional status. There is a general expectation that increasing the frequency of feeding should improve the nutritional status of infants particularly after an episode of diarrhoea [215]. This practice may have diminishing returns as often the total daily amount consumed by infants does not increase beyond 4 or 5 meals per day [109].

Also, mothers who experienced food insecurity and were unable to diversify their infants' diets, opted to continue exclusive breastfeeding well beyond the upper recommended limit of 6 months. This potentially exposed their infants to significant deficits in their energy and nutrient intake at a critical time in their growth and development, with the immediate risk of severe wasting and adverse long-term nutritional and developmental outcomes. These findings highlight the need for more targeted complementary feeding education messages that address the specific challenges that rural African mothers face. HCWs in LMICs should also be encouraged to employ communication strategies that foster discussion with carers about their challenges with infant feeding and rearing, in order to identify and address them in a timely manner before the onset of growth faltering.

The overall prevalence of maternal depressive symptoms was 13%, which was similar to the prevalence reported in a neighbouring rural Gambian community. This finding was not unexpected in the context of maternal exposure to several psychosocial stressors including poverty, food insecurity, marital discord, death & ill health of their infants or husbands and a heavy workload in both the antenatal and postnatal periods. However, maternal depressive symptoms were not significantly associated with severe wasting in infancy in our study. In our study, the lack of association between maternal depressive symptoms and severe wasting could be partly explained by the fact that mothers rarely reported feelings of low mood or being unable to cope with their daily lives even in the context of adverse events that distressed them. We interpreted this reluctance to admit to not coping as a response to societal expectations for mothers to demonstrate resilience even in the face of adversity. In addition, we observed that adequate support networks were key to promoting resilience in mothers and their infants living under adverse psychosocial conditions, therefore enabling mothers to optimise their child care practises as has been reported before in this community [216]. The support of the husband was critical to this process in providing financial support for the family but also in the emotional support that they provided to the mother and infant. Although most child growth interventions have focused on maternal involvement as the bulk of child care is undertaken by them [115], the role of the father is vital in the growth and development of children and not limited to the provision of resources [48, 217]. The evolving roles of fathers even in LMICs as partners in child care [218], presents opportunities to involve them in the counselling of infant feeding and other child care practices that promote infant and child growth and development.

Encouragingly we found that the younger fathers in our study acknowledged and were keen to engage in their evolving roles as fathers, and were keen to be learn and support mothers in child care. This is a theme that needs to be explored further.

The support from the maternal grandmothers was also key to supporting infant feeding through provision of resources when mothers faced food insecurity. Although mothers often lived in the same compound as the paternal grandmothers, their role in child care was often limited to carrying the infants while their mothers attended to their domestic or farming chores as they were often frail. Previous work in this rural Gambian community showed a clear beneficial effect of maternal grandmothers on both child growth and

survival but not with paternal grandmother [132]. Peer support was also important in giving practical support with child care when mothers were incapacitated due to bereavement or illness. Peers were also a useful source of advice on infant feeding strategies particularly if they had been trained as peer supporters. The role of peer support groups within LMIC communities has been shown to result in sustained improvements in infant feeding practices [131].

Strengths and limitations

A key strength of this mixed methods study approach is that it enabled us to investigate infant feeding and rearing practises in great depth and explore the complex phenomenon of the pathways to severe wasting in infants in this rural Gambian community. In addition, our iterative study design enabled us to visit the homes of the respondents 2-3 times from the formative phase to the interview phase. This helped us to build a rapport with the carers the key household members, which facilitated the sharing of sensitive information around family dynamics and infant rearing. It also allowed us to check whether initial responses were captured accurately. This prolonged engagement with participants enhances the credibility and dependability of findings in qualitative studies [163].

A main limitation of this study is that data on many of the exposures were collected retrospectively, and often more than 12 months after the child had growth faltered with the associated challenges of recall bias for example the carers of cases may have been able to recall information on the exposures of interest in greater detail. Alternatively, having a severely malnourished child may have resulted in carers exaggerating certain aspects of care for example the timing and frequency of complementary feeds. We have attempted to mitigate these influences by using the prospectively collected data where it was available, as actual exposure variables (complementary feeding, wealth indicators, infant morbidity, parity). In addition, the inability of this study to capture many known risk factors of severe wasting may be because the minimum calculated sample size for this study may have led to a lack of power to detect statistically significant difference. However, the findings from the mixed methods approach still provide useful information that can guide the development of strategies to prevent wasting in infants in this rural Gambian community.

A limitation of qualitative research is that it is context-specific and therefore the findings are not generalizable to other contexts [162]. The findings of this study in one rural district of The Gambia may therefore not be applicable to other settings within The Gambia or the West African sub-region. However, the themes raised can be explored in other settings. The findings can also be used to generate theory that can be explored in other rural African settings with similar cultural and socioeconomic backgrounds.

6.6 Conclusion

In rural Gambia, inappropriate complementary feeding practices were associated with severe wasting in infants. Adverse psychosocial circumstances constrained mothers from adhering to the recommended infant feeding and rearing practices. Interventions that promote maternal resilience including: gender empowerment, maternal psychosocial support, and fostering the involvement of fathers in infant and child health and nutrition promotion strategies in this rural Gambian community, would help to prevent severe wasting in infants.

6.6 Conclusion

In rural Gambia, adverse psychosocial circumstances constrained mothers from adhering to the recommended infant feeding and rearing practices. Interventions that promote maternal resilience including: gender empowerment, maternal psychosocial support, and fostering the involvement of fathers in infant and child health and nutrition promotion strategies in this rural Gambian community, would help to prevent severe wasting in infants.

6.7 Declarations

6.7.1 Ethics approval and consent to participate

This study was approved by the Gambia Government/MRC Joint Ethics Committee (SCC no 1395) and by the London School of Hygiene and Tropical Medicine Ethics Committee.

Written informed consent was obtained from individual carers. Further information on this can be found in the Supplementary Material.

6.7.2 Consent for publication

Not applicable.

6.7.3 Availability of data and materials

The anonymised dataset analyzed in this study is available from the corresponding author on reasonable request.

6.7.4 Competing interests

The authors declare that they have no competing interests.

6.7.5 Funding

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6.7.6 Authors' contributions

The author contributions included the following: HMN, SEM, AMP, TJF, MKD conceptualised and designed the study; HMN, NCT, AD, SS, BS undertook and coordinated the data collection; HMN, MM, CSM performed the data analysis and drafted the initial manuscript; all authors reviewed and revised the manuscript.

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6.7.8 Authors' information

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SCM is an Associate Professor in Oxford University, based at the KEMRI-Wellcome Trust Research Programme in Kilifi, Kenya. Her social science research portfolio areas are the interface between health systems and households (including exploring household treatment seeking behaviour, gender relations and affordability of health care); and the interface between health researchers and communities (including consent, community engagement, payments and benefits for research communities, and social science ethics).

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AJF was the lead statistician for the MRC International Nutrition Group with a great deal of experience in nutrition epidemiology. AMP supervised HMN in all aspects of her work and is Head of the MRC International Nutrition Group at LSHTM and the Nutrition Theme Leader at the MRC The Gambia Unit and and has undertaken a vast amount of research in maternal and child nutrition in sub-Saharan Africa.

Tables

Table 6.1: Comparison of characteristics between cases and controls

Characteristics	Cases N=77	Controls N= 203	Chi Squared test, p
Children			
Parity, median (IQR)	4 (3, 7)	4 (2, 6)	0.36*
WHZ at growth faltering time point in cases, mean (SD)	**-3.6 (0.5)**	**-0.6 (1.1)**	**<0.005****
WHZ at 12 months, mean (SD)¥	**-2.1 (1.3)$^{\alpha}$**	**-0.8 (1.1)$^{\beta}$**	**<0.005****
Age in years at time of interview, mean (SD)	2.9 (0.8)	2.9 (0.8)	-
Male, n (%)	48 (58)	134 (61)	0.69
Carers			
Paternal age, mean, SD	47.3 (9.7)	48.7 (12.1)	0.38**
Maternal age, mean, SD	35 (6.4)	34 (6.7)	0.29**
Maternal depression, n (%)	10 (13)	36 (12.4)	0.89
Carer from 0-12months- mother, n (%)	77 (100)	199 (98)	0.21
One or more children under 5y in household (other than index child) n (%)	75 (97.4)	196 (96.6)	0.72
Mothers have freedom to move around without escort	76 (98.7)	198 (99.5)	0.23
Education level of mother			
No formal education	23(30)	40 (20)	
Arabic school	40 (52)	122 (61)	
Less than primary school	8 (10)	24 (12)	
Completed primary school	6 (8)	9 (5)	
Completed secondary school	0	4 (2)	0.21
Wealth indices			
Farming	65 (86)	163 (82)	
Business	4 (5)	7 (3)	
Salary	2 (3)	1 (1)	
Other	5 (6)	28 (14)	0.15
Guaranteed monthly income	6 (8)	10 (5)	0.36
Number of rooms in for sleeping household, median (IQR)	2 (2,3)	2 (2,3)	0.28*
Number of people in household past 6 months, median (IQR)	6 (4,8)	5 (4,7)	0.16*
Wealth quintiles, n (%)	PC1 PC2	PC1 PC2	PC1 PC2
1 (Poorest)	30(39) 34 (44)	63 (31) 74 (37)	
2	19 (25) 2 (3)	54 (27) 3 (2)	
3	1(<1) 26 (34)	8 (4) 72 (35)	
4	11 (14) 3 (4)	41 (20) 11 (5)	
5 (Wealthiest)	16 (21) 12 (16)	37 (18) 43 (21)	0.40* 0.15*
Infant feeding practices			

Ever breastfed¥	76 (100)	194 (100)	0.86
Age breastfeeding stopped, months, median (IQR)	24 (20, 24)	24 (20, 24)	0.59*
Age of introducing complementary foods, months, mean (SD) (prospective data collection)	5.2 (1.2)	5.1 (1.3)	0.72**
Commonest complementary food-coos (maize meal) porridge, n (%)	54 (65.1)	155 (70.1)	0.12
Mode of feeding-spoon n (%)	75 (97)	198 (98)	0.95
Frequency of complementary feeds, mean (SD)	**4 (1.0)**	**3 (0.9)**	**0.002****
Frequency of feeding per day, n (%)			
X1	0 (0)	1 (1)	
X2	6 (7)	17 (8)	
X3	26 (34)	114 (58)	
X4	30 (40)	46 (23)	
X5	11 (15)	15 (8)	
Greater than X5	3 (4)	5 (2)	0.09
Scheduled feeding, n (%)	52 (70)	142 (72)	0.81
Own bowl at feeding time, n (%)	75 (97)	198 (98)	0.94
Decision-maker for feeding of child			
Mother alone	68 (90)	188 (95)	
Mother and father	6 (7)	8(4)	
Mother and mother in law	2 (3)	1 (<1)	
Mother and other	0 (0)	1 (<1)	0.31
Decision-maker for medical care of child			
Mother alone	51 (67)	135 (68)	
Mother and father	23 (30)	60 (30)	
Mother and mother in law	2 (3)	3 (2)	
Mother and other	0	1 (<1)	0.86
Illness episodes in index child			
Diarrhoea, median (IQR)	2 (1, 4)	2 (1,4)	-
Morbidity, median (IQR)	10 (6, 14)	9 (6,13)	0.66*
Number who died in first 12 months, n (%)	4 (5)	2 (<1)	-
Number died after 12 months, n (%)	1(<1))	1 (<1)	-
Sibling			
No sibling deaths, n (%)	22 (29)	65 (32)	0.58
Age in months of sibling who most recently died, median (IQR)	**6 (2, 7)**	**2 (1, 5)**	**0.01***
Environmental			
Distance from MRC clinic in km, median (IQR)	17 (10, 23)	13 (5, 23)	0.32*
Population size of village, median (IQR)	768 (528, 1265)	768 (610, 1265)	0.53*
Toilets, n (%)¥			
Flushing	0 (0)	1 (<1)	
VIP latrine	1 (1)	2 (1)	
Traditional pit latrine	70 (92)	190 (95)	
No toilet	5 (7)	5 (3)	0.50

Water source past 6m, n (%)			
Piped water in house	0 (0)	1 (<1)	
Covered public well	0 (0)	3 (2)	
Public tap	69 (91)	173 (87)	
Open public well	5 (7)	10 (5)	
Open well in compound	0 (0)	1 (<1)	
Deep tube well	2(3)	11 (6)	0.67
Accessibility to water, n (%)			
Less than 30 minutes	75 (97)	192 (96)	
30 minutes or more	2 (3)	7 (4)	0.70
Ability to fetch drinking water daily, n (%)	75 (97)	196 (98)	0.54
Number of daily trips for water, median (IQR)	5 (4,6)	5 (4,6)	-
Water purification for drinking water done	26 (34)	76 (38)	0.52
Water purification method, n (%)¥			
Filtration through cloth	25 (96)	76 (100)	
Filtration through ceramic	1 (4)	0 (0)	0.09
Hand washing ¥			
Water alone	7 (9)	17 (9)	
Water and soap	70 (91)	181 (91)	
Water and mud or clay	0 (0)	1 (<1)	0.82

$^{\alpha}$ N=53 $^{\beta}$ N=169 *Wilcoxon Ranksum test ** Student T-test ¥Missing data
PC1: Principal component analysis 1, PC2: Principal component analysis 2

Table 6.2: Characteristics of cases during first 12 months of life

Characteristics			**N=77**
Age at first WHZ <-3, mean (SD)			6.5 (3.6)
Age of introduction complementary foods, mean (SD) (prospective)			5.2 (1.2))
Village of residence	**Village size**	**Distance from MRC clinic in km**	**N (%)**
Keneba	Large	0	4 (5)
Kantong Kunda	Medium	5	12 (15)
Manduar	Medium	8	2 (3)
Tankular	Medium	10	6 (8)
Kuli Kunda	Large	12	8 (10)
Joli	Medium	13	5 (6)
Bajana	Medium	16	2 (3)
Karantaba	Large	17	1 (1)
Jiffarong	Large	19	12 (15)
Burong	Medium	21	4 (5)
Sankandi	Medium	23	3 (4)
Nyorro Jattaba	Large	24	6 (8)
Jattaba	Large	26	5 (6)
Kemoto	Medium	27	5 (6)
Dumbuto	Medium	30	2 (3)
Batelling	Small	42	2 (3)

Table 6.3: Univariable analysis of risk factors for severe wasting in infants

Variables	Unadjusted OR (95% CI)	P value
Carer factors		
Current carer (Mother vs other)	1.03 (0.30-3.46)	1.00
Father lives in household	0.73 (0.44-1.22)	0.23
Carer education	0.79 (0.52-1.20)	0.33
Maternal age (younger)	1.02 (0.98, 1.06)	0.34
Paternal age	1.00 (0.96-1.01)	0.48
Monthly income for mother	2 (0.65-6.12)	0.23
Children under 5y in household	1.39 (0.96-2.01)	0.09
Maternal depression	1.18 (0.54-2.61)	0.68
Mother decides on medical care	1.09 (0.62-1.90)	0.78
Principal component 1 (socioeconomic measure)	1.07 (0.89-1.29)	0.48
Principal component 2 (socioeconomic measure)	0.86 (0.68-1.10)	0.19
Infant factors		
Parity	1.01 (0.90-1.14)	0.82
Increasing age breastfeeding stopped	1.00 (0.93-1.10)	0.93
Increasing age of introducing complementary feeds (prospective)	1.09 (0.86-1.39)	0.47
Mother decides on type complementary food for infant	1.09 (0.62-1.90)	0.78
Increasing frequency of complementary feeds (1-8)	**1.51 (1.13-2.01)**	**0.005**
Mother feeds infant	0.81 (0.26-2.50)	0.72
Scheduled feed times	1.11(0.61- 2.04)	0.74
Illness episodes in infancy		
Diarrhoea episodes	0.96 (0.84-1.09)	0.51
Morbidity	1.02 (0.96-1.07)	0.53
Sibling factors		
No sibling death	1.04 (0.55-1.94)	0.91
Environmental factors		
Number of people sleeping in household	1.90 (0.99-1.21)	0.09
Number of rooms in household	1.24 (1.00-1.60)	0.10
Source of water less than 30 minutes	0.75 (0.14-4.00)	0.74
Drinking water for household fetched daily	0.59(0.10-3.56)	0.57
Increasing trips for fetching drinking water	0.94 (0.82-1.10)	0.47
Water treatment	0.85 (0.48-1.50)	0.57
No toilet in compound	3 (0.78-11.51)	0.11
Traditional pit latrine	0.74 (0.29-1.89)	0.53
Hand washing with soap	1.10 (0.44-2.72)	0.84

Table 6.4: Multivariable analysis of risk factors for severe wasting (after fitting model)

Variables	OR, 95% CI	P value
Carer factors		
Maternal depression	1.37 (0.32-6.00)	0.67
Maternal age (younger)	1.03 (0.91-1.18)	0.64
Paternal age (younger)	0.95 (0.88-1.02)	0.15
No sibling deaths	0.47 (0.11- 2.00)	0.30
Principal component 1 (lower socioeconomic measure)	1.23 (0.88-1.72)	0.23
Principal component 2 (lower socioeconomic measure)	1.19 (0.71-1.98)	0.50
Infant factors		
Parity	0.99 (0.76-1.28)	0.92
Increasing age of stopping breastfeeding	1.05 (0.90-1.19)	0.53
Increasing age of starting complementary feeds (prospective)	0.89 (0.71-1.11)	0.30
Increasing feed frequency of complementary foods (1-8)	**2.06 (1.17-3.62)**	**0.01**
Non-scheduled feeding	2.21 (0.56-8.64)	0.26
Mother feeds infant	0.45 (0.05-4.00)	0.47
Mother makes decision to seek health care	1.42 (0.47-4.34)	0.54
Illness episodes in infancy		
Diarrhoea episodes (fewer)	0.82 (0.62-1.09)	0.51
Morbidity (fewer)	1.02 (0.96-1.07)	0.53
Environmental factors		
Water treatment	**0.31 (0.09- 1.04)**	**0.06**
Number of trips per day to fetch water	0.88 (0.64-1.21)	0.44
Number of people sleeping in house	1.02 (0.81-1.30)	0.85

6.8 Additional information on mixed methods study design

6.8.1 Study design

The mixed methods research approach has been used in the sciences for centuries [219], but was only recognised as a distinct method of inquiry in the social sciences in the 1950's, with the evolution of the concept of triangulation [220]. This is in line with the paradigm shift within the social sciences from metaphysical to a more pragmatic approach (one of the 4 main paradigms of mixed methodology) [221], that encourages the integration of these 2 distinct research methodologies [221, 222]. This approach allows for both assertions that there is a single "real world" and that all individuals have their own unique interpretations

of that world [222]. In Study III, qualitative research was used to build on the quantitative research findings [223], and provide an in-depth understanding of the psychosocial, cultural and environmental factors that influence infant feeding and rearing practices that impact on the nutritional status of infants in this rural district in The Gambia.

6.8.2 Selection, roles and training of the field team

I used a field team to administer the questionnaires and conduct the interviews in this study primarily due to the language barrier. Although I had been working in this community for three years as a Paediatrician, and could conduct a medical consultation in one of the local tribal languages-Mandinka, with the support of an interpreter, I was still not fluent in this or any of the other local languages. It was therefore not feasible for me to conduct the IDIs myself.

The field team consisted of two male senior field workers and a female study nurse. The field workers and the study nurse had each received at least 12 years of formal schooling. They were all proficient in all the local languages including Mandinka, Fula, Jola and Wollof, and spoke and wrote English sufficiently. I had worked with the field workers on a previous childhood malnutrition project. In addition, both field workers had worked on various MRC nutrition-related projects in the West Kiang and neighbouring districts for over 7 years. I had worked with the female study nurse for three years in the MRC Keneba clinic and Nutrition Rehabilitation Unit. Although she had no prior experience of undertaking qualitative research, she had worked as a counsellor in a HIV treatment facility in The Gambia for over 10 years. She therefore, already had very good communication skills, in particular the ability to listen to patients. Other key characteristics of the study nurse were that she was a Gambian, married with children, a devout Muslim who dressed conservatively. As this was a predominantly Muslim community where a woman's status was enhanced by being married, this made it easier for her to build a rapport and trust with the mothers and the extended family within their compounds during the visits. Being a mother herself, gave mothers the confidence to discuss the sensitive issues around the challenges of caring for infants in this setting.

From February to March 2015, I trained the study nurse on "Qualitative Research in Health" using course material that I had received during my own training. The topics covered were:

differences between qualitative and quantitative interviews, difference between medical interviews and qualitative interviews, how to conduct qualitative interviews and observations, how to write field notes and debriefing. She had also recently completed Phase I and II of a course on Communication Skills in Health and Research that was conducted at the MRC Keneba field station, delivered by an experienced team from the KEMRI-Wellcome Trust Research Programme in Kenya. The topics covered included: basic introduction to research including research ethics, informed consent processes, communication skills including picking up on non-verbal cues, how to handle challenges and dilemmas encountered in the field.

Prior to commencement of field activities for this study, I held several meetings with the field staff to discuss:

- the background of the study;
- the aims and objectives of the study;
- the consenting procedures;
- administration of the questionnaires;
- data entry and management (with support from one of the junior data managers);
- the interview guide;
- shifting of roles for the study team from health care providers who promote good nutrition to listening to carers views and challenges on infant nutrition.

For the formative (quantitative) phase, all the questionnaires were piloted and refined over a period of four weeks before the study begun, to ensure that the tools were acceptable to the population. During the period of developing the IDI topic guides, the study nurse also undertook six pilot IDIs, which I discussed with her at great length to ensure that she understood the methodology and the study objectives well. While the two field workers administered the questionnaires during the, I observed the interactions between them and the participants and was also able to observe the family dynamics of the participants. I continued having regular discussions with the field team in relation to the questionnaires, with a particular focus on the translation of terms and phrases to ensure that the original

and intended meaning was maintained. I also had weekly debriefing sessions with the whole field team. These meetings were very interactive and the field team raised issues of concern with regard to the logistics of the study and data collection tools, which we revised accordingly. They also sought clarification on a range of issues. We reviewed the questionnaires and interview topic guides in great details to ensure that the questions asked were appropriate for this setting, when translated to the local languages and if not these were rewritten to ensure that we did not offend whilst still being able to obtain the information that we needed.

As this study involved repeated visits to some of the mothers, it was important for the study nurse to feel comfortable and have good working relationships with the mothers. In the initial visits, the whole field team and I visited all the mothers together to ensure that respondents were familiar with the entire field team. From these visits, it was evident that the mothers were comfortable with the study nurse. Although, I was present during most the field visits during the formative phase of the study, the study nurse revisited the mothers on her own for the structured observations and IDIs. She then, had a debriefing session with me after every 2-3 interviews. During these sessions, various aspects of the visit were discussed including the dynamics of the respondents, observations of interest, emerging issues of interest, overall positive aspects of the visit, any arising challenges or dilemmas, and clarifications required in relation to the interview questions or topic guide. This process was particularly useful in ensuring that evolving themes of interest were immediately incorporated into subsequent visits.

6.8.3 Data collection

Quantitative

Mental Health Questionnaire

The Edinburgh Depression Scale (EDS) consists of ten questions and a woman can rate her depression symptoms on a scale of 0 (none) to 3 (severe). The total score ranges from 0 to 30 and scores of ≥12 are suggestive of depression. We utilised the principles of the WHO translation protocol with *"emphasis on the conceptual and cultural equivalence and not on the linguistic equivalence" [203].* I had extensive discussions with the study team about the

purpose and meaning of the questions in the modified version of the EDS. The team then, consulted and agreed on the most appropriate translations in the most common language-Mandinka. Four members of the field team undertook forward translation of the modified EDS. One field worker was then assigned to administering the tool in Mandinka. He undertook nine pilot interviews that were audio recorded with the tool, in order to facilitate the back translation into English of the tool by an independent translator who was fluent in both English and Mandinka. In addition, it enabled us to assess acceptability of the tool and formed part of the training of the field worker. The field team had further discussions about the conceptual and cultural meanings of the translations and addressed any discrepancies that had arisen in the conceptual equivalence of the translations. Further revisions were made to the Mandinka translated tool. The final version of the tool also incorporated the feedback and reflections from the field worker and the participants in the pilot.

Qualitative

In-depth interview

IDIs are ideal for investigating sensitive and confidential information, as well as seeking individual interpretations and responses [198]. The method utilises guided but open-ended interviews that reflect on ways that respondents understand their situations and how or why they do certain things [224].

I supervised the study nurse during every stage of the study. This involved having debriefing sessions with her after every 2-3 interviews, in order to discuss the conduct of the interview as well as any emerging themes. The interview topics and open-ended questions were carefully derived by me from the study questions, and refined following feedback from the study nurse during the phase of piloting the tool. Further questions evolved during the iterative process of data collection and analysis.

I also conducted IDIs with a study nurse involved in nutritional rehabilitation at the MRC Keneba clinic and the two senior field workers who were all Gambian but from a different region in The Gambia. The purpose of this was to explore their perceptions and health care providers/ researchers in the area on the barriers and facilitators of appropriate infant feeding and child rearing practices in this population. This helped in the further development of the IDI topic guide.

6.8.4 Data management

Quantitative

Demographic and clinical data were securely extracted from the ENID and KEMReS databases respectively at MRC Keneba. The data from the questionnaires was manually entered into a secure SQL server study database. Anonymised patient data sets were used for data analysis to ensure confidentiality. All the databases were password protected with restricted access to the research team members. The data was stored in the MRC Keneba data department and routinely backed up.

Qualitative

Audio-recordings of all the interviews were downloaded into a password protected study folder on the MRC Keneba secure server that was only accessible to the research team. The raw data was stored in its original format. Audio-recordings from all the interviews that were conducted in Mandinka and Fula were translated into English and transcribed verbatim using standardised transcription principles [165].

I took notes of the key issues from the interviews on blank templates of the interview topic guides during debriefing sessions. This enabled me to remain on top of the large volume of data that was being generated and to ensure that I was able to incorporate new and emerging themes into subsequent visits. I also noted down any issues of interest.

Most of the transcriptions were done by a former teacher (independent transcriber), who was recruited on a temporary basis to undertake this work. Before he started working on the transcriptions, I explained to him the background of the study and got him to transcribe 4 pilot interviews that I reviewed and gave him feedback on, in particular the format. One of the field workers from the research team transcribed the interviews in Fula as the independent transcriber was not fluent in Fula, one of the local languages.

6.8.5 Ethical considerations

Institutional review process and community engagement

As a requirement for all studies undertaken within the MRC Unit, The Gambia, this study underwent the institutional review process that involved submitting the proposal to two

scientific committees within MRC: the Scientific Administration Meeting at the MRC Keneba field station that comprises of the senior scientists and senior field staff and the Scientific Coordinating Committee in Fajara that comprises of the senior scientists and government representatives. After it was approved by these committees it was submitted to the joint MRC/The Gambia government ethics committee. I also obtained approval from the LSHTM ethics committee. All these committees verified the scientific rigour and ethical standards of the work. The informed consent documents were also carefully assessed by these committees. At every stage the study documents were revised incorporating the feedback from these committees. Data collection only commenced once approval was obtained from all of these committees.

Informed consent

Once I received ethics approval for this study, my field team and I conducted a series of village meetings in most of the 36 villages within the West Kiang District. In these meetings, the we met with the village "Alkalo" (village head) and the other village elders, carrying a bag of kola nuts for each of the villages that we visited, as is the local tradition. The village "Alkalo" then, called the villagers to gather around to listen to what we had come to share with them. Most of the people present in these meetings were men. The research team explained the background and aims of the study to them and gave them the opportunity to ask questions or make comments. In all the villages that we visited, the elders were all very receptive and approved of us conducting the study in their villages. Once this approval was obtained the field team and I then, approached the individual primary carers (mostly mothers) and explained the study to them again. Participation in the study was voluntary and informed consent was obtained from all participants for both the quantitative questionnaires and the IDIs. Respondents were also made aware of their right to discontinue participation at any time during the study with no adverse consequences to themselves or their families. The ICDs were only available in English but the content was explained to the mothers in their respective local languages (Mandinka and Fula). The mothers all received breakfast after the interviews but this offer was only mentioned at the end of the sessions, in order to ensure that they agreed to participate voluntarily.

Reparation for participants

Due to the inconvenience of not being able to prepare breakfast for themselves during the home visits, each of the participants was given breakfast (1 loaf of bread, mayonnaise, a tin of sardines, teabag, a tablespoon of milk powder and a tablespoon of sugar). This was primarily to compensate them for time lost. The amount of food given as compensation was decided based on consultations with other MRC Keneba researchers who had utilised similar strategies for their studies. In addition, any children or their caregivers who were identified to be sick during the field visits were transported to the MRC Keneba field station for treatment. In addition, during the IDI's the study nurse identified 10 families that were experiencing significant food insecurity. We therefore contacted the NaNA on their behalf. They supported us in submitting an application to the World Food Programme team in The Gambia, who were due to initiate a programme to support families at the severe end of the food insecurity spectrum. We also submitted a photograph of one of our participants to an MRC Unit The Gambia research photograph competition and won first prize. The prize money was used to purchase food items for the mother and her family and for other families that were deemed to be experiencing significant food insecurity.

Confidentiality

We tried to ensure confidentiality throughout the study including finding a quiet spot in the compound to conduct the interviews. This has not always been possible. On some occasions one of the field workers would try and engage the husbands of the respondents in conversation, in order to distract them from the interviews, particularly when sensitive topics around maternal mental health, malnutrition in the index infant and/or the death of an infant were being discussed. We employed a great deal of sensitivity when asking these questions that could cause distress.

To maintain anonymity, participant identifiers were removed from all data collected and replaced by codes and pseudo-names for the purposes of thesis write-up or manuscript for submission. Furthermore, transcripts and audio-recordings were stored in securely locked cabinets and password-protected computers. Only the individuals directly related to the

study were allowed to access the data. On completion of this work, data collected was archived in accordance with the MRC policies.

6.8.6 Reflexivity

Reflexivity is a vital aspect of qualitative research. Alvesson and Skolberg define it as *"the awareness that the researcher and the object of study affect each other mutually and continually in the research process" [225].* They suggest there are two key elements to reflexive research – interpretation and reflection [225]. It therefore entails frequent and continual reflection of the manner in which one's own beliefs, values, views, position, and interests influence the research process, shape the data being collected and how it is interpreted [225]. I maintained notes of my reflections of the research process at every stage. These included my reflections on my position as a researcher from the MRC Unit, working as a paediatrician in this community with a keen interest in the caring for malnourished children, married with her own young children, from a different rural region of Africa and a more privileged background, not fluent in the local language, from a different faith, in relation to the research participants and how this affected my interactions with the carers and my interpretation of the data. The study nurse conducting the interviews also regularly shared her reflections with me during the debriefing sessions. She reflected on how her position as a nurse working for the MRC Unit, married with children, of the same faith as the participants but who was from a different region of The Gambia with a more privileged background, would also influence the dynamics with the carers. These reflections helped us to develop strategies that aimed to mitigate these power dynamics, in order to encourage the mothers to share their perspectives and not focus on giving us the "right" set of responses. Some of the strategies included spending time with the carers as they undertook their daily activities on at least 2 occasions before the IDIs. This helped to develop a rapport and build trust with the carers. I also convened regular de-briefing sessions with the study team where any challenging or fulfilling interactions were discussed, in addition to reflections on roles and positionality of all the team members in the data collection and analysis process.

6.8.7 Strengths and limitations

Study design

A key strength of this mixed methods study approach is that it enables the researcher to explore the meaning of a phenomenon through description [162]. For this study, the use of quantitative questionnaires in the formative phase, informal observations and IDIs with a diverse group of independent respondents facilitated triangulation of the findings that enhanced the comprehensiveness and credibility of the study [163, 164]. It ensured that all aspects of infant feeding and rearing practices as well as the pathways to severe wasting in infants in this rural Gambian community were investigated [164].

Another strength of this work is the iterative study design. The homes of some of the participants were visited 2-3 times from the formative phase to the interview phase. This helped the researchers to build a rapport with the mothers and members of their households, which facilitated the sharing of sensitive information by carers during the research process. Prolonged engagement with participants enhances the credibility of findings in qualitative studies [163]. Krefting, in proposing methods for ensuring rigor in qualitative research states that, *"the researcher spends sufficient time with the informants to identify reappearing patterns"* [163]. Increased engagement with participants grants the researcher opportunities to check perspectives whilst the participants become more comfortable with the researcher [163]. However, this extended engagement also had its challenges as on some occasions the regular presence of the study teams in homesteads gave participants and other family members opportunities to share their or the family's medical problems in the hope that we would be able to provide medical treatment for them in the community. In all these instances, we referred the ill participants or family members to the clinic and those requiring urgent attention were immediately transported to MRC Keneba clinic in the study vehicle and the nurse or doctor on call for acute admissions was alerted of their referral.

The iterative study design also assured dependability of the findings [163]. As the quantitative questionnaires were completed before the IDIs were commenced, the study nurse reviewed the participants' questionnaire responses before visiting their homes and relayed some of the initial responses to them during the interviews, in order to check

whether their initial responses were captured accurately. In this way the participants' responses to the on-going analysis became part of the research data [226].

Chapter 7 General discussion, conclusion and future research

7.1 Main findings

In this PhD, I set out to evaluate the secular trends and timing of growth faltering in early childhood in a select group of children in rural Gambia who grew up within the three core study villages of MRC Keneba. I also explored maternal and infant factors associated with SAM in infancy and described the changes in the energy regulating hormones during nutritional rehabilitation of rural Gambian children.

Four decades of nutrition and public health interventions have resulted in a halving of underweight (from 39% to 22%) and stunting (from 57% to 30%) at 2 years. In addition, growth in the first 2 years was less susceptible to seasonal changes in the more recent decades. However, most concerning was the finding that despite these interventions, patterns of postnatal growth faltering between 3 and 21 months of age and the prevalence of wasting at 2 years were unchanged. These patterns of growth faltering are similar to those from an analysis of cross-sectional anthropometric data from under 2's in LMICs [13], which suggests that some common adverse factors exist in all these countries that need to be explored in order to develop more effective interventions.

I explored these factors in my third study that involved a cohort of mothers and infants who had received intensive health worker involvement in the antenatal and postnatal period, but despite this, a number of the infants developed severe wasting in infancy. I found that some mothers were not able to adhere to the recommended infant feeding practices due to inadequate social support networks (most importantly her husband), infant feeding difficulties (e.g. the perception of inadequate amounts of breast milk or an infant who refuses to eat the complementary feeds offered in the context of food insecurity where dietary variation is not possible) and maternal psychosocial stressors (including difficult marital relationships). In the context of LMICs, these findings highlight the fact that blanket interventions to communities may not reach the most vulnerable and therefore there is a

need to provide more targeted nutrition-specific and nutrition-sensitive interventions in order to tackle growth faltering. A recent Cochrane review has shown that the greatest effect on birth outcomes are noted when antenatal nutritional supplements are given to undernourished mothers. My findings contribute to the evidence that shows that there is an urgent need to address the underlying determinants of growth faltering i.e. nutrition-sensitive interventions, in order to enhance and sustain the impact of the nutrition-specific interventions in LMICs [130].

In my second study, I found that there was a very high degree of variability in the nutritional recovery of children with SAM who had no complications and those with MAM, who were predominantly orally fed and managed in a community setting. In fact, some children experienced some deterioration in WAZ despite intensive nutritional rehabilitation, even though none of them were noted to be oedematous at baseline. As in other observational studies, baseline leptin, IGF-1, IGBFBP3 were low at baseline and increased with increasing WAZ in the first 2 weeks [149]. Conversely, baseline ghrelin, cortisol and sOBR were negatively associated with WAZ [149]. After two weeks, there was increased variability in WAZ and hormone levels in individual children therefore, only baseline C-peptide predicted the changes in WAZ over 28 days of interventions. These findings therefore suggested that the optimal period for the change from a pro-inflammatory state to anabolic state appears to be in the first 2 weeks of outpatient nutritional rehabilitation and thereafter there appeared to be a resurgence of the pro-inflammatory state, which would have been the period after completion of antibiotic courses, therefore diminishing nutritional recovery.

7.2 Strength of the work

This work had a number of strengths that included:

The design of all the three studies was hypothesis-driven and addressed public health issues that were relevant for The Gambia and other SSA countries. This therefore ensured that the studies were designed with a great deal of scientific rigor while taking into consideration the immediate translation of the findings to inform policy.

Data collection for two of these studies (Research Paper I and II) was done under 'normal' conditions i.e. routine anthropometric measurement for routine clinic visits and nutritional rehabilitation under standard outpatient conditions. The findings of these studies are therefore applicable to similar community level facilities and can therefore help in the development of practical interventions.

The use of research tools (modified EDS) and techniques (qualitative methods) for Research Paper III, that were not common place in this research setting, whose focus was on basic science/discovery research. In the process of undertaking this work, I was able to train and build expertise in these areas within the field station. Subsequent research studies have utilized these skills with more projects on maternal and child nutrition incorporating tools to assess maternal mental health status and qualitative methods to explore factors that hinder progress in this area of research/health.

I undertook these studies at a research facility (MRC Keneba field station) that for nearly six decades has produced high quality evidence to inform nutrition interventions in LMICs. I therefore worked with a very knowledgeable team who critiqued my study proposals and designs therefore, making them scientifically rigorous but also practical in their application in the field. I also received excellent support and supervision that have helped me develop as a clinical scientist.

This work also facilitated collaborations with world leading investigator scientists in social sciences, epidemiology and anthropology in Kenya (CSM, MKM) and the USA (RM), who also assured the scientific rigour of the study design, data collection, analysis and manuscript preparation.

7.3 Limitations of the work

There were a number of limitations with this work that included:

For one of the studies (Research Paper I), I used routinely collected clinic anthropometric data that would have not been subject to the rigorous procedures of a scientific study e.g. paired measurements and double entries, minimising missing data. I was also not able to

control for variations in the measurement methods with each decade. However, over the years, both the clinic and field staff at the MRC Keneba field station have been trained on taking anthropometric measurements according to a standard operating procedure on a regular basis. In addition, the measuring equipment is checked and calibrated on a regular basis and faulty equipment is replaced in a timely manner. This therefore provided some degree of validity for these measurements. Also, each child would have had over 5 measurements and during analysis significant outliers were excluded.

The sample size for Research Paper II was small and therefore my findings were not conclusive. However, this was intended to be an exploratory study to describe the dynamics of energy regulating hormones during nutritional rehabilitation in a community setting that had only previously been done once in SSA. Secondly, it also aimed to explore a hypothesis about the biology of the important pleiotropic hormone leptin, which if confirmed would have given us new and previously unexplored insights that could have led to a new understanding of its biology and therefore application for the prevention and management childhood undernutrition.

A major limitation of Research Paper III, was that I retrospectively collected data on infant feeding, care practices and illness episodes with the limitation of recall bias [171]. I was however able to validate these findings with data that was prospectively collected from the trial and where there were major discrepancies, I used the prospectively collected data to mitigate the effect of recall bias.

In addition, in Research Paper I, I did not evaluate the secular trends in catch-up growth beyond 2 years or describe the trends in developmental outcomes over the four decades, which would help to fully understand the long-term impact of the persisting patterns of growth faltering in the first 24 months.

7.4 Practical implications of the study findings

In Research Paper I, the timing and trends in growth faltering suggest that the current health and nutrition interventions including free access to primary health care services have not adequately addressed this problem. This study supports the global health strategy of targeting interventions in the critical window of “1000 days” particularly in vulnerable

populations in LMICs [46]. However, these strategies need to be sustainable to ensure that children continue to grow adequately right through to adulthood to break the cycle of intergenerational adverse outcomes [21, 47, 185]. The evidence currently suggests that there is no “magic bullet” that can mitigate growth faltering and that a package of interventions that include both nutrition-specific and nutrition-sensitive interventions are required [115]. In this respect, this study showed that improvements in WASH conditions at a community level did not mitigate growth faltering, which several trials have also found [227]. This is possibly because, this level of intervention under conditions of poor housing and unhygienic environments, where domestic animals live at very close proximity to humans is not adequate to prevent children from being exposed to multiple infections in early childhood, especially once they become mobile [228]. These exposures most commonly result in subclinical EED and chronic systemic inflammation for which there is currently no effective treatment [228-230]. This therefore suggests that a higher threshold for WASH would need to be met in order to prevent these exposures in early childhood that include significantly improving housing and living conditions (i.e. provision of piped water into households) whilst addressing other social determinants of growth faltering that requires a multi-sectorial approach [193].

Research Paper III, explored the determinants of SAM in infants at an individual and family level in a cohort that received intensive health interventions with weekly home visits by study field workers, accompanied by free transport and access to primary health care services 24 hours a day. In this cohort, I found that mothers/ families that were not amenable to these interventions were those that experienced adverse psychological (e.g. marital discord), social (e.g. lack of support networks) and economic (e.g. food insecurity) circumstances. This study therefore suggests that in addition to public health interventions at the community level, targeted interventions at the most vulnerable families in a community (e.g. food rations, provision of farm inputs, relationship peer counseling) are also required.

Finally, the findings of Research Paper II suggest that in CMAM, baseline C-peptide can be explored in a larger prospective study as a possible predictor of nutritional recovery in addition to other potential biomarkers. In addition, it suggests that the continuing exposure to the unhygienic environmental conditions during nutritional rehabilitation may prolong

the period of nutritional rehabilitation due to the recurrence of chronic inflammation. Therefore, CMAM should incorporate strategies that limit these exposures either through education, which may have limited value of its own in the context of poverty, or combine this with the provision of clean water, handwashing equipment and sanitation.

7.5 Overall conclusion

Growth faltering in early childhood in rural Gambia remains a significant public health problem that has not been amenable to intensive primary health, public health and nutrition interventions. At an individual and family level, adverse psychosocial factors constrained mothers from adhering to the recommended infant feeding and rearing practices and this was associated with severe wasting in infancy. In addition, children undergoing outpatient management of SAM and MAM showed great variability on WAZ after 14 days of nutritional rehabilitation. Baseline C-peptide was predictive of future response to nutritional recovery but would not be a useful clinical marker in isolation.

7.6 How well were the original objectives met?

The overall aim was to evaluate growth faltering in early childhood in rural Gambia. Using the three studies I was able to describe the secular trends and timing of growth faltering in early childhood, the maternal and family determinants of severe wasting in infants and the hormone changes during outpatient nutritional rehabilitation in rural Gambia. Therefore, the original objectives were satisfactorily met.

7.7 Lessons learnt and skills acquired

Lessons learnt

Maternal mental health is a condition that is rarely explored in this community unless a mother presents frankly psychotic. In research Study III, although the pilot work that we undertook with the modified EDS showed that the tool was accepted in the community, during the administration of the modified EDS mothers often played down their symptoms of mental distress. Further exploration of this issue during the IDIs yielded very shallow responses as well. This therefore appears to be a subject that the mothers are not willing to

discuss. In future, incorporating mental health information into the village sensitization sessions and learning from the community about ways to approach and discuss it with them would be prudent.

Skills acquired

- Developing research proposals and protocols, designing a database, applying for scientific and ethics approval, managing a study budget, undertaking field work and clinical research according to the standard operating procedures of the study protocols, data collection using questionnaires, designing analysis plan.
- Manuscript preparation and submission to peer reviewed journals and responding to reviewers' comments in a timely manner, employing diplomacy when points of view differ.
- Working with collaborators: negotiations of terms and conditions and an understanding of collaborative agreements.
- Engaging the community and policy makers during proposal development, prior to commencing study activities and sharing scientific findings from studies.

Skills enhanced

- Quantitative data management and data analysis in Excel and Stata.
- Qualitative study design, training of study team, in-depth interviews and debriefing with study team, data management, synthesis and analysis and manuscript preparation.
- Managing a study team and clinical team and developing leadership skills.
- Presenting scientific data at regional, national and international conferences and networking with leaders in the field of nutrition in LMICs and potential collaborators.

7.8 Further research

This work highlighted areas that need to be explored in future research in addressing the determinants of growth faltering in children in LMICs and the nature of interventions.

The persistence of growth faltering in early childhood despite public health and nutrition interventions suggests that future research should explore the impact on growth of children

in this rural community, of a combined package of interventions that include significant improvements to housing, ready access to clean water and sanitation within households (infrastructure) coupled with education on hygiene and safe infant feeding practices (behaviour) that can be delivered using community health workers and peer support groups with support supervision from the nurses at the local health facilities in the context of a cluster randomized controlled trial. During the planning and development of these interventions, it would be key to undertake some formative research to explore the community perceptions of the relevance, acceptability and sustainability of these interventions.

At an individual/family level future research into a targeted package of interventions for vulnerable families within a community that addresses food insecurity, maternal psychosocial support, involvement of fathers in child health promotion strategies, improving access to infant nutrition messages and gender empowerment of women should be explored. The effectiveness of this package of interventions in reducing the rates of undernutrition in this community can be evaluated using a comparative study using historical controls due to the ethical challenges of not providing an intervention to vulnerable households. The acceptability and sustainability of this package of interventions would also need to be explored during the development of this work with the community as well as the relevant stakeholders such as the Gambian Ministry of Health, the Gambian National Nutrition Agency and international agencies such as UNICEF and World Food Programme (who are involved in nutrition strategies for vulnerable populations in The Gambia).

Further exploration of the causes of high variability in WAZ after the first 14 days of outpatient nutritional rehabilitation is also warranted. A longitudinal study that assesses specific markers of systemic and enteric inflammations, as well as closer monitoring of dietary protein and energy consumed, and further exploration of baseline C-peptide as a possible predictor of nutritional recovery, would help in the development of CMAM protocols, in order to enhance and sustain nutritional recovery in the community.

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Chapter 8: Appendices

Appendix I: Timeline for PhD activities

Activity	Nov 2012- April 2014 Year 1	May 2014- April 2015 Year 2	May 2015- April 2016 Year 3	May 2016- May 2017 Year 4
Literature review				
Proposal development for research Study I & II				
Application for ethics approval, preparation of protocols, setting up database and data collection for Study II				
Upgrading report and seminar				
Interruption of studies for 6 months for maternity leave				
Data cleaning and data analysis research Study I & II				
Writing scientific papers for research Study I & II				
Application for ethics approval, preparation of protocols, setting up database and data collection for Study II				
Data cleaning and data analysis research Study III				
Writing scientific paper for research Study III				
Writing of thesis				

Appendix II: Photographs from fieldwork in West Kiang

A. Mother breastfeeding her baby during the interview

B. Animal feeding from cooking pot

C. Pit latrines

   i. Gravel flooring

ii. Plastered flooring

ii. Slab-flooring

D. Water source in village

Appendix III: Research Paper I supplementary material

Growth faltering persists in rural Gambian children despite four decades of interventions

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Supplementary material- contents

1. Supplementary study population

The UK Medical Research Council has supported a research centre in the remote rural Gambian village of Keneba since 1949. Studies initially consisted of demographic records of births, deaths, marriages and migrations collected by village recorders and an annual survey of health and nutritional status in Keneba and 3 nearby villages: Manduar, Kantong Kunda and Jali [151]. In 1974 the Dunn Nutrition Unit commenced more intensive research and provided full-time clinical services to 3 of the villages (Jali declined to participate).

2. Supplementary methods

Anthropometry

The anthropometry measurements were performed in the clinic by trained clinic staff. Weight measurements were performed with the infants unclothed and recorded in kilograms to two decimal places. In the early decades, birth weights and subsequent weights were recorded using manual Salter spring balance and tared sling (Salter Industrial Measurements Ltd, West Bromwich, United Kingdom) and Todd Scales, Cambridge, UK. In the later decades electronic Seca 336 high precision portable baby weighing scales were used. All the weighing scales were calibrated regularly. Lengths were measured on Holtain infantometer (Holtain, Crymmych, UK) in the early decades but more recently on Kiddimetre (Raven Equipment, Great Dunmow, Essex, UK). The Leicester plastic stadiometer was used to measure maternal heights in the later years. Tape measures were used to measure the infants' mid upper arm circumferences and head circumferences. Sex and age-adjusted z-scores for weight for age (WAZ), length for age (LAZ), weight for length (WLZ), mid upper arm circumference (MUACZ), head circumference (HCZ) and birth weight (BWTZ) were calculated by comparison to the WHO 2006 growth standards [1]. Stunting, wasting and underweight are defined respectively as height-for-age, weight-for-length, weight-for-age of 2 or more standard deviations below the WHO reference median.

We define growth faltering in terms of the fall in z-scores between 3 and 21 months in order to avoid both the complications of catch-up growth in the first 3 months and the inevitably poorer estimates of the curves at the extremes of the data range.

Statistical Methods

Effects of age and season on repeated growth parameters were fitted using random effects models. Models for males and females and each decade were fitted separately. To describe secular changes in growth by decade i.e. rates of stunting, wasting and underweight in 2 year olds plotted in Figure 4, we fitted random effects logistic regression of the binary variable on the first four orthogonal polynomials in age and the first pair of Fourier terms for season (see below).

To describe the effect of season on growth, seasonal patterns of body size were obtained by "Fourier regression" [172, 231]. Briefly, Fourier regression represents the seasonal pattern as a Fourier series whose higher order terms are regarded as high-frequency noise and discarded. The resulting truncated series is a linear combination of trigonometric functions of θ, the angle representing the phase of the year when the measurement was made, and whose coefficients are readily estimated as part of the regression model. We fitted the first three pairs of Fourier terms and controlled for age by including the first three orthogonal polynomials in age (*age*1*, age*2*, age*3) in the model. Thus the j^{th} observation for the i^{th} individual is given by:

$$Y_{ij} = \beta_0 + \beta_1 age1_{ij} + \beta_2 age2_{ij} + \beta_3 age3_{ij} + \sum_{k=1}^{3}\left[\alpha_k \sin\left(k\theta_{ij}\right) + \beta_k \cos\left(k\theta_{ij}\right)\right] + \tau_i + \varepsilon_{ij}$$

where the α_k and γ_k are the coefficients for the Fourier terms and the βs the remaining regression coefficients; τ_i is the random effect due to the i^{th} individual and ε_{ij} is the error term. We appreciate that seasonal patterns may themselves vary with age. Allowing for this would have unduly complicated the analysis and is unnecessary for the purpose of this paper. The seasonal patterns we estimate are therefore averaged across all age groups.

In order to quantify the children's susceptibility to seasonal changes (as plotted in Figure 5) we estimated the amplitude of the seasonal pattern, which we define as the square root of half the sum of the squared Fourier coefficients:

$amplitude = \sqrt{\sum_k [\alpha_k^2 + \gamma_k^2]\,/2}$.[172]

The delta method was used to estimate the standard error of these estimates (employing Stata's post-estimation command *nlcom*).

To describe the changes in body size with age, plots of mean z-score versus age (Figure 2) were produced by fitting age with 10-knot cubic regression splines and controlling for season by including the first pair of Fourier terms.

Estimates of mean values and their standard errors calculated at particular ages for each sex and decade were taken from the predicted values yielded by the above regression models. We quantify growth faltering as the drop in z-score over the 18-month interval starting at 3 months of age. These estimates are all simple linear combinations of the regression coefficients and their standard errors calculated using the variance-covariance matrix for the regression coefficients i.e. the Fisher information matrix (employing Stata's post-estimation command *lincom*).

The estimation of age specific disease incidence over the different decades was done by dividing the total number of children diagnosed with the disease in each age group as numerator, by total number of children 2 years of age or under who were seen in the clinic.

We perform no formal statistical hypothesis tests. With such large volumes of observational data almost any difference examined would be significant so statistical significances provide poor means of discriminating between important and trivial patterns in the data. Instead we focus on estimating effect sizes and their confidence intervals. All analysis was performed using Stata 12. (StataCorp, College Station,TX).

3. Supplementary tables

Table 1: Summary of previous studies that have assessed trends in growth faltering in African children

Study	Study design (interval)	Country	N (age group)	Main findings
Said-Mohammed et al. 2015[232]	Systematic review (1970-2013)	South Africa	50 studies (under 6 years)	NCHS reference, from 1993 to 2003 the prevalence of stunting increased by 2.9 % (z-test, $p < 0.05$). WHO standard, the prevalence of stunting decreased by 5.9 % between 1999 and 2013 (z-test, $p < 0.001$). However, the 2008 National Income Dynamic Study (NIDS) showed an increase of 6.8 % from the 2005 National Food

				Consumption Survey (z-test, p < 0.001).
Gray S et al. 2010[41]	Longitudinal study (1998-2004)	Uganda	123	Noticeable declines in weight velocity occurred in the fourth month and after the sixth month. Weight gain was static after the second year, when upward of 40% of children were clinically underweight.
Kalanda BF et al. 2005[39]	Longitudinal study	Malawi	(0-1 y)	Low birthweight infants were shorter and lighter throughout infancy than either normal birthweight or international reference values. At 12 months, placental or peripheral malaria at delivery (adjusted odds 1.8; 1.0, 3.1), number of infant illness episodes (AOR = 2.1; 1.2, 3.6) and maternal illiteracy (AOR = 2.7; 1.5, 4.9) were independently associated with low weight for age. Maternal short stature (AOR = 1.8; 1.1. 3.2), male sex (AOR = 2.4; 1.4, 4.1), number of infant illness episodes (AOR = 2.6; 1.5, 4.4), and birth in the rainy season (2.1; 1.2, 3.7) were independently associated with stunting. Placental or peripheral malaria at delivery (AOR = 2.2; 1.1, 4.4) and number of illness episodes (AOR = 2.2; 1.1, 4.5) were independently associated with thinness.
Hauspie RC et al 1989[37]	Longitudinal study	Democratic Republic of Congo	4030 (0-4 y)	Growth weight velocity slows down below average in the rainy seasons.

Table 3: Summary of randomised trials of nutrition interventions in The Gambia aimed at improving growth

Study	**Study design** **(intervention)**	**Country**	**N** **(mother/ infant/children or mother-infant pairs)**	**Main findings**
Johnson et al. 2016 [197]	RCT (Oral 1. Iron-folate (FeFol = standard care)	The Gambia	620	Despite evidence of between-arm differences in some fetal biometry, z-scores at birth were not greater in the intervention arms than the FeFol arm (e.g., birth weight z-scores: FeFol -0.71, MMN -0.63, PE -0.64, PE + MMN -0.62; group-wise p = .796). In

	2. Multiple micronutrients (MMN) 3. Protein-energy (PE) 4. MMN+PE)			regression analyses, intervention associations with birth weight and head circumference were modified by maternal weight gain between booking and 30 weeks gestation (e.g., PE + MMN associations with birth weight were +0.462 z-scores (95% CI [0.097, 0.826]) in the highest quartile of weight gain but -0.099 z-scores (-0.459, 0.260) in the lowest).
Goldberg et al. 2013 [233]	RCT (Oral calcium)	The Gambia	525	No significant effect of calcium supplementation on infant growth. (Not designed with birth weight as an outcome therefore, it wasn't powered to look at birthweight.)
van de Merwe et al. 2013 [87]	RCT (Oral long chain polyunsaturated fatty acids (PUFA))	The Gambia	172	PUFA supplementation resulted in a significant increase in plasma n-3 LC-PUFA concentrations ($P < 0.001$ for both DHA and EPA) and mid upper arm circumference (MUAC) (effect size: 0.31 z scores; 95% CI: 0.06, 0.56; $P = 0.017$) at 9 mo of age. At 12 mo, MUAC remained greater in the intervention group, and we observed significant increases in skinfold thicknesses ($P \leq 0.022$ for all). No other significant differences between treatment groups were detected for growth or LMRs at 9 mo or for secondary outcomes.
Unger S 2013 (Thesis) [234]	RCT (Oral MMN)	The Gambia	1101	Multiple micronutrient supplementation was associated with a small increase in height-for-age z-scores 24wk after recruitment (effect size for MMN groups combined: 0·084 SD/24wk, 95%CI: 0·005, 0·168; p=0·037; equivalent to 2-5mm depending on age).
Williams et al. 2007 [235]	RCT (Oral glutamine)	The Gambia	93	Gambian infants showed a seasonal deterioration in growth and persistently elevated acute phase protein concentrations and intestinal permeability. Oral supplementation with glutamine did not improve growth (x +/- SE: weight gain, 60 +/- 19 and 69 +/- 20 g/mo; length gain, 1.01 +/- 0.05 and 0.95 +/- 0.03 cm/mo) or intestinal permeability [lactulose: mannitol ratio: 0.29 (95% CI: 0.23, 0.35) and 0.26 (95% CI: 0.21, 0.32)] in the glutamine and placebo groups, respectively.
Darboe et al. 2007 [85]	RCT (Oral vitamin A)	The Gambia	197	Apart from a transient difference in length between the treatment groups at 6 months (in favour of WHO dose of Vitamin A), there was no detectable effect of the supplementation regimen on growth.
Jarjou et al. 2006 [236]	RCT (Oral calcium)	The Gambia	125	No significant differences were detected between the groups in breast-milk calcium concentration, infant birth weight, or growth or bone mineral status during the first year of life.
Krähenbühl et al. 1998 [84]	RCT (High fat or high carbohydrate biscuit)	The Gambia	90	Neither the high fat nor the high carbohydrate supplement had an effect on weight or height gain. The high fat supplement did slightly increase adipose tissue mass.

Ceesay et al. 1997 [237]	RCT (High energy groundnut biscuit)	The Gambia	2047	Maternal weight gain increased by 201 g (P < 0.001) in the hungry season, by 94 g (P < 0.01) in the harvest season (November to May), and by 136 g (P < 0.001) over the whole year. The odds ratio for low birthweight babies in supplemented women was 0.61 (95% CI 0.47 to 0.79, P < 0.001). Head circumference was significantly increased (P < 0.01), but by only 3.1 mm. Birth length and duration of gestation were not affected.
Hoare S et al. 1996 [238]	RT (Oral high energy supplement)	The Gambia	40	With a 50% increase in energy intake and a 100% increase in protein intake there was a rapid and highly significant (P < 0.001) gain in weight within a fortnight whether the supplement was given immediately or 2 weeks after presentation. Rates of weight increase were similar whether supplementation was provided early or late, but over the full 28 d (of intervention and non-intervention) children who received late supplementation had greater overall weight gain (P < 0.02) than those supplemented early.
Bates et al. 1993 [83]	RCT (Oral zinc)	The Gambia	110	Body weights and arm circumferences showed a linear increase, plus a seasonal effect (rainy season faltering). For body weight there was no significant overall effect of the supplement. For arm circumference, a very small (2%) but significant (P < 0.01) difference favoured the supplemented group.
Prentice AM et al. 1987 [82]	RCT (Oral energy-dense supplement)	The Gambia	379	Supplementation was ineffective during the dry season but highly effective during the wet season: +225 +or- 56 grams, p=0.001 (unadjusted) or +200 +or- 53 grams, p=0.001 (adjusted for sex, season, and parity) by between-child multiple regression analysis; +231 +or- 65 grams, p=0.001 by within-mother analysis. The proportion of babies of low birthweight (2501 grams) decreased from 23.7 to 7.5%, p=0.002.

Appendix IV: Research Paper II supplementary material

Hormonal Correlates and Predictors of Nutritional Recovery in Malnourished African Children

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Supplementary material- contents

1. Supplementary background

Insulin-like-growth factor-1 and 2 (IGF-1 and 2) are the primary growth-promoting factors from fetal life until 6 months of age [1]. Thereafter the growth hormone (GH)-IGF-1 axis, and its interplay with the key insulin-like-growth factor binding protein-3 (IGFBP3), play an important role in regulating and coordinating postnatal growth [2]. Leptin is a pleiotropic hormone that plays key roles in energy homeostasis, appetite, immune regulation, modulation of intestinal barrier function and haematopoiesis [3]. Ghrelin is an orexigenic hormone that can also stimulates the release of GH, and modulates digestive properties, sleep and muscle integrity [4]. Paradoxically, ghrelin levels are significantly higher in severely wasted children compared to controls, even in the context of poor appetite but decline with nutritional rehabilitation [5, 6].

2. Supplementary methods

Study population

At presentation, children were assessed and treated for any medical conditions by a paediatrician or medical officer. A medical and dietary history (including breastfeeding practices), sociodemographic profile, and physical examination was completed at enrolment.

Note that growth in this population is generally poor so that, while the control group can be considered better nourished than SAM or MAM, they do not represent normal healthy growth according to international reference growth curves. WHZ scores against an internal reference generated using pooled anthropometric data from the Keneba database were: Controls 0.0, MAM -1.0 and SAM -1.7.

SAM was defined by one of three criteria: 1) WHZ less than -3; and/or 2) mid upper arm circumference (MUAC) less than 115 mm; or 3) bilateral pedal oedema with other clinical features of kwashiorkor [7]. MAM was defined as WHZ less than between -2 and -3 [8].

Nutritional composition of WHO-F75

WHO F-75 is used in Phase 1 of the treatment of children over 6 months of age, suffering from severe acute malnutrition (SAM). It contains 75 kcalories and 0.9 grams of protein per 100 ml. Ingredients include concentrated milk powder, food oil and dextrin vitamin complexes to provide a reduced amount of proteins, fats and sodium, but rich in carbohydrates. [9]

Study interventions

The SAM children were initially managed in the nutritional rehabilitation unit (NRU) at MRC Keneba. Their care was supervised by the study nurse and 3 auxiliary nurses supported by a medical officer and paediatrician. On admission they received F-75 (starter formula) then, F100 (catch up formula) and subsequently, Plumpy'Nut (Nutriset) ready-to-use-therapeutic food (RUTF) once their appetite and clinical condition had improved. During the initial stabilisation phase, all children received a 1 week course of oral or intravenous antibiotics. The children were encouraged to take oral feeds from the outset, which most of them tolerated. Only four children who did not initially tolerate the oral feeds had nasogastric feeds for 24-48 hours. All the children over 1 year of age received oral mebendazole 250 mg on admission for treatment for parasitic infections. Children who presented with diarrhoea received oral zinc 20 mg per day for 10-14 days and the anaemic children with SAM also

received one iron/folic acid combined tablet (Iron 60mg, Folate 400 µg), if <10kg and two tablets if >10kg, every two weeks after completing treatment for all the acute infections according to the Gambian guidelines. Children were discharged home from the NRU to the community management of malnutrition programmes (CMAM) when they were able to tolerate oral feeds including Plumpy'Nut and were gaining weight. Carers of children with MAM received nutritional counselling from the study nurse and received 28 days' worth of RUTF. Carers of the controls received nutritional counselling from the study nurse but the children did not receive any nutritional supplements. All the children in the study had anthropometric measurements done by the study field workers on alternate days from Day 0-28, then at 6 months from baseline.

Biological sample collection and analysis

A maximum of 5ml of blood per child was collected at each time point. Blood samples were collected at 10am (pre-meal) and 1 hour later (post-meal), to account for potential circadian variation in all hormones measured. The saliva samples were collected using SalivaBio's Children's Swab (Salimetrics, Pennsylvania) and stored at -70°C. Blood samples were placed immediately on ice and processed promptly, and plasma stored at -70° until analysis. The saliva samples were collected using SalivaBio's Children's Swab (Salimetrics, Pennsylvania) and stored at -70°C. Samples were shipped on dry ice to the University of Colorado at Boulder, USA for analysis.

The assays that used for the analysis of leptin, leptin soluble receptor (sOBR), IGF-1 and IGFBP3 were from R&D Systems (R&D Systems, Minneapolis, USA) [10-13], for total ghrelin Merck Millipore (Merck Millipore, Darmstadt, Germany) [14], for cortisol and C-peptide ALCPO (ALPCO, New Hampshire, USA) [15, 16] and for salivary C-reactive protein (CRP) from Salimetrics (Salimetrics, Pennsylvania, USA) [17]. These assays use the quantitative sandwich enzyme-linked immunosorbent assay technique (ELISA). Monoclonal antibodies specific for the antigens had been pre-coated onto their respective microplates. Standards and samples were pipetted into the wells and any respective antigens present were bound by the immobilized antibody. After washing away any unbound substances, enzyme-linked monoclonal antibodies (horseradish peroxidise enzyme in the total ghrelin and salivary assays) specific for the respective antigens were added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells

and colour developed in proportion to the amount of antigen bound in the initial step. The colour development was stopped and the intensity of the colour was measured spectrophotometrically and compared to reference standards [10-17]. The intra and inter-assay coefficients of variation (CV) were 3% and 4.2% for leptin, 2.2% and 5.3% for sOBR, 4.3% and 8.3% for IGF-1, 5% and 6.5% for IGBP3, 3.9% and 4.6% for total ghrelin and 3.3% and 7.6% for C-peptide, 6.3% and 10.4% for cortisol, 9.6%, 10.6 % for insulin and 4.7% for salivary CRP respectively.

Clean catch urine samples (obtained when a child passes urine directly into a sterile pot after their perineum has been cleaned, in our case with medicated soap and clean water) [18], were collected and stored in a laboratory refrigerator at temperatures between 2-8°C. Analysis of urine was done using Combur 9 test strips (Roche Diagnostics Limited, Switzerland) urine and the results were interpreted using a coloured scale [19]. A urine dipstick result that was positive for nitrites and leucocytes; nitrites and blood or leucocytes and blood was reported as a urine infection. The urine dipstick analyses were performed within 12 hours of collecting sterile urine samples.

Anthropometry

Lengths were measured on a Raven Kiddimetre (Raven Equipment, Great Dunmow, Essex, UK). Weight measurements were done with infants unclothed and recorded to the nearest 10 grams using electronic Seca 336 high precision portable baby weighing scales. All the weighing scales were calibrated regularly. The MUAC was recorded to the nearest millimetre using MUAC tapes. The knee heel length was measured using a knenometer (Chasmors Ltd, London, UK) and recorded to the nearest millimetre.

Statistical analysis

A mixed effects model was used to assess for differences in hormone levels between nutritional groups at baseline and over time, allowing for interaction between nutritional groups and time points, adjusted for age and gender, which accounted for repeated measurements in each individual child i.e. pre- and post-prandial levels and over time. A piecewise linear random slope model was used to assess the change in weight, MUAC, knee

heel, WHZ, weight-for-age Z-scores (WAZ) and height-for-age Z-scores (HAZ) over time and allow for variation in growth rate between individual children. We considered three time intervals: 0-14 days, 14-28 days and 29-180 days. A random slope was allowed for each time interval. The Wald test was used to test for interaction between time and nutritional group at both time intervals. Interaction terms, age at recruitment and sex were not included in the final model if there was no evidence at 5% level of significance.

3. Supplementary results

The median salivary CRP levels were lower in the controls (median [IQR] 2.9 [2.4, 4.1]) compared to MAM (median [IQR] 4.9 [2.8, 10.3]) and SAM (median [IQR] 5.6 [4.1, 9.9]); p=0.04. All the participants completed up to the 6-month follow-up visit. Children with SAM all received at least a week's course of antibiotics.

The average length of admission for children with SAM was 11 days and 18 (90%) were discharged to the CMAM programmes on or before Day 14. Only 7 (35%) of children with SAM had achieved a WHZ >-2 on Day 28, but the majority 18 (90%) were no longer severely wasted i.e. WHZ >-3. Eleven (61%) of children with MAM had a WHZ >-2 on Day 28.

Hormone status at baseline

There was a very strong correlation (r <0.9 in all cases) between the pre- and post-prandial values with no significant deviation from the Y=X line (see Bland-Altman plots in Supplementary Figure 2). The strength of these correlations validates the precision of the assays and shows high discrimination ratios indicating that each of the indices has the potential to be good predictors of response.

The IGF-1: IGFBP3 molar ratio was only significantly lower in SAM compared to the controls (geometric mean ratio 0.7 [95% CI: 0.6, 0.9], p<0.05).

Anthropometric changes over time

At the lower end two SAM and eight MAM children showed a slight deterioration in WAZ despite the intensive intervention, whilst at the upper end three children gained close to +2 Z-scores (probably indicative of some recovery-associated water retention). There was a non-significant tendency for girls to recover better than boys (+0.70 vs +0.38 WAZ, p=0.07).

Hormone changes over time

For the SAM group, there were also significant decreases in sOBR (geometric mean ratio 0.8 [95%CI: 0.7, 0.9], p<0.001) and ghrelin (geometric mean ratio 0.7 [95%CI: 0.6, 0.9] p<0.001)

over this time interval. However, from Day 0-28 of nutritional rehabilitation, the increases in total leptin, IGF-1 and IGFBP3 were only significant in the SAM group: total leptin (geometric mean ratio 1.6 [95%CI: 1.1, 2.3], p<0.001); IGF-1 (geometric mean ratio 2.2 [95%CI: 1.7, 2.8], p<0.001); and IGFBP3 (geometric means ratio 1.5 [95%CI: 1.3, 1.7], p<0.001). Conversely, there were significant decreases in total ghrelin in both MAM and SAM (geometric mean ratio 0.8 [95% CI: 0.6, 0.9], p=0.007 and 0.7 [95% CI 0.6, 0.9], p<0.001). In the SAM group, significant decreases were also found for sOBR (geometric mean ratio 0.8 [95%CI: 0.7, 0.9], p<0.001) and molar excess of sOBR: total leptin (geometric mean ratio 0.5 [95%CI: 0.3, 0.7], p<0.001).
There was weak evidence of declining cortisol levels from Day 0-28 in the SAM group (geometric mean ratio 0.8, [95%CI: 0.6, 1.1], p=0.03).

4.Supplementary discussion

In line with our findings, prior observational studies in low income countries have found that at baseline, SAM children have lower leptin, insulin, IGF-I and IGFBP-3; and higher basal cortisol, GH, soluble leptin binding receptor (sOBR) and IGFBP-1 compared to their non-malnourished counterparts [6, 20-22]. It has been hypothesised that low levels of leptin may stimulate the hypothalamic-pituitary-adrenal and hypothalamic–pituitary-growth hormone axes to maintain high cortisol and GH levels for lipolysis to provide fuel for the brain and other vital organs during nutritional deprivation, whilst maintaining low levels of IGF-1 and insulin until the onset of nutritional recovery [21-24]. These normalize to the levels in non-malnourished children within 2 weeks of intensive nutritional rehabilitation associated with rapid weight gain [6, 20-22].

A recent pilot study of children aged 18±4m being rehabilitated from SAM in our centre in rural Gambia, with age and sex matched community controls, found that in both groups a significant postprandial rise in leptin levels was found (Nweneka, Prentice *et al*, unpublished). We hypothesised, on the basis of Stein's prior finding of very high sOBR: leptin ratios in malnutrition [22], that this rapid rise of postprandial leptin was due to a circulating reservoir of leptin bound to the soluble binding receptor (sOBR) that is released into the circulation acutely with feeding. As no other studies, have shown acute post-prandial rises in plasma leptin we were concerned that the initial study resulted from a methodological artefact and sought replication in the current study. Our concerns were validated as we failed to replicate

an acute leptin response. Although there was no evidence of an immediate effect of feeding on the hormone levels during nutritional rehabilitation, our other findings on the more chronic responses of leptin and sOBR were consistent.

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11. Quantikine ELISA Human Leptin sR Immunoassay

http://www.rndsystems.com/pdf/dobr00.pdf Accessed 27/01/2017

12. Quantikine ELISA Human IGF-I Immunoassay https://resources.rndsystems.com/pdfs/datasheets/dg100.pdf Accessed 27/01/2017
13. Quantikine ELISA Human IGFBP-3 Immunoassay https://resources.rndsystems.com/pdfs/datasheets/dgb300.pdf Accessed 27/01/2017
14. Human ghrelin (total) ELISA kit 96-Well Plate (Cat. # EZGRT-89K) https://www.merckmillipore.com/INTL/en/product/Human-Ghrelin-%28total%29-ELISA,MM_NF-EZGRT-89K?ReferrerURL=https%3A%2F%2Fwww.google.com%2F&bd=1 Accessed 27/01/2017
15. ALPCO Cortisol ELISA https://www.alpco.com/pdfs/11/11-CORHU-E01.pdf Accessed 27/01/2017
16. ALPCO C-peptide ELISA https://www.alpco.com/pdfs/80/80-CPTHU-E01.1.pdf Accessed 27/01/2017
17. Salimetrics Salivary C-Reactive Protein ELISA kit https://www.salimetrics.com/assets/documents/1-3302.pdf Accessed 27/01/2017
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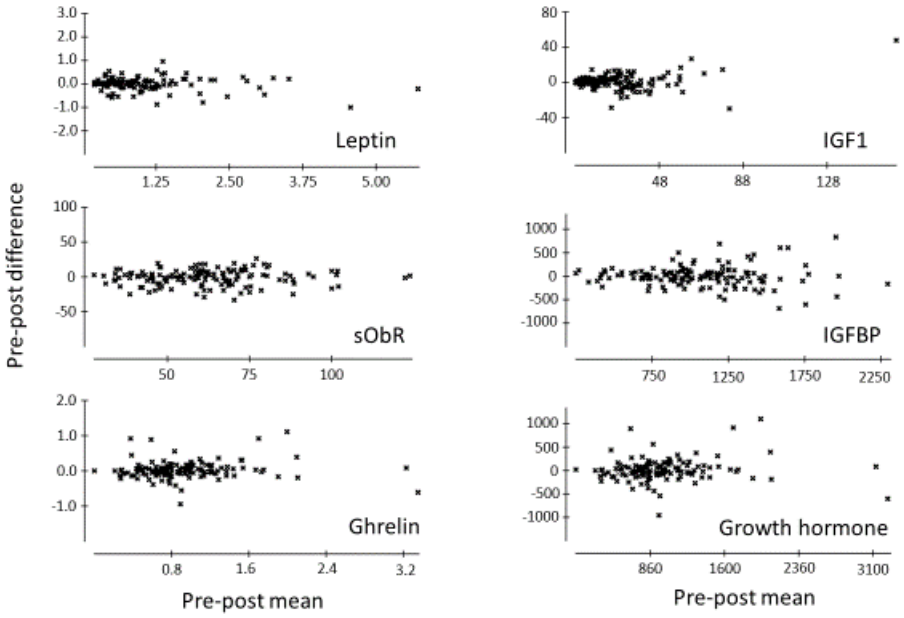
6. Supplementary figure



Supplementary figure 5.1: Correlation between the pre- and post-meal hormone differences

7. Supplementary figure Legends

Correlation between the pre- and post-meal hormone differences

I. Pre- and post-meal differences by pre-post mean in leptin levels.

J. Pre- and post-meal differences by pre-post mean in Soluble leptin receptor (sOBR) levels.

K. Pre- and post-meal differences by pre-post mean in total ghrelin levels.

L. Pre- and post-meal differences by pre-post mean in insulin like growth factor 1 (IGF-1) levels.

M. Pre- and post-meal differences by pre-post mean in insulin like growth factor binding protein 3 (IGFBP3) levels.

Appendix V: Research paper III supplementary material

Maternal psychosocial stressors and severe wasting in rural Gambian infants: a mixed methods approach

Nabwera HM, [1,2] Moore SE,[3] Mwangome MK,[4] Molyneux CS,[4,5] Darboe MK,[1] Camara-Trawally N,[1] Sonko B,[1] Darboe A[1], Singhateh S[1], Fulford AJ,[1,2] Prentice AM[1,2]

6. Medical Research Council Unit, The Gambia, P. O. Box 273, Banjul, The Gambia
7. Department of Population Health, London School of Hygiene and Tropical Medicine, Keppel street, London, WC1E 7HT, United Kingdom
8. Division of Women's Health, King's College London, 10th floor North Wing, St Thomas' Hospital, Westminster Bridge Road, London, SE1 7EH, United Kingdom
9. Kenya Medical Research Institute-Wellcome Trust Research Programme, P.O.Box 230-80108, Kilifi, Kenya
10. University of Oxford, Nuffield Department of Medicine, Henry Wellcome Building for Molecular Physiology, Old Road Campus, Headington, Oxford OX3 7BN

Supplementary material- contents

1. Supplementary methods

Study population

The Early Nutrition and Immune Development Trial (ENID, ISRCTN49285450) is a randomised trial designed to investigate the effects of combined pre-natal and infancy nutritional supplements on infant immune development. In the pre-natal arm, pregnant women (from <20weeks gestation) were randomized to 4 intervention arms [194]. From 6 months of age All pregnant women underwent voluntary counselling and testing for HIV as part of routine antenatal care and those found to be HIV infected were not recruited into the ENID trial, but were referred to the closest HIV care facility [197]. In total 875 mother-infant pairs were recruited into this trial from 2010-2015 [194].

Data collection

Quantitative

Mental Health Questionnaire

The Edinburgh Depression Scale (EDS) consists of ten questions and a woman can rate her depression symptoms on a scale of 0 (none) to 3 (severe). The total score ranges from 0 to 30 and scores of ≥12 are suggestive of depression. The reported sensitivity is 79% and specificity 85% [200, 202]. We utilised the principles of the WHO translation protocol with *"emphasis on the conceptual and cultural equivalence and not on the linguistic equivalence"[203].*

Data analysis

Quantitative

Principal component analysis

For the PCA, all the sociodemographic variables were converted to binary variables where missing values of distinct binary variables were replaced by the means of all summarized "0" values (asset not present) and "1" values (asset present). Initially all the variables that were based on the 10 indicators of childhood poverty as stated in the multi-dimensional poverty index report [239], were added to the PCA. This generated 30 principal components. Using the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy [174], variables with a KMO value of <0.50 or missing values were dropped from the model. The final PCA had 6 variables that were measures of assets, education and distance from the drinking water sources (Table 4). The overall value was 0.62 (mediocre) (Table 5). The first two principal components where the associated eigenvalue was greater than one were selected assuming that the first principal component was a measure of economic status (Figure 1) [240, 241].

"The eigenvalue (variance) for each principal component indicates the percentage of variation in the total data explained"[241].

2. Supplementary table

Table 1: Inclusion and exclusion criteria for the ENID randomized trial [194].

Inclusion criteria for ENID trial		**Exclusion criteria for ENID trial**	
Women Resident in West Kiang and aged between 18 – 45 y at August 1st 2008 Planning to remain resident in West Kiang for the next 36 months	Infants All infants born to women enrolled into the pre-natal arm of the study	Women Currently enrolled in another MRC study	Infants Major congenital malformations
		Current pregnancy (beyond 20 wk on ultrasound assessment)	**Severe malnutrition (weight-for-height Z-score < -3)**
		Severe anaemia (haemoglobin < 7 g/dL)	
		Known sickle cell disease	
		Reported onset of menopause	
		Known HIV infected	

Table 2: Sample size estimation

Number of cases ->				
33	**40**	**50**	**75**	**132**

prop. controls exposed						
	0.1	5.81	5.10	4.43	3.50	2.66
	0.2	4.18	3.72	3.29	2.70	2.15
	0.3	3.69	3.31	2.95	2.45	1.98
	0.4	3.55	3.18	2.83	2.36	1.92
	0.5	3.62	3.23	2.85	2.36	1.91
	0.6	3.93	3.45	3.02	2.45	1.96
	0.7	4.68	4.00	3.41	2.69	2.09
	0.8	7.06	5.61	4.49	3.28	2.39
	0.9	128.24	23.84	11.88	6.01	3.48

Alpha 5%; Beta 90%; Controls per case 3

Table 3: Kaiser-Meyer-Olkin measure of sampling adequacy of final principal component analysis model

Variable	KMO* measure
Motorcycle	0.77
Car	0.62
TV	0.60
Electricity	0.59
Cart	0.57
Bicycle	0.52
Overall	0.61

*Kaiser-Meyer-Olkin

Table 4: Eigenvectors in final principal component analysis model

Variable	Comp1	Comp2	Comp3	Comp4	Comp5	Comp6
Electricity	0.58	-0.24	-0.12	-0.20	0.14	-0.73
TV	0.55	-0.22	-0.03	-0.24	-0.26	0.67
Cart	0.26	0.61	-0.24	-0.33	-0.62	0.01
Bicycle	0.20	0.68	-0.10	0.41	0.57	-0.06
Motorcycle	0.46	-0.16	0.24	0.71	-0.43	0.09
Car	0.22	0.19	0.88	-0.34	0.12	0.08

3. Supplementary figures

Figure 1: Scree plot of eigenvalues from principal component analysis

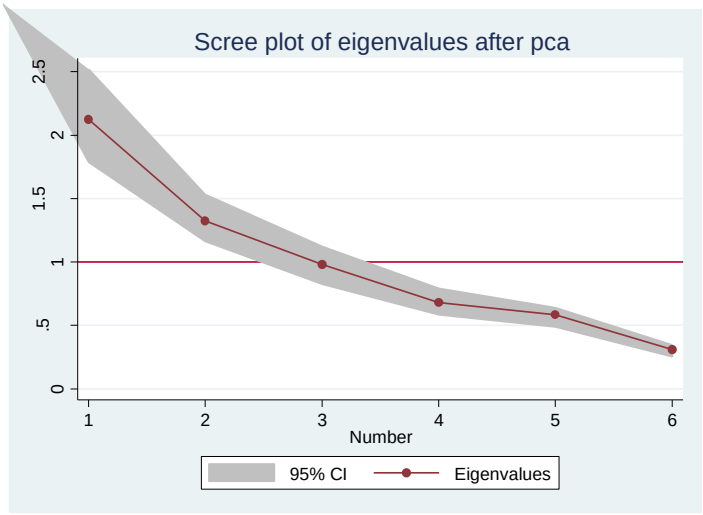
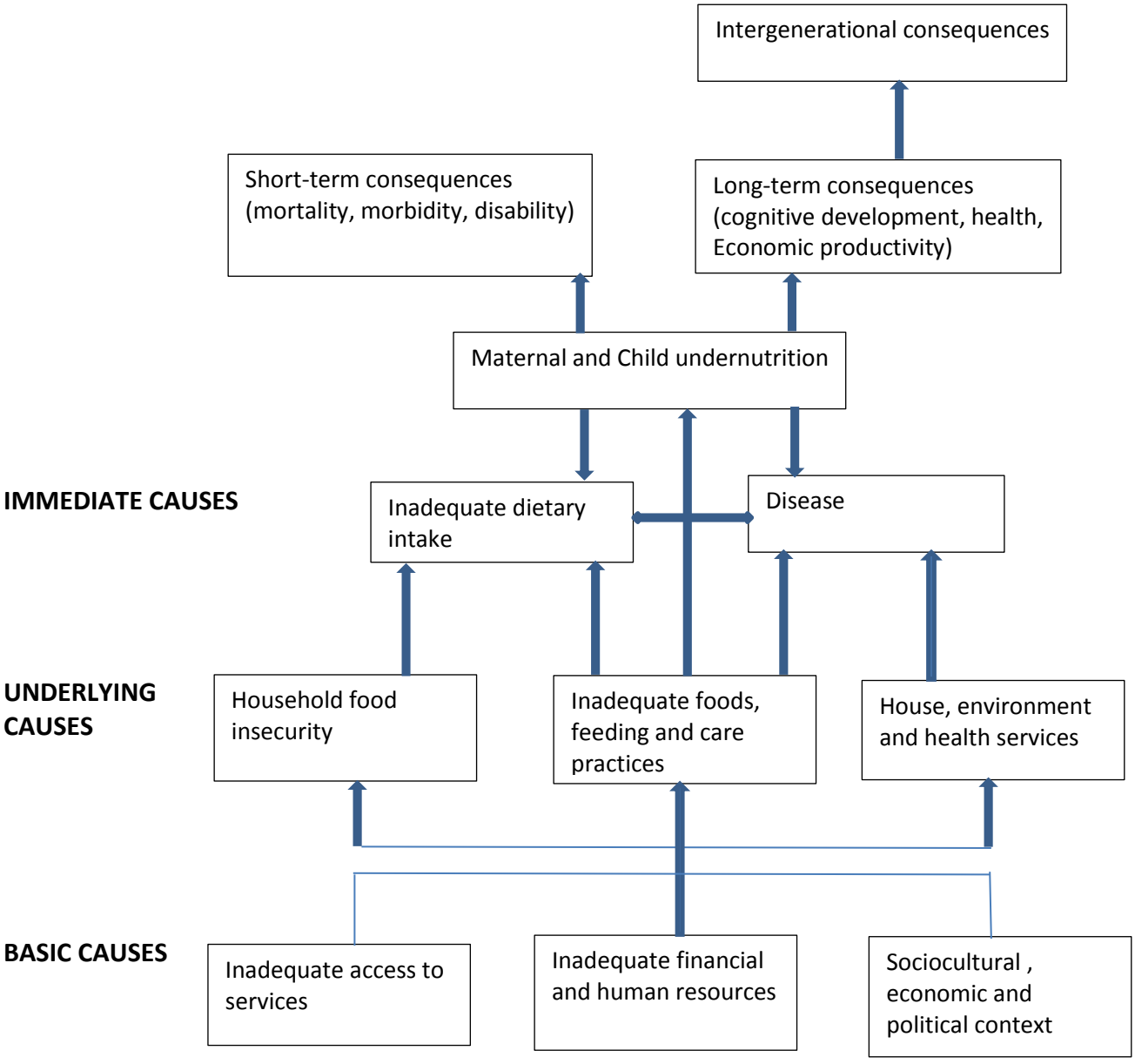


Figure 2: UNICEF conceptual framework for undernutrition (adapted) [191]



Appendix VI: Information and consent sheets

A. Research study II

MEDICAL RESEARCH COUNCIL UNIT, THE GAMBIA

Evaluation of hormonal regulation of growth in rural Gambian children

Participant Information Sheet

Version number 02 9th October, 2012

SCC 1306

(If necessary, to be read to participants in their own language)

What is the purpose of this study?

The growth of children in rural Gambia is often affected by lack of nutritious food and many infections. As a result, a child can become malnourished and have long-term health problems. Although many health facilities in the Gambia are able to offer support to children who are malnourished, the outcomes are often poor. We believe that measuring the levels of chemical signals in the blood called "hormones" will give us information that will enable us to improve the way in which we provide care for children who are malnourished and improve their long-term health.

Before you decide to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to listen to this carefully and discuss it with others if you wish. Please ask if there is anything which is unclear or if you would like more information. Take time to decide whether or not you wish to take part.

What does participation in the study mean for you?

The following will be required if you agree to take part:

At enrolment, your child will be allocated to one of the 3 groups depending upon their nutritional status that will be based on the results of the height, weight, mid upper arm circumference when they attend the MRC Keneba clinic.

Relevant section only to be read to/read by parent guardian:

Group 1, well nourished

You will be asked to collect a stool and urine sample from him/her today. The study nurse or field worker will also collect some saliva from your child's mouth, a small volume of blood (2.5ml, equivalent to half a teaspoon full) from your child's arm before (2.5ml) and after (2.5ml) feeding. No further samples will be required from your child.

Group 2, moderately malnourished

As for Group 1, except that further samples including blood as stated above, will be collected again on Day 14 and 28. Your child will also receive a food supplement with advice on how to give it to your child and how to improve their diet. Your child will have further measurements done every 2 days for a period 4 weeks to monitor their growth. If your child's growth is noted to be getting worse during these visits then, your child will be admitted to the nutritional supplement centre at the MRC Keneba for more intensive nutritional support.

Group 3, severely malnourished

As for Group 2, except that your child will be admitted to the nutritional supplementation centre for intensive nutritional rehabilitation.

At the end of the study, some of the blood samples collected will be transferred to a laboratory overseas for analysis. This is because we do not have the equipment required for measuring all of the factors we are interested in The Gambia. All of the information obtained will then be sent back to the investigators in The Gambia.

All information which is collected during the course of this study will be kept strictly confidential and you will only be identified by an ID number.

It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. If you have any questions, please ask. We hope that you will agree to participate.

Thank you very much.

Principal investigator: Dr Helen Nabwera
Career Development Fellow, MRC Keneba.
Mobile number: 786 4520

MEDICAL RESEARCH COUNCIL UNIT, THE GAMBIA

Evaluation of hormonal regulation of growth in rural Gambian children

Consent Form:

Version number 02 9th October 2012

SCC 1306

The information sheet has been read to me and I understand it / I have read and understood the information sheet.

I understand what participation in the study means for my child.

I understand that the information regarding my child that is collected in the course of this study will remain confidential.

I understand that laboratory tests will be done on the urine, stool, saliva and blood samples from my child, and that some of these samples may later be sent overseas for laboratory testing. I also understand that part of the blood samples collected will be stored for future analyses conducted by the investigators running this study.

I understand that if my child gets sick during the study period, I can go to the clinic where study staff are providing care, and that we will be examined and treated for free.

I understand that I am free to allow my child to take part in the study or refuse, and that I can withdraw my child from the study at any time, and without giving any reason. Deciding not to take part or to withdraw from the study will not affect the care that I or any of my family are normally entitled to.

I have had a chance to ask questions and have them answered.

Signature or thumb print of volunteer: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

This form has been read by / I have read the above to _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
(write name of volunteer)
in a language that she understands. I believe that she has understood what I explained and that she has freely agreed to take part in the study.

Signature of field worker: _

Name of field worker: _

Date: |___|___| / |___|___| / |___|___|___|___|

B. Research study III

PARTICIPANT INFORMATION SHEET (FOR ADULT)

Version 01 Date 21st July 2014

Study Title: Evaluation of the risk factors for severe acute malnutrition in children recruited to a supplementation trial in rural Gambia

SCC:	1395	Protocol:	

Sponsor: Medical Research Council, International Nutrition Group

What is informed consent?

You are invited to take part in a research study. Participating in a research study is not the same as getting regular medical care. The purpose of regular medical care is to improve one's health. The purpose of a research study is to gather information. It is your choice to take part and you can stop any time.

Before you decide you need to understand all information about this study and what it will involve. Please take time to read the following information or get the information explained to you in your language. Listen carefully and feel free to ask if there is anything that you do not understand. Ask for it to be explained until you are satisfied. You may also wish to consult your spouse, family members or others before deciding to take part in the study.

If you decide to join the study, you will need to sign or thumbprint a consent form saying you agree to be in the study.

Why is this study being done?

The growth of children in rural Gambia is often affected by lack of nutritious food and many infections. As a result, a child can become malnourished and have long-term health problems. Although many health facilities in the Gambia are able to offer support to children who are malnourished, the outcomes are often poor. Your child participated in the ENID study that involved you and your child taking nutrition supplements and having regular contact with the health services at MRC Keneba. Despite this, some children in the study became malnourished. We therefore believe that by looking at how other factors such as hygiene, access to clean water and infant feeding practices affect the growth of rural Gambian children will enable us to improve our understanding of how to prevent and manage malnutrition in your children.

Before you decide to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to listen to this carefully and discuss it with others if you wish. Please ask if there is anything which is unclear or if you would like more information. Take time to decide whether or not you wish to take part.

The results of the study will be made available to your community.

What does this study involve?

The following will be required if you agree to take part:

At enrolment, a member of the study team will spend time with you asking a series of questions about your family, how you fed your child, where and how you access your water and what toileting facilities you have access to. Following this, the team member will spend time with you observing how you carry out your household activities over a period of 2-4 days. We may also invite you to attend an interview at a place that would be convenient for you, in order to get more information about your experiences of caring for infants in rural Gambia.

It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. If you have any questions, please ask. We hope that you will agree to participate.

In case the investigator discovers you are sick and decides that you cannot participate in the study because of that, you will receive immediate care at the study site and then be referred to the appropriate health facility.

If the research study needs to be stopped, you will be informed and you will have your normal medical care.

What will happen to the samples taken in this study?

No samples will be taken from you.

What harm or discomfort can you expect in the study?

During the interviews, you may be required to remember very sad moments especially if your baby is/was very sick or died. If this happens, we will ensure that these caregivers are seen by a nurse counselor in the MRC Keneba clinic to provide support and counseling.

What benefits can you expect in the study?

The benefits to you will be that through meeting and discussing issues of hygiene and infant feeding with trained medical staff, you will have better knowledge on how to care for your babies to ensure that they remain health and grow well. You and your family will also receive free health care at the MRC Keneba clinic.

Will you be compensated for participating in the study?

You will not get paid for participation, but you will get either transport by MRC or get the costs for the transport reimbursed.

What happens if you refuse to participate in the study or change your mind later?

You are free to participate or not in the study and you have the right to stop participating at anytime without giving a reason. This will not affect the medical care that you would normally receive.

In case you decide to withdraw your participation during the study we will not work on your samples without your permission, but any information already generated from the samples will be kept. The study doctor may also ask for tests for your safety.

Should any new information become available during the study that may affect your participation, you will be informed as soon as possible.

If you are injured in the study what compensation will be available?

We will be responsible to provide for treatment caused by procedures of the research study.

If medical treatment is required as an emergency, please refer to your health centre or clinic and contact the field worker who gave his/her telephone number to you or contact Dr Helen Nabwera on 7864520.

How will personal records remain confidential and who will have access to it?

All information that is collected about you in the course of the study will be kept strictly confidential. Your personal information will only be available to the study team members and might be seen by some rightful persons from the Ethics Committee, Government authorities and sponsor.

Who should you contact if you have questions?

If you have any queries or concerns you can contact Dr Helen Nabwera or Mr Seedy Singhateh on 7864520 or 7951676 and you can always call the personal numbers of the study staff given to you.

Please feel free to ask any question you might have about the research study.

Who has reviewed this study?

This study has been reviewed and approved by a panel of scientists at the Medical Research Council and the Gambia Government/MRC Joint Ethics Committee, which consists of scientists and lay persons to protect your rights and wellbeing.

CONSENT FORM

VERSION 01 DATE 21ST JULY 2014

SCC 1395

Participant Identification Number: |__|__|__|__|__|__|__|__|__|__|__|__|

_____
(Printed name of participant)

☐ I have read the written information **OR**

☐ I have had the information explained to me by study personnel in a language that I understand,

and I
confirm that my choice to participate is entirely voluntarily,

confirm that I have had the opportunity to ask questions about this study and I am satisfied with the answers and explanations that have been provided,

understand that I grant access to data about me to authorised persons described in the information sheet,

have received time to consider to take part in this study,

agree to take part in this study.

Tick as appropriate

I agree to further research on my samples as described in the information sheet Yes ☐ No ☐

Participant's signature/ thumbprint* ☐

_____
Date (dd/mmm/yyyy) Time (24hr)

Printed name of witness* _____

Printed name of person obtaining consent _____

I attest that I have explained the study information accurately in _____ to, and was understood to the best of my knowledge by, the participant. He/she has freely given consent to participate *in the presence of the above named witness (where applicable).

Signature of person obtaining consent

_____
Date (dd/mmm/yyyy) Time (24hr)

** Only required if the participant is unable to read or write.*

PARTICIPANT INFORMATION SHEET (FOR CHILD)

Version 01 Date 21st July 2014

Study Title: Evaluation of the risk factors for severe acute malnutrition in children recruited to a supplementation trial in rural Gambia

SCC:	1395	Protocol:	

Sponsor: Medical Research Council, International Nutrition Group

What is informed consent?

You are invited to let your child take part in a research study. Before you decide you need to understand why the research study is being done and what it will involve. Please take time to read the following information or get the information explained to you in your language. Listen carefully and feel free to ask if there is anything that is not clear or you do not understand. You may also wish to consult your spouse, family members, friends or others before deciding to let your child take part in the study.

If you decide to allow your child to join the study, you will need to sign or put a thumbprint on a consent form saying you agree for your child to be in the study.

Why is this study being done?

The growth of children in rural Gambia is often affected by lack of nutritious food and many infections. As a result, a child can become malnourished and have long-term health problems. Although many health facilities in the Gambia are able to offer support to children who are malnourished, the outcomes are often poor. Your child participated in the ENID study that involved you and your child taking nutrition supplements and having regular contact with the health services at MRC Keneba. Despite this, some children in the study became malnourished. We therefore believe that by looking at how other factors such as hygiene, access to clean water and infant feeding practices affect the growth of rural Gambian children will enable us to improve our understanding of how to prevent and manage malnutrition in your children.

Before you decide to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to listen to this carefully and discuss it with others if you wish. Please ask if there is anything which is unclear or if you would like more information. Take time to decide whether or not you wish to take part.

The results of the study will be made available to your community.

What does this study involve?

<u>The following will be required if you agree to take part:</u>

At enrolment, a member of the study team will spend time with you asking a series of questions about your family, how you fed your child, where and how you access your water and what toileting facilities you have access to. Following this, the team member will spend time with you observing how you carry out your household activities over a period of 2 days. We may also invite you to attend an interview at a place that would be

convenient for you, in order to get more information about your experiences of caring for infants in rural Gambia.

It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. If you have any questions, please ask. We hope that you will agree to participate.

In case the investigator discovers your child is sick and decides that he/she cannot participate in the study because of that, he/she will receive immediate care at the study site and then be referred to the appropriate health facility.

If the research study needs to be stopped, you will be informed and your child will have the normal medical care.

What will happen to the samples taken in this study?

No samples will be taken from your child.

What harm or discomfort can you expect in the study?

During the interviews, you may be required to remember very sad moments especially if your baby is/became very sick or died. If this happens, we will ensure that you are seen by a nurse counselor in the MRC Keneba clinic to provide support and counseling.

What benefits can you expect in the study?

The benefits to children in the study will be that through meeting and discussing issues of hygiene and infant feeding with trained medical staff, you will have better knowledge on how to care for your babies to ensure that they remain health and grow well. You and your family will also receive free health care at the MRC Keneba clinic.

Will you be compensated for your child's/ward's participation in the study?

You will not get paid for participation of your child in the study, but you will get either transport by MRC or get the costs for the transport reimbursed.

What happens if you refuse to participate in the study or change your mind later?

You are free to let your child participate or not in the study and you have the right to stop his/her participating at anytime without giving a reason. This will not affect the medical care that your child would normally receive.

In case you decide to withdraw your child's participation during the study we will not work on your child's samples without your permission, but any information already generated from the samples will be kept. The study doctor may also ask for tests for your child's safety.

Should any new information become available during the study that may affect your child's participation, you will be informed as soon as possible.

What compensation will be available if your child is injured during the study?

We will be responsible to provide for treatment caused by procedures of the research study.

If medical treatment is required as an emergency, please refer to your health centre or clinic and contact the field worker who gave his/her telephone number to you or contact Dr Helen Nabwera on 7864520.

How will your child's information be kept and who will be allowed to see it?

All information that is collected about your child in the course of the study will be kept strictly confidential. Your child's personal information will only be available to the study team members and might be seen by some rightful persons from the Ethics Committee, Government authorities and sponsor.

Who should you contact if you have questions?

If you have any queries or concerns you can contact Dr Helen Nabwera or Mr Seedy Singhateh on 7864520 or 7951676 and you can always call the personal numbers of the study staff given to you.

Please feel free to ask any question you might have about the research study.

Who has reviewed this study?

This study has been reviewed and approved by a panel of scientists at the Medical Research Council and the Gambia Government/MRC Joint Ethics Committee, which consists of scientists and lay persons to protect your rights and wellbeing.

CONSENT / ASSENT FORM

Participant's Name _____

Participant's Identification Number: |__|__|__|__|__|__|__|__|__|__|

_____ **OR**

_____

(Printed name of parent) (Printed name of guardian)

☐ I have read the written information **OR**

☐ I have had the information explained to me by study personnel in a language that I understand,

and I

confirm that my choice to let my child participate is entirely voluntarily,

confirm that I have had the opportunity to ask questions about this study and I am satisfied with the answers and explanations that have been provided,

understand that I grant access to data about my child to authorised persons described in the information sheet,

have received time to consider to let my child and I take part in this study,

agree to allow my child take part in this study.

Tick as appropriate

I agree to further research on my child's samples as described in the information sheet Yes ☐ No ☐

Participant's signature/ thumbprint* for **assent** (child aged 12-17 years)

_____

Date (dd/mmm/yyyy) Time (24hr)

Participant's parent/guardian signature/thumbprint*

_____

Date (dd/mmm/yyyy) Time (24hr)

Printed name of witness* _____

Printed Name of Person obtaining consent _____

I attest that I have explained the study information accurately in _____ to, and was understood to the best of my knowledge by, the participant/parent/guardian. He/she has freely given consent to participate *in the presence of the above named witness (where applicable).

Signature of Person obtaining consent _____

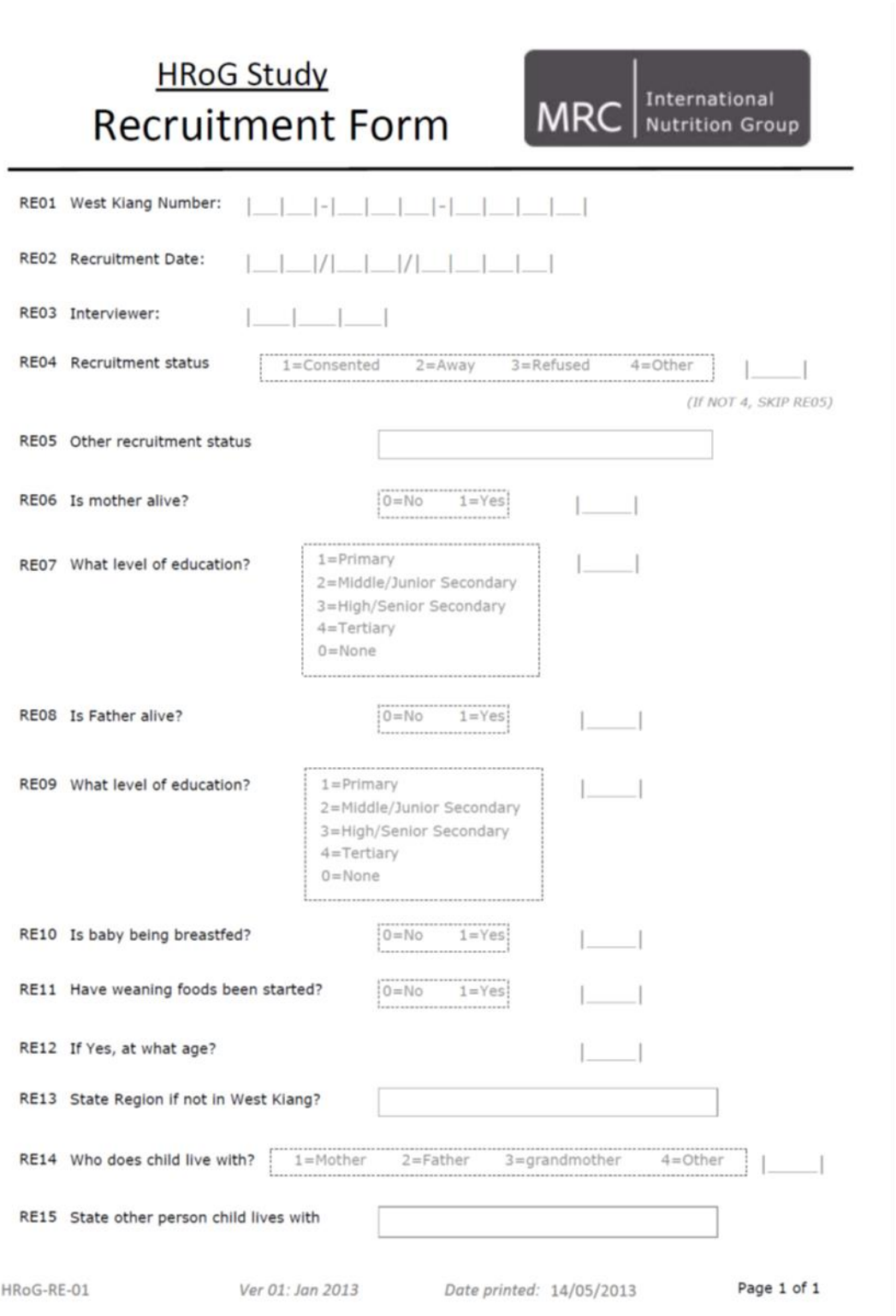
_____

Date (dd/mmm/yyyy) Time (24hr)

** Only required if the participant is unable to read or write.*

Appendix VII: Data collections forms

A. Research study II



HRoG Study

Recruitment Form

MRC International Nutrition Group

RE01 West Kiang Number: |__|__|-|__|__|__|-|__|__|__|__|

RE02 Recruitment Date: |__|__|/|__|__|/|__|__|__|__|

RE03 Interviewer: |____|____|____|

RE04 Recruitment status 1=Consented 2=Away 3=Refused 4=Other |_____|

(If NOT 4, SKIP RE05)

RE05 Other recruitment status

RE06 Is mother alive? 0=No 1=Yes |_____|

RE07 What level of education? |_____|
1=Primary
2=Middle/Junior Secondary
3=High/Senior Secondary
4=Tertiary
0=None

RE08 Is Father alive? 0=No 1=Yes |_____|

RE09 What level of education? |_____|
1=Primary
2=Middle/Junior Secondary
3=High/Senior Secondary
4=Tertiary
0=None

RE10 Is baby being breastfed? 0=No 1=Yes |_____|

RE11 Have weaning foods been started? 0=No 1=Yes |_____|

RE12 If Yes, at what age? |_____|

RE13 State Region if not in West Kiang?

RE14 Who does child live with? 1=Mother 2=Father 3=grandmother 4=Other |_____|

RE15 State other person child lives with

HRoG-RE-01 *Ver 01: Jan 2013* *Date printed:* 14/05/2013 Page 1 of 1

HRoG Study
Anthropometry Form

MRC International Nutrition Group

AT01. **Child's Subject ID:** _____ **Child's WKNO:** _____

AT02. Visit Date: |__|__|/|__|__|/|__|__|__|__|

AT03. Has it been decided to withdraw the baby from the studcy? 0=No 1=Yes |_____|

(If 0, skip to AT07)

AT04. If YES, what is the reason? |_____|

1=Mother/Guardian decision
2=Moved away
3=Died
0=Other

AT05. If other, what is the reason? _____

AT06. Visit Day _____

		Measurement 1	Measurement 2	Measurement 3
AT07.	Weight (kg)	|__|__|__|.|__|__|	|__|__|__|.|__|__|	|__|__|__|.|__|__|
AT08.	Length (cm)	|__|__|__|.|__|__|	|__|__|__|.|__|__|	|__|__|__|.|__|__|
AT09.	Knee-Heel Length (cm)	|__|__|__|.|__|__|	|__|__|__|.|__|__|	|__|__|__|.|__|__|
AT10.	HC (cm)	|__|__|__|.|__|__|	|__|__|__|.|__|__|	|__|__|__|.|__|__|
AT11.	MUAC (mm)	|__|__|__|.|__|__|	|__|__|__|.|__|__|	|__|__|__|.|__|__|

AT12. Measurements performed by: |____|____|____|

A613. Has Test meal been given to the Subject? 0=No 1=Yes |_____|

(If 0, End Form here)

AT14. Test meal start time (hh:mm) 24hr |__|__|:|__|__|

AT15. Test meal end time (hh:mm) 24hr |__|__|:|__|__|

HRoG Study
Anthropometry Form
Home Visit

MRC International Nutrition Group

AT01. Child's Study ID: **Child's WKNO:**

AT02. Visit Date: |__|__|/|__|__|/|__|__|__|__|

AT03. Has it been decided to withdraw the baby from the study? 0=No 1=Yes
(If 0, skip to AT07)

AT04. If YES, what is the reason? |_____|
1=Mother/Guardian decision
2=Moved away
3=Died
0=Other

AT05. If other, what is the reason?

AT06. Visit Day

		Measurement 1	Measurement 2	Measurement 3
AT07. Weight	(kg)	|__|__|__|.|__|__|	|__|__|__|.|__|__|	|__|__|__|.|__|__|
AT08. Length	(cm)	|__|__|__|.|__|__|	|__|__|__|.|__|__|	|__|__|__|.|__|__|
AT09. Knee-Heel Length	(cm)	|__|__|__|.|__|__|	|__|__|__|.|__|__|	|__|__|__|.|__|__|
AT10. HC	(cm)	|__|__|__|.|__|__|	|__|__|__|.|__|__|	|__|__|__|.|__|__|
AT11. MUAC	(mm)	|__|__|__|.|__|__|	|__|__|__|.|__|__|	|__|__|__|.|__|__|

AT12. Measurements performed by: |____|____|____|

PENDING STOOL SAMPLE

AT13. Has stool sample been collected? 0=No 1=Yes |_____|

AT14. Stool sample processing time (hh:mm) 24hr |__|__|:|__|__|

AT15. Stool Sample processed by |____|____|____|

SC21. Saliva Sample processed by |____|____|____|

BLOOD SAMPLE

SC22. Blood Sample collected by |____|____|____|

SC23 Baseline sample collected(2.5ml)? 0=No 1=Yes |_____|

(If 1, skip to SC25)

SC24. 1 hour posttest meal sample collected(2.5ml)? 0=No 1=Yes |_____|

(If 1, skip to SC25)

SC25. If no to any of the above, Please state reason

SC26. Blood sample processing time (hh:mm) 24hr |__|__|:|__|__|

SC27. Blood Samples processed by |____|____|____|

B. Research study III

Medical Research Council Unit, The Gambia

Evaluation of the risk factors for malnutrition in children recruited to a supplementation trial in rural Gambia

Version 02, 14th August 2014 Modified, SCC 1395

Edinburgh Depression Scale

Questions and filters	Coding categories
We would like to know something about how you feel in your day-to-day life. Please answer each question for how you have felt in the last 7 days, not only today	
Have you been able to laugh and see the funny side of things	|__|
	As much as you always could 0
	Not quite so much as you used to 1
	Definitely not so much as you used to 2
	Not at all 3
Have you looked forward with enjoyment to things	|__|
	As much as you used to 0
	A bit less than you used to 1
	Definitely less than you used to 2
	Hardly at all 3
Have you blamed yourself unnecessarily when things went wrong	|__|
	Yes, most of the time 3
	Yes, some of the time 2
	Not very often 1
	Not at all 0
Have you been anxious or worried for no good reason	|__|
	No, not at all 0
	Hardly ever 1
	Yes, sometimes 2
	Yes, very often 3
Have you felt scared or panicked for no good reason	|__|
	Yes, quite a lot 3
	Yes, sometimes 2
	No, not much 1
	No, not at all 0
Have you been coping with your daily routine	|__|
	Yes, I have been coping as well as ever 0
	Yes, most of the times I have coped quite well 1
	No, sometimes I have not coped as well as usual 2
	No, most of the time I have not been able to cope at all 3
Have you had difficulty sleeping	|__|
	Yes, most of the time 3
	Yes, sometimes 2
	Not very often 1
	No, not at all 0
Have you felt sad	|__|
	Yes, most of the time 3
	Yes, quite often 2
	Not very often 1
	No, not at all 0
Have you been so unhappy that you have been crying	|__|
	Yes, most of the time 3
	Yes, quite often 2
	Not very often 1
	No, never 0
Do you like to be by yourself	|__|
	Yes, most of the time 3
	Yes, some of the time 2
	Not very often 1
	Not at all 0

Medical Research Council Unit, The Gambia

Evaluation of the Risk Factors for Malnutrition in Children Recruited to a Supplementation Trial in Rural Gambia

QUESTIONNAIRE, SCC 1395

Version 03, 15th Sept. 2014

WestKiang No: #Name? Date of Birth: #Name? Study ID:

Visit date: |__|__|/|__|__|/|__|__|__|__| Fieldworker Name:

Section 1: Demographic and Socio-economic Information

1. Who is the child's current primary carer?

☐ Mother [01] ☐ Father [02] ☐ Brother [03] ☐ Sister [04] ☐ Maternal Grandmother [05]
☐ Paternal Grandmother [06] ☐ Maternal Grandfather [07] ☐ Paternal Grandfather [08] ☐ Maternal Auntie [09] ☐ Paternal Auntie [10]
☐ Maternal Uncle [11] ☐ Paternal Uncle [12] ☐ Co-wife [13] ☐ No Relation [14] ☐ Other Relation [15]

2. Who was the child's primary carer between 0-12 months of age?

☐ Mother [01] ☐ Father [02] ☐ Brother [03] ☐ Sister [04] ☐ Maternal Grandmother [05]
☐ Paternal Grandmother [06] ☐ Maternal Grandfather [07] ☐ Paternal Grandfather [08] ☐ Maternal Auntie [09] ☐ Paternal Auntie [10]
☐ Maternal Uncle [11] ☐ Paternal Uncle [12] ☐ Co-wife [13] ☐ No Relation [14] ☐ Other Relation [15]

3. Where does the child's mother live?

☐ In household [1] ☐ Outside of household within the Gambia [2] ☐ Abroad [3] ☐ Whereabouts unknown [4] ☐ Died [5]

4. Where does the child's father live?

☐ In household [1] ☐ Outside of household within the Gambia [2] ☐ Abroad [3] ☐ Whereabouts unknown [4] ☐ Died [5]

5. How many children younger than 5 years live in the household? |___|___|

6. Are all the child's siblings alive? If not, please give the estimated age and cause of death of the deceased siblings. ☐ Yes [1] ☐ No [0]

	Age of Death	Cause of Death		Age of Death	Cause of Death
1			7		
2			8		
3			9		
4			10		
5			11		
6			12		

7. What level of formal education has the primary carer had?

☐ No formal education [1] ☐ Arabic School [2] ☐ Less than primary school [3] ☐ Completed primary school [4]
☐ Completed secondary school [5] ☐ Post-secondary school [6] ☐ Don't know [7]

8. What is the carer's main source of income?

☐ Farming [1] ☐ Business [2] ☐ Salary [3]
☐ Trading [4] ☐ Remuneration from children [5] ☐ Other, specify _____ [6]

9. Is the above income guaranteed every month? ☐ Yes [1] ☐ No [0]

10. How many rooms in your household are used for sleeping? |___|___|

11. How many people have been sleeping regularly in your household over the past 6 months? |___|___|

12. Does the mother/carer have the freedom to move around the community to access resources such as markets, shops, clinics, and companions...

☐ Alone [1]? ☐ With child [2]? ☐ with another same-aged woman [3]? ☐ Only with a husband or elder [4]? ☐ Never [5]?

13. Who decides what medical care to give the child?

☐ Mother alone [1] ☐ Jointly with husband [2] ☐ Jointly with mother-in-law [3] ☐ Jointly with another person [4] ☐ Mother has no say [5]

Section 2: Infant Feeding Practices

1. Is child currently breastfed? ☐ No [0] ☐ Partial breastfeeding [1] ☐ Exclusive breastfeeding [2]

2. If no, has the child ever been breast fed? ☐ Yes [1] ☐ No [0]

3. If yes, what type of breastfeeding was it? ☐ Partial breastfeeding [1] ☐ Exclusive breastfeeding [2

4. If Yes, what age was the child when breastfeeding was stopped? |___|___| months

5. If No, what feeds did the child receive soon after birth? _____

6. At what age did you introduce complementary feeds? |___|___| months

7. What complementary feeds did the child receive before 12 months of age? _____

8. Please list the types of food that you give to the child regularly?

1		2		3		4		5	

9. Who decides what food to feed the child? ☐ Mother alone [1] ☐ Jointly with husband [2] ☐ Jointly with mother-in-law [3] ☐ Jointly with another person [4] ☐ Mother has no say [5]

10. Who feeds the child most of the time?

☐ Mother [01] ☐ Father [02] ☐ Brother [03] ☐ Sister [04] ☐ Maternal Grandmother [05]
☐ Paternal Grandmother [06] ☐ Maternal Grandfather [07] ☐ Paternal Grandfather [08] ☐ Maternal Auntie [09] ☐ Paternal Auntie [10]
☐ Maternal Uncle [11] ☐ Paternal Uncle [12] ☐ Co-wife [13] ☐ No Relation [14] ☐ Other Relation [15]

11. What do you use to feed the child? ☐ Hand [1] ☐ Spoon [2] ☐ Other, please specify_____ [3]

12. How many times a day is the child fed on complementary feeds? |___|___|

13. Regarding feeding times for the child: ☐ Scheduled [1] ☐ Fed when hungry [2]

14. How do you assess to see whether the child is hungry? _____

_____

15. How do you assess to see whether the child is full? _____

_____

16. At most feeding times: ☐ Own bowl [1] ☐ Share with other children [2] ☐ Share with adults [3]

Section 3: Water, Sanitation and Hygiene

1. Over the past 6 months, what was the main source of water for the members of your household

☐ Piped into house [1] ☐ Covered well in house or compund [2] ☐ Piped into yard [3] ☐ Covered public well [4]
☐ Public tap [5] ☐ Open public well River or stream [6] ☐ Open well in house or yard [7] ☐ Deep tube well [8]
☐ Rainwater [9] ☐ Shallow tube well [10] ☐ Bought (tank, bottles, etc) [11] ☐ Borehole [12]
☐ Other, please specify_____ [13]

2. How long does it take to go there, get water, and come back?

☐ Less than 15 minutes [1] ☐ 15 - 29 minutes [2] ☐ 30 - 59 minutes [3] ☐ 1 - 3 hours [4] ☐ More than 3 hours [5]

3. Do you or other members from your household go and fetch drinking water for the household every day? ☐ Yes [1] ☐ No [0]

4. If yes, on average, how many trips do you and members from your household make to fetch water each day? |___|___|

5. Do you usually treat drinking water at home? ☐ Yes [1] ☐ No [0]

6. If yes, which method do you use the most to treat drinking water at home? (Tick all that applies)

☐ Leave water in sun to disinfect [1] ☐ Boil [2] ☐ Filter through a cloth [3] ☐ Filter through ceramic or other filter [4]
☐ Chlorine liquid, powder, or tablets Alum [5] ☐ Other chemical or additive, specify_____ [6]

7. If you use chlorine liquid, powder or tablets, which type do you most commonly use? (Tick only one)

☐ Certeza [1] ☐ Watermaker [2] ☐ Aquatabs PurR [3] ☐ AquaGuard [4] ☐ WaterGuard [5] ☐ Don't know [6]
☐ Other chemical or additive, specify_____ [7]

8. What kind of facility does your household most commonly use to dispose off human fecal waste? [Show pictures to confirm the identity of the facility used.

☐ Flush toilet [1] ☐ Pour flush toilet [2] ☐ Ventilated improved pit (VIP) latrine [3] ☐ Traditional pit toilet [4] ☐ Bush [5]
☐ Field [6] ☐ Ground [7] ☐ Stream [8] ☐ No facility [9] ☐ Other, specify_____ [10]

9. When do you usually wash your hands? [Tick all that applies. Do not probe.]

☐ Before eating [1] ☐ After handling domestic animals [2] ☐ Before cooking [3] ☐ After cleaning child who defecated [4]
☐ Before you nurse or prepare baby's food [5] ☐ After you defecate [6] ☐ Never [7] ☐ Other, specify_____ [8]

10. When you wash your hands, what do you usually use? [tick only one]

☐ Water only [1] ☐ Water and soap [2] ☐ Water and ashes [3] ☐ Water and mud or clay [4]
☐ Other, please specify_____[5]

Evaluation of the risk factors for severe acute malnutrition in children recruited to a supplementation trial in rural Gambia

SCC 1395

DIRECT OBSERVATIONAL GUIDE

Observation No: ☐☐☐

Observer: _____ Date of Observation: ___/___/___ (dd/mm/yy)

Age of care giver (in years) _____

Residence _____

Level of education _____

Gender _____

Age of child (in months) _____

- Time started _____

Socioeconomic

- Describe the compound ie number of houses, animals in the compound

- Number of children under 5 years in the compound

Infant feeding practices

- What food is given to the infants in the compound?

- How long does it take to prepare food for infants in the compound?

- How is food for infants prepared?

- Do infants eat on the same plate/bowl as the adults or do they have separate plates/bowls?

- Is the feeding of infants in the compound supervised or do they feed themselves?

- Are the hands of the infants washed with soap/ash/mud and water before feeds? How about the index case?

- Does the person serving/feeding the infants wash their hands with soap/ash/mud and water? How about the one serving/feeding the index case?

- Do the infants finish the food that they are served? If not, what happens to the food?

- Does the index child feed themselves or does someone feed them?

- If the infants are fed, describe what happens before feeding is stopped? How about the index case?

- Does the index child complete the food that they are served?

- How often are infants fed in the compound?

- How often are infants breastfed in the compound?

- Is the index child still breastfeeding?

Water, sanitation and hygiene

- How long does it take for a member of the household to fetch water from the water sources?

- What water storage facilities are available for drinking water?

- Is drinking water treated before storage? Describe

- What containers are used for giving drinking water to the infant?

- How are faeces disposed of in the compound?

- Are there any hand washing facilities for people to use after using the toilet?

- Do people wash their hands after using the toilet? If yes, what do they use?

- How is the infant cleaned when they soil and how are the faeces disposed?

- Do people wash their hands after handling the faces of infants? If yes, what do they use?

- How are the faeces of the index case handled?

- Time ended:- _____

Any other comments/thoughts/reflections:

Medical Research Council Unit, The Gambia

Evaluation of the risk factors for malnutrition in children recruited to a supplementation trial in rural Gambia

In depth interview guide

Version 03, 26th February 2015

[Before turning on the recorder]

- Introduce yourself
- Go through the information leaflet and consent form (consent form completed prior quantitative questionnaires)
- Go over areas to cover
- Explain that you will note down anything that you want to come back to
- Reiterate that there is no right or wrong answer
- That all the information that she gives will be kept confidential and will only be shared with those involved in the study
- That the data collected will be anonymised so they will not be identified
- That any information that she provides that indicates she or someone else is at risk of harm will have to be shared with the relevant health care providers (MRC Keneba doctor/nurse) for hers and the child's or other adult's safety.

Introduction: I have come back to discuss with you in more details the issues around your experiences of looking after an infant (child under 12 months) in this community.

Infant feeding practises

- What is your understanding of how an infant should be fed?
- What was your experience of feeding your child when they were an infant?
- How do other mothers in this village feed their infants?
- Where do mothers get advice on how to feed their infants?
- Where did you get your advice from?
- What factors influenced the choices that you made with regard to feeding your infant?
- Were there any factors that limited your choices?
- Tell me about your experience with health care workers during pregnancy and the time when your child was an infant?
- Was the infant feeding information from health care workers available to you?
- Was it helpful? Can you give me an example?
- What factors prevented you from adhering to this information?

Hygiene

- In you view, how does hygiene in the household affect the health growth of an infant?
- What are the challenges of maintaining good hygiene in this environment?
- How would you prepare and serve the food for an infant?
- How does hand washing affect your infant's health and growth?
- Does the availability of clean water and good toilet facilities in the household have an effect on the health and growth of an infant? How?
- What factors limit a mother's/carer's ability of maintaining hygiene in her household?

Parenting skills

- Who is primarily responsible for looking after infants in this community?
- What is the role of fathers in the care of children under 12 months of age?
- In your view, do you think having fathers more involved in the day to day care of an infant would improve their health and growth?
- How does the structure a family affect an infant's health the growth e.g. polygamy (co-wives), living with grandparents etc
- What factors limited/limit your ability to care for your child during infancy?
- Have you or anyone close to you experienced the death of a child?
- How did you/they manage? How did this affect yours/their ability to look after the other children (particularly infants)

Miscellaneous

- Have you or anyone close to you suffered with any mental health concerns such as learning difficulties or depression?
- How did this affect your/their ability to care for their infants?
- Does the educating of a mother (Arabic school/English school) have an effect on how she cares for her infant? How?

Is there anything you would like to ask me?

Thank you for your time

[Turn off the recorder]

Debrief

- Inform the participant that findings will be fed back to the community and made public after the study has been completed

Once the participant has left or you have left the participant's compound, please make **field notes**:

- Any notable themes

- Social characteristics of setting
- Participant characteristics
- Your perception of the person/thoughts/emotions
- Notable events during interview
- Note any suggested changes to the topic guide

Appendix VIII: Scientific and ethics approval

A. Research study II

The Gambia Government/MRC Joint
ETHICS COMMITTEE

C/o MRC Unit: The Gambia, Fajara
P. O. Box 273, Banjul
The Gambia, West Africa
Fax: +220 – 4495919 or 4496 513
Tel: +220 – 4495442-6 Ext. 2308

8 November 2012

Dr Helene Nabwera
MRC Unit, The Gambia
Keneba Field Station

Dear Dr Nabwera

SCC 1306v2, Evaluation of hormonal regulation of growth in rural Gambian children

Thank you for submitting your letter dated 5 November 2012 for consideration by the Gambia Government/MRC Joint Ethics Committee's Chair.

I am please to approve your project proposal which will be forwarded to the Ethics Committee for notification at its November meeting.

With best wishes

Yours sincerely

Mr Malamin Sonko
Acting Chairman, Gambia Government/MRC Joint Ethics Committee

Additional documents submitted for review:-

- Participant Information Sheet, Version 2.0 – 9 October 2012
- Consent form, Version 2.0 – 9 October 2012

The Gambia Government/MRC Joint
ETHICS COMMITTEE

C/o MRC Unit: The Gambia, Fajara
P. O. Box 273, Banjul
The Gambia, West Africa
Fax: +220 – 4495919 or 4496 513
Tel: +220 – 4495442-6 Ext. 2308

8 November 2012

Dr Helene Nabwera
MRC Unit, The Gambia
Keneba Field Station

Dear Dr Nabwera

SCC 1306v2, Evaluation of hormonal regulation of growth in rural Gambian children

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With best wishes

Yours sincerely

Mr Malamin Sonko
Acting Chairman, Gambia Government/MRC Joint Ethics Committee

Additional documents submitted for review:-

- Participant Information Sheet, Version 2.0 – 9 October 2012
- Consent form, Version 2.0 – 9 October 2012

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United Kingdom
Switchboard: +44 (0)20 7636 8636
www.lshtm.ac.uk

Observational / Interventions Research Ethics Committee

Helen Nabwera
PhD student
LSHTM

5 April 2013

Dear Ms. Nabwera,

Study Title: **Evaluation of hormonal regulation of growth in rural Gambian children**
LSHTM ethics ref: **6354**

Thank you for your letter of 27 March 2013, responding to the Interventions Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
LSHTM ethics application	2	
Protocol	2	February 2013
Information Sheet & Consent form	3	24/02/2013
Anthropometry Form	1	January 2013
Recruitment Form	1	January 2013
Sample Collection Form	1	January 2013

After ethical review

Any subsequent changes to the application must be submitted to the Committee via an E2 amendment form. All studies are also required to notify the ethics committee of any serious adverse events which occur during the project via form E4. At the end of the study, please notify the committee via form E5.

Yours sincerely,

Professor John DH Porter
Chair
ethics@lshtm.ac.uk
http://intra.lshtm.ac.uk/management/committees/ethics/

B. Research study III

8 October 2014

Dr Helen Nabwera
Nutrition Theme
MRC Unit, The Gambia
Keneba

Dear Dr Nabwera

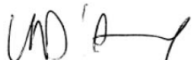
L2014.62, Amendment to protocol SCC 1395, Evaluation of the risk factors for severe acute malnutrition in children recruited to a supplementation trial in rural Gambia

Thank you for submitting your letter dated 22 September 2014 for consideration by the SCC at its meeting held on 6 October 2014.

We are pleased to approve your request which will be forwarded to the Ethics Committee for consideration at its meeting on 31 October 2014.

With best wishes

Yours sincerely



The Gambia Government/MRC Joint
ETHICS COMMITTEE

C/o MRC Unit: The Gambia, Fajara
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Tel: +220 – 4495442-6 Ext. 2308
Email: ethics@mrc.gm

29 October 2014

Dr Helen Nabwera
Nutrition Theme
MRC Unit, The Gambia
Keneba

Dear Dr Nabwera

SCC 1395v2, Evaluation of the risk factors for severe acute malnutrition in children recruited to a supplementation trial in rural Gambia

Thank you for submitting your response letter dated 20 October 2014 addressing the issues raised by The Gambia Government/MRC Joint Ethics Committee at its meeting held on 29 August 2014.

Your responses have been reviewed and found satisfactory. I am now pleased to approve the commencement of this project.

With best wishes

Yours sincerely

Mr Malamin Sonko
Chairman, Gambia Government/MRC Joint Ethics Committee

Documents submitted for review:-

- Response letter – 20 October 2014
- SCC application form, version 1.0 – 21 July 2014
- Response Letter – 14 August 2014
- Response Letter (from Professor Andrew Prentice)
- Informed Consent Document (child),version 01 – 21 July 2014
- Informed Consent Document (adult),version 02 – 20 October 2014
- In depth interview guide, version 01 – 21 July 2014
- Questionnaire, version 01 – 23 June 2014
- WHO Self reporting questionnaire, version 01 – 21 July 2014
- Protocol, version 1.0 – 17 June 2014
- CV:Catherine Molyneux
- Modified Edinburgh Depression Scale, version 02 – 14 August 2014

The Gambia Government/MRC Joint
ETHICS COMMITTEE

C/o MRC Unit: The Gambia, Fajara
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17 November 2014

Dr Helen Nabwera
Nutrition Theme
MRC Unit, The Gambia
Keneba

Dear Dr Nabwera

L2014.62, Amendment to protocol SCC 1395, Evaluation of the risk factors for severe acute malnutrition in children recruited to a supplementation trial in rural Gambia

Thank you for submitting your letter dated 22 September 2014 for consideration by The Gambia Government/MRC Joint Ethics Committee at its meeting held on 31 October 2014.

We are pleased to approve your request.

With best wishes

Yours sincerely

Mr Malamin Sonko
Chairman, Gambia Government/MRC Joint Ethics Committee

Documents submitted for review:-
- SCC approval letter – 8 October 2014
- Request letter – 22 September 2014

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Observational / Interventions Research Ethics Committee

Dr Helen Nabwera
LSHTM

9 January 2015

Dear Dr Nabwera

Study Title: Evaluation of the risk factors for severe acute malnutrition in children recruited to a supplementation trial in rural Gambia

LSHTM Ethics Ref: 8619

Thank you for responding to the Observational Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Protocol / Proposal	Questionnaire_ENID Malnutrition_v1.1HNabwera.pdf	23/06/2014	01
Protocol / Proposal	In-depth interview guide_ENID Malnutrition_HNabwera.docx	21/07/2014	1
Protocol / Proposal	Modified Edinburgh Depression Scale_Nabwera_SCC 1395.pdf	14/08/2014	1
Local Approval	SCC 1395_Nabwera_Approved_18Aug14.pdf	18/08/2014	1
Investigator CV	cv_HNabwera.docx	22/08/2014	1
Protocol / Proposal	OBSERVATION TOOL_HNabwera.docx	08/09/2014	1
Information Sheet	lshtm_consent_HNabwera.pdf	15/09/2014	1
Protocol / Proposal	StudyProtocol_HNabwera_Risk factors for malnutrition.docx	15/09/2014	1
Information Sheet	lshtm_pis_HNabwera.pdf	15/09/2014	1
Local Approval	SCC 1395v2_Nabwera_approved_29Oct14.pdf	29/10/2014	02
Covering Letter	LSHTM_HNabwera_Response to Ethics Committee_November2014.pdf	17/11/2014	01
Protocol / Proposal	StudyProtocol_HNabwera_Risk factors for malnutritionv3.docx	20/11/2014	02
Information Sheet	lshtm_pis_HNabwera_v2.docx	20/11/2014	02

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

Professor John DH Porter
Chair

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