Improved child development outcomes in rural Uganda: long-term follow-up of a randomized maternal education trial

Prudence Atukunda

University of Oslo

Dissertation for the degree of Doctor of Philosophy (PhD)

Department of Nutrition

Institute of Basic Medical Sciences

Faculty of Medicine

University of Oslo

Oslo, February 2020

Series of dissertations submitted to the
Faculty of Medicine, University of Oslo

ISBN 978-82-8377-694-2

Cover: Hanne Baadsgaard Utigard.
Print production: Reprosentralen, University of Oslo.

Table of Contents

Acknowledgements

Any journey, who you travel with, is much more imperative than the last stop. The work in this thesis was done at the greatly motivating work place of the Department of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, and funded by the Norwegian State Educational Fund ("Statens Lånekasse for utdanning"). Additional financial support was obtained from the Johan Throne Holst Foundation for Nutrition Research, TNO's Early Research Program "Personalized Health" and Kristiania University College.

As a neurodevelopment practitioner, I found the introduction to areas of nutrition, child development, growth and maternal mental health combined with gut bugs, fascinating! I am forever grateful for the lifetime opportunity to do a PhD in a field that is so passionate to me. This thesis results from teamwork of many people in Norway, The Netherlands and Uganda; they all contributed in immeasurable ways. I want to express my sincere gratitude to every one of those who made this work possible.

Special gratitude goes to the team of supervisors who are also great mentors and friends: Ane Cecilie Westerberg, Per Ole Iversen and Archileo Kaaya. I do thank you for your support and reliance, and for all you have done and still do for me. You involve, mentor, encourage, inspire and share my enthusiasm from ideas to results and most of all you do care. Meeting you was the best life-changer, I have grown tremendously, both as a scientist and personally. You are all sources of inspiration from now and the entire future that awaits me. Ane Cecilie Westerberg, it was sometime in Kampala in March 2014, when what started as a conversation ended up into this incredible work, you identified me as potential PhD candidate, shared it with Per Ole Iversen and just like a dream, I got a PhD position at the University of Oslo.

Special thanks goes to Remco Kort and Tim J van den Broek who analyzed and kept track of the microbiota samples; Guus Roeselers for his contribution to design the gut microbiota study in its early phase; Jens Petter Berg, Anne-Marie Siebke Trøseid and Marit Daneid for keeping track of the urine samples and performing the urine iodide analysis. Carrying out human samples studies in a rural setting is demanding as well as challenging, you have been vital to bring this to completion.

Great thanks to all the collaborators, especially Lien My Diep for your valuable contribution in statistical guidance, interpreting and writing of the articles. Grace Kyamazima Mehangye Muhoozi, that call you made to me will forever be a mark on my life: Mentor, friend, co-author and a star who shone in my life unexpectedly, thank you for introducing me to the field of nutrition. I am highly indebted to your guidance, fun when all seemed tough and your expertise in the field as we walked hills to reach out to the children and their mothers.

Kristin R and Mikkel Kardel my family, your support progressed this thesis. Appreciation to colleagues at the Department of Nutrition; Wenche Barth Eide, Lillian K, friends; Ajebit, Anselm, Fatin, Mustafa and Prof Baguma P, you have become family to me. Annicke Stranda Haslestad you gave all your time to select and ship study kits to Uganda.

The team in the field; Paul Kakwangire, Emmanuel Tumusiime, Namanya Joshua, Ruth Ninsiima and Abor Isaac, thank you. I am also thankful to all the children and their parents, you did sacrifice a lot to contribute to the scientific knowledge presented here.

Lastly, to Edward and Jenina Rwambuka my inspirational parents and Friberg family, thank you for the love, support and encouragement during the study. Charity Tugumisirize my big sister and Jotham you believed in me. To my siblings special thanks too, you prayed for me and showed much love when I was low.

Oslo, February 2020

Prudence Atukunda

List of Papers

In this thesis, the papers will be referred to by their Roman numerals.

Paper I

Atukunda P, Muhoozi GKM, van den Broek TJ, Kort R, Diep LM, Kaaya AN, Iversen PO, Westerberg AC.

Child development, growth and microbiota: follow-up of a randomized education trial in Uganda.

Journal of Global Health 2019;9:010431. doi: 10.7189/jogh-09-010431.

Paper II

Atukunda P, Muhoozi GKM, Diep LM, Berg JP, Westerberg AC, Iversen PO.

The association of urine markers of iodine intake with development and growth among children in rural Uganda: a secondary analysis of a cluster-randomized education trial.

Submitted.

Paper III

Atukunda P, Muhoozi GKM, Iversen PO, Westerberg AC.

Nutrition, hygiene and stimulation education for impoverished mothers in rural Uganda: Effect on maternal depression symptoms and their associations to child development outcomes.

Nutrients 2019, 11(7), E1561. doi: 10.3390/nu11071561.

Abbreviations

ASQ	Ages and Stages Questionnaire
BDI	Beck Depression Inventory
BSID-III	Bayley Scales of Infant and Toddler Development, third edition
CES-D	Center for Epidemiologic Studies Depression Scale
CNS	Central Nervous System
HAZ	Height-for-age z-score
HC I	Health Centre I
ICC	Intra-Cluster Correlation
ICR	Iodide/Creatinine Ratio
LMIC	Low- and Middle-Income Countries
MDG	Millennium Development Goal
MSEL	Mullen Scales of Early Learning
RCT	Randomised Controlled Trial
SDG	Sustainable Development Goal
SUN	Scaling Up Nutrition
UIC	Urine Iodide Concentration
UDHS	Uganda Demographic and Health Survey
VHT	Village Health Team
WASH	Water, Sanitation and Hygiene
WISC-R	Wechsler Intelligence Scale for Children-Revised

Summary

Background: Uganda, being a low-and-middle income country, struggles with high stunting (low height-for-age) rates which may result in impaired child development. Although there are several causative factors of child stunting and inadequate development, low nutrient supply, poor hygiene and lack of stimulation, are main promoters. In addition, a dysregulated gut microbiota, inadequate iodine status and maternal depression may play a role. Up until 2013, few if any maternal education intervention studies had been conducted within the framework of a community-based randomized controlled trial (RCT), and long-term follow up studies were not performed. We therefore conducted a cluster-RCT in 2013-14 comprising a six months' maternal education intervention primarily to reduce child stunting. The study included 511 mother-child pairs and started when the children were 6-8 months. Whereas we found no effects on growth when the children were 20-24 months, several developmental outcomes were markedly improved in the intervention group compared to the controls.

Aims: Given these promising data on developmental outcomes we performed a follow-up study when the children were 20-24 and 36 months (current thesis). In addition to assessments of developmental outcomes and growth, we investigated child gut microbiota, iodine status and maternal depression symptoms.

Methods: The maternal education in the original trial focused on nutrition, hygiene and stimulation. Anthropometry was measured using WHO-standards whereas development outcomes were assessed with three independent neuropsychological tools. We used 16S rRNA gene sequencing to study gut bacteria whereas a colorimetric method was used to determine urine iodide concentration (a marker of iodine intake). Maternal depression symptoms were self-reported.

Results: The intervention significantly improved child development outcomes at 20-24 and at 36 months. Linear growth faltering was significantly less at 36 months in the intervention compared with the control group. The intervention did not lead to any significant changes in gut microbiota or iodine intake, but iodine intake was associated with child cognitive scores. The intervention reduced maternal depression symptoms and this reduction was associated with improved child cognitive- and language development.

Conclusion: Our maternal education intervention had long-lasting positive effects on child development and perhaps linear growth. Possible explanations for these findings include adequate iodine intake and reduced maternal depression while gut microbiota was not affected by the intervention. To identify the mechanism(s) underlying the detrimental effects of child undernutrition on growth and development requires multiple approaches including mechanistic studies and well-conducted community-based RCTs.

1. Introduction

1.1. Child development

Child development refers to a series of physical, language, thought and emotional changes from childhood to early adulthood [1]. A good start in early life (appropriate childcare practices and feeding) of every child is emphasized [2, 3]. Normal development is reflected in the child's ability to grow up to where their cognitive, social, emotional and educational needs are met [4, 5], and this is reflected by various indicators of cognitive, gross motor, fine motor, personal and social development, general understanding, language, visual as well as audio development [5]. Nations whose children achieve their full holistic development outcomes attain a fast drive to both short- and long-term human, social, and economic development [6]. Different ages are characterized by development of specific functions, with major developmental steps taking place especially during the first 1000 days of life with major changes in neural function. Postural development starts at about three months of age [7], and at six months, infants develop the ability to adapt their postural activity. At nine months, infants may be able to try standing up against furniture and walk sideways [8]. During this period, children become conscious that objects exist when they are removed and out of sight. The first word appears at this stage (8-12 months) [9]. The child begins to respond selectively to words, demonstrates intentional behavior, initiates actions, realizes objects that existed when out of sight and will try to look for them (object permanence), is interested and understands words and says words like "mama", "dada". Children's' ability to attain these developmental milestones may be influenced by social and cultural factors [10].

A child at 18 months will be able to run, stiffly walk backwards, attempts to kick a ball, climb on furniture, crude turning of book pages, and use a spoon well. Most children can take off pieces of clothing**.** By this age, children's vocabulary is about ten words, and using them with gestures. The infant can vocalize "no", and can point to pictures of common objects in their surroundings. It is during this age that the child starts to explore the environment. There is rapid acquisition of receptive language skills, but limited expressive communication [9].

At 36 months, the child has attained cognitive developmental milestones that e.g. include matching an object in a hand. Language has developed to a limited vocabulary of 500-3,000 words and the child is only able to form three- to four-word sentences, and can answer "yes"

and “no” to questions appropriately. The child is able to make cognitive judgments about what is heard [11]. The child often engages in imaginary play, and in turn further develops social emotional and cognitive skills [9].

1.1.1. Global perspectives of child development

Globally, about 200 million children under five years do not meet their development potential [12], which is linked to various factors [13]. Thus, several initiatives to address inadequate child development and growth are proposed. This is not only essential for the child’s wellbeing, but ultimately an indispensable investment toward future human capital to achieve desired global development objectives [2, 14]. Adequate nutrition, fostering caregiving and early learning opportunities during childhood together comprise the recipe for the best chances of child thriving [2]. In 2000, The UN emphasized this urgency in the Millennium Development Goals (MDG), e.g. in MDG 1: eradication of extreme poverty and hunger; MDG 4: reduce child mortality; and MDG 5: improving maternal health [15]. Currently, there is a worldwide call by the UN Sustainable Development Goals (SDG), WHO, UNICEF and the World Bank Nurturing Care Framework on developing and implementing required measurements for early child development [16, 17]. Vehemently, it is crucial to accelerate and track progress towards achieving specific goals related to early child development, especially in low- and middle-income countries (LMIC) [3].

Previous findings have identified the problems of child undernutrition and poor child stimulation [12]. These adversely affect brain structure, function, and have lasting impaired child development (cognitive and emotional) [12]. In the aftermath of the 2007, Lancet Child Development in Developing Countries Series on child development among children below 5 years [12], trends showed an encouraging, yet insufficient, progress in reducing risks for poor child development. For example, in 2010 about 43% of children below five years risked not fulfilling their development potential while, in 2016 this number increased to 56% [18]. Global challenges to improve child development especially in LMICs are still at large and will likely affect both human capital and health even in the coming decade [14]. Improving child development outcomes during early childhood necessitates thorough and wide-range as well as immediate actions to scale up effective holistic interventions. Moreover, there is a vast need to increase knowledge of evidence-based interventions on early child development and maternal health [3, 19].

1.2. Child stunted growth

In LMICs, impairment of linear growth (stunting) is a serious problem. Child stunting is defined as height-for-age more than two standard deviations below the WHO child growth standard's median [20], and is considered a proxy for chronic undernutrition. Stunting often starts in utero and continues postnatally during the first two years of child life [21]. Intrauterine growth restriction, a condition where the fetus is not growing at a normal rate inside the womb, affects many children in LMICs [22]. In 2014, sub-Saharan Africa reports indicated a decline in child stunting, from 42% in 2013 to 34% in 2014 [21]. According to the UN, the highest stunting prevalence is found in East Africa (43%), West Africa (34%) and South-Central Asia (35%) [23]. Despite a global decline in the proportional prevalence of child stunting the last decade, the prevalence in West and Central Africa increased from 22.4 million in 2000 to 28.9 million in 2018 due to population growth [24].

Due to the high figures of stunting among children, especially in LMICs, many policy-makers came up with policies aiming at reducing stunting rates**.** WHO in 2012 adopted a resolution on maternal, infant and young child nutrition which had six global targets to reduce the high burden of disease associated with undernutrition [25]. This had its focus and attention on the critical period from conception to 24 months of age. It also included a global target to reduce the number of stunted children under five years of age by 40% in 2025 [25]. As of 2014, the Food and Agriculture Organization of the UN estimated that in sub-Saharan Africa, 214 million people were undernourished, yielding a prevalence of 23.8%, making Africa the region with the highest prevalence of undernourishment [26].

1.3. Conceptual frameworks: The multilayered nature of child malnutrition and development

Following more or less successful ways of preventing and treating malnutrition in the 1970s and 80s, UNICEF recognized the need for a more holistic approach to this global health problem. As a result, a new strategy primarily targeting children and women of childbearing age in developing countries was presented in 1990, and included an analytical component (the triple A cycle) and a conceptual framework [27]. Briefly, the triple A cycle proposed a critical evaluation (Assessment) of the current situation to obtain information (Analysis) that could elicit a response (Action). This approach could be applied across the various levels involved in combating malnutrition, e.g. (i) diagnosis of nutrient-deficiencies among individuals and in

vulnerable populations (Assessment); (ii) identifying causes of these deficiencies, such as food insecurity and disease, by health care workers (Analysis); and (iii) subsequent implementation of proposed activities by community leaders and ministries to alleviate the problem (Action). Importantly, to succeed over time this approach would need to be repeated, hence the term "cycle".

Furthermore, UNICEF noted that this new strategy should be widely applicable and not restricted to certain geographical regions with specific guidelines and interventions, hence a conceptual framework was designed. Central to this framework was the realization that malnutrition is a multifaceted problem that needs a multilevel approach. The top level of the framework is the manifestation of malnutrition, e.g. disease or death (Figure 1). Then the immediate cause(s) (determinants) are identified, e.g. inadequate nutrition, hygiene and stimulation. To eradicate or reduce nutrient-deficiencies in a longer term, the next level of causes should be identified. Such underlying causes might be household food insecurity and/or poor sanitation and may require assistance from e.g. community leaders or NGOs. Their ability to respond would be dependent on access to appropriate resources such as workforce and funding and this would be the responsibility of ministries, governments etc. In turn, these latter stake-holders would rely on all potential resources, thus lack of resources was considered as basic causes of malnutrition.

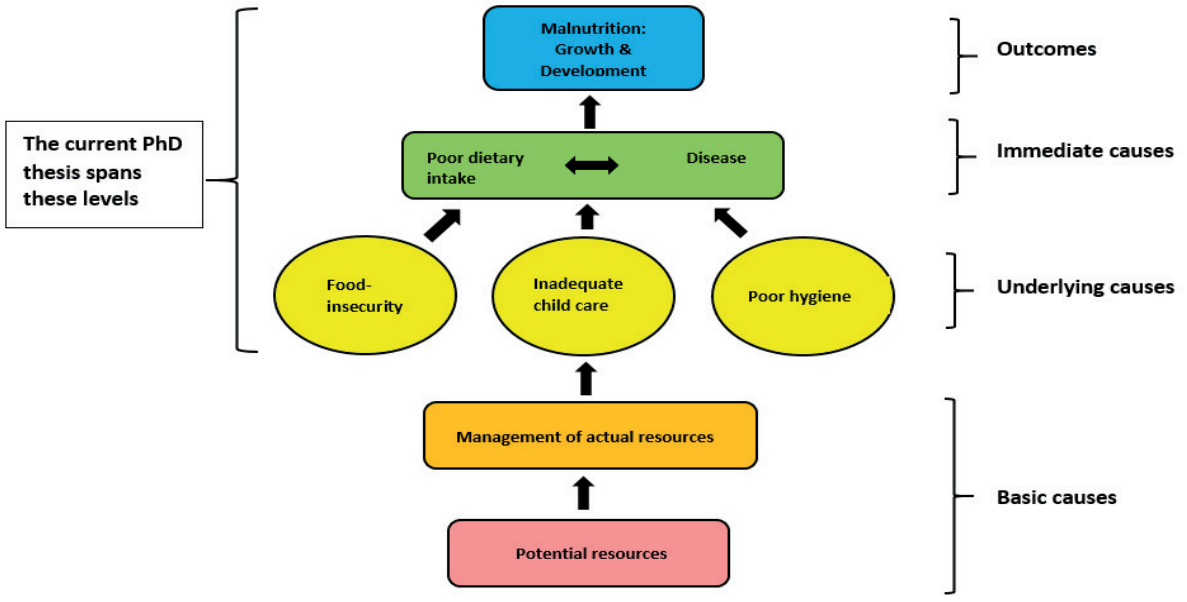


Figure 1: Modified illustration of the 1990 UNICEF conceptual framework [27].

In 2008, the journal Lancet published a series of five papers by the Maternal and Child Undernutrition Study Group reporting on the progress worldwide to fight malnutrition. The focus was how malnutrition in various forms (e.g. micronutrient-deficiencies) would impact on mortality in children and health later in life [28, 29]. The Group then reviewed the results from various interventions [30] before recommendations were given on how to address the malnutrition problem at a national [31] and international level [32]. An important message from this series was the need to focus on nutrition during the first 1000 days of life. Furthermore, interventions should aim to improve the underlying determinants of undernutrition, such as poverty, poor education, disease burden and lack of women's empowerment [30].

The Maternal and Child Undernutrition Study Group reassessed in 2013 the issues presented in its Lancet 2008 series. Notably, in this Lancet 2013 series the Group also included aspects of overnutrition (overweight/obesity) in addition to undernutrition. Among the main findings was that micronutrient deficiencies were still prevalent globally, and child and maternal obesity was on the increase. While the rates of stunting had declined in many countries, it was still increasing in Africa [33]. Next, the effects of various interventions and associated costs were reviewed and based on this nutrition-sensitive interventions were suggested on how to save lives and reduce morbidity associated with malnutrition. Specific emphasis was put on the role of women and their potential for empowerment [34]. Importantly, a new framework was presented that included a more detailed description of proposed components (e.g. breastfeeding, child stimulation, feeding practices, maternal empowerment and hygiene) that would need to be addressed to achieve optimal nutrition from early childhood on [33]. Specifically, this framework re-emphasized the conclusion from the UNICEF 1990 report that malnutrition and its related problems have to be addressed by a multilevel approach. In line with this, the new framework also included means to achieve optimum fetal and child growth and development, and not only the determinants of malnutrition. Moreover, it explained the interlinks between the determinants and how they are affected by underlying factors, such as food security, caregiving resources, and environmental conditions. These underlying factors in turn are shaped by economic and social conditions as well as national and global resources and depend on the prevailing political governance. The importance of a multilevel involvement was further elaborated, pointing to the significance of including the agricultural sector, social welfare systems and education institutions to relieve the burden of malnutrition [35]. Underpinning further the role of a multilevel approach to malnutrition was the elaboration on how

environments such as governance, political systems and resource prioritizations, are crucial to involve if we are to prevent malnutrition [36]. Whereas focus had been on nutritional aspects only in the UNIFEC framework and in the Lancet 2008 series, the Group also included child development in 2013. However, despite a wealth of data on nutritional aspects and growth in children, direct data on how nutrition can impact developmental issues were not detailed. Rather, the Group concluded that child development interventions alone had little impact on nutrition, but combined with nutritional interventions could act synergistically on developmental outcomes and perhaps improve nutrition [35].

1.3.1 Determinants of child development and growth

In this thesis, the focus is on three major determinants for child development and growth in LMICs and include nutrition, hygiene (linked to gut infections) [37], and stimulation (including maternal mental health, in particular maternal depression) [38].

1.3.1.1 Childhood nutrition

Adequate nutrition is critical to ensure a healthy development and growth pattern. This includes focus on breastfeeding issues and later on adequate complementary feeding.

1.3.1.2. Breastfeeding

Exclusive child breastfeeding during the first six months is recommended by the WHO to promote child development, optimal growth and to prevent infant morbidity and mortality in developing countries [39, 40]. Exclusive breastfeeding improves several child health outcomes, including cognitive development [41], protection against gastrointestinal and respiratory infections, as well as adequate child growth [42, 43]. About 66% of Ugandan children are reported to be exclusively breastfed [44]. Globally, UNICEF findings on breastfeeding show that about 66% of children aged 1-5 months are exclusively breastfed while 87% continue breastfeeding to 1 year and 43% continue breastfeeding to 2 years [45]. Continued breastfeeding once complementary feeding starts, is recommended until the child is 24 months of age and possibly beyond [46-48].

1.3.1.3. Complementary feeding

Complementary feeding is the process when an exclusively breastfed infant is introduced to foods and liquids while breastfeeding continues. During this period, provision of timely, safe and adequate complementary foods to breastfed infants is critical in preventing undernutrition

[49]. If complementary feeding is introduced too early, the infant may be at risk of overweight/obesity as breastfeeding is also protective against overnutrition, possibly in part due to reduced intake of energy-dense foods and drinks [50]. The age of six months is the recommended period to commence complementary feeding because then breast milk is no longer able to meet the nutritional needs (e.g. in terms of energy, protein and micronutrients) of infants growing at a rapid rate [51, 52]. In addition to ensuring adequate dietary intakes, complementary feeding also promotes the development of organs and their functions. For example, whereas breastmilk is important for the defence against infections from birth onwards [53], recent studies in animals and humans, where complementary foods were directed at a healthy microbiota profile, reported increased levels of biomarkers for growth, bone formation, neurodevelopment and immune function [54]. Introduction of complementary feeding also promotes differentiation and proliferation of intestinal stem cells in order to adjust to changes of stopping/reducing breastfeeding [55]. Moreover, the introduction of foods encourages the sense of taste and smell, enables the infant to develop motor skills such as the ability to chew and handle solid foods as well as appreciate other foods [56]. The skills of self-feeding will develop by about nine months [57, 58]. In 2003, PAHO/WHO [59] developed ten guiding principles for complementary feeding of the breastfed child, which should be adapted to local feeding practices and conditions [59]. In rural Uganda, there is a tradition of mainly giving nutrient poor diluted porridges based on finger millet and maize to infants and toddlers which may negatively impact on nutritional status during weaning [60].

1.3.1.4. Nutritional status, role for child development and growth

Adequate nutrition is of great importance to the structural and functional development of the brain and indirectly for children's behaviour as well as experience [61, 62]. Between the last trimester of pregnancy until the age of two years, significant development of the brain depends on sufficient supply of macro- and micronutrients as building blocks [61, 63], and for maintenance of the nervous and brain tissue [62, 64]. Similarly, children at risk of intrauterine growth restriction have an increased risk for poorer neurodevelopmental outcomes later [65].

Chronic undernutrition and micronutrient deficiency linked to cognitive deficits are widely reported [66]. Several intervention trials have studied the role of nutrition on child development outcomes. Table 1 gives a brief overview of some recently performed

randomized trials including nutrition and child development among low- and middle income countries (LMICs).

Table 1: Overview on recent randomized trials on nutrition supplementation interventions and the impact on child development in LMICs

Author	Age of participants	Study site & number of participants	Type of intervention	Effect on child development
Pitchik *et al,* 2018 [67]	20-39 months	Tanzania; n=198	Prenatal vitamin A and zinc supplementation	No significant effect on any child development outcome assessed by Caregiver Reported Early Childhood Development Index
Locks *et al,* 2017 [68]	6-15 months	Tanzania; n=247 (sub-sample)	Zinc and multivitamins	No significant effect between study groups on BSID-III cognitive, language and motor development
Christian *et al,* 2016 [69]	6-24 months	Bangladesh; n=734	Iron-folic acid	No difference between study groups on BSID-III cognitive, language, and motor development
Prado *et al*, 2016 [70]	6-18 months	Malawi; n=1932	Lipid-based nutrient supplements	No significant difference between study groups on motor, language, socio-emotional, or executive function assessed by Kilifi Developmental Inventory, Griffins Mental Development Scale, Merril–Plamner scales and MacArthur Communication Development Inventory.
Yousafzai *et al,* 2014 [71]	2.5 months	Pakistan; n=1489	Multiple micronutrient powder	Significant effect on BSID-III cognitive, language, and social-emotional scales
Taljaard *et al,* 2013 [72]	6-11 years	South Africa; n=414	Micronutrients beverages	Significant effect on cognition assessed by Kaufman Assessment Battery for Children version II and the Hopkins Verbal Learning Test
Baumgartner *et al,* 2012 [73]	6-11 years	South Africa; n=926	Iron supplementation and fatty acids (DHA/EPA)	Significant improvement on verbal, non - verbal learning and memory assessed by Hopkins Verbal Learning Test and Kaufman Assessment Battery for Children
Murray *et al,* 2012 [74]	12-13 months	Nepal; n=734	Iron plus folic acid supplementation	No significant effect on intellectual, executive and motor function at 7 and 9 years, later assessed by Universal Non-verbal Intelligence Test go/no-go task, a Stroop (numbers) test, and a Backward Digit Span test and Movement Assessment Battery for Children
Manno *et al,* 2012 [75]	6-18 months	Zambia; n=743	Rich micronutrient porridge	No significant effect on mental and motor development assessed by BSID-II
Phuka *et al,* 2012 [76]	6 months	Malawi; n=163	Lipid-based nutrient supplement	No significant difference on Griffiths' developmental scores and mean developmental quotients

Undernutrition presents in the forms of stunting (low height/length-for-age or shortness), underweight (low weight-for-age) or wasting (low weight-for-height or thinness). Among all the three forms, stunting is often regarded as the most devastating form because of its severe short- and long-term child health consequences [77]. For example, stunting during early childhood is associated with increased morbidity, impaired cognitive development, educational

attainment that consequently leads to low adult work capacity, increased weight gain and metabolic diseases in adulthood [29, 33]. Globally more than 156 million children below five years of age are stunted [78, 79]. Approximately one million child deaths are attributed to stunting each year and 800,000 deaths to wasting (60% of which are attributable to severe wasting) [28]. Current global reports indicate a substantial progress in reducing the number of stunted children, but sadly not in Africa [80]. Unfortunately, there has been less progress in relation to reducing the number of wasted children [80]. Notably, the world is behind course to meet the World Health Assembly goals of a 40% reduction in the prevalence of stunting and wasting to <5% by 2025 [80]. Indeed the influence of childhood nutrition on growth is widely recognised. For example, zinc and multiple micronutrients supplementation positively impacts on child growth [81]. In addition, protein is also positively associated improved child growth. In fact, the importance of additional protein in catch-up growth interventions was highlighted by the WHO/FAO/UNO 2007 guidelines [82, 83].

1.3.1.5. The role of iodine in child development

Insufficient iodine intake during early childhood is linked to impaired child development especially cognitive development, at least in part due to impaired brain development, including the processes of myelination, cell migration, differentiation and maturation [84, 85]. Notably, moderate-to-severe maternal iodine deficiency during pregnancy leads to reduced birth weight, and increased infant mortality [85]. A 2013 systematic review examining the relationship between iodine and mental development among children five years old and below, indicated a substantial impact of iodine on child mental development across different study designs [12, 86, 87]. Iodine supplementation trials assessing development among 6 to 14 years old children have reported mixed findings following primarily prenatal supplementation of mothers [88]. For example, in Bangladesh, no significant difference was reported between supplemented vs. non-supplemented groups on intelligence [88] whereas a positive association was reported on cognitive-perceptual tasks among children of supplemented mothers in Malawi and Albania [89, 90]. A review on several micronutrients among four African countries identified iodine to be among the most prevalent micronutrient deficiency that needs to be included in public health prevention strategies [91].

1.3.2. Child development and growth – associations to hygiene and infections

The initiative on improving water, sanitation and hygiene (WASH) to reduce infections is recommended for interventions aimed at promoting adequate child development and growth [92]. In the LMICs, it is estimated that about 1.1 billion people do not have access to clean water [93].Thus, when introducing other foods and water during weaning, it may result in chronic environmental enteropathy, low child weight and stunting [94].

Malnourished children have greater incidences of infections, longer duration and increased severity of diarrheal illnesses [95]. Inadequate dietary intake during illness further weakens the immune response system and increases susceptibility to more infections. This is associated with weight loss, lowered immunity, and mucosal damage [42]. Subsequently this leads to a vicious cycle of adverse nutritional status and increased susceptibility to infection (Figure 2) [96].

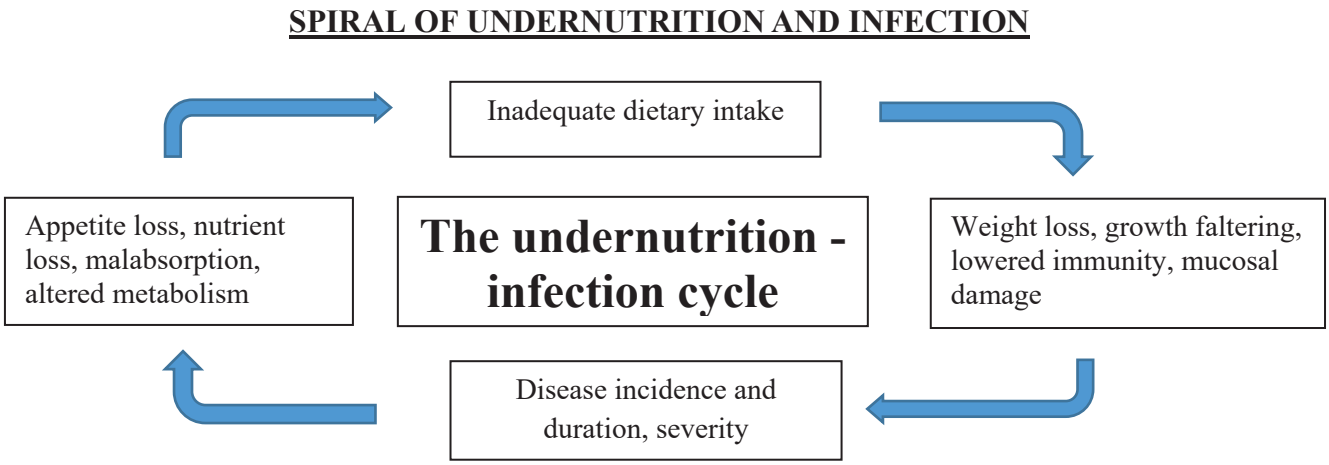


Figure 2: The "vicious cycle" of undernutrition and infection. Adapted from Katona & Katona [42].

Children in LMICs are prone to a high burden of gut infections [94, 97]. Persistent gut infections among children often lead to reduced intestinal nutrient absorption and delayed brain development. In turn, this may impair child cognition, educational achievement and linear growth [92, 98]. The interplay between gut infections and child development and growth is complex [92]. For example, child infections may cause disturbances in small intestinal structure resulting into compromised gut barrier function and chronic systemic inflammation. This increased intestinal permeability is negatively associated with cognitive development and child growth [92]. A recent systematic review pointed out the adverse influence of childhood gut infections on cognitive function and educational loss [97]. Especially, children with infections

had low development scores in the cognitive domains of learning, memory and intelligence compared to their counterparts without infections [97]. In line with this, a 1-day increase in diarrhea per month was associated with a decrease in Wechsler Intelligence Scale for Children–Revised (WISC-R) scores at 12 and 24 months [99]. Moreover, Giardia and soil-transmitted helminth (geohelminth) infections are common in tropical environments and may cause intestinal mucosal damage. Giardia infection is associated with poor nutritional status of young children in LMICs and it has been associated with decreased WISC-R scores [99]. Geohelminths seem to be implicated in restricting child growth, causing maldigestion and malabsorption [100]. Furthermore, rural Gambian infants between the ages of 3 and 15 months were found to have a small intestinal mucosal enteropathy for 75% of the time, but showed clinical manifestations of diarrhoea, for only 7.3% of the time. The presence and severity of the enteropathy could explain 43% of the long-term growth faltering whereas the prevalence of diarrhoea was not significantly associated with growth failure [101, 102]. Another study from Gambia showed that intestinal permeability (measured as lactulose: mannitol ratio in blood) more than doubled between 12 weeks and one year of age and this was negatively associated with growth [103]. Growth faltering was associated with impaired small intestinal barrier function, leading to endotoxemia and systemic inflammation. The infants also showed evidence of chronic low-level immune-stimulation, evaluated by white blood cell counts [103]. Also, mean plasma levels of IgA, IgG and IgM increased above expected values after 8 weeks age and all correlated negatively with growth and positively with intestinal permeability [103]. Raised plasma concentrations of endotoxin and endotoxin antibody implicated intestinal involvement. Moreover, elevated endotoxin core antibody concentrations were closely associated with raised intestinal permeability and lactulose uptake, adding further evidence for the premise of a “leaky” gut barrier [103].

1.3.2.1. Gut microbiota

Previous studies have provided insights into how the central nervous system (CNS) and development may be influenced by the microbiome and gut health [104, 105]. This is often referred to as the microbiome-brain-gut axis. The network of communication between the gut microbiota and brain includes the sympathetic and the parasympathetic braces of the autonomous nervous system, the enteric nervous system and the neuroendocrine and neuro-immune systems [106]. Increasing data supports the role played by commensal organisms in the gut in facilitating early programming and later responsivity of the CNS.

Microbial colonization of the human gut begins at birth, overlapping with the critical period of brain development [107]. Both nutrition and enteric infections influence the establishment of a more stable microbiome, which normally occurs during the first three years of life [106]. Microbiota colonization is influenced to a great degree by mode of delivery and feeding patterns [108, 109]. The gut microbiota of breast-fed infants appears to be more diverse and heterogeneous with an ability to modulate the innate immune transcriptome according to a recent metagenomic study [110]. A study of fecal microbiota from children living in rural communities in Malawi and from children distributed across the USA reported several differences of microbiota between the two child-groups already at the age of three years [104]. These results suggested a program of functional maturation of the human gut microbiota, driven in part by breast milk, and that this program varied between Western compared to non-Western diets. Notably, the taxonomic composition of the gut microbiota in Malawian subjects was more diverse and clustered together unlike the Western gut microbiota. These data also indicated that when defining nutritional requirements for different age groups, the gut microbiota needs to be considered [104]. Indeed, previous research has indicated the ability of the gut microbiota to influence the brain functionality and behaviour [111-113], and an important regulator of several cognitive functions [114]. Consequently, suggestions have been put forward on how the gut microbiota may be used to develop new therapies to improve various brain functions [115-117]. In LMICs, intestinal microbiota modulation is suggested as an optimal and safe approach to facilitating child growth and development especially among the vulnerable populations struggling with child undernutrition [118].

1.3.3. The role of stimulation for child development

Child stimulation is the set of structured, age-appropriate activities included in a child's daily living as well as the family's daily child play routines [119, 120]. These activities can range from feeding and dressing to bathing and play. These types of child stimulation are science-based activities that when practiced systematically and sequentially facilitate cognitive, physical, social and emotional development, especially from birth to six years [120]. The child first year's relationship with the caregivers is characterized by learning to recognize and interpret verbal as well as nonverbal communication cues from each other. Without this reciprocal process, adverse outcomes in the emotional bonding, attachment and emotional functioning transpires [121, 122]. Child cognitive, language, social and emotional development

largely depends on an effective communication between children and caregivers [121, 123]. A disruption, inconsistent as well as non-responsive interaction in this relationship, grossly impairs child development outcomes [122, 124]. Table 2 provides an overview of recently performed randomized trial on child stimulation and child development in LMICs.

Table 2: Randomized trials on child stimulation interventions and child development in LMICs

Author	Age of participants	Study site & number of participants	Type of intervention	Effect on child development
Pitchik *et al,* 2018 [67]	20-39 months	Tanzania; n=198	Integrated environmental, educational, parenting, and stimulation interventions	Significant effect on child development outcomes motor and cognitive/language development assessed by Caregiver Reported Early Childhood Development Index
Worku *et al*, 2018 [125]	3-59 months	Ethiopia: n= 78	Play stimulation	Significant effect on child language, personal-social and social-emotional assessed by Denver II-Jimma, and social-emotional by the Ages and Stages Questionnaire
Hartinger *et al,*2017 [126]	6-35 months	Peru; n=534	Home-based interventions, integrated household intervention package and early child development intervention	Significant effect on all assessed child development gross, fine motor and communication
Helmizar *et al,* 2017 [127]	6-9 months	Bangladesh; n=355	Psychosocial stimulation	Significant difference on cognitive and motor development
Singla *et al,* 2014 [128]	12-36 months	Uganda; n=319	Community-based parenting programme (psychosocial stimulation, and maternal care)	Significant effect on cognitive and language
Yousafzai *et al,* 2014 [129]	2.5 months	Pakistan; n=1489	Community-based cluster-randomised responsive stimulation	Significant effect on cognitive, language, and motor development assessed by BSID-III
Aboud *et al*, 2013 [130]	4-14 months	Bangladesh; n=463	Responsive feeding and play	Significant effect on child development cognitive and language assessed by BSID-III
Vazir *et al*, 2013 [131]	3 months	India; n=200	Responsive feeding and psychosocial stimulation	Significant effect on motor and mental development assessed by BSID-II
Draper *et al,* 2012 [132]	2-4 years	South Africa; n=118	Gross motor skills child stimulation	Significant effect on gross motor and cognitive function assessed by Test of Gross Motor Development-2
Nahar *et al,* 2012 [133]	6-24 months	Bangladesh; n=507	Psychosocial stimulation	Significant effect on mental development but not motor development assessed by BSID-II
Potterton *et al,* 2010 [120]	Below 2 years	South Africa; n=122	Basic home stimulation (daily living and developmentally appropriate play activities)	No significant difference between groups in child development assessed by BSID-II

1.3.3.1. Maternal depression

Maternal depression may lead to an inadequate child development [134]. Specifically, maternal depression may disrupt the early child-mother attachment, which is critical to the healthy development of the child [135]. Depression is common (10–19%) [136] among mothers of young children and is associated with impaired child development, underweight as well as stunting [137]. Postpartum distress significantly contributes to cognitive and socio-emotional

delay in infants from birth to 1 year of age [138]. The severity and duration of maternal depression increases the behavior- and vocabulary problems of the children [138]. According to Bowlby's attachment theory, infants of depressed mothers often react to their mothers with anger, distress, withdrawal behavior, avoidance, and disruptions in the ability to regulate their own emotions [139, 140]. Consequently, these infants tend to cry more than babies of non-depressed mothers [140]. Subsequently, this leads to inadequate mental (cognitive), motor, and language skills development [140]. They also have a less capacity to concentrate, fewer abilities across a broad spectrum of emotional skills, more negative responses to their environment, more behavioral difficulties and a higher risk of psychiatric disorders during adolescent years than those of non-depressed mothers [141, 142]. These consequences are not restricted to infancy, but can extend into toddlerhood, school-age and even adulthood [87].

Maternal depression in LMICS is a greatly under-researched topic despite estimated indicators showing that among LMICs, maternal depression is three times more prevalent than in high income countries, with prevalence ranging from 10 to 41% [143, 144]. This calls for screening to identify and intervene on early postpartum depression to decrease the negative effects on child development outcomes as well as the devastating effects on new mothers [145]. Mental health services are rare in many LMICs [144]. This has prompted suggestions to include mental health services in community-based strategies to curb maternal depression and improve maternal child care [146].

1.4. The Uganda child health perspective

UN has increasingly recognized nutrition as a basic pillar for social and economic development. Unfortunately, Uganda's effort and progress to achieve the MDGs, especially MDG 4 and 5 on child and maternal health, was insufficient [147, 148]. Currently, Uganda is part of the Scale Up Nutrition (SUN) movement launched in 2010 to work toward improving global nutrition [149]. Lately, Uganda has implemented the UN SDG of 2015.

Despite the national and international efforts to combat undernutrition, Ugandan children are still being affected by various nutrition problems. According to the Uganda Demographic Health Survey (UDHS) of 2016, the prevalence of stunting among children < 5 years was 33% [44, 150]; see also Figure 3. Thus, about 2.3 million young children in Uganda may be chronically undernourished. Approximately 20% of Ugandan children aged 6 months to 5 years suffer from vitamin A deficiency [151]. More so, iron-deficiency anemia affects three-quarters of children 6–59 months old [151]. It is well known that anemia among children leads to a

significant slowdown in cognitive development, decreased physical activity, and reduced resistance to disease [152, 153]. Zinc deficiency is projected to about 20 to 70% in young children [154-156].

Child health indicators in Uganda

Indicator		Number or %
Neonatal mortality rate	per 1,000 live births	27
Infant mortality rate	per 1,000 live births	43
Under-five mortality rate	per 1,000 live births	64
Maternal mortality rate	per 100,000 live births	343
Births attended to by a skilled health person		64%
Basic child immunization by 1 year		49%
Total fertility rate per woman		5.4

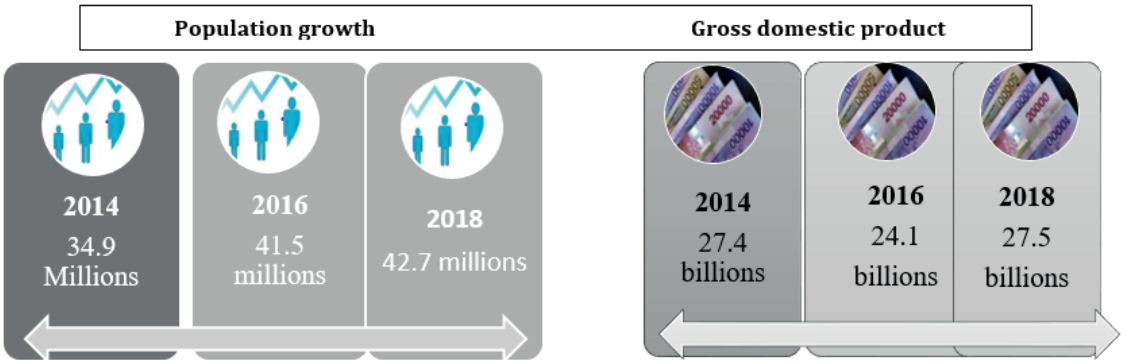


Figure 3: Uganda child health indicators 2017, population growth trends and gross domestic product projections [44, 157-160].

Due to undernutrition (Figure 4), Uganda has suffered from great costs with adverse effects on economic growth and general welfare [161]. Undernutrition among Uganda's children affects both the individual, their households, communities and the nation as a whole in terms of physical and mental problems and increased burden of disease [161].

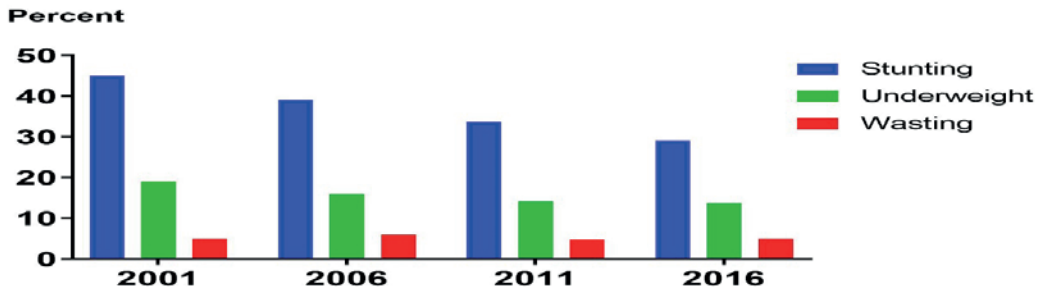


Figure 4: Nutritional status, trends in Uganda among 6-59 months children; Uganda Demographic Surveys 2001-2016.

2. Rationale for the current thesis

In 2013, when we initiated this research project, the South-Western region of Uganda was considered to be food secure and referred to as the country's food basket [162]. Despite this, persistent high levels of undernutrition among its children affected both their growth and cognitive development [161]. Specifically the reported rates of stunting were high [33, 139]. At that time there was much less focus on gut microbiota, iodine and maternal depression as explanatory variables for child undernutrition and development. Hence, we did not start to collect data regarding these three factors at the start of the original RCT.

The etiology of stunting is complex with overwhelming costs (personal and financial) for the affected children. Thus, a randomized trial comprising a maternal education intervention focussing on nutrition, hygiene and stimulation was implemented, directed towards the multiple determinants of child growth and development. Notably, we only provided education, we did not give any foods or food-supplements to the participants. In this original trial, we primarily wanted to reduce stunting among the children. The intervention lasted six months and was implemented when the children were aged 6-8 months. Whereas we were unable to demonstrate any appreciable effects on physical growth when the children were 20-24 months, several developmental outcomes were markedly improved among children in the intervention group compared to the controls [163].

Given these promising data on developmental outcomes, we decided to perform a follow-up study when the children were 36 months, and this forms the basis of the current thesis. In addition to assessments of developmental outcomes and growth, we here also investigated (i) child gut microbiota and (ii) their iodine status, in addition to (iii) maternal depression symptoms, since these three factors may impact on both development and growth, in particular in low-resource settings. Importantly, in this follow-up study we only collected and analysed data, we did not perform any specific intervention. Notably, when this follow-up study was designed in 2015 we were unable to identify any similar study that had included the long-term impact of a maternal education intervention on child development and growth in sub-Saharan Africa.

3. Aim

The aim of the present PhD thesis was to investigate long-term effects of a maternal education intervention on development and growth among children in rural Uganda. We also wanted to examine the possible effects of this intervention on child gut microbiota, iodine status as well as on maternal depression symptoms, as these three factors may affect child development and growth. To achieve this aim, we used the follow-up study to address the following research questions.

3.1. Research questions

Can education about nutrition, hygiene and child stimulation:

(i) improve development outcomes (cognitive, language and motor) (Paper I)

(ii) improve growth (Paper I)

(iii) impact on the gut microbiota, leading to better development and growth (Paper I)

(iv) impact on iodine status, leading to better development and growth (Paper II)

(v) impact on self-reported maternal depression symptoms, leading to better development and growth (Paper III)

4. Methodology

4.1. Study area

This study was conducted in the neighbouring districts of Kabale and Kisoro in South-Western Uganda because of high stunting rates [135, 147,153]. Administratively, Kabale is divided into 24 sub-counties and Kisoro into 14 sub-counties (Figure 5). Both districts are mountainous and hilly with spectacular valleys and located at an average height of 1,219- 2,347 m above sea level. The topography is mainly green, interlocking and with heavily cultivated hills [164, 165]. The land is heavily fragmented, and each household has on average 6-7 plots of land on several hills. These pieces of land are often over-cultivated and facing depletion of soil fertility causing too low food productivity to satisfy the nutritional needs of families.

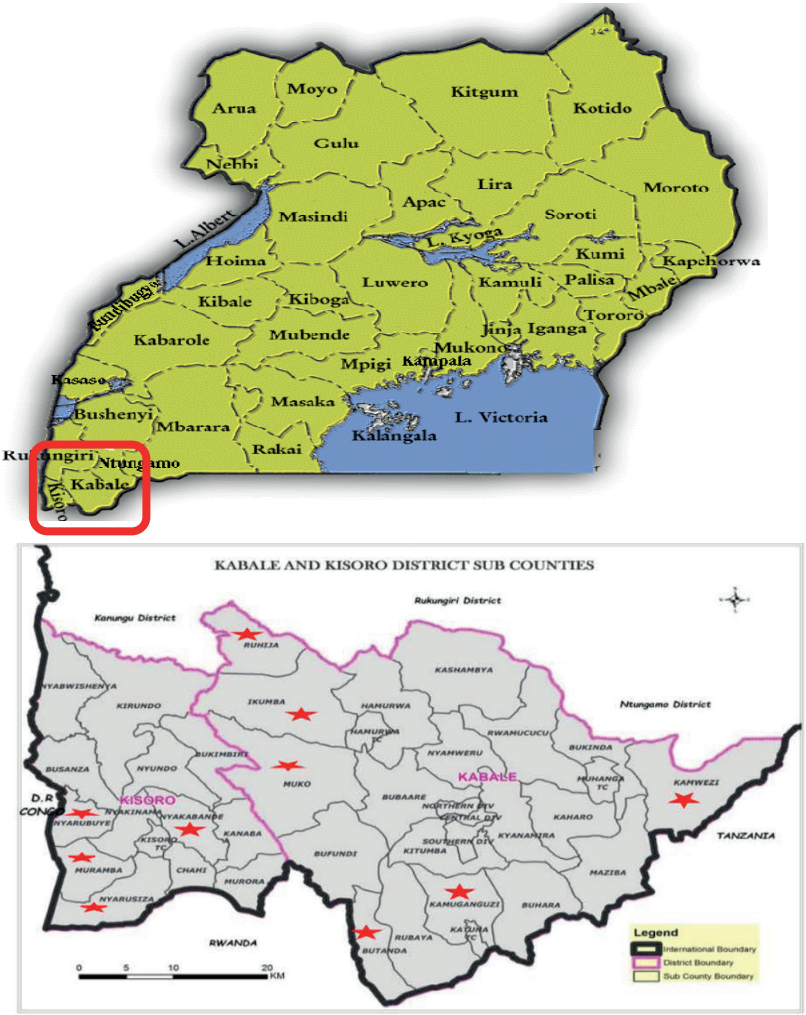


Figure 5: Map of Uganda districts. The study districts Kabale and Kisoro are encircled in red. The asterisks denote the 10 study clusters. Modified from Uganda Travel Guide.

The regional referral hospital for the two districts is located in Kabale, while Kisoro has a district hospital in the town centre. The two districts have a network of health centres (HC) II, III and IV at the various levels. HC IV, also known as health sub-district, is normally located at the county level and is equipped with basic preventive, curative and rehabilitative care and secondary level referral services. It also handles life-saving medical, surgical and obstetrical emergency care such as blood transfusion, and surgical emergency interventions. The basic unit in the community is HC I with a team of 9-10 people known as the Village Health Team (VHT). The VHT mainly facilitates community mobilization and empowerment for health [166].

4.1.1. Kabale District

Kabale district occupies approximately 1,827 sq. km. According to the 2014 population and housing census, Kabale district had a population of 534,160 people, largely (86%) living in a rural setting. People are predominantly of the Bakiga tribe, but also of the Batwa (pygmies), Banyarwanda and Bahororo tribes [157].

4.1.2. Kisoro District

Kisoro district occupies a total area of about 729.7 sq. km. Kisoro district had a population of 287,179 according to the 2014 national housing and population census and with annual population growth rate estimated at 2.21% [157]. The people in the district are mainly of the Bafumbira- Hutu, Tutsi and Twa (pygmies) tribes. A section of the district is inhabited by their neighbours, the Bakiga [165].

4.2. Study population and selection of participants in the original RCT

The randomisation and selection of study participants in the original trial has been detailed [163]. Briefly, simple random sampling was done to allocate 10 sub-counties (clusters) in each district (6 from Kabale and 4 from Kisoro districts) to either intervention or control. District town centres were excluded to ensure a uniform population in terms of socioeconomic status and child feeding practices. All villages in each sub-county (intervention or control) were listed alphabetically and by use of random numbers, villages to participate were obtained. Computer-generated random numbers were then used to obtain the villages and finally, complete enumeration was used to obtain households with children (6-8 months). Intervention villages did not share common geographical boundaries with control villages to prevent "contamination" of intervention-contents between the two groups.

Infant exclusion criteria were congenital malformations or physical handicap among children that would influence food intake, growth to interfere with taking the anthropometric measurements, mental or brain illness as evidenced by mother or health worker. In addition, if the family was likely to migrate during the study period and/or if the mother denied to consent to the study project, they were excluded.

4.3. Study population and selection of participants in the follow-up study

In the current follow-up study, we primarily wanted to examine if the developmental benefits of the education intervention lasted over time. Hence, the child had to be 20-24 months during the period of January-May 2015 to be included in the current follow-up study since developmental milestones at this age may predict IQ at 5-6 years when children are about to start school [17].

4.4. Calculation of sample size for the follow-up study

For Paper I and II we reasoned that an increase in the Bayley Scales of Infant and Toddler Development, 3rd edition (BSID III) cognitive development scores between 6-8 months and 20-24 months by 0.5 SD (corresponding to 7.5 points) would be regarded as clinically significant [71]. To detect this difference with a $p < 0.05$ and a power of 80%, the sample size had to be 126 in total (63 in both study groups). To allow for about 1/5 of dropout rate, 155 households were required for this follow-up study. With an intra-cluster correlation (ICC) of 0.01 [167] and dropouts, the mean number of children per sub-county was 15. Among the eligible 155 households, we randomly selected 77 children from the intervention group and the 78 children from the control group at 20-24 months.

For Paper III, a mean difference of about 1.5 SD in BDI-II scores between intervention and control groups at 36 months was deemed clinically important. Thus, with a power of 0.8 and $p < 0.05$ a minimum of 44 mother/child pairs per group were required. To account for an ICC of 0.01 and dropouts, a total of 155 children were included. Among these 155, we randomly selected 77 mother/child pairs from the parental trial intervention group and 78 children from the parental trial control group. Notably, the numbers (and mother/child pairs) in the intervention (n=77) and control (n=78) groups are the same as in Paper I and II.

4.5. The education intervention in the original RCT

An education intervention emphasizing nutrition, hygiene (including oral hygiene) and stimulation was delivered to mothers in the intervention group as described previously [163]. In short, cooking and oral hygiene demonstrations together with making of play toys to promote child stimulation were parts of the education intervention package. The intervention lasted six months in which each group of mothers received three main education sessions (with a nutrition education team) followed by monthly village meetings. Our strategy with the intervention was to promote behaviour change through providing information and prompt practice (demonstrations). The intervention is detailed below.

4.5.1. Nutrition education

The nutrition package was centred on PAHO/WHO guiding principles of complementary feeding of a breastfed infant (quality and quantity of complementary feeds) [59]. The main emphasis was on;

- The importance of breastfeeding and a demonstration of how to position and attach the infant to the breast.
- The need to allow emptying one breast before changing to the other breast so that the infant could benefit from both the fore and hind breast milk.
- Breastfeeding eight or more times in a day including at night.
- All mothers were asked to start complementary feeding if they had not done so, since all infants were between 6 and 8 eight months of age.
- In complementary feeding, they were advised to start with soft foods in small amounts at a time and gradually increase the portion and the thickness of the food.
- Providing food that is rich in variety of nutrients and the importance of combining a variety of foods in one dish.
- To give infants complementary foods 2-3 times a day and increase the frequency of feeding to 3-4 times a day as the child grew.
- Providing nutritious healthy snacks (such as fruit) to the infant in between the main meals.
- Interaction and responsiveness while feeding the infants by talking, smiling and encouraging them to eat more without forcing them; to exercise patience and make feeding session a time for joy and bonding.

- To allow the infants to eat finger foods which they can hold with their hands.
- Continued breastfeeding until the child was 24 months of age.
- Breastfeeding more frequently, providing more fluids during illness (especially in diarrhoea and fever) of the infant, and giving foods that are more nutritious after recovery.

4.5.1.1. Cookery demonstrations

The cookery activities involved:

- Dishes which could combine up to 13 different foods in one obtained in their local environments.
- Inexpensive formulated recipes using locally available foods with emphasis on animal protein obtained from silverfish (*Rastrineobola argentea)* locally known as *Mukene.*
- Soymilk making, scraping meat (muscle), preparation of pumpkin seed powder and silverfish powder to incorporate in the infant's food, addition of oil/fat and sugar to porridges to increase the energy content.
- Preparation of enriched porridge recipe1 and 2 which were enriched with the ingredients of; cooking oil, sugar, silver fish powder, milk, pumpkin seed powder and eggs; in combinations of two or more.
- Preparation of scrambled eggs preferred to the boiled eggs or omelette, which are rather hard for the infants to consume.

4.5.2. Hygiene education

Themes of emphasis included:

- The importance of living in a clean home environment for the good health of the family particularly the young children.
- The basic requirement to always wash hands and utensils with clean water and soap during food preparation and infant feeding.
- The prerequisite to clean food before preparation to make it free of soil and other contaminants.
- Mothers were encouraged to carry water and a piece of soap to the field/gardens to wash hands before feeding the infants.

- Mothers were warned on giving leftover foods to the infants, since safety of such food was not possible and safe for the infants to consume later.
- Licking spoons as they fed the babies (to test the temperature) was discouraged to avoid transmission of infections from the mother to the infant.

4.5.3. Child stimulation

The child play and stimulation emphasized:

- The importance of age graded child play activities and the role of mothers, other family members to engage in child stimulation.
- The significance of play to promote healthy development of the child.
- Explanation of the three development domains (cognitive, language and motor domains).

We explained to the mothers that the aim of play was to develop imagination creativity and social skills in the child [168]. The mothers were encouraged to use "name and identify" child's body parts to facilitate the child's understanding during his/her daily routine related to his body [169]. Practically, mothers engaged children in some of child play activities such as hiding favourite items for children to find; screwing and unscrewing bottles and imaginary play. Mothers also hand-made "easy to make" toys (from local materials) which were recommended as appropriate for children; shakers, empty transparent bottles with screws and food pellets inside, baby dolls made from cloth or banana fibres.

Language development was defined as verbal and non-verbal communication (expressive and receptive language) [170]. 'We Talk' slogan was used to show mothers the importance of talking to the child so that they learn to talk back and in the process develop language skills [170]. Mothers were encouraged using communication development aides such as imitation, roleplaying games, songs and music, to facilitate the child's ability to communicate emotions, thoughts, needs and interests [126, 171]. The mothers were encouraged to set aside time to purposefully talk to the children, call them by their name and to respond to them in word and/by gesturing; mention household and personal items while pointing at them, naming domestic animals, imitating their words and actions.

For motor development, the "Learn whereas playing' slogan was emphasized. The concept of

gross motor skills was explained as the use of coordination and control of the body to facilitate the development of security, speed, and accuracy [172] in daily performance of tasks in a child's life (larger movements like walking and kicking). Fine motor skills were defined as the ability to perform complex skills for more proficient tasks of daily living [126] (smaller movements like writing, tying shoelaces, and unbuttoning clothes). The following activities were emphasized:

- Giving child items to hold with their fingers, for example handing a pencil and paper for them to scribble.
- Matching lids with same size colour and shape games.
- Threading with beads
- Poking straws into holes.
- Stacking cups

The recommended toys included balls, bottle lids, cups, big beads, threads, ropes, shakers, pencils and paper. Furthermore, the mothers were encouraged to empower each other, by meeting regularly in their groups to practice and evaluate their childcare skills. We also advised them to be active with their sub-county activities for easy identification by government programs targeting women.

4.5.4. Booster sessions of the educational components after the intervention period

To prolong the effects of, and adherence to, the education intervention after the 6-months' intervention period had ended and until the children were aged 36 months (current follow-up study), we administered booster sessions to groups of 6-12 women from the original trial cohort of 511 women. These sessions (each lasting about 6 hours) were provided by the education team every third month and started three months after end of the intervention period, hence a maximum of 8 booster sessions were given. The sessions were reminders of the education activities taught during the intervention period and re-emphasized the importance of (i) making nutritious meals; (ii) hand-washing and hygienic preparations, and (iii) child stimulation.

4.5.5. Routine health care practices

The intervention group received routine health care and the education intervention while the control group received only routine health care. The routine health care consisted of the recommended regular anthropometric measurements, immunizations, deworming, vitamin A

supplementation, malaria-prophylaxis and iron-deficiency anemia prevention. Importantly, when the children were aged 20-24 months we found that mothers in the intervention group had gained significantly more knowledge and better practices related to child feeding, hygiene and stimulation [163] compared to the control mothers, indicating that the contents of our education intervention differed markedly from routine health care.

4.6. Assessment tools and data collection for the follow-up study

4.6.1. Questionnaires

For demographic characteristics of the study population (obtained when the children were aged 6-8 and 20-24 months), we had a general questionnaire which asked information on socio-demographic characteristics, child care/feeding practices and morbidity status. Household socio-economic status was determined by use of the simple poverty score-card for Uganda [173]. Dietary diversity was determined by the use of the individual (child) dietary diversity score [174] while household food security was assessed by the household food insecurity access scale [175].

4.6.2. Measurements of development outcomes

Child development assessments were performed by bachelor degree holders in psychology blinded of randomization allocation. This data collection team participated in training sessions to ensure uniform and standardized assessment procedures. Assessments were administered in the local language and conducted in hired, secluded rooms in the villages without interruptions to minimize distractions.

We used the BSID III to evaluate child cognitive, language and motor development [176]. BSID-III scale is known to be the most comprehensive tool to measure development in children up to 42 months [176]; it has been adapted for appropriate use among children in rural Uganda [128] and has been used in similar settings [177]. Unfamiliar items in the stimulus and picture booklets were easy to replace with familiar objects in the Ugandan context; for example, red apples were replaced with tomatoes and a vacuum cleaner with a mop. To maintain functional equivalence with the original stimuli, replacement items were chosen based on their size, colour, and shape. Children's performance was scored according to the guidelines in the administration manual and the raw scores converted to composite scores according to the reference material. The reference mean-score is 100 with a SD of 15.

Children scoring less than 70 (< 2SD below the mean for a US reference population) were considered to be severely delayed while scores between 70 and 85 were mildly delayed in development [176].

The Ages and Stages Questionnaire (ASQ) was used to generate caregiver reported quantitative measures of communication, gross motor, fine motor, personal social and problem solving abilities [178, 179]. The ASQ was able to capture and establish some adaptive behaviours reported by the caregiver that would not easily be established by the BSID-III. The ASQ was also used to capture social–emotional abilities of the child since we did not administer the social-emotional scale of BSID-III due to time constraint.

Mullen Scales of Early Learning (MSEL) [180] was introduced at 36 months to assess early intellectual development and readiness for school. MSEL has been validated for use in rural Uganda [181, 182] and is a comprehensive measure of cognitive functioning between the ages of birth to 68 months [180]. We used the four cognitive scales (visual reception, fine-motor, receptive and expressive language) of MSEL. Each individual scale has T-scores (mean 50, SD 10) [180]. The MSEL has strong concurrent validity with other cognitive and language measures. It has been widely used in child assessments [183].

The administration of the tests was performed by personnel fluent in English and the local language. All the three tools were pretested before use.

4.6.3. Measurements of growth

Length/height, weight, mid-upper arm circumference and head circumference were measured according to the WHO guidelines [184]. Trained nutritionists performed the anthropometric measurements. In addition, refresher training were given before each study time point and inter-rater reliability determined at the end of each refresher training period. To avoid bias, the team that assessed growth in the original RCT was replaced by a new team, which was blinded to group allocation for later anthropometric assessments after intervention. The two groups did not meet in the field and the ICC between the two teams was ranging from 0.63 to 0.75 ($P < 0.0001$). WHO child growth standards were used as the reference for growth. We computed the following z-scores as (observed value – median value of the reference population)/SD value

of the reference population [20]. Stunting, wasting and underweight were defined as z-scores of < -2SD from the median of the reference population [185].

4.6.4. Stool sampling and gut microbiota determination

Fecal samples were collected from 145/155 (94%) of children whereas a rectal swab sample was collected from the remaining 10 (6%) children (equally distributed between intervention and control groups). These samples were collected by a graduate student of laboratory technology. Reportedly, rectal swap samples provide reliable data on gut microbiota composition [186]. The stool samples were shipped to The Netherlands for further analysis. Upon arrival, cotton swabs were stored at −80°C until further processing. Microbial compositional analyses of all fecal samples occurred via DNA extraction followed by targeted amplification and sequencing of the V4 hypervariable region of the 16S rRNA gene using an Illumina MiSeq sequencing platform [187, 188]. The cotton swabs were cut from the sticks and directly re-suspended in 96 well Axygen 2-mL deep well plates (Axygen, MA, USA), containing 350 µL of lysis buffer (Mag Mini DNA Isolation Kit; LGC Ltd, UK), 500 µL zirconium beads (0.1 mm; BioSpec products, OK, USA) and 600 µL of phenol saturated with Tris-HCl (pH 8.0; Carl Roth GmbH, Germany). Mechanical disruption of bacterial cells was performed by bead beating for 2 min in a mini-beadbeater-8 cell disruptor (Merlin Bio-products, The Netherlands). After bead beating, the samples were cooled on ice prior to a 10 min centrifugation step (10,000 rpm). After another phenol extraction step of the aqueous phase, 500 µL of the aqueous phase were transferred to a new centrifugation tube prefilled with 1000 µL binding buffer (Agowa GmbH, Germany) and 20 µL magnetic beads (Agowa). After mixture, the suspension was left for 10 min to allow binding of the chromosomal DNA to the magnetic beads. After washing the beads according to the Agowa Mag mini DNA extraction protocol, the DNA was extracted from the beads with 63-µl elution buffer (Agowa) according to the manufacturer's instructions. A set of different custom scripts and open-source software packages, all optimized for metagenomic data analysis, were used to analyse sequence data and infer microbial community composition [188-190].

4.6.5. Sampling and measurements of urine metabolites

Urine were sampled by use of small containers, transferred to tubes and kept at 4ºC for no more than 24 h before being frozen at -20ºC. A graduate student of laboratory technology collected 155 and 148 samples of morning spot urine (volumes ranged from 2.5 to 4 mL) at 20-24 and at

36 months, respectively. The samples were then shipped on dry ice to Oslo University Hospital for analysis at the Department of Medical Biochemistry. We measured the concentration of the anion iodide (oxidized form of iodine). Briefly, urine iodide was analyzed by a colorimetric method based on ammonium persulfate digestion prior to the Sandell-Kolthoff reaction, as described by Ohashi et al. [191] and with an analytical coefficient of variation (CV) of 6% at 0.9 μmol/L [192].

Creatinine in urine was measured with enzymatic colorimetry using Cobas 6000 (Roche, Basel, Switzerland; CV 3%) [193]. Urine was collected as spot urine samples which were passed out when the childrens' bladders were full. It was not feasible to collect diurnal urine samples. The urine iodide concentration (UIC) was corrected for differences in urine dilution/concentration by calculating the individual urine iodide/creatinine ratio (ICR) which was used as a measure of iodine status in addition to UIC [194].

4.6.6. Maternal depression

Maternal depression symptoms were assessed using both Beck Depression Inventory II (BDI-II) scores [195] and Center for Epidemiologic Studies Depression Scale (CES-D) scores [196]. The BDI-II is a self-reported tool for assessing symptoms of depression on a 4-point scale from 0 to 3 with 21 questions, giving a possible range of 0-63. A score of 10 or above is considered to be indicative of probable depression [197]. Similarly, CES-D asks the mothers to report, on a 4-point scale (0=rarely/none of the time to 3=all of the time), the frequency of symptoms for 20 scale assessment items. A total score of 16 or higher is considered to indicate depression in the general population [196]. The BDI-II and CES-D have been validated for use in Uganda [198, 199]. In addition, CES-D has been used extensively in psychological and epidemiologic studies of postnatal women [200]. Both tools were used because BDI-II has evaluated many different populations in Uganda [198, 199] while CES-D has been extensively used in epidemiological studies [128, 201]. The inter-rater reliability for the BDI-II was at least 0.80 across all measurement time points while that for CES-D was at least 0.85. We report scores when the children were 12-16, 20-24 months and at 36 months. The BDI-II and the CES-D tools were not included at baseline assessment in order not to burden the mothers too much since the education protocol was quite comprehensive and the number of various assessments was large. Consequently, at baseline we only identified maternal depression symptoms by the following categorized interview question included in our social demographic questionnaire:"

How sad did you get with the birth of this child?" Their responses were categorized as not sad (score=0) or sad (score=1). After completion of the original RCT, we experienced that the mothers were willing also to undertake a more thorough assessment of their mental health, hence we included the BDI-II and CES-D tools from the 12-16 months' time point and onwards.

4.7. Data analysis

All quantitative data were analysed using Stata/SE (StataCorp. 2015, Stata Statistical Software: Release 14. College Station, Stockholm, Sweden) [202] and SPSS version 22.0 (IBM SPSS Statistics, IBM Corp., Armonk, NY) [203].

There were no significant differences in any sociodemographic variables or in any developmental outcomes between the intervention and control group in the follow-up study when these children were aged 6-8 months. Therefore we did not make any adjustments for data obtained at 6-8 months when we analysed development outcomes at 36 months. We found that the stunting occurrence was higher in the intervention vs the control group at 6-8 months in the follow-ups study, but, there were no difference in stunting occurrence between the two study groups at age 20-24 months. Therefore, in the analysis of height-for-age z-score (HAZ) at age 36 months we did not adjust for stunting occurrence at 6-8 months. However, we have later re-analyzed the HAZ data at age 36 months after adjustments for stunting occurrence and HAZ at 6-8 months.

Paper I: We used a mixed effect linear regression to compare the intervention with the control group and estimated ICC, and adjusted for clusters. Differences between the two study groups are given as mean (SD or 95% CI). Gut microbiota 16S rRNA amplicon sequencing data analysis was performed using R version 3.3.2 (R Core Team, 2016) [204, 205]. The 16S rRNA amplicon sequencing data was rescaled and transformed using Wisconsin double and square root transformations. The PERMANOVA procedures, Shannon and 1-Simpson's diversity indices were performed as implemented in the 'vegan' package [206]. All PERMANOVA analyses were performed using the Bray-Curtis distance measure. All phyla and genera were included in the statistical analysis.

Paper II: Differences between the two study groups in concentrations of urine compounds were tested by Mann-Whitney U tests for each time point, as the data was not normally distributed.

For the association analyses, we used mixed models to investigate the effect of ICR on growth and development outcomes in the intervention and control groups separately and possible interaction effects of time and ICR. We adjusted for values obtained at 6-8 months for the outcome of interest. The association of ICR with child development and growth was expressed as the regression coefficient with 95% confidence interval and its corresponding p-value.

Paper III: We used a mixed effect linear regression to compare the intervention with the control group and estimated ICC. The sub-county (cluster), village, mother, and mothers within villages were the random intercepts, while the time points and group affiliation (intervention or control) were the random slope and fixed variables in the model. We fitted data by the maximum likelihood method and used a log likelihood-ratio test to determine the overall effect of the intervention for the entire study period. Differences between the two study groups are given as mean (SD or 95% CI). We did not adjust for stunting occurrence measured at 6-8 months because there was no reason to believe that stunting would affect maternal depression scores. For the secondary outcomes (association analyses) we used pooled data from the two study groups (intervention and control) to examine associations between maternal depression symptoms using the BDI-II and CES-D scores and the development outcomes by the BSID-III scores using mixed effects linear regression models to handle three data points per mother. Time, maternal depression scores, and interaction were treated as fixed variables, the sub-county as cluster and reml as a fitting method. These analyses were adjusted for group affiliation.

4.8. Research approvals

Ethical approval was obtained from the Uganda National Council of Science and Technology Ref HS 1809, after being reviewed by The AIDS Support Organisation Research Ethics Committee (No. TASOREC/06/15-UG-REC-009). The Norwegian Regional Committee for Medical and Health Research Ethics also approved the study (No. 2013/1833), including an amendment for this follow-up study. The original RCT was registered at ClinicalTrials.gov ID NCT02098031. Written informed consent was obtained from mothers who participated in the study. The consent form was translated into the local language and all participants would either sign or thumb-print the consent form. Participants were informed that they were free to withdraw from the study at any time. The rights of the participating individuals were protected as described by the World Medical Association Declaration of Helsinki.

5. Summary of results

Amendments to Papers I and III can be found in the List of corrections below (point 5.4.).

5.1. Paper I

Atukunda P, Muhoozi GKM, van den Broek TJ, Kort R, Diep LM, Kaaya AN, Iversen PO, Westerberg AC. Child development, growth and microbiota: follow-up of a randomized education trial in Uganda. *Journal of Global Health* 2019;9:010431.

Paper I describes the follow-up study of our open cluster-randomized controlled trial (see section 4.2. above). The main purpose was to assess the education intervention effect on child development, growth and microbiota at 20-24 and at 36 months.

All child development outcomes (i.e. BSID-III cognitive, language and motor composite scores) were significantly higher in the intervention compared to the control group at both time points. Similarly, mean scores on the ASQ scale (i.e. communication, gross motor, problem solving, and personal social development) were significantly higher in the intervention compared with the control group at 20-24 months. At 36 months, only the ASQ fine motor scores were significantly higher in the intervention group compared with the controls. At 36 months, the MSEL fine motor, language (receptive and expressive), cognitive and early learning composite standard scores were significantly higher in the intervention compared to the controls.

The mean HAZ declined in both study groups during the study period, indicating linear growth faltering. However, this decline was significantly less at 36 months in the intervention compared with the control group. There were no significant differences in the other mean anthropometric measures at 36 months.

The intervention did not lead to any significant changes in the gut microbiota composition or the Shannon diversity index compared with the control group at the phylum level, at 20-24 or at 36 months. As expected the Shannon diversity index increased significantly in both study groups from 20-24 to 36 months, indicating increased gut microbiota diversity, while there was no significant change in the overall genera distribution from 20-24 to 36 months. In line with

this, there was no change in the variable 1-Simpson index between the two study groups at the two time points, and this variable increased from 20-24 to 36 months, again indicating increased gut microbiota diversity.

In conclusion, the maternal education intervention had marked positive effects on child development whereas the effect on linear growth was small at three years. Moreover, the intervention had no apparent effect on gut microbiota composition or -diversity.

5.2. Paper II

Atukunda P, Muhoozi GKM, Diep LM, Berg JP, Westerberg AC, Iversen PO.
The association of urine markers of iodine intake with development and growth among children in rural Uganda: a secondary analysis of a randomized education trial.
Submitted.

Paper II describes the follow-up results of UIC and ICR at 20-24 and at 36 months. It also includes analyses of possible associations between UIC and ICR, and child development and growth separately in the two study groups (intervention and control).

The median UIC for both study groups at 20-24 and at 36 months were similar ($p > 0.05$) and within the normal range of 100 to 199 μg/L. The BSID-III cognitive score was significantly and positively associated with ICR at 20-24 months in the intervention group. The ASQ gross motor score was significantly and negatively associated with ICR at 20-24 months among the controls. The ICR was not significantly associated with growth in the two study groups at either time point.

In conclusion, the intervention led to a positive association between ICR and child cognitive score at 20-24 months, whereas no positive assocation with ICR and growth was detected. Iodine sufficiency may be important for child cognition in this setting.

5.3. Paper III

Atukunda P, Muhoozi GKM, Iversen PO, Westerberg AC.
Nutrition, hygiene and stimulation education for impoverished mothers in rural Uganda: Effect on maternal depression symptoms and their associations to child development outcomes. *Nutrients*. 2019;11(7):1561.

Paper III describes the effect of the intervention among the follow-up sample mothers' self-reported maternal depression symptoms. It also focuses on the associations between the self-reported maternal depressive symptoms and child development.

The intervention significantly reduced self-reported maternal depression symptoms' scores at 20–24 and at 36 months, respectively, measured by both CES-D and BDI-II. There was a significant negative association of BDI-II scores and BSID-III cognitive and language scores at 20–24 and at 36 months, indicating a negative effect of maternal depression symptoms on child development scores. CES-D associations with BSID-III cognitive and language scores showed similar trends. Lower BSID-III motor scores were significantly associated with more depression symptoms at 36 months for both BDI-II and CES-D.

In conclusion, the maternal group education intervention reduced maternal depression scores. Moreover, the depression scores were inversely associated with child cognitive and language development outcomes.

5.4. List of corrections

Paper I:

In table 1 in Paper I the following corrections should be noted:

- The data of BSID-III composite scores and ASQ scores from the parental trial were obtained when the children were 20-24 months (and not when they were 6-8 months-baseline as stated in the heading to this table).
- The data of BSID-III composite scores and ASQ scores from the follow-up study were obtained when the children were 36 months (and not when they were 20-24 months as stated in the heading to this table).

Moreover, since the BSID-III and ASQ data from the follow-up study are also given in tables 2 and 3, they should not have been included in table 1, for the sake of clarity. Similarly, the BSID-III and ASQ data from the parental study have been reported previously (table 3 in [163]), so they should not have been included in table 1 either. Notably, these corrections have no impact on the data analyses and findings. Below we have provided a revised table 1.

In the Result-section to Paper I the paragraph with the sub-title: "Study participants", should read:

One hundred and fifty-five mother-child pairs were included at 20-24 months (Figure 1). By 36 months, eight of them were lost to follow-up (three in the intervention group and five in the control group). There were no significant differences in the characteristics between the two study groups in the parental cohort (data obtained at baseline, i.e. at 6-8 months) or between the two study groups in the follow-up cohort (data obtained at 20-24 months; Table 1). Moreover, there were no significant differences in any characteristics between the parental study and the follow-up study in either of the two study groups, thus no adjustments for baseline differences were made in subsequent analyses.

After publication of Paper I, the HAZ data at 36 months were re-analyzed after adjustment for both stunting occurrence and HAZ obtained at 6-8 months. However, these adjustments had no significant impact on the HAZ values at 36 months, so the intervention group had significantly less growth faltering.

In table 5 the p-value for WHZ at 36 months is given with a negative sign, which is a typographical error.

Revised Table 1 for Paper I: Study population characteristics for the parental trial and the follow-up study

Characteristics	Parental trial (data obtained at 6-8 months)		Follow-up study (data obtained at 20-24 months)		
	Intervention (n=263)	Control (n=248)	Intervention (n=77)	Control (n=78)	*P*-value
Children (*n*, %)					
Males	139 (52.9)	123 (49.6)	44 (57.1)	41 (52.6)	0.75
Females	124 (47.1)	125 (50.4)	33 (42.9)	37 (47.4)	0.40
Age at inclusion (months)	7.4 (0.8)	7.3 (0.9)	21.4 (1.0)	21.2 (1.0)	0.24
Stunting*	55 (20.9)	70 (28.0)	32 ***(41.6)***	46 ***(59.0)***	0.06
Underweight*	25 (9.5)	36 (14.5)	6 ***(7.8)***	8 ***(10.3)***	0.37
Wasting*	12 (4.6)	12 (4.8)	3 ***(3.9)***	2 ***(2.6)***	0.50
Illness at study time (*n*, %)					
Yes	94 (35.7)	71 (28.6)	47 (61.0)	40 (51.3)	0.21
No	169 (64.3)	177 (71.4)	30 (39.0)	38 (48.7)	0.38
Maternal data					
Maternal education (years)	4.9 (2.8)	4.9 (2.8)	5.5 (2.5)	5.0 (2.6)	0.20
Maternal age (years)	26.1 (5.8)	26.8 (6.3)	26.2 (6.1)	27.4 (6.4)	0.27
Number of children per mother	3.4 (2.2)	3.3 (2.2)	3.4 (2.2)	3.3 (2.2)	0.25
Household data					
Household head age (years)	31.3 (7.7)	32.6 (19.4)	30.2 (7.3)	33.1 (10.9)	0.06
Household head education (years)	6.4 (3.1)	5.9 (3.1)	6.6 (3.3)	6.5 (3.4)	0.29
Household size (n)	5.5 (2.1)	5.5 (2.1)	5.7 (2.2)	5.8 (2.2)	0.76
Household poverty score	47.8 (11.7)	47.6 (11.4)	49.0 (11.6)	46.3 (12.3)	0.18
Sanitation composite score	7.2 (1.9)	7.3 (1.9)	7.0 (1.8)	7.1 (1.9)	0.83

Values are means (SD) unless otherwise stated. *z- score values are < -2SD of the median of the reference population. P-values are for comparisons between the two study groups in the follow-up study. There were no significant differences in any of the characteristics between the two study groups in the parental study. Values in italics, underlined and bold denotes corrections of errors appearing in table 1in Paper I.

Paper III:

In table 1 in Paper III the following corrections should be noted:

- The data of BSID-III composite scores from the parental trial were obtained when the children were 20-24 months (and not when they were 6-8 months as stated in the heading to this table).
- All data from the follow-up study was collected when the children were 20-24 months (and not when they were 12-16 months as stated in the heading to this table), except the data for the BSID-III composite scores that were obtained when the children were 36 months.

Moreover, since the BSID-III data from both the parental study and the follow-up study have been reported previously ([163] and Paper I, respectively), they should not have been included in table 1, for the sake of clarity. Notably, these corrections have no impact on the data analyses and findings. Below we have provided a revised table 1.

Point 3.1 in the Result-section of Paper III (2nd paragraph), should read:

There were no significant differences in the characteristics between the two study groups in the parental cohort (data obtained at baseline) or between the two study groups in the follow-up cohort (data obtained at 20-24 months; Table 1). Moreover, there were no significant differences in any characteristics between the parental study and the follow-up study in either of the two study groups, thus no adjustments for baseline differences were made in subsequent analyses.

We did not adjust for stunting occurrence measured at 6-8 months because there was no reason to believe that stunting would affect maternal depression scores.

Revised Table 1 for Paper III: Study population characteristics for the parental trial and the follow-up study

Characteristics	Parental trial (data obtained at baseline)		Follow-up study (data obtained at 20-24 months)		
	Intervention (n=263)	Control (n=248)	Intervention (n=77)	Control (n=78)	*P*-value
Children (*n*, %)					
Males	139 (52.9)	123 (49.6)	44 (57.1)	41 (52.6)	0.75
Females	124 (47.1)	125 (50.4)	33 (42.9)	37 (47.4)	0.40
Age at inclusion (months)	7.4 (0.8)	7.3 (0.9)	21.4 (1.0)	21.2 (1.0)	0.24
Stunting*	55 (20.9)	70 (28.0)	32 (41.6)	46 (59.0)	0.06
Underweight*	25 (9.5)	36 (14.5)	6 (7.8)	8 (10.3)	0.37
Wasting*	12 (4.6)	12 (4.8)	3 (3.9)	2 (2.6)	0.50
Maternal data					
Maternal education (years)	4.9 (2.8)	4.9 (2.8)	5.5 (2.5)	5.0 (2.6)	0.20
Maternal age (years)	26.1 (5.8)	26.8 (6.3)	26.2 (6.1)	27.4 (6.4)	0.27
Number of children per mother	3.4 (2.2)	3.3 (2.2)	3.4 (2.2)	3.3 (2.2)	0.25
Household data					
Household head age (years)	31.3 (7.7)	32.6 (19.4)	30.2 (7.3)	33.1 (10.9)	0.06
Household head education (years)	6.4 (3.1)	5.9 (3.1)	6.6 (3.3)	6.5 (3.4)	0.29
Household size (n)	5.5 (2.1)	5.5 (2.1)	5.7 (2.2)	5.8 (2.2)	0.76
Household poverty score	47.8 (11.7)	47.6 (11.4)	49.0 (11.6)	46.3 (12.3)	0.18
Sanitation composite score	7.2 (1.9)	7.3 (1.9)	7.0 (1.8)	7.1 (1.9)	0.83

Values are means (SD) unless otherwise stated. *z- score values are < -2SD of the median of the reference population. P-values are for comparisons between the two study groups in the follow-up study. There were no significant differences in any of the characteristics between the two study groups in the parental study.

6. General discussion

6.1. Summary of main findings

- This thesis is based on the follow-up study of children aged three years of a cluster-RCT aiming at improving child growth and development. The intervention was on maternal education emphasising nutrition, hygiene and stimulation and conducted when the children were 6-8 months. Whereas the original trial included 511 mother/child pairs, the follow-up study comprised 155 such pairs. Nevertheless, the two study groups (intervention and control) in the follow-up study did not differ significantly from the original two study groups in terms of several socio-demographic factors, thus probably comprising a representative sample of the original trial.
- The intervention significantly improved BSID-III scores at both the 20-24 and the 36 months' assessment. Moreover, at 20-24 months, the intervention also led to improvements in most of the ASQ and the MSEL scores. At 36 months, only the ASQ fine motor scores had improved whereas the MSEL fine motor, language, cognitive and early learning composite standard scores were significantly higher in the intervention compared to the controls.
- Linear growth decreased in both study groups during the study period. However, this linear growth faltering was significantly less at 36 months in the intervention compared with the control group. The intervention did not affect significantly the other anthropometric measures.
- The intervention did not lead to any significant changes in infant gut microbiota, neither the average relative bacteria abundance nor the bacteria species diversity compared with the control group. As expected, the microbiota diversity, and indicator of gut microbiota maturation, increased at 36 months in both study groups.
- The intervention did not affect UIC. ICR was positively associated with BSID-III cognitive composite score in the intervention group at 20-24 months whereas the ASQ gross motor score was negatively associated with ICR at 20-24 months among the controls. Thus, these associations did not show a consistent pattern.
- The intervention reduced self-reported maternal depression symptoms at 20–24 and at 36 months when assessed with two independent tools. Notably, the reduced depression symptoms were associated with improved child cognitive- and language development outcomes.

6.2. Methodological considerations

6.2.1. Study design aspects

This follow-up study was based on a cluster-RCT. In the original trial, we chose to have education as the only intervention component and did not provide any supplements or money. We reasoned that this approach would support intervention-sustainability over time. Randomization has known advantages of eliminating potential bias, especially of confounding and selection in group assignment [207]. Moreover, cluster-RCTs are commonly used in non-therapeutic intervention studies for generalizability of the results [208], in resource-constrained settings as ours where one needs to have a robust and pragmatic approach to be able to complete interventions and long-term follow-up studies. Our study had a long follow-up time of the respondents and with relatively low rate of loss to follow-up in both study groups. We believe that this was partly due to the careful selection criteria and randomization procedure as detailed in Paper I, and partly due to involvement of the lay helpers (VHT leaders and mother leaders) [163]. The VHT leader/mother leader was selected by consensus in the groups and attended all the three main education sessions with the mothers. They assisted the mothers to identify, empower and collaborate with each other with a main goal of implementing the taught skills regarding child health and development. We also employed intention-to-treat analysis, widely accepted and recommended as the most valid analytic approach for prospective intervention studies [209, 210]. Still, the low number of participants may limit the generalizability of the results.

Some bias can occur even in well-conducted trials, e.g. selection bias and contamination. To reduce selection bias, we included allocation concealment in which the randomization sequence was unknown from the researchers and the participants at recruitment. Sub-counties were allocated into control and intervention groups after inclusion into the study and with either written and thumb print consent obtained. The randomization technique using computer-generated sequentially numbered, is recognized as a simple, cheap, effective and straightforward method of allocating study participants while maintaining allocation concealment [211]. Even though the intervention and control villages were not close to each other, study contamination could have taken place, e.g. in situations where study control participants have relations in the intervention villages, or meet up in common gatherings, market places as well as in hospitals.

At the time of designing the original trial (in 2013) we did not include collection of biological samples of stool and urine and scoring of maternal depression symptoms at baseline when children were 6-8 months due to financial, logistical and practical constraints. Thus, we lack data for a long-term comparison of trend analysis on these outcomes.

6.2.2. Research tools to measure development outcomes

We chose to use the three independent child development scales BSID-III, ASQ and MSEL because they (i) supplement each other and (ii) they give a broad view of possible effects on child development, and (iii) they have acceptable reliability and validity. Notably, these child development assessment scales are widely used globally in assessment of child development [212].

BSID-III is commonly referred to as a "gold standard" that is feasible for the assessment of child neurodevelopmental status even in nutritional intervention studies in low resourced communities [213, 214]. The highly significant ($p = 0.0001$) ICC of 0.75 for BSID-III, suggests excellent agreement among the study personnel in the obtaining BSID-III scores. Limitations in the use of BSID-III include the need to be adapted for use in specific local settings, its administration requires specific training and it is may be expensive for low-funded clinical studies.

The ASQ is a parental completed child development screening tool with 21 age-specific questionnaires. It can assess child development from 1 to 60 months of age. ASQ does not require a professional technical training to complete the screening. An important ASQ limitation is the inability to identify specific risks for child development outcomes [215].

MSEL has four cognitive scales (visual reception, fine-motor, receptive and expressive language). It is used to assess children from birth to 68 months. The MSEL has been previously used to evaluate neurodevelopment among Ugandan children [181]. MSEL is a detailed objective assessment of child development gross, fine motor, expressive, receptive language, and visual reception in children [212]. MSEL easily identifies various risk factors for poor child development. However, it requires child development assessors with training and experience in the assessment of young children [216].

We used conventional anthropometric tools to assess growth, as recommended by the WHO [20]. A limitation is that we were not equipped to perform detailed analyses of biomarkers of growth or of body composition, which may provide a better understanding of how interventions may impact on early growth as well as on short- and long-term health outcomes [217].

As possible determinants of child development and growth, we examined biomarkers of gut microbiota and urine iodide concentrations. The microbiota data was based on bacterial 16S rRNA amplicon sequencing, a standardized method. In line with this, the statistical handling of the obtained data also followed state-of-the art methodology. Methodological aspects of the iodine data are discussed below in section 6.3.4.

We believe that the inclusion of assessment of maternal depression symptom scores strengthened our study. Two independent self-reported depression scales (BDI and CES-D) were used. Our intervention was not specifically designed to reduce these symptoms, but the assessment allowed us to investigate the association between maternal depression symptoms and early child development. Notably, these tools only score self-reported depression symptoms and do not allow for a definite diagnosis of depression.

6.3. Interpretation of findings

This section starts with general reflections of our findings in the follow-up study on child development and growth in the light of previous findings in these research topics. Then the possible roles of our results on child gut microbiota, iodine status and maternal depression symptoms are discussed in relation to child development and growth.

6.3.1. Child development

Several protective- and risk factors for undernutrition are often related to developmental outcomes [87]. Shared risk factors include nutrient deficiencies, intrauterine growth retardation, economic and social conditions, determinants that can be identified in the UNICEF 1990 conceptual framework as well as the framework proposed in the Lancet 2013 series. Impaired development risk factors also include inadequate learning opportunities and inadequate quality of caregiver-child interactions (e.g. caregiver-child stimulation) [87]. Shared protective factors include breastfeeding and maternal education. Thus, early deficits of child nutritional status and development have led to suggestions of implementing long-lasting interventions, for example

integrating nutritional and other approaches to promote overall child development [218]. In line with this, focus has shifted from policies and programs on single issues to more integrated approaches and wider-reaching interventions across the life span. These would enable each child to develop as well as possibly mitigate the impact of constraints under which their development may be occurring [219].

Our intervention continued to improve child development outcomes (cognitive, motor and language) in this longitudinal follow-up study (Paper I). However, we are not able to discern which of the components of our maternal education package (nutrition, hygiene, stimulation) that impacted most on development. Possibly it was the combination of these components that was effective. In support of this, according to the systematic review by Grantham-McGregor et al., evidence from several efficacy trials, with combined nutrition and child development intervention activities have shown additive benefits for child development [220]. Our findings together with these previous studies thus indicate a synergy-like relationship between child development and combined intervention benefits. One limitation of our education intervention to address child development is our focus on underlying causes (as per UNICEF conceptual framework terminology) only, and to a certain extent omitting the multi-layered nature of determinants important for child development as well as the need for multisectoral approach to address inadequate development outcomes.

Our study findings contribute to the wider knowledge which has acknowledged the benefits of adequate child nutrition together with stimulation. Interventions that include child nutrition and stimulation facilitate high brain plasticity in early years of life and offer repair from early brain insults [128, 129]. Thus, despite early nutritional deficiencies that lead to impaired neurodevelopmental process [128, 129], early childhood interventions, including stimulation, may provide a buffer to promote new neuronal development and synapse growth [221, 222].

Rapid development in brain structure and function supporting acquisition of child development outcomes skills is profound during early childhood and continues into adolescence [218, 223]. There is evidence that interventions targeting cognitive, language, and socioemotional development may also be of benefit beyond the first 1,000 days of life (commonly denoted the "critical window of opportunity") [33, 87]. Indeed, our intervention conducted during early childhood (i.e. at the child-age of 6-8 months) showed marked benefits of development even

up to 36 months. In fact, early interventions until the age of 36 months are proposed to be the best stage to prevent impaired child development [218], which is supported by our findings (Paper I). Albeit being outside our resources, adding digital imaging and/or objective neurophysiological tests to our measurement portfolio would have been desirable.

Child stimulation activities that favour development outcomes are usually easy to do and can effortlessly be integrated in daily child play routines [224, 225]. Moreover, they involve rather simple, feasible actions and colourful play materials often made by parents, siblings and other caregivers including the fathers. Simple caregiver-child interaction is acknowledged to improve child development [226-228]. Appropriate caregiver-child interaction using suitable play materials promotes the caregiver's motivation and aspirations for later school achievement and this in turn results in improved mental development scores of children [66, 131, 229]. Notably, in our study language scores improved less than cognitive and motor scores. This could be because language development play materials are not easily integrated in child play activities. Notably, most of the study mothers were burdened with so much small-scale farming and household work and thus lacked the required sufficient time to fully engage children in activities to promote language skills.

6.3.1.1. Maternal child care empowerment and child development

The Lancet 2013 series emphasized maternal empowerment as key to child care. Maternal empowerment often leads to household wellbeing, which in turn benefits child development outcomes [230]. In LMICs, women are commonly the main primary caregivers to the children [230]. Community interventions aimed at maternal empowerment influence child care practices which consequently lead to improved child health outcomes. In 2017, Britto et al. in the Lancet strongly recommended the inclusion of early child care caregiver empowerment interventions in order to benefit child development [231].

WHO and UNICEF Care for Child Development, an intervention to support caregivers in child care including age appropriate play and communication, have shown benefits on children's cognitive, social and language development [232]. Mothers who benefit from such interventions in child care support have improved sensitivity and responsiveness to their child's needs [232]. Moreover, positive responsive care giving is linked to responsive feeding, child stimulation, and learning opportunities as well as profound benefits of maternal mental health

[71]. In hindsight, the original study should have planned for a systematic engagement during the entire three-year study-period of other important caregivers, e.g. the fathers and the grandparents.

6.3.2. Child growth

In this section the focus is on linear growth (referred to below as growth only) and its impairment, i.e. stunting. Despite a decline in the global prevalence of stunting, still approximately one in four children below five years are stunted, and this poses a hindrance to the international agreed target on halving the number of stunted children by 2030 [233].

Findings from our original trial indicated that by 6-8 months about 25% of the total cohort of children were already stunted [163]. The corresponding percentage of stunted children (when aged 6-8 months) of the total follow-up study cohort was 27 (Paper II). Similar observations have been reported in other studies performed in LMICs [49, 234, 235]. These findings highlight the cost of the *in utero* and early life conditions' lasting influence on child growth. At 36 months we observed a smaller reduction in growth faltering in the intervention compared with their counterparts in the control arm (Paper I), even after adjusting for stunting occurrence and HAZ, suggesting that our intervention may have had a protective effect against growth faltering over time. However, the change was small and no similar changes over time for the other anthropometric markers were found. Further follow-up studies are needed to clarify if this education intervention may confer growth benefits in the longer-term. Studies have suggested that height catch-up normally occurs between 24 months and mid-childhood [236, 237]. Our findings at 36 months indicate a possible catch-up in growth even after early childhood growth faltering. Possible explanations for these findings at 36 months, include: (i) existing community programs targeting stunted children at two and three years; (ii) children at three years in rural Uganda freely move, visit and eat with the neighbourhoods, exposing them to supplementary food in addition to meals in their own households; and (iii) our measurements at 20-24 and at 36 months could have been taken in a time-period where food was readily available, i.e. season-dependent. In support of this, other studies have found effect of seasonality on child height-for-age scores. For example, in Tanzania [238], Gambia [237] and Malawi [239] child stunting was associated with the rainy seasons commonly related to household food insecurity.

Collectively, these studies and our data propose an additional window of opportunity where growth-faltering resulting from various life cycle events as well as from intergenerational effects can be restored [240]. In turn, this additional window of opportunity could be harnessed by interventions in areas that have high stunting prevalence. Notwithstanding this, it is not clear which mechanism(s) may benefit intergenerational catch-up growth recognised through interventions at this later age [241]. Whether possible benefits from such interventions that promote catch-up growth may result in better health outcomes in general later in life, is also unknown [242-244].

Generally, interventions with a combination of information, education and communication focusing on nutrition, hygiene and appropriate complementary feeding practices, are often effective public health measures. These address child-feeding practices aimed at promoting good child health, especially child nutritional status in LMICs [245, 246]. Supporting this assertion are the findings of a Bangladesh study whose results of an intervention that combined nutrition, water, sanitation, and handwashing improved length-for-age z-scores of children [247]. Contradictory findings were reported in India [248, 249] where a water, sanitation, and handwashing intervention did not prevent growth faltering. A summary of intervention trials in South-Asia concluded that educational interventions aimed at improving child stunting have shown effective behavioral changes, but with less and often only a modest change on linear growth [250].

6.3.3. Gut microbiota, child development and growth

The first years of life are characterized by rapid changes in both the gut microbiota and the developing infant brain [251]. Lately, the role of gut microbiota in modulating brain development and behavior is increasingly acknowledged [252, 253]. In a landmark study by Koenig et al. it was suggested that diet plays an important role in gut microbiota composition and diversity in the first three years of life [254]. In line with this, in our study by 36 months, the gut microbiota had matured to resemble an adult-like phenotype (Paper I). This maturation took place concomitant with diet changes such as weaning to solid foods.

Studies have identified that differences exist between the gut microbiota of African compared with European children. For example, rural African children's gut microbiota had more *Bacteroides* and less *Firmicutes*, possibly due to higher consumption of vegetarian foods [255,

256]. We found that both *Bacteroides* and *Firmicutes* were dominant at 20-24 and at 36 months (Paper I). Dostal et al. reported that iron supplementation study among rural South African children did not affect dominant bacterial groups including *Bacteroides* and *Firmicutes* [257]. They also concluded that in addition to nutrition interventions, gut microbiota is likely to be influenced by other factors within the child environment [257].

In our study, we postulated that the intervention with focus on nutrition and hygiene could facilitate adequate gut maturation and establishment. Several diet changes have been directed at modulating gut microbiota establishment in interventions on child behavior [258]. Importantly, animal studies have demonstrated a causative role of an altered gut microbiota in undernourished children, and correction to an adequate gut microbiota may promote healthy growth [259]. However, we did not find any statistical difference in gut microbiota composition or diversity between the two study groups (Paper I). We therefore do not know whether our maternal education had a specific effect on the establishment of the gut microbiota at three years. Notably, our intervention was not designed primarily to study gut microbiota *per se*, and we did not collect microbiota data at the 6-8 months' inclusion assessment (i.e. at baseline), which could have enabled us to explore trend analysis of the microbiota development between the two study groups. Although the microbiota diversity was similar in the two study groups, we recently reported that *Lactobacillus* species isolated from the gut microbiota could bind the fungi-derived aflatoxins, possible inhibitors of linear growth. Possibly *Lactobacillus* species may provide remedy to child stunting, if included in child complementary foods in this setting [260]. In addition to microbiota diversity and abundance, biological factors, such as signaling molecules, could link microbiota to child development and growth. However, we recently failed to demonstrate any effect of the intervention on urine markers of short chain fatty acids and kynurenine metabolites, known markers of gut-brain signaling [261]. To address the role of gut microbiota specifically, tailor-made nutrition interventions focusing on promoting the gut microbiota alteration and establishment during early childhood may be an important target to promote child growth and development, in particular in LMICs [262, 263]. Also, we only studied bacteria derived from the gut. Possibly, other bacteria-habitats, e.g. from the oral cavity, may have yielded more information about possible links between the microbiota and child development and growth.

6.3.4. Iodine, child development and growth

Insufficient iodine is one of the major causes of preventable CNS development deficits including impaired cognitive function [264]. Many regions report to be iodine deficient; these include countries in Africa, Asia, the Eastern Mediterranean region and parts of Eastern Europe [265]. To address iodine deficiency, several nations have implemented universal salt iodisation as a preferred strategy. In some settings iodine fortification in other condiments for example in edible vegetables, cereal grains, water, fish sauces, oils and fats, have been successfully performed [266].

Due to the important impact iodine deficiency can have on child neurodevelopment, this topic has been quite extensively studied. In their systematic review and meta-analysis, Bougma et al. reported the impact of iodine deficiency on development among children below five years, especially mental development. In fact, insufficient child iodine status contributed to a loss of between 6.9 and 10.2 IQ points compared with children having normal iodine status [86]. The included intervention studies in this review consistently found positive effects of iodine on mental outcome of children regardless of study design, geographical location or population under study [86]. In contrast, studies that assessed maternal iodine status in iodine deplete areas reported mixed effects on child development outcomes [267]. For example, whereas the ALSPAC cohort in the UK indicated that children of iodine-deficient mothers (iodine-to-creatinine ratio below 150 μg/g) had significantly higher risk of suboptimal cognitive outcomes with a mean lower total IQ [267]. In the Netherlands, a study from the Generation R cohort did not find a significant relationship between maternal low urinary iodine concentration and child development outcome of non-verbal IQ [268].

Apparently, there is no iodine deficiency in Uganda, probably due to universal salt iodisation [265]. This is supported by our findings that most children in both study groups a UIC > 100 μg/L (Paper II). A possible explanation for this could be that both groups consumed iodised salt [161]. Notably, our intervention was not designed to enhance child iodine consumption specifically, dietary/nutrient intake was not recorded, and baseline-data of iodine status was not collected.

We showed that in the intervention group the ICR was associated with child cognitive development, but not with the anthropometric markers. Although we cannot exclude that this association was by chance only, our finding is supported by others [269].

There are some limitations to our approach that merit attention. We did not measure dietary iodine intake directly. Hence, we have no information about the content of iodine in the consumed foods, supplements, household salt or breastmilk. Instead we used UIC as a marker for iodine intake, however this provides an estimate of iodine intake at the population and not at the individual level. UIC may also vary according to hydration status, hence we used ICR. The ICR was based on only one spot urine sample per time point, and not several spot samples or 24 h sampling (not feasible in our setting), which is recommended [270]. Furthermore, there was no significant correlation between UIC and ICR at 24 (Spearmans rho -0.17, p=0.84) or at 36 (Spearmans rho 0.03, p=0.74) months, suggesting that one or both of these markers of iodine intake are unspecific. Moreover, we did not measure thyroid hormones, which can be normal even with mildly reduced UIC.

A recent systematic review identified inconsistencies in the associations between iodine status and child development [271]. Despite using global child developmental assessments like the BSID, Bell et al suggested that in regions with mild to moderate iodine deficiency, the association between cognitive development and iodine status might be small. Moreover, the BSID-III may not be a sensitive tool for scoring developmental differences in specific cognitive tasks and/or skills [272]. Our reported associations of ICR and BSID-III cognitive scores do not prove a causal relationship. The negative association between ICR and the ASQ gross motor score at 20-24 months in the control group might have been due to chance alone as we performed multiple statistical tests. This could at least in part explain some of the inconsistency in these association analyses Also, due to a small sample size, some of the cognitive association data had 95% confidence intervals there were wide, indicating uncertainty. Collectively then, more trials are needed with specific interventions targeting iodine status including iodine supplementation, to explore these associations further.

Notwithstanding the many global iodine supplementation and fortification intervention programs, there still exists a gap in the desired iodide status among many populations. This presents an additional burden in particular to impoverished children, especially neurocognitive

development deficits even in cases of mild iodine deficiency [267]. Moreover, even a small reduction in the mean population IQ may affect the economic development and well-being of the affected societies [273, 274]. This warrants pragmatic interventions to address child iodine deficiency.

6.3.5. Maternal depression, child development and growth

Maternal depression and poor mother–child interaction may compromise the child attachment process. Consequently, maternal depression can lead to poor child health and impair child developmental and growth outcomes [146]. Maternal depression and its adverse psychological distress on children are common in LMICs and it is a well-known hindrance to desired child development outcomes and possibly also growth.

Interestingly we found that the maternal education intervention reduced the incidence of self-reported maternal depression symptoms (Paper III). Improved nutritional status and/or growth may possibly have lessened the burden to mothers in the intervention group who were depressed at study start, so that they later reported fewer depression symptom scores.

The reduction in maternal depression symptom scores was associated with improved child development outcomes at both the 20-24 and the 36 months' assessments when analyzed for the whole study cohort with mixed effects linear regression. In comparison to other interventions, including several child care practices and general parental programs in communities, similar observations have been reported. For example, in their systematic review, Gilmore and McAuliffe observed that community-based strategies, including malaria prevention, health education, breastfeeding promotion, newborn care and psychosocial support, presented a means for relief from depression among mothers in LMICs [146]. Barnes and colleagues' meta-analysis reported a negative association between maternal depression and child development [275]. The USA Community Child Health Network multicenter study reported higher risk of developmental delays among children whose mothers experienced more negative life events and greater negative impact of these events, but postpartum maternal depression did not explain this [276]. Furthermore, results from the Spanish Pelotas birth cohort supported the negative impact that maternal depression has on children's socioemotional competences until early adolescence [277]. Contrary to our findings, a South African birth cohort study reported high maternal distress symptoms, but no association with early child

developmental outcomes [278]. Although this study's development assessments were performed already at six months of age, the authors suggested that the effects can be more manifest at a later child age [278]. Similar findings were reported by the P-MaMiE study in Ethiopia where maternal depression was not associated with child cognitive, language or motor development at 12 months of age [279]. These discrepancies in study findings may at least in part be explained by different study designs and assessment tools. Also of note is the fact that we did not target maternal depression specifically with our intervention and we report on maternal depression symptom scores and not on actually diagnosed depression disease. One may question if the observer effect (the Hawthorne effect) could explain the reduction in depression symptoms, simply because of the attention the mothers received by the researchers. However, this is unlikely because the depression symptoms were not assessed by the researchers themselves, but they were self-reported using reliable tools.

The association between maternal depression and child growth impairment was explored by Smith et al. using data from 137 LMICs throughout the world [280]. Reportedly, maternal depression accounted for about 7.2 million cases of stunting in these countries. This finding is supported by several other studies [281, 282]. Whereas the exact mechanism(s) explaining the association between maternal depression and child stunting has not been finally identified, likely explanations include inadequate feeding and lack of appropriate maternal care-giving.

In summary, several lines of evidence support the negative impact of maternal depression on child development and growth, in particular in LMICs. Strategies to treat maternal depression using e.g. lay community health workers could be effective in resource-limited settings and inclusion of maternal depression treatment in community programs will be an addition to the required solutions aimed at addressing inadequate child development in LMICs [280]. Ultimately, this may boost the drive needed for LMICs economic progress towards the achievement of the SDGs [16].

6.3.6. Child development and growth – any link(s)?

Previous research has indicated that improvements in developmental outcomes are associated with growth among children below two years, and that these improvements would be even bigger with integration of nutrition, environmental, educational, and stimulation interventions [283]. Sixty-eight intervention studies implemented in LMICs showed the existence of a

positive association between growth with cognitive and motor development [283]. These findings concluded that effective interventions which reduce growth restriction could improve child developmental outcomes. This is somewhat at variance with our data since we only found a minor impact on growth faltering and only when the children where three years (Paper I). Notably, many children were stunted already at baseline, i.e. when they were 6-8 months. Others have proposed that interventions benefitting growth may be independent of child development, hence what impacts on development and growth outcomes may occur through different mechanism(s) [61]. In line with this and similar to our findings, interventions that improved development outcomes have not always led to improved growth [37, 61, 284]. Interestingly, the concept of stunting being a marker for undernutrition was recently questioned, based on a study from Asia showing that there was no correlation between nutritional status and height [285]. However, neither our study nor previous research findings have adequately addressed the mechanism(s) of how combined interventions benefit more on child development compared to growth outcomes [220].

6.3.7. Multilevel approach to address causes of child malnutrition and development

When the original RCT was initiated in October 2013, our main aim was to test if the education intervention would reduce the stunting rate, which was high in the two study-districts. In addition, we wanted to examine if this intervention could also impact positively on child development as undernutrition impairs development outcomes. Mainly due to the positive findings on child development up until the age of 20-24 months, we decided to do a follow-up (current thesis) of these children after yet another year to examine possible continuity of this positive trend.

With reference to the UNICEF 1990 framework (Figure 1), growth and development would correspond to outcomes (manifestations) [27]. So, the purpose was primarily to address inadequate nutrition, poor hygiene and child stimulation, as these were main underlying causes of poor child growth and development in the study areas. Moreover, we specifically evaluated the possible role of iodine, microbiota and maternal depression, because they are related to nutrition, hygiene and stimulation, respectively, in this setting. Our approach to address these aims were to develop and implement the RCT, which would correspond to a combination of nutrition specific interventions/programs and nutrition sensitive programs/approaches, as proposed in the 2013 Lancet framework [33].

Despite the positive findings of our previous research and the current follow-up study, in particular regarding child development, there would be a need to also include broader issues if our intervention was to be sustained and have a potential for impact at scale. For example, inclusion of stake-holders/leaders at local, national and possibly at the international level is needed to commit adequate resources to support implementation of the proposed intervention activities. In addition, this would facilitate increased knowledge about the causes and appropriate actions needed to successfully remedy the problems of poor growth and reduced stimulation among children at risk. Such a multilevel approach would fit with the need to identify basic causes within the UNICEF framework and also address important parts of the 2013 Lancet framework.

7. Conclusions

The present thesis using data from a follow-up study of a cluster-RCT involving a maternal education intervention, makes the following conclusions:

- The maternal education intervention, with focus on nutrition, hygiene, and stimulation and delivered to impoverished mothers, improved child development outcomes of the cognitive, language and motor domains.
- Possibly our maternal intervention including nutrition education, but without providing actual food handouts or supplements, may have reduced child growth faltering.
- Our maternal intervention had no apparent effect on the gut microbiota composition or diversity among the children.
- Child iodine intake was associated with cognitive development, but not with anthropometric markers.
- The education intervention significantly reduced maternal depression symptom scores. The reduction in the self-reported maternal depression was associated with improved child development.

8. Future research

The current findings point to several aspects that require further studies. Firstly, revealing the mechanism(s) underlying the detrimental effects of child undernutrition requires multiple approaches (e.g. mechanistic studies and well-executed community randomized trials) to address developmental and growth. Secondly, the association between gut microbiota and child development, especially in LMICs, should be more specifically addressed with targeted interventions, e.g. with diets and possibly in the future with fecal transplantation. Thirdly, to further address the effects of iodine on child health, regular population monitoring and assessments need to be conducted. Fourthly, more research on interventions to alleviate poor maternal mental health to improve child development and growth should be performed. Finally, several follow-ups of this cluster RCT, e.g. when the children start at school and when becoming adults, should be performed to examine the long-term effects of the maternal education intervention in early childhood on future school achievements and productivity in adulthood.

9. References

1. Alderman, H. and L. Fernald, *The nexus between nutrition and early childhood development.* Annu Rev Nutr, 2017. 37: 447-476.
2. Black, M.M., R. Perez-Escamilla, and S.F. Rao, *Integrating nutrition and child development interventions: scientific basis, evidence of impact, and implementation considerations.* Adv Nutr, 2015. 6: 852-859.
3. Richter, L.M., et al., *Investing in the foundation of sustainable development: pathways to scale up for early childhood development.* Lancet, 2017. 389: 103-118.
4. Center for Disease Control and Prevention, *Child Development Basics*. 2019.
5. Institute of Medicine and National Research Council 2015, *Transforming the workforce for children birth through age 8: A unifying foundation.* 2015.
6. Jensen, S.K., et al., *Enhancing the child survival agenda to promote, protect, and support early child development.* Semin Perinatol, 2015. 39: 373-386.
7. Hadders-Algra, M., *Development of postural control during the first 18 months of life.* Neural Plasticity, 2005. 12: 99-108.
8. Gajewska, E., et al., *Achieving motor development milestones at the age of three months may determine, but does not guarantee, proper further development.* Sci World J, 2013. 2013.
9. Dosman, C.F., D. Andrews, and K.J. Goulden, *Evidence-based milestone ages as a framework for developmental surveillance.* Ped Child Health, 2012. 17: 561-568.
10. Findlay, L., D. Kohen, and A. Miller, *Developmental milestones among Aboriginal children in Canada.* Ped Child Health, 2014. 19: 241-246.
11. Justice, L.M., *Communication sciences and disorders: An introduction*. 2006: Pearson/Merrill Prentice Hall.
12. Grantham-McGregor, S., et al., *Developmental potential in the first 5 years for children in developing countries.* Lancet, 2007. 369: 60-70.
13. Jones, G., et al., *How many child deaths can we prevent this year?* Lancet, 2003. 362: 65-71.
14. Milner, K.M., et al., *Counting outcomes, coverage and quality for early child development programmes.* Arch Dis Child, 2019. 104: S13-S21.
15. United Nations General Assembly. 2000. "United Nations Millennium Declaration." United Nations General Assembly, New York. http://www.un.org/millennium /declaration/ares552e.htm.
16. United Nations (2015). *Transforming Our World: The 2030 Agenda for Sustainable Development*. New York: UN Publishing.
17. UNICEF. Nurturing care for early childhood development: a framework for helping children survive and thrive to transform health and human potential. 2018.
18. Lu, C., M.M. Black, and L.M. Richter, *Risk of poor development in young children in low-income and middle-income countries: an estimation and analysis at the global, regional, and country level.* Lancet Glob Health, 2016. 4: e916-e922.
19. Veena, S.R., et al., *Association between maternal nutritional status in pregnancy and offspring cognitive function during childhood and adolescence; a systematic review.* BMC Pregnancy Childbirth, 2016. 16: 220.
20. World Health Organization. WHO Child Growth Standards: Length/height-for-age, Weight-for-age, Weight-for length, Weight-for-height and Body Mass Index for-age: Methods and Development. http://www.who.int/childgrowth/standards/Technical_report.pdf 2006.

21. De Onis, M. and F. Branca, *Childhood stunting: a global perspective.* Matern Child Nutr, 2016. 12: 12-26.
22. Carducci, B. and Z.A. Bhutta, *Care of the growth-restricted newborn.* Best practice & research Clin Obsted Gynecol, 2018. 49: 103-116.
23. United Nations Children's Fund, *UNICEF–WHO–World Bank: joint child malnutrition estimates* UNICEF, New York; WHO, Geneva; The World Bank, Washington, DC, 2014
24. United Nations Children's Fund, World Health Organization & The World Bank UNICEF–WHO–World Bank: joint. *Child malnutrition* estimates UNICEF, New York; WHO, Geneva; The World Bank, Washington, DC March 2019 edition. https://data.unicef.org/topic/nutrition/malnutrition/.
25. WHO/UNICEF, *Sixty-fifth World Health Assembly in Resolutions and Decisions 2012: Geneva.*WHO/UNICEF, Countdown to 2015 Maternal, New born and Child Survival; Uganda Maternal and Child Health data 2012.
26. FAO, World Food Programme. *strengthening the enabling environment for food security and nutrition.* Rome: FAO, 2014: 2014.
27. UNICEF, *Strategy for improved nutrition of children and women in developing countries: A UNICEF Policy Review Unicef.* 1990, E/ICEF/1990/L. 6, New York.
28. Black, R.E., et al., *Maternal and child undernutrition: global and regional exposures and health consequences.* Lancet, 2008. 371: 243-260.
29. Victora, C.G., et al., *Maternal and child undernutrition: consequences for adult health and human capital.* Lancet, 2008. 371: 340-357.
30. Bhutta, Z.A., et al., *What works? Interventions for maternal and child undernutrition and survival.* Lancet, 2008. 371: 417-440.
31. Bryce, J., et al., *Maternal and child undernutrition: effective action at national level.* Lancet, 2008. 371: 510-526.
32. Morris, S.S., et al., *Effective international action against undernutrition: why has it proven so difficult and what can be done to accelerate progress?* Lancet, 2008. 371: 608-621.
33. Black, R.E., et al., *Maternal and child undernutrition and overweight in low-income and middle-income countries.* Lancet, 2013. 382: 427-451.
34. Bhutta, Z.A., J.K. Das, and A. Rizvi, *The Lancet Nutrition Interventions Review Group, and the Maternal and Child Nutrition Study Group. Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost?* Lancet, 2013. 382: 452-477.
35. Ruel, M.T., et al., *Nutrition-sensitive interventions and programmes: how can they help to accelerate progress in improving maternal and child nutrition?* Lancet, 2013. 382: 536-551.
36. Gillespie, S., et al., *The politics of reducing malnutrition: building commitment and accelerating progress.* Lancet, 2013. 382: 552-569.
37. Prado, E.L., et al., *Do effects of early life interventions on linear growth correspond to effects on neurobehavioural development? A systematic review and meta-analysis.* Lancet Glob Health, 2019. 7: e1398-e1413.
38. Liu, Y., et al., *Maternal depressive symptoms and early childhood cognitive development: a meta-analysis.* Psychol Med, 2017. 47: 680-689.
39. Vaivada, T., M.F. Gaffey, and Z.A. Bhutta, *Promoting early child development with interventions in health and nutrition: a systematic review.* Pediatrics, 2017. 140: e20164308.

40. Victora, C.G., et al., *Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect.* Lancet, 2016. 387: 475-490.
41. Kramer, M.S., et al., *Breastfeeding and child cognitive development: new evidence from a large randomized trial.* Arch Gen Psych, 2008. 65: 578-584.
42. Katona, P. and J. Katona-Apte, *The interaction between nutrition and infection.* Clin Infect Dis, 2008. 46: 1582-1588.
43. Ali, S.S. and S.G. Dhaded, *The impact of nutrition on child development at 3 years in a rural community of India.* Int J Prev Med, 2014. 5: 494.
44. UBOS. *Uganda Demographic and Health Survey* 2016, Key Indicators Report Kampala, Uganda Bureau of Statistics and ICF, 2017.
45. UNICEF, World Health Organization. *Global Breastfeeding Scorecard, 2017: Tracking Progress for Breastfeeding Policies and Programmes.* New York. 2017.
46. ProPAN, P., *Process for the promotion of child feeding. cataloguing-in-publication PHL.* Washington, DC: PAHO, 2004
47. World Health Organization, UNICEF. *Complementary feeding: report of the global consultation, and summary of guiding principles for complementary feeding of the breastfed child*. Geneva, WHO. 2003.
48. World Health Organization. *Summary of guiding principles for complementary feeding of the breastfed child.* Geneva, Switzerland: 2002.
49. Dewey, K.G. and B.S. Vitta, *Strategies for ensuring adequate nutrient intake for infants and young children during the period of complementary feeding.* Washington: Alive & Thrive, 2013. 7.
50. Spaniol, A.M., et al., *Breastfeeding reduces ultra-processed foods and sweetened beverages consumption among children under two years old.* BMC Publ Health, 2020. 20: 1-9.
51. Imdad, A., M.Y. Yakoob, and Z.A. Bhutta, *Impact of maternal education about complementary feeding and provision of complementary foods on child growth in developing countries.* BMC Publ Health, 2011. 11: S25.
52. Arikpo, D., et al., *Educational interventions for improving primary caregiver complementary feeding practices for children aged 24 months and under.* Cochrane Database of Systematic Reviews, 2018(5).
53. Meltzer, H.M., et al., *Benefit and risk assessment of breastmilk for infant health in Norway: Opinion of the Steering Committee of the Norwegian Scientific Committee for Food Safety.* 2013.
54. Gehrig, J.L., et al., *Effects of microbiota-directed foods in gnotobiotic animals and undernourished children.* Science, 2019. 365: 4732.
55. Chen, X., et al., *Differentiation and proliferation of intestinal stem cells and its underlying regulated mechanisms during weaning.* current protein and peptide Science, 2019. 20: 690-695.
56. Fewtrell, M., et al., *Complementary feeding: a position paper by the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) Committee on Nutrition.* J Ped Gastroenterol Nutr, 2017. 64: 119-132.
57. Rapley, G., *Baby-led weaning: the theory and evidence behind the approach.* J Health Visiting, 2015. 3:144-151.
58. Brown, A., S.W. Jones, and H. Rowan, *Baby-led weaning: the evidence to date.* Curr Nutr Rep, 2017. 6: 148-156.
59. PAHO/WHO, *Guiding Priciples for Complementary Feeding of the Breastfed Child.* Division of Health Promotion and Protection, 2003 (Washington D.C.).

60. Isingoma, B.E., et al., *Improving the nutritional value of traditional finger millet porridges for children aged 7-24 months in Bujenje County of Western Uganda.* Afr J Food Sci, 2015. 9: 426-436.
61. Dewey, E.P.a.K., *Nutrition and Brain Development in Early Life, in Alive and Thrive*. Washington, D.C. 2012.
62. Prado, E.L. and K.G. Dewey, *Nutrition and brain development in early life.* Nutr Rev, 2014. 72: 267-284.
63. Nyaradi, A., et al., *The role of nutrition in children's neurocognitive development, from pregnancy through childhood.* Front Hum Neurosci, 2013. 7: 97.
64. Uauy, R. and A.D. Dangour, *Nutrition in brain development and aging: role of essential fatty acids.* Nutr Rev, 2006. 64: S24-S33.
65. Levine, T.A., et al., *Early childhood neurodevelopment after intrauterine growth restriction: a systematic review.* Pediatrics, 2015. 135: 126-41.
66. Perkins, J.M., et al., *Understanding the association between stunting and child development in low-and middle-income countries: next steps for research and intervention.* Soc Sci Med, 2017. 193: 101-109.
67. Pitchik, H.O., et al., *Prenatal nutrition, stimulation, and exposure to punishment are associated with early child motor, cognitive, language, and socioemotional development in Dar es Salaam, Tanzania.* Child Care Health Dev, 2018. 44: 841-849.
68. Locks, L.M., et al., *The effect of daily zinc and/or multivitamin supplements on early childhood development in Tanzania: results from a randomized controlled trial.* Matern Child Nutr, 2017. 13: e12306.
69. Christian, P., et al., *Effects of prenatal multiple micronutrient supplementation on growth and cognition through 2 y of age in rural Bangladesh: the JiVitA-3 trial.* Am J Clin Nutr, 2016. 104: 1175-1182.
70. Prado, E.L., et al., *Effects of maternal and child lipid-based nutrient supplements on infant development: a randomized trial in Malawi.* Am J Clin Nutr, 2016. 103: 784-793.
71. Yousafzai, A.K., et al., *Effect of integrated responsive stimulation and nutrition interventions in the Lady Health Worker programme in Pakistan on child development, growth, and health outcomes: a cluster-randomised factorial effectiveness trial.* Lancet, 2014. 384: 1282-1293.
72. Taljaard, C., et al., *Effects of a multi-micronutrient-fortified beverage, with and without sugar, on growth and cognition in South African schoolchildren: a randomised, double-blind, controlled intervention.* Br J Nutr, 2013. 110: 2271-2284.
73. Baumgartner, J., et al., *Effects of iron and n-3 fatty acid supplementation, alone and in combination, on cognition in school children: a randomized, double-blind, placebo-controlled intervention in South Africa.* Am J Clin Nutr, 2012. 96: 1327-1338.
74. Murray-Kolb, L.E., et al., *Preschool micronutrient supplementation effects on intellectual and motor function in school-aged Nepalese children.* Arch Ped Adolesc Med, 2012. 166: 404-410.
75. Manno, D., et al., *Rich micronutrient fortification of locally produced infant food does not improve mental and motor development of Zambian infants: a randomised controlled trial.* Br J Nutr, 2012. 107: 556-566.
76. Phuka, J.C., et al., *Developmental outcomes among 18-month-old Malawians after a year of complementary feeding with lipid-based nutrient supplements or corn-soy flour.* Matern Child Nutr, 2012. 8: 239-248.
77. De Onis, M., et al., *The W orld H ealth O rganization's global target for reducing childhood stunting by 2025: rationale and proposed actions.* Matern Child Nutr, 2013. 9: 6-26.

78. Khara, T., et al., *Children concurrently wasted and stunted: A meta-analysis of prevalence data of children 6–59 months from 84 countries.* Matern Child Nutr, 2018. 14: e12516.
79. UNICEF, *Levels and trends in child malnutrition UNICEF-WHO-World Bank Group joint child malnutrition estimates: key findings of the 2015 edition.* New York: UNICEF, WHO, World Bank Group, 2015.
80. IFPRI, *Global Nutrition Report 2015: Actions and accontability to advance nutrition and sustainable development.* Retrieved from Washington, DC, 2015.
81. Dewey, K.G. and D.R. Mayers, *Early child growth: how do nutrition and infection interact?* Matern Child Nutr, 2011. 7: 129-142.
82. Meyer, R., et al., *The impact of the elimination diet on growth and nutrient intake in children with food protein induced gastrointestinal allergies.* Clin Translat Allerg, 2016. 6: 25.
83. World Health Organization, United Nations University. Protein and amino acid requirements in human nutrition. World Health Organization; 2007 Dec 15.
84. Melse-Boonstra, A. and N. Jaiswal, *Iodine deficiency in pregnancy, infancy and childhood and its consequences for brain development.* Best Pract Res Clin Endocrinol Metab, 2010. 24: 29-38.
85. Zimmermann, M.B., P.L. Jooste, and C.S. Pandav, *Iodine-deficiency disorders.* Lancet, 2008. 372: 1251-1262.
86. Bougma, K., et al., *Correction: Bougma, K., et al. Iodine and Mental Development of Children 5 Years Old and Under: A Systematic Review and Meta-Analysis. Nutrients 2013, 5, 1384–1416.* Nutrients, 2014. 6: 5770-5771.
87. Walker, S.P., et al., *Inequality in early childhood: risk and protective factors for early child development.* Lancet, 2011. 378: 1325-1338.
88. Huda, S.N., S.M. Grantham-McGregor, and A. Tomkins, *Cognitive and motor functions of iodine-deficient but euthyroid children in Bangladesh do not benefit from iodized poppy seed oil (Lipiodol).* J Nutr, 2001. 131: 72-77.
89. Shrestha, R.M., *Effect of iodine and iron supplementation on physical, psychomotor and mental development in primary school children in Malawi*. 1994: Thesis, Wafgeningen University.
90. Zimmermann, M.B., et al., *Iodine supplementation improves cognition in iodine-deficient schoolchildren in Albania: a randomized, controlled, double-blind study.* Am J Clin Nutr, 2006. 83: 108-114.
91. Harika, R., et al., *Are Low Intakes and Deficiencies in Iron, Vitamin A, Zinc, and Iodine of Public Health Concern in Ethiopian, Kenyan, Nigerian, and South African Children and Adolescents?* Food Nutr Bull, 2017. 38: 405-427.
92. Harper, K.M., et al., *Environmental enteric dysfunction pathways and child stunting: A systematic review.* PLoS Negl Trop Dis, 2018. 12: e0006205.
93. Shah, A., *Poverty facts and stats.* Global Issues, 2013. 7.
94. McCormick, B.J. and D.R. Lang, *Diarrheal disease and enteric infections in LMIC communities: how big is the problem?* Trop Travel Med Vacc, 2016. 2: 11.
95. Guerrant, R.L., et al., *Malnutrition as an enteric infectious disease with long-term effects on child development.* Nutr Rev, 2008. 66: 487-505.
96. Rodríguez, L., E. Cervantes, and R. Ortiz, *Malnutrition and gastrointestinal and respiratory infections in children: a public health problem.* Int J Environ Res Publ Health, 2011. 8: 1174-1205.

97. Pabalan, N., et al., *Soil-transmitted helminth infection, loss of education and cognitive impairment in school-aged children: A systematic review and meta-analysis.* PLoS Negl Trop Dis, 2018. 12(1).
98. Oriá, R.B., et al., *Early-life enteric infections: relation between chronic systemic inflammation and poor cognition in children.* Nutr Rev, 2016. 74: 374-386.
99. Berkman, D.S., et al., *Effects of stunting, diarrhoeal disease, and parasitic infection during infancy on cognition in late childhood: a follow-up study.* Lancet, 2002. 359: 564-571.
100. Goto, R., C.N. Mascie-Taylor, and P.G. Lunn, *Impact of intestinal permeability, inflammation status and parasitic infections on infant growth faltering in rural Bangladesh.* Br J Nutr, 2009. 101: 1509-1516.
101. Lunn, P.G., *The impact of infection and nutrition on gut function and growth in childhood.* Proc Nutr Soc, 2000. 59:147-154.
102. Lunn, P., C. Northrop-Clewes, and R. Downes, *Intestinal permeability, mucosal injury, and growth faltering in Gambian infants.* Lancet, 1991. 338: 907-910.
103. Campbell, D., M. Elia, and P. Lunn, *Growth faltering in rural Gambian infants is associated with impaired small intestinal barrier function, leading to endotoxemia and systemic inflammation.* J Nutr, 2003. 13: 1332-1338.
104. Yatsunenko, T., et al., *Human gut microbiome viewed across age and geography.* Nature, 2012. 486: 222.
105. Guernier, V., et al., *Gut microbiota disturbance during helminth infection: can it affect cognition and behaviour of children?* BMC Infect Dis, 2017. 17: 58.
106. Smith, M.I., et al., *Gut microbiomes of Malawian twin pairs discordant for kwashiorkor.* Science, 2013. 339: 548-554.
107. Al-Asmakh, M., et al., *Gut microbial communities modulating brain development and function.* Gut Microbes, 2012. 3: 366-373.
108. Rutayisire, E., et al., *The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: a systematic review.* BMC Gastroenterol, 2016. 16: 86.
109. Romano-Keeler, J. and J.-H. Weitkamp, *Maternal influences on fetal microbial colonization and immune development.* Ped Res, 2015. 77: 189-195.
110. Schwartz, S., et al., *A metagenomic study of diet-dependent interaction between gut microbiota and host in infants reveals differences in immune response.* Genome Biol, 2012. 13: r32.
111. Diaz Heijtz, R., et al., *Normal gut microbiota modulates brain development and behavior.* Proc Natl Acad Sci USA, 2011. 108: 3047-52.
112. Cenit, M.C., Y. Sanz, and P. Codoñer-Franch, *Influence of gut microbiota on neuropsychiatric disorders.* World J Gastroenterol, 2017. 23: 5486.
113. Cryan, J.F. and T.G. Dinan, *Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour.* Nat Rev Neurosci, 2012. 13: 701.
114. Parashar, A. and M. Udayabanu, *Gut microbiota regulates key modulators of social behavior.* Eur Neuropsychopharmacol, 2016. 26:78-91.
115. Wallace, C.J. and R. Milev, *The effects of probiotics on depressive symptoms in humans: a systematic review.* Ann Gen Psych, 2017. 16: 14.
116. Jenkins, T.A., et al., *Influence of tryptophan and serotonin on mood and cognition with a possible role of the gut-brain axis.* Nutrients, 2016. 8: 56.
117. Sarkar, A., et al., *Psychobiotics and the manipulation of bacteria–gut–brain signals.* Trend Neurosci, 2016. 39: 763-781.

118. Gough, E.K., et al., *The impact of antibiotics on growth in children in low and middle income countries: systematic review and meta-analysis of randomised controlled trials.* BMJ, 2014. 348: 2267.
119. Bradley, R.H., L.M. McKelvey, and L. Whiteside-Mansell, *Does the quality of stimulation and support in the home environment moderate the effect of early education programs?* Child Dev, 2011. 82: 2110-2122.
120. Potterton, J., et al., *The effect of a basic home stimulation programme on the development of young children infected with HIV.* Dev Med Child Neurol, 2010. 52: 547-551.
121. Black, M.M. and F.E. Aboud, *Responsive feeding is embedded in a theoretical framework of responsive parenting.* J Nutr, 2011. 141: 490-494.
122. Landry, S.H., et al., *A responsive parenting intervention: the optimal timing across early childhood for impacting maternal behaviors and child outcomes.* Dev Psychol, 2008. 44: 1335.
123. Kretchmar, M.D. and D.B. Jacobvitz, *Observing mother-child relationships across generations: Boundary patterns, attachment, and the transmission of caregiving.* Fam Process, 2002. 41: 351-374.
124. Petterson, S.M. and A.B. Albers, *Effects of poverty and maternal depression on early child development.* Child Dev, 2001. 72: 1794-1813.
125. Worku, B.N., et al., *Effects of home-based play-assisted stimulation on developmental performances of children living in extreme poverty: a randomized single-blind controlled trial.* BMC Ped, 2018. 18: 29.
126. Hartinger, S.M., et al., *Impact of a child stimulation intervention on early child development in rural Peru: a cluster randomised trial using a reciprocal control design.* J Epidemiol Comm Health, 2017. 71: 217-224.
127. Helmizar, H., et al., *Local food supplementation and psychosocial stimulation improve linear growth and cognitive development among Indonesian infants aged 6 to 9 months.* Asia Pac J Clin Nutr, 2017. 26: 97.
128. Singla, D.R., E. Kumbakumba, and F.E. Aboud, *Effects of a parenting intervention to address maternal psychological wellbeing and child development and growth in rural Uganda: a community-based, cluster-randomised trial.* Lancet Glob Health, 2015. 3: e458-e469.
129. Aboud, F.E. and A.K. Yousafzai, *Global health and development in early childhood.* Annu Rev Psychol, 2015. 66: 433-57.
130. Aboud, F.E., et al., *Effectiveness of a parenting program in Bangladesh to address early childhood health, growth and development.* Soc Sci Med, 2013. 97: 250-258.
131. Vazir, S., et al., *Cluster-randomized trial on complementary and responsive feeding education to caregivers found improved dietary intake, growth and development among rural Indian toddlers.* Matern Child Nutr, 2013. 9: 99-117.
132. Draper, C.E., et al., *Impact of a community-based programme for motor development on gross motor skills and cognitive function in preschool children from disadvantaged settings.* Early Child Dev Care, 2012. 182: 137-152.
133. Nahar, B., et al., *Effects of a community-based approach of food and psychosocial stimulation on growth and development of severely malnourished children in Bangladesh: a randomised trial.* Eur J Clin Nutr, 2012. 66:701-709.
134. Slomian, J., et al., *Consequences of maternal postpartum depression: A systematic review of maternal and infant outcomes.* Womens Health, 2019. 15: 1745506519844044.

135. Lefkovics, E., I. Baji, and J. Rigó, *Impact of maternal depression on pregnancies and on early attachment.* Infant Ment Health J, 2014. 35: 354-365.
136. Gelaye, B., et al., *Epidemiology of maternal depression, risk factors, and child outcomes in low-income and middle-income countries.* Lancet Psych, 2016. 3: 973-982.
137. Surkan, P.J., et al., *Maternal depression and early childhood growth in developing countries: systematic review and meta-analysis.* Bulletin World Health Org, 2011. 89: 607-615.
138. Ali, N.S., et al., *Impact of postpartum anxiety and depression on child's mental development from two peri-urban communities of Karachi, Pakistan: a quasi-experimental study.* BMC Psych, 2013. 13: 274.
139. Ainsworth, M.S., *Infant–mother attachment.* Am Psychologist, 1979. 3: 932.
140. Ainsworth, M.D.S., et al., *Patterns of attachment: A psychological study of the strange situation*. 2015: Psychology Press.
141. Letourneau, N.L., L. Tramonte, and J.D. Willms, *Maternal depression, family functioning and children's longitudinal development.* J Ped Nursing, 2013. 28: 223-234.
142. Burke, L., *The impact of maternal depression on familial relationships.* Int Rev Psych, 2003. 15: 243-255.
143. World Health Organization. *Maternal mental health and child health and development in low and middle income countries, Report of the WHO-UNFPA meeting held in Geneva, Switzerland 30 January–1 February 2008*
144. World Health Organization. *Maternal mental health and child health and development in low and middle income countries: report of the meeting, Geneva, Switzerland, 30 January-1 February, 2008.*
145. Halbreich, U. and S. Karkun, *Cross-cultural and social diversity of prevalence of postpartum depression and depressive symptoms.* J Affect Disord, 2006. 91: 97-111.
146. Gilmore, B. and E. McAuliffe, *Effectiveness of community health workers delivering preventive interventions for maternal and child health in low-and middle-income countries: a systematic review.* BMC Publ Health, 2013. 13: 847.
147. UBOS, *Uganda demographic and health survey 2016: key indicators report*. UBOS, and Rockville Maryland 2016.
148. Kabakyenga, J.K., et al., *Influence of birth preparedness, decision-making on location of birth and assistance by skilled birth attendants among women in south-western Uganda.* PloS One, 2012. 7(4).
149. Schuftan, C. and T. Greiner, *The Scaling Up Nutrition (SUN) Initiative.* Right to Food and Nutrition Watch, 2013: p. 22-23.
150. UBOS. Uganda Demographic and Health Survey 2006, Kampala, Uganda Bureau of Statistics and Macro-DHS Inc., 2007.
151. Paul, J.-Y., et al., *Banana21: from gene iscovery to deregulated golden bananas.* Fronti Plant Sci, 2018. 9: 558.
152. Ministry Of Health *Uganda, Child Survival Strategy for Uganda–2008-2015*. Draft. Kampala, Uganda: Uganda MOH
153. Vaivada, T., M.F. Gaffey, and Z.A. Bhutta, *Promoting Early Child Development With Interventions in Health and Nutrition: A Systematic Review.* Pediatrics, 2017. 140.
154. Kikafunda, J.K., et al., *Risk factors for early childhood malnutrition in Uganda.* Pediatrics, 1998. 102: e45-e45.
155. Profile, F.N.C., *The Republic of Uganda.* Nutrition and Consumer Protection Division, FAO: Rome, Italy, 2010.
156. Hess, S.Y., *National risk of zinc deficiency as estimated by national surveys.* Food Nutr Bull, 2017. 38: 3-17.

157. Uganda Bureau of Statistics. *National Population and Housing Census 2014 Uganda Bureau of Statistics. Uganda Bureau of Statistics.* https://www.ubos.org/explore-statistics/statistical-datasets/2468.
158. UBOS. *Uganda Demographic and Health Survey 2011*. Kampala, Uganda Bureau of Statistics and ICF Inc., 2012.
159. UBOS. *International ICF. Uganda Demographic and Health Survey 2011. 2012* Kampala, Uganda & Calverton, Maryland, USA: UBOS & ICF International Inc
160. Uganda GDP per capita 1982-2018 Data 2019-2020 https://tradingeconomics.com/uganda/gdp-per-capita.
161. FANTA, I., *The analysis of the nutrition situation in Uganda.* Food and Nutrition Technical Assistance II Project, USAID, 2010.
162. Mugisha, J., et al., *Factors enhancing household nutrition outcomes in potato value chain in South-Western Uganda.* J Sustain Dev, 2017. 10: 215-230.
163. Muhoozi, G.K., et al., *Nutrition, hygiene, and stimulation education to improve growth, cognitive, language, and motor development among infants in Uganda: A cluster-randomized trial.* Matern Child Nutr, 2018. 14: e12527.
164. Kasozi, S., B. Bashaasha, and V. Ochwoh, *Economics of sorghum production and soil fertility management in Kabale Highlands, Uganda.* J Food Agricul Environ, 2005. 3: 105.
165. Kisoro District Local Government. Kisoro District. Kisoro, Uganda P. O. Box 123 | Tel: +256 772-456-916 E-mail: info@kisoro.go.ug.
166. Ministry of Health, R.o.U. *VHT / Community Health Extension Workers*. 2019 https://health.go.ug/community-health-departments/vht-community-health-extension-workers.
167. Campbell, M., A. Donner, and N. Klar, *Developments in cluster randomized trials and Statistics in Medicine.* Stat Med, 2007. 26: 2-19.
168. Bruner, J.S. and H. Haste, *Making Sense (Routledge Revivals): The Child's Construction of the World*. 2010: Routledge.
169. Chang, S.M., et al., *Integrating a parenting intervention with routine primary health care: a cluster randomized trial.* Pediatrics, 2015. 136: 272-280.
170. Vos, R.C., et al., *Developmental trajectories of receptive and expressive communication in children and young adults with cerebral palsy.* Dev Med Child Neurol, 2014. 56: 951-959.
171. Wolf, J., et al., *Impact of a child stimulation intervention on early child development in rural Peru: a cluster randomised trial using a reciprocal control design.* 2017. J Epidemiol Community Health 71: 217-224.
172. Donnelly, J.E., et al., *Physical activity, fitness, cognitive function, and academic achievement in children: a systematic review.* Med Sci Sports Exerc, 2016. 48: 1197.
173. Schreiner, M., *A simple poverty scorecard for Uganda.* http://www.simplepovertyscorecard.com/UGA_2012_ENG.pdf.
174. Swindale, A. and P. Bilinsky, *Household dietary diversity score (HDDS) for measurement of household food access: indicator guide.* Washington, DC: Food and Nutrition Technical Assistance Project, Academy for Educational Development, 2006.
175. Coates, J., A. Swindale, and P. Bilinsky, *Household Food Insecurity Access Scale (HFIAS) for measurement of food access: indicator guide.* Washington, DC: food and nutrition technical assistance project, academy for educational Development, 2007. 34.
176. Bayley, N., *Bayley scales of infant and toddler development*. 2006: PsychCorp, Pearson.
177. Cromwell, E.A., et al., *Validity of US norms for the Bayley Scales of Infant Development-III in Malawian children.* Eur J Paediatr Neurol, 2014. 18: 223-230.

178. van Heerden, A., et al., *Support for the feasibility of the ages and stages questionnaire as a developmental screening tool: a cross-sectional study of South African and Zambian children aged 2-60 months.* BMC Pediatr, 2017. 17:55.
179. Shrestha, M., et al., *The feasibility of the Ages and Stages Questionnaire for the assessment of child development in a community setting in Nepal.* Child Care Health Dev, 2019. 45:394-402.
180. Dumont, R. and J.O. Willis, *Mullen Scales of Early Learning: Ags Edition.* Encyclopedia of Special Education, 2008: p. 1393-1394.
181. Boivin, M.J., et al., *Malaria illness mediated by anaemia lessens cognitive development in younger Ugandan children.* Malaria J, 2016. 15: 210.
182. Collins, S.M., et al., *Quality of caregiving is positively associated with neurodevelopment during the first year of life among HIV-exposed uninfected children in Uganda.* J Acq Imm Defic Syndr, 2018. 77: 235.
183. Boivin, M.J. and B. Giordani, *Neuropsychology of Children in Africa.* Perspectives on Risk and Resilience. 1st ed. Michigan: Springer, 2013.
184. World Health Organization, 2006. WHO child growth standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development.
185. De Onis, M., M. Blossner, and W.H. Organization, *WHO global database on child growth and malnutrition*. 1997, Geneva: World Health Organization.
186. Budding, A.E., et al., *Rectal swabs for analysis of the intestinal microbiota.* PloS One, 2014. 9:e101344.
187. Caporaso, J.G., et al., *Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms.* ISME J, 2012. 6: 1621-1624.
188. Roeselers, G., et al., *Microbial biogeography of drinking water: patterns in phylogenetic diversity across space and time.* Environ Microbiol, 2015. 17: 2505-2514.
189. Langille, M.G., et al., *Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences.* Nat Biotechnol, 2013. 31: 814.
190. Schloss, P.D., et al., *Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities.* Appl Environ Microbiol, 2009. 75: 7537-7541.
191. Ohashi, T., et al., *Simple microplate method for determination of urinary iodine.* Clin Chem, 2000. 46: 529-536.
192. Bath, S.C., et al., *Gestational changes in iodine status in a cohort study of pregnant women from the United Kingdom: season as an effect modifier.* Am J Clin Nutr, 2015. 101:1180-1187.
193. Kulasingam, V., et al., *Pediatric reference intervals for 28 chemistries and immunoassays on the Roche cobas® 6000 analyzer—A CALIPER pilot study.* Clin Biochem, 2010. 43: 1045-1050.
194. Jooste, P., et al., *Endemic goitre in the absence of iodine deficiency in schoolchildren of the Northern Cape Province of South Africa.* Eur J Clin Nutr, 1999. 53: 8.
195. Beck, A.T., R.A. Steer, and G.K. Brown, *Beck depression inventory-II.* San Antonio, 1996. 78(2): p. 490-498.
196. Lewinsohn, P.M., et al., *Center for Epidemiologic Studies Depression Scale (CES-D) as a screening instrument for depression among community-residing older adults.* Psychol Aging, 1997. 12: 277.
197. Ovuga, E., J. Boardman, and D. Wasserman, *Undergraduate student mental health at Makerere University, Uganda.* World Psych, 2006. 5: 51.

198. Natamba, B.K., et al., *Reliability and validity of the center for epidemiologic studies-depression scale in screening for depression among HIV-infected and-uninfected pregnant women attending antenatal services in northern Uganda: a cross-sectional study.* BMC Psych, 2014. 14: 303.
199. Ovuga, E., J. Boardman, and D. Wasserman, *The prevalence of depression in two districts of Uganda.* Soc Psych Psyciat Epidemiol, 2005. 40: 439-445.
200. Pietikäinen, J.T., et al., *Sleeping problems during pregnancy—a risk factor for postnatal depressiveness.* Arch Wom Ment Health, 2019. 22: 327-337.
201. Siddaway, A.P., A.M. Wood, and P.J. Taylor, *The Center for Epidemiologic Studies-Depression (CES-D) scale measures a continuum from well-being to depression: Testing two key predictions of positive clinical psychology.* J Affect Disord, 2017. 213: 180-186.
202. STATACorpLLC, *StataSE. 14 (64-bit) ed*, in *Lakeway Drive*, STATACorp, Editor. 2015: Texas, USA
203. IBMCorp, *IBM SPSS Statistics for Windows*, in *Armonk*, I. Corp, Editor. 2016: New York, USA
204. Core Team, R., *R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing*, 2013.
205. Team RC. A language and environment for statistical computing Vienna, Austria. Retrieved from https://www.r-project. org/. 2017.
206. Oksanen, J., et al., *vegan: community ecology package http://CRAN.* R-project. org/package= vegan, 2013.
207. Mazor, K.M., et al., *Cluster randomized trials: opportunities and barriers identified by leaders of eight health plans.* Med Care, 2007. 45: S29-S37.
208. Gums, T., B. Carter, and E. Foster, *Cluster randomized trials for pharmacy practice research.* Int J Clin Pharm, 2016. 38: 607-14.
209. Hernán, M.A. and S. Hernández-Díaz, *Beyond the intention-to-treat in comparative effectiveness research.* Clin Trials, 2012. 9: 48-55.
210. Godwin, M., et al., *Pragmatic controlled clinical trials in primary care: the struggle between external and internal validity.* BMC Med Res Method, 2003. 3: 28.
211. Doig, G.S. and F. Simpson, *Randomization and allocation concealment: a practical guide for researchers.* J Crit Care, 2005. 20: 187-191.
212. Gridley, N., et al., *Psychometric properties of child (0–5 Years) outcome measures as used in randomized controlled trials of parent programs: A systematic review.* Clin Child Fam Psychol Rev, 2019. 22: 388-405.
213. Aisah, S. and K.N. Siregar. *The cognitive screening in children under five years old in developing countries: a systematic literature review*. In: *Proceedings of the International Conference on Applied Science and Health*. 2018.
214. Raab, M., C.J. Dunst, and D.W. Hamby, *Efficacy trial of contrasting approaches to the response-contingent learning of young children with significant developmental delays and multiple disabilities.* J Edu Dev Psychol, 2017. 7: 12-28.
215. Halle, T., et al., *Understanding and Choosing Assessments and Developmental Screeners for Young Children Ages 3-5: Profiles of Selected Measures. OPRE Report# 2011-23.* Administration for Children & Families, 2011.
216. Koura, G.K., et al., *Usefulness of child development assessments for low-resource settings in francophone Africa.* J Dev Behav Ped, 2013. 34(7).
217. Owino, V.O., et al., *Measuring growth and medium-and longer-term outcomes in malnourished children.* Matern Child Nutr, 2019. 15: e12790.

218. Alderman, H., et al., *Evidence of impact of interventions on growth and development during early and middle childhood.* Child and Adolescent Health and Development. 3rd edition, 2017.
219. Fine, A. and M. Kotelchuck, *Rethinking MCH: The life course model as an organizing framework.* US Department of Health and Human Services, Health Resources and Services Administration, Maternal and Child Health Bureau, 2010: p. 1-20.
220. Grantham-McGregor, S.M., et al., *Effects of integrated child development and nutrition interventions on child development and nutritional status.* Ann NY Acad Sci, 2014. 1308: 11-32.
221. Daniel, A.I., et al., *Psychosocial stimulation interventions for children with severe acute malnutrition: a systematic review.* J Glob Health, 2017. 7: 010405.
222. Ahun, M.N., et al., *Child development in rural Ghana: Associations between cognitive/language milestones and indicators of nutrition and stimulation of children under two years of age.* Can J Public Health, 2018. 108: e578-e585.
223. Bundy, D., et al., *Evidence of Impact of Interventions on Growth and Development during Early and Middle Childhood.* In: Child and Adolescent Health and Development. 3rd edition. Washington (DC): The International Bank for Reconstruction and Development / The World Bank; 2017 Nov 20. Chapter 7.
224. Mavilidi, M.-F., *Effects of integrating movements into the learning task on preschool children's cognition and learning.* 2017. Thesis. University of Wollongong.
225. Honig, A.S., *Language insights for caregivers with young children.* Early Child Dev Care, 2017. 187: 527-541.
226. Fay-Stammbach, T., D.J. Hawes, and P. Meredith, *Parenting influences on executive function in early childhood: A review.* Child Dev Perspect, 2014. 8: 258-264.
227. Black, M.M., et al., *Early childhood development coming of age: science through the life course.* Lancet, 2017. 389: 77-90.
228. Richter, L.M., et al., *Investing in the foundation of sustainable development: pathways to scale up for early childhood development.* Lancet, 2017. 389: 103-118.
229. Pérez-Escamilla, R., S. Segura-Pérez, and M. Lott, *Feeding guidelines for infants and young toddlers: A responsive parenting approach.* Nutr Today, 2017. 52: 223-231.
230. Siddhanta, A. and A. Chattopadhyay, *Role of women's empowerment in determining child stunting in Eastern India and Bangladesh.* Soc Sci Spectrum, 2017. 3: 38-51.
231. Britto, P.R., et al., *Nurturing care: promoting early childhood development.* Lancet, 2017. 389: 91-102.
232. World Health Organization. Care for child development: improving the care of young children. 2012
233. FAO, UNICEF, WFP and WHO *The State of Food Security and Nutrition in the World 2019. Safeguarding against economic slowdowns and downturns.* Rome, FAO. Licence: CC BY-NC-SA 3.0 IGO., 2019.
234. Aguayo, V.M., N. Badgaiyan, and K. Paintal, *Determinants of child stunting in the R oyal K ingdom of B hutan: an in-depth analysis of nationally representative data.* Matern Child Nutr, 2015. 11: 333-345.
235. Aguayo, V.M. and P. Menon, *Stop stunting: improving child feeding, women's nutrition and household sanitation in South Asia.* Matern Child Nutr, 2016. 12: 3-11.
236. Millward, D.J., *Nutrition, infection and stunting: the roles of deficiencies of individual nutrients and foods, and of inflammation, as determinants of reduced linear growth of children.* Nutr Res Rev, 2017. 30: 50-72.
237. Nabwera, H.M., et al., *Growth faltering in rural Gambian children after four decades of interventions: a retrospective cohort study.* Lancet Glob Health, 2017. 5: e208-e216.

238. Rogawski McQuade, E.T., et al., *Seasonal food insecurity in Haydom, Tanzania, is associated with low birthweight and acute malnutrition: Results from the MAL-ED Study.* Am J Trop Med Hyg, 2019. 100: 681-687.
239. Chikhungu, L.C. and N.J. Madise, *Seasonal variation of child under nutrition in Malawi: is seasonal food availability an important factor? Findings from a national level cross-sectional study.* BMC Publ Health, 2014. 14: 1146.
240. Georgiadis, A. and M.E. Penny, *Child undernutrition: opportunities beyond the first 1000 days.* Lancet Publ Health, 2017. 2: e399.
241. Christian, P. and E.R. Smith, *Adolescent undernutrition: Global burden, physiology, and nutritional risks.* Ann Nutr Metab, 2018. 72: 316-328.
242. Das, J.K., et al., *Nutrition in adolescents: physiology, metabolism, and nutritional needs.* Ann NY Acad Sci, 2017. 1393: 21-33.
243. Prentice, A.M., et al., *Critical windows for nutritional interventions against stunting.* Am J Clin Nutr, 2013. 97: 911-918.
244. Bhargava, A., *Protein and micronutrient intakes are associated with child growth and morbidity from infancy to adulthood in the Philippines.* J Nutr, 2015. 146: 133-141.
245. Panjwani, A. and R. Heidkamp, *Complementary feeding interventions have a small but significant impact on linear and ponderal growth of children in low- and middle-income countries: A Systematic review and meta-analysis.* J Nutr, 2017. 147: 2169s-2178s.
246. Humphrey, J.H., et al., *Independent and combined effects of improved water, sanitation, and hygiene, and improved complementary feeding, on child stunting and anaemia in rural Zimbabwe: a cluster-randomised trial.* Lancet Glob Health, 2019. 7: e132-e147.
247. Luby, S.P., et al., *Effects of water quality, sanitation, handwashing, and nutritional interventions on diarrhoea and child growth in rural Bangladesh: a cluster randomised controlled trial.* Lancet Glob Health, 2018. 6: e302-e315.
248. Patil, S.R., et al., *The effect of India's total sanitation campaign on defecation behaviors and child health in rural Madhya Pradesh: a cluster randomized controlled trial.* PLoS Med, 2014. 11: e1001709.
249. Clasen, T., et al., *Effectiveness of a rural sanitation programme on diarrhoea, soil-transmitted helminth infection, and child malnutrition in Odisha, India: a cluster-randomised trial.* Lancet Glob Health, 2014. 2: e645-e653.
250. Dewey, K.G., *Reducing stunting by improving maternal, infant and young child nutrition in regions such as South Asia: evidence, challenges and opportunities.* Matern Child Nutr, 2016. 12: 27-38.
251. Yang, I., et al., *The Infant Microbiome: Implications for Infant Health and Neurocognitive Development.* Nurs Res, 2016. 65: 76-88.
252. Clarke, G., et al., *Priming for health: gut microbiota acquired in early life regulates physiology, brain and behaviour.* Acta Ped, 2014. 103: 812-819.
253. Borre, Y.E., et al., *Microbiota and neurodevelopmental windows: implications for brain disorders.* Trends Mol Med, 2014. 20: 509-18.
254. Koenig, J.E., et al., *Succession of microbial consortia in the developing infant gut microbiome.* Proc Natl Acad Sci USA, 2011. 108: 4578-4585.
255. Ley, R.E., et al., *Microbial ecology: human gut microbes associated with obesity.* Nature, 2006. 444: 1022-3.
256. De Filippo, C., et al., *Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa.* Proc Natl Acad Sci USA, 2010. 107: 14691-14696.

257. Dostal, A., et al., *Effects of iron supplementation on dominant bacterial groups in the gut, faecal SCFA and gut inflammation: a randomised, placebo-controlled intervention trial in South African children.* Br J Nutr, 2014. 112: 547-556.
258. Heilskov Rytter, M.J., et al., *Diet in the treatment of ADHD in children - a systematic review of the literature.* Nord J Psychiatry, 2015. 69: 1-18.
259. Blanton, L.V., et al., *Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children.* Science, 2016. 351: 3311.
260. Wacoo, A.P., et al., *Aflatoxins: Occurrence, exposure, and binding to lactobacillus species from the gut microbiota of rural ugandan children.* Microorganisms, 2020. 8: 347.
261. Iversen, P.O., et al., *No associations between microbiota signaling substances and cognitive, language and motor development among three-year-old rural Ugandan children.* Acta Ped, 2020 in press.
262. Cowardin, C.A., et al., *Mechanisms by which sialylated milk oligosaccharides impact bone biology in a gnotobiotic mouse model of infant undernutrition.* Proc Natl Acad Sci USA, 2019. 116: 11988-11996.
263. Patnode, M.L., et al., *Interspecies competition impacts targeted manipulation of human gut bacteria by fiber-derived glycans.* Cell, 2019. 179: 59-73. e13.
264. Santos, J.A.R., et al., *Iodine fortification of foods and condiments, other than salt, for preventing iodine deficiency disorders.* Cochrane Database of Systematic Reviews, 2019. 2: p. Cd010734.
265. Pearce, E.N., M. Andersson, and M.B. Zimmermann, *Global iodine nutrition: where do we stand in 2013?* Thyroid, 2013. 23: 523-528.
266. Land, M.A., et al., *Iodine fortification of foods and condiments, other than salt, for preventing iodine deficiency disorders.* Cochrane Database of Systematic Reviews, 2013(9).
267. Bath, S.C., et al., *Effect of inadequate iodine status in UK pregnant women on cognitive outcomes in their children: results from the Avon Longitudinal Study of Parents and Children (ALSPAC).* Lancet, 2013. 382: 331-337.
268. Ghassabian, A., et al., *Maternal urinary iodine concentration in pregnancy and children's cognition: results from a population-based birth cohort in an iodine-sufficient area.* BMJ Open, 2014. 4: e005520.
269. Robinson, S.M., et al., *Preconception maternal iodine status is positively associated with IQ but not with measures of executive function in childhood.* J Nutr, 2018. 148: 959-966.
270. König, F., et al., *Ten repeat collections for urinary iodine from spot samples or 24-hour samples are needed to reliably estimate individual iodine status in women.* J Nutr, 2011. 141: 2049-2054.
271. Bell, M.A., A.P. Ross, and G. Goodman, *Assessing infant cognitive development after prenatal iodine supplementation.* Am J Clin Nutr, 2016. 104: 928S-934S.
272. Pauen, S., *Early childhood development and later outcome*. 2012: Cambridge University Press.
273. Schneider, W. and G. Jones, *Intelligence, Human Capital, and Economic Growth: A Bayesian Averaging of Classical Estimates (BACE) Approach.* Illinois State University. Department of Psychology, 2006.
274. Völzke, H., et al., *Ensuring effective prevention of iodine deficiency disorders.* Thyroid, 2016. 26: 189-196.
275. Barnes, J. and J. Theule, *Maternal depression and infant attachment security: A meta-analysis.* Infant Ment Health J, 2019. 40: 817-834.

276. Schmeer, K.K., et al., *Maternal postpartum stress and toddler developmental delays: Results from a multisite study of racially diverse families.* Dev Psychobiol, 2019. 62:62-76.
277. Maruyama, J.M., et al., *Impact of maternal depression trajectories on offspring socioemotional competences at age 11: 2004 Pelotas Birth Cohort.* J Affect Disord, 2019. 253: 8-17.
278. MacGinty, R.P., et al., *Associations of antenatal maternal psychological distress with infant birth and development outcomes: Results from a South African birth cohort.* Compr Psychiatry, 2019. 96: 152128.
279. Servili, C., et al., *Maternal common mental disorders and infant development in Ethiopia: the P-MaMiE Birth Cohort.* BMC Publ Health, 2010. 10: 693.
280. Smith Fawzi, M.C., et al., *Lifetime economic impact of the burden of childhood stunting attributable to maternal psychosocial risk factors in 137 low/middle-income countries.* BMJ Glob Health, 2019. 4: e001144.
281. Farías-Antúnez, S., M.O. Xavier, and I.S. Santos, *Effect of maternal postpartum depression on offspring's growth.* J Affect Disord, 2018. 228: 143-152.
282. Anato, A., et al., *Maternal depression is associated with child undernutrition: A cross-sectional study in Ethiopia.* Matern Child Nutr, 2019: e12934.
283. Sudfeld, C.R., et al., *Linear growth and child development in low- and middle-income countries: a meta-analysis.* Pediatrics, 2015. 135: e1266-75.
284. Yousafzai, A.K., et al., *Effects of responsive stimulation and nutrition interventions on children's development and growth at age 4 years in a disadvantaged population in Pakistan: a longitudinal follow-up of a cluster-randomised factorial effectiveness trial.* Lancet Glob Health, 2016. 4: e548-e558.
285. Scheffler, C., et al., *Stunting is not a synonym of malnutrition.* Eur J Nutr, 2020. 74: 377-386.

Paper I

journal of global health

Child development, growth and microbiota: follow-up of a randomized education trial in Uganda

Prudence Atukunda[1], Grace K M Muhoozi[1,2], Tim J van den Broek[3], Remco Kort[3,4], Lien M Diep[5], Archileo N Kaaya[6], Per O Iversen[1,7,8,*], Ane C Westerberg[9,*]

[1] Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway
[2] Department of Human Nutrition and Home Economics, Kyambogo University, Kampala, Uganda
[3] Netherlands Organization for Applied Scientific Research (TNO), Microbiology and Systems Biology, Zeist, the Netherlands
[4] Department of Molecular Cell Biology, VU University Amsterdam, the Netherlands
[5] Oslo Centre for Biostatistics and Epidemiology, Oslo University Hospital, Oslo, Norway
[6] School of Food Technology, Nutrition and Bioengineering, Makerere University, Kampala, Uganda
[7] Department of Haematology, Oslo University Hospital, Oslo, Norway
[8] Division of Human Nutrition, Stellenbosch University, Tygerberg, South Africa
[9] Institute of Health Sciences, Kristiania University College, Oslo, Norway
[*] Equal authorship

Background Undernutrition impairs child development outcomes and growth. In this follow-up study of an open cluster-randomized intervention trial we examined the effects of an education package delivered to mothers in rural Uganda on their children's development, growth and gut microbiota at 36 months of age.

Methods The parental trial included 511 mother-child pairs recruited when the children were 6-8 months. In that trial, a nutrition, stimulation and hygiene education was delivered to mothers in the intervention group while the control group received routine health care. A follow-up sample of 155 pairs (intervention n = 77, control n = 78) were re-enrolled when the children were 24 months. Developmental outcomes were assessed with the Bayley Scales of Infant and Toddler Development (BSID-III) composite scores for cognitive (primary endpoint), language and motor development. Development outcomes were also evaluated using the Ages and Stages Questionnaire (ASQ) and the Mullen Scales of Early Learning (MSEL). Other outcomes included growth and gut microbiota composition.

Results The demographic characteristics were not different ($P > 0.05$) between the intervention and control groups and similar to those of the parental study. The intervention group had higher BSID-III scores than controls, with mean difference 10.13 (95% confidence interval (CI): 3.31-17.05, $P = 0.002$); 7.59 (1.62-13.66, $P = 0.01$); 9.00 (2.92-15.40, $P = 0.005$), for cognitive, language and motor composite scores, respectively. An improvement in the intervention compared to the control group was obtained for both the ASQ and the MSEL scores. The mean difference in height-for-age z-score was higher in the intervention compared to the control group: 0.50 (0.25-0.75, $P = 0.0001$). Gut microbiota composition did not differ significantly between the two study groups.

Conclusions The maternal education intervention had positive effects on child development and growth at three years, but did not alter gut microbiota composition. This intervention may be applicable in other low-resource settings.

Trial registration ClinicalTrials.gov registration number NCT02098031.

Correspondence to:
Per Ole Iversen
Department of Nutrition
P.O. Box 1046 Blindern
0317 Oslo
Norway
p.o.iversen@medisin.uio.no

Undernutrition among children in developing countries is a major, global health challenge causing more than one-third of under-five deaths [1]. About 200 million children below five years worldwide who are undernourished do not meet their development potential [2]. In addition to stunting, poor cognitive stimulation, and adverse environmental conditions, low maternal education is recognized as risk factors for impaired child development [3]. In line with this, undernourished children

are at risk of impaired structural development of the central nervous system (CNS) and extra- neuronal tissue [4]. Adequate childhood nutrition thus promotes healthy growth and development outcomes [5-7].

The underlying causes of chronic undernutrition are complex. In addition to inadequate food quantity and quality of food, a combination of poor sanitation and hygiene resulting in sustained exposure to enteric pathogens plays an important role [8]. Pregnancy and the first two years of life are important periods for interventions to improve child growth and cognitive development, and can be considered both a window of vulnerability as well as a window of opportunity [9]. The development of the gut microbiota is mostly accomplished within the first three years of life. Interventions directed towards an appropriate maturation of the gut microbiota and its associated metabolic potential, may support healthy growth and cognitive development [10]. In line with this, there are new insights into how the CNS and cognitive development may be influenced by the gut microbiota through the so-called "gut-brain axis" [11]. Moreover, a number of studies report systematic differences in the composition between rural Africa and urban Europe, indicating that the gut microbiota is tailored to local diet, specific nutritional requirements of the host and hygiene practices [12-14]. However, a possible role of gut microbiota in undernutrition and child development has not been adequately addressed. Inadequate caregiving skills and stimulation can also adversely impact development and growth of small children, in particular in low-resource settings. Integration of nutrition and stimulation in a Pakistani trial highlighted a potential for child development and linear growth benefits [5].

In 2013 we initiated a cluster-randomized controlled trial to examine the effect on growth and development of a 6-month intervention comprised of nutrition, stimulation and hygiene education among impoverished mothers of children aged 6-8 months in rural districts of Uganda [15]. The intervention consisted of educating mothers aimed at (i) increasing dietary diversity to improve nutrient intake as well as continued breastfeeding, (ii) improving hygiene and sanitation practices, and (iii) enhancing stimulation based on a social-cognitive learning theory to improve development. Whereas this intervention did not alter child growth at the age of 20-24 months, cognitive, language and motor development improved markedly [15]. In order to examine if these findings were sustainable over time, we decided to perform a follow-up study. Notably, a long term follow-up of such a nutrition education intervention has previously not been done in a resource-constrained setting as Uganda. We now examined development, growth and gut microbiota composition among a subsample of these children at the age of 36 months.

METHODS

Study design and approvals

This is a follow-up study of a two-armed, open cluster-randomized education intervention regarding nutrition, stimulation and hygiene among impoverished mothers of children aged 6-8 months in the Kisoro and Kabale districts of South-Western Uganda. Details of the intervention have recently been published [15]. All mothers gave written or thumb-printed, informed consent to participate and could decline an interview or assessment at any time. The study was approved by The AIDS Support Organisation Research Ethics Committee (No. TASOREC/06/15-UG-REC-009) and by the Uganda National Council for Science and Technology (No. UNCST HS 1809) as well as by the Norwegian Regional Committee for Medical and Health Research Ethics (No. 2013/1833). The trial was registered with clinicaltrials.gov (NCT02098031). We report the data according to the CONSORT guidelines.

Randomisation of the parental and follow-up participants

For the parental trial we used proportionate sampling, 10 sub-counties (ie, clusters) were obtained (6 out of 19 in Kabale and 4 out of 14 in Kisoro) to participate in the study. We used a three-stage procedure to identify households for the study. First, by simple random sampling, three sub-counties in Kabale were allocated to the intervention group and the other three to the control group. Similarly, two sub-counties were allocated to the intervention and the other two to the control group in Kisoro district. Second, all the villages in each participating sub-county (intervention or control) were listed alphabetically and assigned numbers in an ascending order. By use of computer-generated random numbers, villages to whose assigned number matched with the random numbers were selected. The intervention villages did not share common geographical boundaries with control villages to minimize contamination of the intervention contents between the two study groups. Third, by complete enumeration, all consenting households with children aged 6-8 months within a participating village were recruited to the study. If a household had

more than one eligible child, the youngest was selected, and in the case of twins, we randomly selected one for evaluation. We finally enrolled 511 mother-child pairs in the parental study and they were randomised to the intervention (n = 263) or the control (n = 248) group. The intervention group received the nutrition, hygiene and stimulation education in addition to routine health care while the control group received only routine health care.

The child had to be 20-24 months during the period of January-May 2015 to be included in the current follow-up study since age dynamic gut microbial shifts occur at this age resulting in an adult-like, stable composition [16], and developmental milestones at this age may predict IQ at 5-6 years when children are about to start school [17]. Based on a sample size calculation we then randomly selected participants from each of the two study groups (n = 77 from the intervention group and n = 78 from the control group). Data was collected when the children were 20-24 months and at 36 months. The data collection teams in the follow-up study were masked to group allocation and never had any interaction with the study team that delivered the education intervention in the parental trial.

Contents of the education intervention in the parental trial

The intervention was conducted by the study team at three group meetings over a period of 6 months to 26 groups of mothers (6-10 mothers per group), and was detailed recently [15]. Briefly, it was delivered by a trained education team and included two behavior change techniques: providing information and prompt practice (ie, demonstrations of preparing food and stimulation of the children). The nutrition education curriculum was based on the 10 guiding principles of complementary feeding [18]. Recipes were formulated and cooking demonstrated using locally available foods with emphasis on protein. Moreover, the need to take ill children to hospital for medical attention and to increase the feeding frequency during and after illness was emphasized. Hand-washing before feeding as well as use of clean utensils during food preparation and feeding was part of the hygiene intervention. A novel aspect of this intervention was the focus on oral hygiene, and with distribution of tooth brushes to all household members and demonstration of their use. The education team highlighted the importance of play to improve cognitive, language and motor development. The stimulation intervention was based on social-cognitive learning theory [19]. In addition to the three group meetings, the women met at monthly intervals to practice what they had learnt and ensuring compliance to the intervention [15].

Assessments of outcomes

The child development assessments were performed by three bachelor degree holders in psychology whereas two graduates of laboratory technology collected stool samples. Two bachelor degree holders in nutrition collected the anthropometric data. These three data collection teams participated in training sessions to ensure uniform and standardized procedures. Assessments were administered in the local language and conducted in hired, secluded rooms in the villages without interruptions to minimize distractions. To promote reliability, the child development assessments were administered first, followed by anthropometric measurements, stool sampling and then interviews with the mothers.

The Bayley Scales of Infant and Toddler Development-III (BSID-III), the Ages and Stages Questionnaire (ASQ) and the Mullen Scales of Early Learning (MSEL; Supplementary information) were used [15]. The BSID-III scale is known to be the most comprehensive child development measure for children up to 3.5 years and has been adapted and used in similar settings [20]. The ASQ is a parent/caregiver completed screening scale with excellent psychometric properties which capture and establish a wide range of adaptive behaviors, and previously used in this setting [21]. Both tools were used because we did not include the social-emotional scale of BSID-III. The BSID-III and the ASQ were administered at 20-24 and at 36 months. MSEL was introduced at 36 months to assess early intellectual development and readiness for school, and it has been validated for use in rural Uganda [22]. Inter-observation agreement between the child assessment team was good indicated by an intra-class correlation coefficient (ICC) of 0.75 ($P = 0.0001$) for BSI-III, 0.79 ($P = 0.0001$) for ASQ and 0.77 ($P < 0.001$) for MSEL.

Weight, height, and head circumference (HC) were measured as recommended by WHO [21], with a Seca-scale model 881 (Hamburg, Germany) to the nearest 0.1 kg. Height was measured (to the nearest 0.1 cm) with a Seca board (SO114530). HC was measured with a non-stretchable tape (Seca, S0145630 PAC-50). Anthropometric data were converted to z-scores, height-for-age (HAZ), weight-for-age (WAZ), weight-for-height (WHZ), and head circumference (HCZ), using the Anthro (version 3.2.2) software, a nutritional assessment tool based on WHO standards. A z-score<-2 SD from the me-

dian of the WHO reference standards indicated stunting for HAZ, underweight for WAZ and wasting for WHZ, respectively [23].

We collected stool samples using sterile cotton swabs (COPAN Diagnostics Inc, Murrieta, CA) and frozen at -20°C within 24 hours of collection. The samples were then air-dried and shipped to the Netherlands for further processing and analyses (Supplementary information). These storage conditions have a very limited effect on the microbial composition [24]. All 16S rRNA amplicon paired end reads (n = 560) of the gut microbiota samples sequenced in this study can be accessed at Sequence Read Archive (SRA) SUB4476421.

Statistical analyses

The primary outcome in the current follow-up study was cognitive development assessed with the BSID-III at 36 months. Previous intervention studies in similar low-resource-settings report a mean difference of about 0.5 SD in child development score between intervention and control groups [5,25]. To detect a difference between the two study groups in the BSID-III cognitive composite score at 36 months of 0.5 SD (corresponding to 7.5 points) with a power of 0.8 and α of 0.05, 63 children per group was required. To account for an intra-cluster correlation of 0.01 and dropouts, the mean number of children per sub-county was 15, thus a total of 155 children were included [15,26]. Among these 155 we randomly selected the 77 children from the intervention group and the 78 children from the control group at 20-24 months. Child development outcomes and growth were analyzed using Stata/SE (StataCorp. 2015, Stata Statistical Software: Release 14. College Station, Stockholm, Sweden) and SPSS version 22.0 (IBM SPSS Statistics, IBM Corp., Armonk, NY). Significance level was set at $P < 0.05$. We used a mixed effect linear regression to compare the intervention with the control group and estimated ICC. Differences between the two study groups are given as mean (SD or 95% CI).

All statistical analysis of gut microbiota on the 16S rRNA amplicon sequencing data was performed using R version 3.3.2 (R Core Team, 2016) [27]. The 16S rRNA amplicon sequencing data was rescaled and transformed using Wisconsin double and square root transformations. The PERMANOVA procedures, Shannon and 1-Simpson's diversity indices were performed as implemented in the 'vegan' package [28]. Whereas increasing values for the Shannon diversity index indicate more diversity, the opposite is true for the 1-Simpson's index. All PERMANOVA analyses were performed using the Bray-Curtis distance measure. All phyla and genera were included in the statistical analysis.

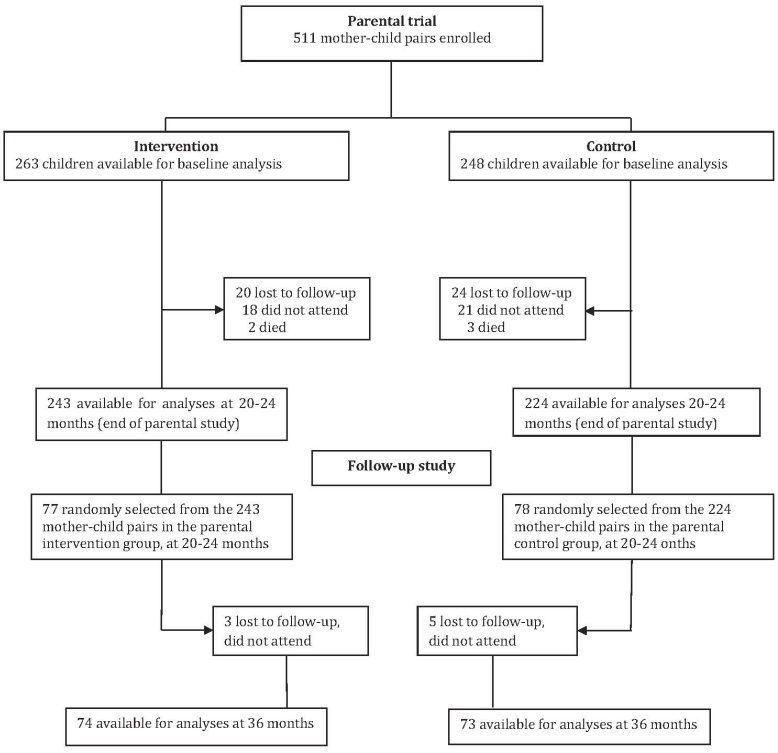


Figure 1. Profile of the parental trial and the follow-up study.

RESULTS

Study participants

One hundred and fifty-five mother-child pairs were included at 20-24 months (**Figure 1**). By 36 months, eight of them were lost to follow-up (three in the intervention group and five in the control group). There were no significant differences in the characteristics between the parental cohort (data obtained at baseline) and the follow-up cohort (data obtained at 20-24 months; **Table 1**), thus no adjustments for baseline differences were made in subsequent analyses.

Development outcomes

Overall, the intervention significantly improved all child development outcomes (ie, cognitive, language and motor composite scores) based on the BSID-III at 36 months (**Table 2**). The Cohen's d effect sizes at 36 months were medium (cognitive 0.57, language 0.56 and motor 0.50). The effect of the intervention on the ASQ mean scores for communication, gross motor, problem solving, and personal social development, was sig-

Table 1. Study population characteristics for the parental trial at baseline and at start of the follow-up study*

Characteristics	Parental trial (data obtained at baseline)		Follow-up study (data obtained at 20-24 months)		*P*-value
	Intervention (n = 263)	Control (n = 248)	Intervention (n = 77)	Control (n = 78)	
Children (*n*, %):					
Males	139 (52.9)	123 (49.6)	44 (57.1)	41 (52.6)	0.75
Females	124 (47.1)	125 (50.4)	33 (42.9)	37 (47.4)	0.40
Age at inclusion (months)	7.4 (0.8)	7.3 (0.9)	21.4 (1.0)	21.2 (1.0)	0.24
Stunting†	55 (20.9)	70 (28.0)	32 (18.1)	46 (36.6)	0.06
Underweight†	25 (9.5)	36 (14.5)	6 (8.3)	8 (11.3)	0.37
Wasting†	12 (4.6)	12 (4.8)	3 (4.2)	2 (2.8)	0.50
BSID-III composite score:					
Cognitive	114.9 (21.3)	99.3 (17.1)	116.1 (15.6)	105.9 (15.9)	0.89
Language	98.3 (14.3)	88.4 (9.1)	106.5 (14.8)	98.9 (12.8)	0.50
Motor	113.7 (18.9)	99.1 (14.3)	122.3 (18.7)	113.3 (19.9)	0.49
ASQ scores:					
Communication	40.8 (14.5)	33.8 (15.3)	51.4 (9.9)	48.1 (11.4)	0.36
Gross motor	52.8 (10.3)	46.9 (13.8)	55.6 (7.0)	53.8 (9.7)	0.51
Fine motor	44.6 (9.9)	40.4 (11.5)	47.9 (10.8)	42.5 (13.9)	0.23
Problem solving	49.5 (11.7)	40.6 (13.1)	44.0 (12.3)	40.1 (12.7)	0.24
Personal-social	41.0 (11.3)	36.6 (11.1)	48.7 (10.8)	45.8 (9.9)	0.35
Illness at study time (*n*, %):					
Yes	94 (35.7)	71 (28.6)	47 (61.0)	40 (51.3)	0.21
No	169 (64.3)	177 (71.4)	30 (39.0)	38 (48.7)	0.38
Maternal data:					
Maternal education (years)	4.9 (2.8)	4.9 (2.8)	5.5 (2.5)	5.0 (2.6)	0.20
Maternal age (years)	26.1 (5.8)	26.8 (6.3)	26.2 (6.1)	27.4 (6.4)	0.27
Number of children per mother	3.4 (2.2)	3.3 (2.2)	3.4 (2.2)	3.3 (2.2)	0.25
Household data:					
Household head age (years)	31.3 (7.7)	32.6 (19.4)	30.2 (7.3)	33.1 (10.9)	0.06
Household head education (years)	6.4 (3.1)	5.9 (3.1)	6.6 (3.3)	6.5 (3.4)	0.29
Household size (n)	5.5 (2.1)	5.5 (2.1)	5.7 (2.2)	5.8 (2.2)	0.76
Household poverty score	47.8 (11.7)	47.6 (11.4)	49.0 (11.6)	46.3 (12.3)	0.18
Sanitation composite score	7.2 (1.9)	7.3 (1.9)	7.0 (1.8)	7.1 (1.9)	0.83

ASQ – Ages and Stages Questionnaire, BSID – Bayley's Scales of Infant and Toddler Development
*Values are means (SD) unless otherwise stated.
†z- score values are <-2SD of the median of the reference population.

Table 2. Composite scores derived from the Bayley Scales of Infant and Toddler Development-III scales*

Age of child (months)	Intervention (n = 73-77)†	Control (n = 74-78)†	Between group difference‡	*P*-value§	ICC
Cognitive composite scores:					
20-24	117.84 (20.86)	101.58 (19.14)	16.26 (9.57 to 23.04)	0.0001	0.05
36	116.07 (15.55)	105.94 (15.99)	10.13 (3.31 to 17.05)	0.002	
Language composite scores:					
20-24	100.31 (12.91)	89.00 (9.32)	11.31 (5.43 to 17.28)	0.0001	0.06
36	106.54 (14.79)	98.95 (12.77)	7.59 (1.62 to 13.66)	0.010	
Motor composite scores:					
20-24	113.79 (16.06)	100.04 (15.47)	13.75 (7.80 to 20.01)	0.0001	0.01
36	122.32 (18.74)	113.32 (19.89)	9.00 (2.92 to 15.40)	0.005	

ICC – intra-class correlation coefficient
*Values are means (standard deviation) unless otherwise stated.
†The variation in n was due to missing data because some children did not complete all the tests.
‡Mean differences (95% CI) of Bayley Scales of Infant and Toddler Development-III composite scores.
§*P*-value is for the difference between the two study groups adjusted for clusters.

nificantly higher in the intervention group compared with the controls at 24 months (**Table 3**). At 36 months, the ASQ fine motor scores were significantly higher in the intervention group compared with the controls. The Cohen's d effect sizes at 36 months ranged from small to medium for the ASQ scores (gross motor 0.16, personal social development 0.25, problem solving 0.29, fine motor 0.49 and communication 0.68). Also, the MSEL fine motor, language (receptive and expressive), cognitive and early

Table 3. Mean scores from the Ages and Stages Questionnaire*

Age of child (months)	Intervention (n = 71-74)†	Control (n = 70-73)†	Between group difference‡	*P*-value§	ICC
Communication scores:					
20-24	41.37 (14.04)	31.58 (18.45)	9.79 (3.90 to 15.76)	0.001	0.06
36	51.41 (9.96)	48.11 (11.40)	3.30 (-2.68 to 9.33)	0.28	
Gross motor scores:					
20-24	53.46 (10.76)	46.47 (15.79)	6.99 (2.47 to 11.60)	0.003	0.00
36	55.58 (7.04)	53.80 (9.72)	1.78 (-2.80 to 6.47)	0.44	
Fine motor scores:					
20-24	45.73 (9.93)	42.04 (12.58)	3.69 (-0.27 to 8.01)	0.067	0.07
36	47.93 (10.80)	42.52 (13.94)	5.41 (1.36 to 9.81)	0.010	
Problem solving scores:					
20-24	50.35 (10.19)	38.94 (14.24)	11.41 (7.24 to 15.57)	0.0001	0.02
36	44.02 (12.25)	40.06 (12.69)	3.96 (-0.31 to 8.21)	0.069	
Personal-social development scores:					
20-24	43.24 (10.41)	36.81(10.05)	6.43 (1.99 to 10.85)	0.0001	0.06
36	48.74 (10.83)	45.75 (9.95)	2.99 (-1.54 to 7.49)	0.10	

ICC – intra-class correlation coefficient

*Values are means (standard deviation) unless otherwise stated. ICC- intra-class correlation coefficient.

†The variation in n was due to missing data because some children did not complete all the tests.

‡Mean differences (95% CI) of Ages and Stages Questionnaire scores.

§*P*-value is for the difference between the two study groups adjusted for clusters.

learning composite standard scores were significantly higher in the intervention compared to the controls at 36 months (**Table 4**). In contrast, the MSEL visual reception scores were not different between the two study groups. The corresponding mean Cohen's d effect sizes were: 0.23, 0.44, 0.34, 0.42, 0.42, and 0.36 for MSEL visual reception, fine motor, receptive language, expressive language, cognitive total score and early learning score, respectively.

Growth outcomes

The mean HAZ declined in both study groups during the study period, indicating linear growth faltering (**Table 5**). However, this decline was significantly less at 36 months in the intervention compared with the control group. There were no significant differences in the other mean anthropometric measures (ie, WAZ, WHZ, and HCZ) at 36 months. The Cohen's d effect sizes at 36 months were 1.01, 0.16, -0.46, and 0.30 for HAZ, WAZ, WHZ, and HCZ, respectively.

Gut microbiota composition

The intervention did not lead to any significant changes in the gut microbiota diversity compared with the control group at the phylum level (**Figure 2**). Neither did we observe any significant differences between the two study groups in the Shannon diversity index at the two time points (**Figure 3**). However, as expected the Shannon diversity index increased significantly in both study groups from 20-24 to 36 months, indicating increased gut microbiota diversity, while there was no significant change in the overall genera distribution from 20-24 to 36 months. In line with this, there was no change in the variable 1-Simpson index between the two study groups at the two time points (**Figure 3**), and this variable increased from 20-24 to 36 months, again indicating increased gut microbiota diversity. In support of these findings, the

Table 4. Mullen Scales of Early Learning scores obtained in the two study groups at 36 months*

	Intervention (n = 74)	Control (n = 73)	Between group difference†	*P*-value‡
Visual reception	53.31 (13.63)	50.33 (12.44)	2.98 (-7.24 to 1.27)	0.17
Fine motor	62.84 (15.55)	56.18 (14.91)	6.66 (1.69 to 11.83)	0.009
Receptive language	58.72 (10.33)	55.10 (11.26)	3.62 (0.10 to 7.14)	0.044
Expressive language	60.59 (10.33)	56.25 (10.51)	4.34 (0.95 to 7.74)	0.012
Cognitive total score	235.46 (42.27)	217.85 (41.35)	17.61 (3.98 to 31.24)	0.012
Early learning score	75.64 (29.17)	64.77 (31.67)	10.87 (1.81 to 14.87)	0.013

*Values are means (standard deviation) unless otherwise stated.

†Mean differences (95% confidence interval) of Mullen Scales of Early Learning scores.

‡*P-value* is for the difference between the two study groups adjusted for clusters.

Table 5. Child growth during the study period*

Age of child (months)	Intervention (n = 74-77)†	Control (n = 73-78)†	Between group difference‡	*P*-value§	ICC
Height-for-age z-scores:					
20-24	-1.96 (1.14)	-2.07 (1.20)	0.11 (-0.14 to 0.35)	0.41	0.34
36	-2.15 (1.01)	-2.65 (0.88)	0.50 (0.25 to 0.75)	0.0001	
Weight-for-age z-scores:					
20-24	-0.76 (0.88)	-0.85 (0.88)	0.09 (-0.37 to 0.55)	0.70	0.10
36	-0.98 (0.89)	-1.18 (0.69)	0.20 (-0.27 to 0.66)	0.40	
Weight-for-height z-scores:					
20-24	0.26 (0.94)	0.45 (0.77)	-0.19 (-0.52 to 0.16	0.31	0.04
36	0.44 (0.91)	0.84 (0.74)	0.40 (-0.75 to 0.05	-0.054	
Head circumference z-scores:					
20-24	0.30 (0.93)	0.61 (1.05)	-0.25 (-0.64 to 0.04)	0.079	0.00
36	-0.34 (0.90)	0.05 (1.01)	-0.39 (-0.72 to 0.34)	0.055	

*Values are means (standard deviation) unless otherwise stated. ICC-intra-class correlation coefficient.
†The variation in n is due to missing data.
‡Mean differences (95% confidence interval).
§*P*-value is for the difference between the two study groups adjusted for clusters.

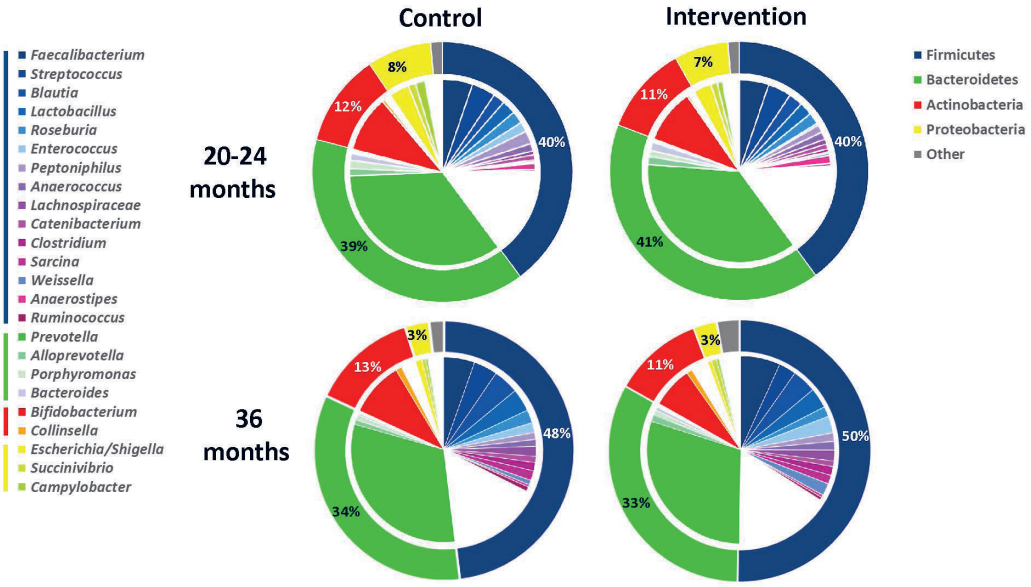


Figure 2. Fecal microbiota compositions based on normalized 16S rRNA amplicon sequencing reads from the control (left pie charts) and intervention (right pie charts) group at 20-24 (upper pie charts) and at 36 (lower pie charts) months. The outer donuts represent the four predominant phyla (legend: right upper corner) and the inner pie charts the most abundant genera within each of these phyla (legend: left). Charts indicate the average relative abundance of phyla and genera in the fecal microbiota of the children with a cut-off value of 0.7%.

PERMANOVA analysis revealed that there was a significant change in the composition of the gut microbiota from 20-24 to 36 months, both at the genus ($P=0.001$) and at the phylum ($P=0.001$) level, but that there was no significant effect ($P=1$) of the intervention on the overall gut microbiota composition.

DISCUSSION

This is probably the first randomized education intervention trial incorporating gut microbiota analysis in rural Sub-Saharan Africa. In the parental trial the 6-month education intervention led to significant improvements in development outcomes when the children reached 20-24 months, without affecting growth [15]. We now show a sustained improvement in the development outcomes even at 36 months and with the use of three independent tools. The intervention also reduced linear growth faltering until 36 months, but had no effect on gut microbiota composition.

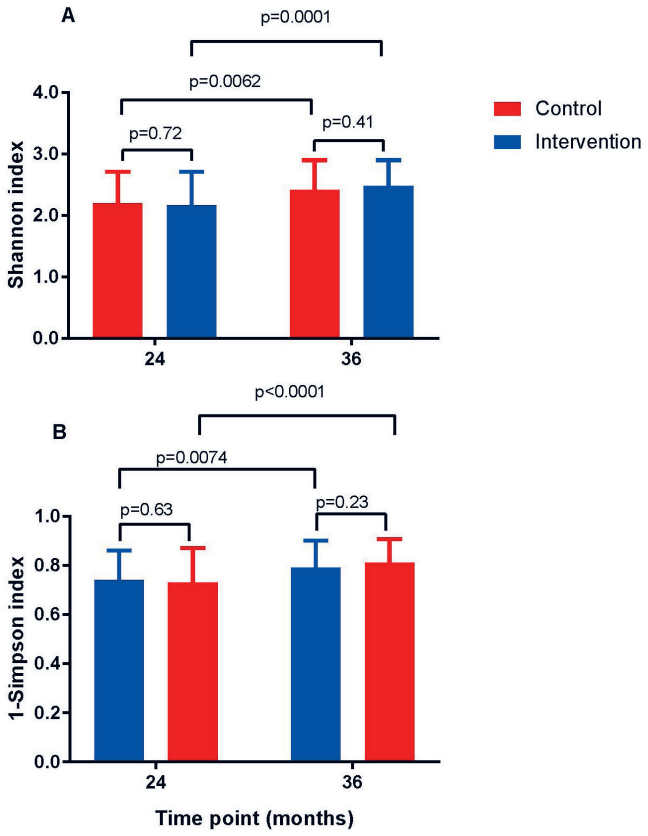


Figure 3. Shannons diversity index (**A**) and 1-Simpsons index (**B**) for gut microbiota diversity obtained from the control (open symbol) and intervention (closed symbol) group among the children at age 20-24 and 36 months. Values are mean ± standard deviation.

Our effect sizes on child development outcomes were comparable/higher than those obtained in previous studies that included nutrition supplementation and child stimulation intervention [5,29,30]. Studies from low income countries using the BSID-III to assess development found that children who received both nutrition and responsive stimulation reported higher cognitive, language and motor skills compared to those who only received either nutrition or stimulation [25,31]. Notably, most of these studies provided micronutrient supplementation and play materials whereas we educated the mothers without supporting them with either food or toys. Mother-child play interaction models promote children's engagement in several activities that enhance development [32]. Moreover, our findings are in accordance with a previous Ugandan study which reported slightly higher cognitive scores three months after stimulation and nutrition education [20].

The baseline stunting levels observed in the two study groups compare favorably with those of previous surveys in Uganda [33]. Corroborating the anthropometric results obtained when the children were 20-24 months [15], most growth indicators were not significantly different between the two study groups at 36 months. The only exception was a smaller reduction in linear growth faltering in the intervention compared with the control group. This could imply that the education intervention may have a protective effect against linear growth faltering over time.

Emerging data suggest links between gut microbiota composition and stunting as well as cognition in childhood [34,35], possibly mediated through cross-talk between microbiota-derived signaling molecules and host tissues [36]. As improved diet and hygienic practices may promote a healthy gut microbiota [37], interventions to enhance nutrition may indirectly impact positively on child growth and development outcomes [38,39]. Previous studies on nutrition and gut microbiota are mostly based on animal models or clinical trials with specific nutrients, pre- or probiotics to modify microbiota diversity [39]. In the present trial we emphasized education of the mothers about preparing nutritious foods, ensuring hygienic meal preparations and maintaining good oral health among their children. Despite acceptable adherence to this intervention [15], we could not detect any significant effects on gut microbiota composition after 20-24 or after 36 months.

Our baseline data on maternal and household characteristics were in line with previous reported data from Uganda [40-42]. Our education intervention consisted of a combined strategy to improve nutrient intakes, hygiene/sanitary practices and stimulation through increased knowledge and empowerment of the mothers. Although it is not possible to exactly specify which component(s) led to the improvement in child development outcomes, the unchanged child diet diversity observed among the households in the intervention group at 20-24 months [15] as well as the unaltered gut microbiota, suggest that the improvements were predominantly resulting from enhanced stimulation and hygiene practices. A systematic review of combined nutrition and stimulation interventions reported that child development was consistently improved through stimulation while growth and nutritional status were usually improved by nutrition [43]. Although this review found little evidence for combined benefits of both nutrition and child stimulation interventions on child development, our findings indicate that having a combination of nutrition, hygiene and child stimulation education may have a potential benefit on child development outcomes.

Strengths and limitations

In this study we adopted a multidisciplinary approach combining aspects of nutrition, hygiene, psychology, microbiology and validated research instruments. Of note, the children were followed for several years. Despite that only about one-third of the mother-child pairs of the parental trial could be re-enrolled for this follow-up study, the latter cohort was well balanced with the baseline characteristics of the parental

cohort. A limitation of our study was lack of baseline data of gut microbiota composition, and we have no information about body composition, dietary intakes or relevant biomarkers among the children or if the mothers in the intervention group continued to stimulate their children in the period between end of intervention and when the children reached the age of three years. ASQ is a maternal report and could possibly be biased. Furthermore, we do not report on maternal mental health which may impact on development and growth of small children, in particular in low-resource settings [44].

CONCLUSIONS

This nutrition, hygiene and stimulation education intervention among mothers of 6-8 months old children had a positive effect on child development and growth until 36 months. We found no significant effects of the intervention on gut microbiota composition. The positive effects from this intervention would call for further research of such an intervention before consideration of scale-up and implementation in other low-income rural settings.

Acknowledgements: We thank Guus Roeselers for his contribution to design of the gut microbiota study in the early phase. Appreciation goes to the mothers and children in Kisoro and Kabale districts whose valuable time enabled us to complete this study, as well as all field staff for their dedicated work.

Funding: The study was supported by the Throne Holst Foundation, the University of Oslo and TNO's Early Research Program "Personalized Health".

Authorship contributions: PA and GKMM were responsible for the concept and design of the study as well as analyzing data and provided critical revisions to the manuscript. TB and RK were responsible for the microbiota analyses and provided critical revisions to the manuscript. LMD was responsible for the statistical analyses and provided critical revisions to the manuscript. AK interpreted the data and provided critical revisions to the manuscript. POI supervised the study and was responsible for the concept and design of the study as well as analyzing data and provided critical revisions to the manuscript. ACW supervised the study and was responsible for the concept and design of the study as well as analyzing data and provided critical revisions to the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Competing interests: All authors completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available upon request from the corresponding author), and declare no conflicts of interest.

REFERENCES

1 Black RE, Allen LH, Bhutta ZA, Caulfield LE, de Onis M, Ezzati M, et al. Maternal and child undernutrition: global and regional exposures and health consequences. Lancet. 2008;371:243-60. Medline:18207566 doi:10.1016/S0140-6736(07)61690-0

2 Countdown to 2030 Collaboration. Countdown to 2030: tracking progress towards universal coverage for reproductive, maternal, newborn, and child health. Lancet. 2018;391:1538-48. Medline:29395268 doi:10.1016/S0140-6736(18)30104-1

3 Lu C, Black MM, Richter LM. Risk of poor development in young children in low-income and middle-income countries: an estimation and analysis at the global, regional, and country level. Lancet Glob Health. 2016;4:e916-22. Medline:27717632 doi:10.1016/S2214-109X(16)30266-2

4 Prado EL, Dewey KG. Nutrition and brain development in early life. Nutr Rev. 2014;72:267-84. Medline:24684384 doi:10.1111/nure.12102

5 Yousafzai AK, Rasheed MA, Rizvi A, Armstrong R, Bhutta ZA. Effect of integrated responsive stimulation and nutrition interventions in the Lady Health Worker programme in Pakistan on child development, growth, and health outcomes: a cluster-randomised factorial effectiveness trial. Lancet. 2014;384:1282-93. Medline:24947106 doi:10.1016/S0140-6736(14)60455-4

6 Aboud FE, Yousafzai AK. Global health and development in early childhood. Annu Rev Psychol. 2015;66:433-57. Medline:25196276 doi:10.1146/annurev-psych-010814-015128

7 Larson LM, Yousafzai AK. A meta-analysis of nutrition interventions on mental development of children under-two in low- and middle-income countries. Matern Child Nutr. 2017;13. Epub 2015 Nov 26. Medline:26607403 doi:10.1111/mcn.12229

8 Kane AV, Dinh DM, Ward HD. Childhood malnutrition and the intestinal microbiome. Pediatr Res. 2015;77:256-62. Medline:25356748 doi:10.1038/pr.2014.179

9 Krebs NF, Lozoff B, Georgieff MK. Neurodevelopment: The impact of nutrition and inflammation during infancy in low-resource settings. Pediatrics. 2017;139 Suppl 1:S50-8. Medline:28562248 doi:10.1542/peds.2016-2828G

10 Gordon JI, Dewey KG, Mills DA, Medzhitov RM. The human gut microbiota and undernutrition. Sci Transl Med. 2012;4:137ps12. Medline:22674549 doi:10.1126/scitranslmed.3004347

11 Sherwin E, Sandhu KV, Dinan TG, Cryan JF. May the force be with you: The light and dark sides of the microbiota-gut-brain axis in neuropsychiatry. CNS Drugs. 2016;30:1019-41. Medline:27417321 doi:10.1007/s40263-016-0370-3

12 De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci U S A. 2010;107:14691-6. Medline:20679230 doi:10.1073/pnas.1005963107

13 Grześkowiak Ł, Collado MC, Mangani C, Maleta K, Laitinen K, Ashorn P, et al. Distinct gut microbiota in southeastern African and northern European infants. J Pediatr Gastroenterol Nutr. 2012;54:812-6. Medline:22228076 doi:10.1097/MPG.0b013e318249039c

14 Cheung YB, Xu Y, Mangani C, Fan YM, Dewey KG, Salminen SJ, et al. Gut microbiota in Malawian infants in a nutritional supplementation trial. Trop Med Int Health. 2016;21:283-90. Medline:26644222 doi:10.1111/tmi.12650

15 Muhoozi GKM, Atukunda P, Diep LM, Mwadime R, Kaaya AN, Skaare AB, et al. Nutrition, hygiene, and stimulation education to improve growth, cognitive, language, and motor development among infants in Uganda: A cluster-randomized trial. Matern Child Nutr. 2018;14:e12527. Medline:28925580 doi:10.1111/mcn.12527

16 Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M. etal. Human gut microbiome viewed across age and geography. Nature. 2012;486:222-7. Medline:22699611 doi:10.1038/nature11053

17 Peyre H, Charkaluk ML, Forhan A, Heude B, Ramus F. Do developmental milestones at 4, 8, 12 and 24 months predict IQ at 5-6 years old? Results of the EDEN mother-child cohort. Eur J Paediatr Neurol. 2017;21:272-9. Medline:27889381 doi:10.1016/j.ejpn.2016.11.001

18 PAHO/WHO. Guiding Priciples for Complementary Feeding of the Breastfed Child. Washington D.C, Division of Health Promotion and Protection; World Health Organisation; 2003.

19 Bandura A. Social cognitive theory: an agentic perspective. Annu Rev Psychol. 2001;52:1-26. Medline:11148297 doi:10.1146/annurev.psych.52.1.1

20 Singla DR, Kumbakumba E, Aboud FE. Effects of a parenting intervention to address maternal psychological wellbeing and child development and growth in rural Uganda: a community-based, cluster randomised trial. Lancet Glob Health. 2015;3:e458-e469. Medline:26144389 doi:10.1016/S2214-109X(15)00099-6

21 Hornman J, Kerstjens JM, de Winter AF, Bos AF, Reijneveld SA. Validity and internal consistency of the Ages and Stages Questionnaire 60-month version and the effect of three scoring methods. Early Hum Dev. 2013;89:1011-5. Medline:24041814 doi:10.1016/j.earlhumdev.2013.08.016

22 Boivin MJ, Sikorskii A, Familiar-Lopez I, Ruiseñor-Escudero H, Muhindo M, Kapisi J, et al. Malaria illness mediated by anaemia lessens cognitive development in younger Ugandan children. Malar J. 2016;15:210. Medline:27076184 doi:10.1186/s12936-016-1266-x

23 Child Growth Standards WHO. Geneva, Switzerland:World Health Organization; 2006.

24 Lauber CL, Zhou N, Gordon JI, Knight R, Fierer N. Effect of storage conditions on the assessment of bacterial community structure in soil and human-associated samples. FEMS Microbiol Lett. 2010;307:80-6. Medline:20412303 doi:10.1111/j.1574-6968.2010.01965.x

25 Gardner JM, Powell CA, Baker-Henningham H, Walker SP, Cole TJ, Grantham-McGregor SM. Zinc supplementation and psychosocial stimulation: effects on the development of undernourished Jamaican children. Am J Clin Nutr. 2005;82:399-405. Medline:16087985 doi:10.1093/ajcn/82.2.399

26 Campbell MJ, Donner A, Klar N. Developments in cluster randomized trials and statistics in medicine. Stat Med. 2007;26:2-19. Medline:17136746 doi:10.1002/sim.2731

27 Team RC. A language and environment for statistical computing Vienna, Austria. Retrieved from https://www.r-project.org/. 2017.

28 Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. Vegan: community Ecology Package. Available: https://cran.r-project.org/web/packages/vegan/index.html. Accessed: 14 April 2019.

29 Prado EL, Sebayang SK, Apriatni M, Hidayati N, Islamiyah A, Siddiq S, et al. Maternal multiple micronutrient supplementation and other biomedical and socioenvironmental influences on children's cognition at age 9-12 years in Indonesia: follow-up of the SUMMIT randomised trial. Lancet Glob Health. 2017;5:e217-28. Medline:28104188 doi:10.1016/S2214-109X(16)30354-0

30 Chang SM, Grantham-McGregor SM, Powell CA, Vera-Hernández M, Lopez-Boo F, Baker-Henningham H, et al. Integrating a parenting intervention with routine primary health care: A cluster randomized trial. Pediatrics. 2015;136:272-80. Medline:26148947 doi:10.1542/peds.2015-0119

31 Yousafzai AK, Obradovic J, Rasheed MA, Rizvi A, Portilla XA, Tirado-Strayer N, et al. Effects of responsive stimulation and nutrition interventions on children's development and growth at age 4 years in a disadvantaged population in Pakistan: a longitudinal follow-up of a cluster-randomised factorial effectiveness trial. Lancet Glob Health. 2016;4:e548-58. Medline:27342433 doi:10.1016/S2214-109X(16)30100-0

32 Bentenuto A, De Falco S, Venuti P. Mother-child play: A comparison of autism spectrum disorder, Down syndrome, and typical development. Front Psychol. 2016;7:1829. Medline:27920745 doi:10.3389/fpsyg.2016.01829

33 UBOS. Uganda Demographic and Health Survey. (2011). Kampala, Uganda:UBOS and Calverton (pp. 2012). Maryland: ICF International Inc.

34 Carlson AL, Xia K, Azcarate-Peril MA, Goldman BD, Ahn M, Styner MA, et al. Infant gut microbiome associated with cognitive development. Biol Psychiatry. 2018;83:148-59. Medline:28793975 doi:10.1016/j.biopsych.2017.06.021

REFERENCES

35 Blanton LV, Barratt MJ, Charbonneau MR, Ahmed T, Gordon JI. Childhood undernutrition, the gut microbiota, and microbiota-directed therapeutics. Science. 2016;352:1533. Medline:27339978 doi:10.1126/science.aad9359

36 Schroeder BO, Backhed F. Signals from the gut microbiota to distant organs in physiology and disease. Nat Med. 2016;22:1079-89. Medline:27711063 doi:10.1038/nm.4185

37 Chassaing B, Vijay-Kumar M, Gewirtz AT. How diet can impact gut microbiota to promote or endanger health. Curr Opin Gastroenterol. 2017;33:417-21. Medline:29019865 doi:10.1097/MOG.0000000000000401

38 Cong X, Xu W, Romisher R, Poveda S, Forte S, Starkweather A, et al. Gut microbiome and infant health: brain-gut-microbiota axis and host genetic factors. Yale J Biol Med. 2016;89:299-308. Medline:27698614

39 Cong X, Henderson WA, Graf J, McGrath JM. Early life experience and gut microbiome: The brain-gut-microbiota signaling aystem. Adv Neonatal Care. 2015;15:314-23. Medline:26240939 doi:10.1097/ANC.0000000000000191

40 FANTA-2. The analysis of the nutrition situation in Uganda. Food and nutrition technical assistance II Project. Washington, DC: AED; 2010.

41 Wamani H, Nordrehaug A, Strøm A, Peterson S, James T, Tylleskar T. Predictors of poor anthropometric status among children under 2 years of age in rural Uganda. Public Health Nutr. 2006;9:320. Medline:16684383 doi:10.1079/PHN2006854

42 Kikafunda JK, Agaba E, Bambona A. Malnutrition amidst plenty: an assessment of factors responsible for persistent high levels of childhood stunting in food secure Western Uganda. AFJAND. 2014;14:9288313.

43 Grantham-McGregor SM, Fernald LC, Kagawa RM, Walker S. Effects of integrated child development and nutrition interventions on child development and nutritional status. Ann N Y Acad Sci. 2014;1308:11-32. Medline:24673166 doi:10.1111/nyas.12284

44 Rahman A, Fisher J, Bower P, Luchters S, Tran T, Yasamy MT, et al. Interventions for common perinatal mental disorders in women in low- and middle-income countries: a systematic review and meta-analysis. Bull World Health Organ. 2013;91:593-601I. Medline:23940407 doi:10.2471/BLT.12.109819

Supplementary information

Child development, growth and microbiota: follow-up of a randomized education trial in Uganda

Prudence Atukunda, Grace K.M. Muhoozi, Tim J van den Broek, Remco Kort, Lien M. Diep, Archileo N. Kaaya, Per O. Iversen, Ane C. Westerberg

Supplementary Methodology

1. Mullen Scales of Early Learning

We used the four cognitive scales (visual reception, fine-motor, receptive and expressive language) of Mullen Scales of Early Learning (MSEL; used to assess children from birth to 68 months). Each individual scale has T-scores (mean 50, SD 10). The MSEL has been administered in local languages to evaluate neurodevelopment among Ugandan children.[1,2] The MSEL sum of cognitive scores had acceptable internal consistency (Cronbach's α=0.89) and inter-observer agreement (ICC=0.72).

2. Gut Microbiota Assessments

2.1. Stool Preparation

We collected fecal samples from 145/155 (94%) children whereas a rectal swab sample was collected from the remaining 10 (6%) children (equally distributed between intervention and control groups) who failed to pass stool voluntarily. Reportedly rectal swap samples provide reliable data on gut microbiota composition.[3] The stool samples were shipped to Netherlands for further analysis. Upon arrival cotton swabs were stored at −80°C until further processing. The cotton swabs were cut from the sticks and directly re-suspended in 96 well Axygen 2-mL deep well plates (Axygen, MA, USA), containing 350 µL of lysis buffer (Mag Mini DNA Isolation Kit; LGC Ltd, UK), 500 µL zirconium beads (0.1 mm; BioSpec products, OK, USA) and 600 µL of phenol saturated with Tris-HCl (pH 8.0; Carl Roth GmbH, Germany). Mechanical disruption of bacterial cells was performed by bead beating for 2 min in a mini-beadbeater-8 cell disruptor (Merlin Bio-products, The Netherlands). After bead beating, the samples were cooled on ice prior to a 10 min centrifugation step (10,000 rpm). After another phenol extraction step of the aqueous phase, 500 µL of the aqueous phase were transferred to a new centrifugation tube prefilled with 1000 µL binding buffer (Agowa GmbH, Germany) and 20 µL magnetic beads (Agowa). After mixture, the suspension was left for 10 min to allow binding of the chromosomal DNA to the magnetic beads. After washing the beads according to the Agowa Mag mini DNA extraction protocol, the DNA was extracted from the beads with 63 µl elution buffer (Agowa) according to the manufacturer's instructions.

2.2. Microbiota bar-coded 16S rRNA Gene Amplicon Sequencing and Data Processing

Sequencing and data processing was performed according to methods described by Zaura et al.[4] To determine the amount of bacterial DNA, a quantitative polymerase chain reaction (qPCR) using primers specific for the bacterial 16S rRNA gene was applied (forward: TCCTACGGGAGGCAGCAGT; reverse: GGACTACCAGGGTATCTAATCCTGTT; probe: 6FAM-CGTATTACCGCGGCTGCTGGCAC-BHQ1).[5] For 16S rDNA amplicon sequencing of the V4 hypervariable region, 1 ng of DNA was amplified as described with the exception that 33 cycles were used instead of 35, using F515/R806 primers.[6,7] Primers included the Illumina adapters (Illumina, the Netherlands) and a unique 8-nt sample index sequence key. The amount of DNA per sample was quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Thermo Fisher Scientific, MA, USA). The amplicon libraries were pooled in equimolar amounts and purified using the IllustraTM GFXTM PCR DNA and Gel Band Purification Kit (GE Healthcare, The Netherlands). Amplicon quality and size was analyzed on an Agilent 2100 Bioanalyzer (Santa Clara, CA, USA). Paired-end sequencing of amplicons was conducted on the Illumina MiSeq platform (Illumina). The sequence data was processed with mothur v.1.31.2[8] in line with the mothur MiSeq SOP. Before merging the read pairs, low quality regions were trimmed using Btrim with a sliding window size of 5 nt and average quality score of 25.[9] After merging, the sequences were filtered by length (range: 243 – 263), while no ambiguous bases were allowed. The unique sequences were

aligned to the bacterial SILVA SEED reference alignment release 102 (available at: http://www.mothur.org/wiki/Silva_reference_files); sequences were filtered using screen.seqs with parameters "optimize=start-end, criteria=90". Chimeric sequences were identified per sample using UCHIME in *de novo* mode and removed from all samples. [10] Next, sequences occurring less than 10 times in the entire dataset were removed. Taxonomic names were assigned to all sequences using the Ribosomal Database Project (RDP) naïve Bayesian classifier with a confidence threshold of 60%, 1000 iterations and the mothur-formatted version of the RDP training set v.9 (trainset9_032012).[11] The full set of sequence data is available on request.

3. References

1. Boivin MJ, Sikorskii A, Familiar-Lopez I, et al. Malaria illness mediated by anaemia lessens cognitive development in younger Ugandan children. *Malari J.* 2016;15:210.
2. Kammerer B IP, Lundy S. Approaches to assessment of very young children in Africa in the context of HIV. In: Boivin MJ, Giordani B,, editors. Neuropsychology of children in Africa: perspectives on risk and resilience. *Specialty topics in neuropsychology*. New York: Springer; 2013.
3. Budding AE, Grasman ME, Eck A, Bogaards JA, et al. Rectal swabs for analysis of the intestinal microbiota. *PLoS One*. 2014; 9:e101344.
4. Zaura E, Brandt B, Prodan A, et al. On the ecosystemic network of saliva in healthy young adults. *ISME J* . 2017;11:1218-31.
5. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina Sequencing Platform. *Appl Environ Microbiol*. 2013;79: 5112–20.
6. Caporaso JG, Lauber CL, Walters WA, et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc Nat Acad Sci USA*. 2011;108 Suppl 1:4516-22.
7. Schloss PD, Westcott SL, Ryabin T, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol*. 2009;75:7537-41.
8. Kong Y. Btrim: A fast, lightweight adapter and quality trimming program for next-generation sequencing technologies. *Genomics*. 2011;98:152–3.
9. Yilmaz P, Parfrey LW, Yarza P, et al. The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. *Nucl Acids Res*. 2014;42(Database Issue):D643-D8.
10. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*. 2011;27:2194-200.
11. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol*. 2007;73:5261-7.

Paper II

II

The association of urine markers of iodine intake with development and growth among children in rural Uganda: a secondary analysis of a randomized education trial

Prudence Atukunda[1], Grace K.M. Muhoozi[1,2], Lien M. Diep[3], Jens P. Berg[4,5], Ane C Westerberg[6] and Per O. Iversen[1,7,8,*]

[1]Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway, [2]Department of Human Nutrition and Home Economics, Kyambogo University, Kampala, Uganda, [3]Oslo Center for Biostatistics and Epidemiology, Oslo University Hospital, Oslo, Norway, [4]Department of Medical Biochemistry, Oslo University Hospital, Oslo, Norway, [5]Institute of Clinical Medicine, University of Oslo, Oslo, Norway, [6]Institute of Health Sciences, Kristiania University College, Oslo, Norway, [7]Department of Haematology, Oslo University Hospital, Oslo, Norway, [8]Division of Human Nutrition, Stellenbosch University, Tygerberg, South Africa

Short title: Iodine and child development and growth

Acknowledgements: We thank the mothers and children in the Kisoro and Kabale districts whose valuable time enabled us to complete this study, as well as all field-staff for their dedicated work. We also thank Ms Anne-Marie Siebke Trøseid for keeping track of the urine samples and Ms Marit Daneid for performing the urine iodide analysis.

Financial support: This research was funded by the Throne Holst Foundation and the University of Oslo. The funders had no role in the design, analysis or writing of this article.

Conflict of Interest: None.

Authorship: P.A. designed and performed the study, collected and analyzed the data and drafted the manuscript. G.K.M.M. designed and performed the study, collected and analyzed the data and read all manuscript drafts. L.M.D. and J.P.B. analyzed the data and read all manuscript drafts. A.C.W and P.O.I. designed and supervised the study, interpreted the data and read all manuscript drafts and they share last authorship. All authors read and approved the final manuscript.

Ethical Standards Disclosure: This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving research study participants were approved by The AIDS Support Organisation Research Ethics Committee (no. TASOREC/06/15-UG-REC-009) and by the Uganda National Council for Science and Technology (no. UNCST HS 1809) as well as by the Norwegian Regional Committee for Medical and Health Research Ethics (no. 2013/1833). All mothers gave written or thumb-printed, informed consent to participate and could decline assessment at any time.The study was registered with Clinical trials.gov ID: NCT02098031.

[*]Corresponding author: Per Ole Iversen, MD, Department of Nutrition, P.O.B 1046 Blindern, 0317 Oslo, Norway; Telephone: +47 22 85 13 91; fax: +47 22 85 13 41; E-mail: p.o.iversen@medisin.uio.no

Abstract

Objective: We wanted to examine the possible association of urine iodide excretion, a marker of iodine intake, with child development- and growth outcomes in a low resource setting.

Design: This is a secondary analysis of a 1:1 cluster-randomized trial with a nutrition/stimulation/hygiene education intervention among mothers of children aged 6-8 months aiming at improving child development and growth. Development was assessed using Bayley Scales of Infant and Toddler Development-III (BSID-III) and the Ages and Stages Questionnaire (ASQ) whereas anthropometry was used to assess growth. Urine iodide concentration (UIC) and the urine iodide/creatinine ratio (ICR) were measured.

Setting: The study was conducted in two rural districts in Southern Uganda.

Participants: We analyzed data from 155 children at the ages 20-24 and 36 months.

Results: The median UIC for both study groups at 20-24 and at 36 months were similar ($P > 0.05$) and within the normal range of 100 to 199 μg/L (0.79-1.60 μmol/L) whereas the intervention group had significantly higher ICR at 20-24 months. The BSID-III cognitive score was positively associated ($P = 0.028$) with ICR at 20-24 months in the intervention group. The ASQ gross motor score was negatively associated ($P=0.020$) with ICR at 20-24 months among the controls. The ICR was not significantly associated with any anthropometrical measure in the two study groups at either time point.

Conclusions: Following the intervention a positive association was noted between ICR and child cognitive score at 20-24 months, whereas no positive association with ICR and growth was detected. Iodine sufficiency may be important for child cognition in this setting.

Keywords: child development, education, growth, iodide, mothers, nutrition, Uganda

Introduction

Iodine is necessary for the production of the thyroid hormones thyroxine and triiodothyronine that are needed for normal growth and development[1]. Globally, insufficient intake of iodine is still one of the most common micronutrient deficiencies[2], despite being one of the most preventable causes of impaired child cognitive development[3]. In line with this, a recent cluster-randomized controlled trial from Ethiopia showed improved mental development among children aged 20-29 months after receiving iodized salt[4]. Notably, iodine deficiency among children below five years is linked not only to poor mental development, but also to stunted growth[1,5]. Yet, discussions on the etiology of childhood stunting involving nutrition seldom include iodine deficiency[6]. Contrary to many other nutrient deficiencies, iodine deficiency occurs in both developed and developing countries[7]. A recent report from the Iodine Global Network database indicated that 129 out of 197 countries have mandatory legislation for the iodization of at least household/table salt or salt for food processing[8]. Moreover, a UNICEF report from 2015 indicated that about 75% of households worldwide use iodized salt[9], henceforth the number of iodine-deficient countries have decreased from 110 in 1993 to 15 in 2016[10].

With the use of urine iodide concentration (UIC; a marker of iodine intake), according to the WHO, the threshold for iodine deficiency among children is UIC < 100 µg/L (<0.79 µmol/L)[11]. Using this threshold, Harika et al. reported large variations in the prevalence of iodine deficiency among children aged 0-19 years in Ethiopia (86%), Nigeria (59% and South Africa (15%)[12]. As a result, several countries with soils and water low in iodine have implemented the use of iodized salt as a cheap public health measure to prevent iodine deficiency disorders[3]. However, despite the progress reported on using iodized salt to combat iodine deficiency, about 41 million newborns per year world-wide remain unprotected from iodine deficiency-induced consequences of brain damage[13].

In Uganda, more than 95% of the households consume iodized salt[14]. In 2002, a study on the severity of iodine deficiency disorders showed that about 30% of the Ugandan general population had been diagnosed with goiter, indicating a severe public health problem[14]. However, the current prevalence of iodine deficiency is unclear since few surveys provide accurate estimates[10]. Specifically, Uganda's districts bordering the Democratic Republic of Congo, with high mountainous terrain, deep valleys, volcanic soils and abundant rainfall, are endemic for iodine deficiency[15]. This is illustrated by Ehrenkranz et al. who found that the prevalence of iodine deficiency was 21% and 23% among newborns in the districts of Kabale and Kisoro, respectively[15].

Although several studies have investigated the adverse outcomes of iodine deficiency on child health[3,16,17], few studies have examined the association of iodine status with development and growth, especially in Sub-Saharan Africa[13,18]. Moreover, health promotion interventions for children have usually overlooked iodine's importance for child health outcomes[6].

We performed a randomized trial to test the effect of a 6-months' intervention consisting of nutrition, stimulation and hygiene education among mothers of children aged 6-8 months in Uganda, on growth and development[19]. Whereas this education intervention did not improve growth at child age of 20-24 months, cognitive, language and motor development improved. We then conducted a follow-up of this trial when the children were 36 months. This presented a unique opportunity to (i) evaluate the effects of the intervention on urine iodide excretion, and (ii) perform a secondary analysis to search for associations of iodine intake, measured as urine iodide excretion, with child development as well as with growth.

Methods

Study area and participants

This is a secondary analysis of a follow-up study of our open cluster-randomized education intervention regarding nutrition, stimulation and hygiene among impoverished mothers of children aged 6-8 months in the Kisoro and Kabale districts of South-Western Uganda; details can be found elsewhere[19]. We report the data according to the CONSORT guidelines.

Randomization and allocation to study groups

The randomization procedure for the original trial is detailed elsewhere[19]. In brief, 10 clusters were first obtained (i.e. sub-counties) from the two study districts by simple random sampling before they were randomized to either intervention (*n* 5) or control (*n* 5). Second, all the villages in each cluster were listed alphabetically and assigned numbers. By use of computer-generated random numbers, villages to whose assigned number matched with the random numbers, were chosen. We accounted for an intra-cluster correlation (a measure of relatedness of clustered data) of 0.01[21]. We finally enrolled 511 mother-child pairs in the original study and they were randomized to an intervention (*n* 263) or a control (*n* 248) group. The intervention group received the nutrition, hygiene and stimulation education in addition to routine health care while the control group received only routine health care.

The child had to be 20-24 months during the study period of January-May 2015 to be included in the current follow-up study since developmental milestones at this age may predict IQ at 5-6 years when children are about to start school[20]. Out of the 511 children in the original trial, 501 were 20-24 months at the time of this follow-up study. Among these 501 children, 155 children were randomly selected to participate in this study. The primary outcome in this follow-up study was cognitive development assessed with the Bayley Scales of Infant and Toddler Development-III (BSID-III) at 36 months. To detect a clinically

relevant difference between the two study groups in the BSID-III cognitive composite score at 36 months of 0.5 SD (corresponding to 7.5 points) with a power of 0.8 and α of 0.05, 63 children per group were required. To account for dropouts a total of 155 children were included. Among these 155 we randomly selected 77 children from the intervention group and 78 children from the control group.

Data were collected when the children were 20-24 months and at 36 months. The data collection teams were masked to group allocation and never interacted with the team that delivered the education intervention in the original trial.

Delivery and content of the education intervention in the original trial

The education intervention duration lasted for 6 months starting when the children were between 6 and 8 months old and has been described[19]. Mothers in the intervention group were periodically followed and reminded of the intervention activities. Following end of the intervention period, 8 booster education sessions were conducted after every three months until the children were 36 months. The intervention education teams emphasized nutrition, child stimulation and hygiene in these booster sessions.

Briefly, the education was delivered to groups of mothers by a team of nutrition educators (bachelor graduates in nutrition) following a nutrition education curriculum based on the 10 guiding principles of complementary feeding[22]. The nutrition educators demonstrated breast feeding practices and cookery. The mothers were advised to start complementary feeding of their children with nutrient-rich foods while breastfeeding continued, to increase the number of feeds to 3-4 times a day, and to provide nutritious healthy snacks between the main meals. The mothers were also encouraged to practice responsive feeding and allow the children to feed themselves. The importance of oral hygiene and sanitation was given special emphasis.

Mothers together with the intervention team engaged in specific play activities and toys that could be useful in developing each of the development domains (cognitive, language and motor). The stimulation intervention was based on social-cognitive learning theory, emphasizing the benefits of the stimulation practices[23]. Mothers in the intervention group also met at monthly intervals to practice what they had learnt, thereby empowering them and ensuring compliance to the intervention[19].

Collection of development and growth data

We have detailed our data collection procedures for child development and anthropometric measurements[19]. In case of child illness, data collection was postponed. Three bachelor degree holders in psychology performed the child development assessments while two bachelor degree holders in nutrition collected the anthropometric data. Assessments were administered in the local language and conducted in hired rooms in the villages without interruptions to minimize distractions. To promote reliability, the child development assessments were administered first, followed by anthropometric measurements, and then urine sampling. The BSID-III and the Ages and Stages Questionnaire (ASQ) were used. The BSID-III scale is known to be the most comprehensive child development measure for children up to 3.5 years and has been adapted and used in similar settings[24]. The ASQ is a parent/caregiver completed screening scale with excellent psychometric properties, which capture and establish a wide range of adaptive behaviors, and previously used in similar settings[25]. For mothers who could not read the translated ASQ tool in the local language, the assessments were conducted together with our blinded data collection team. This team would read the ASQ questions to the mothers and then they would score the results together. Notably, few women could not read the local language; we registered only 5 (3%) mothers out of the 155 mothers. Both tools were used because ASQ assesses the social–emotional

abilities of the child, which are not included in the BSID III test. In addition, ASQ is used to evaluate a range of adaptive behaviors not obtained with the BSID III. Inter-observation agreement between the child assessment team was good indicated by an intra-class correlation coefficient of 0.75 ($P < 0.001$) for BSI-III, and 0.79 ($P < 0.001$) for ASQ.

Nutritional status was evaluated using weight and length, following standard procedures and calibrations recommended by WHO[26]. Weight (to the nearest 0.1 kg) was measured with a Seca-scale model 881 (Hamburg, Germany) whereas recumbent length was measured (to the nearest 0.1 cm) with a length board (Seca, SO114530). The date of birth was obtained from the child health card. These anthropometric data were converted to z-scores for height-for-age (HAZ), weight-for-age (WAZ), weight-for-height (WHZ), head circumference, and mid-upper arm circumreference using the WHO Anthro (version 3.2.2) software[27]. Undernutrition (stunting, underweight and wasting) was defined as a z-score below minus two SD from the median of the WHO reference standards for HAZ (stunting), WAZ (underweight) or WHZ (wasting), respectively[27].

Urine iodide and creatinine concentrations

We collected 155 and 148 samples of morning spot urine (volumes ranged from 2.5 to 4 mL) at 20-24 and at 36 months, respectively. These samples were collected by a graduate student of laboratory technology using small containers that were transferred to tubes and kept at 4ºC for no more than 24 h before being frozen at -20 ºC. They were then shipped on dry ice to Oslo University Hospital for analysis at the Department of Medical Biochemistry. We measured the concentration of the anion (oxidized form of iodine) which is iodide. Briefly, urine iodide was analyzed by a colorimetric method based on ammonium persulfate digestion prior to the Sandell-Kolthoff reaction, as described by Ohashi et al. and with an analytical coefficient of variation (CV) of 6% at 0.9 µmol/L[28].

Creatinine in urine was measured with enzymatic colorimetry using Cobas 6000 (Roche, Basel, Switzerland; CV 3%). Urine was collected as spot urine samples which were passed out when the childrens' bladders were full. It was not feasible to collect diurnal urine samples. The urine iodide concentration was corrected for differences in water intake and hence urine dilution and concentration by calculating the individual urine iodide/creatinine ratio (ICR) which was used as a measure of iodine status in addition to urine iodide concentration (UIC)[29].

Measurements of iodine concentration in drinking water

To determine the concentration of iodine in drinking water, 20 randomly selected samples (10 from intervention villages and 10 from control villages) were collected from the following sources of water: protected springs (*n* 4), unprotected springs (*n* 4), free flowing springs (*n* 3), ponds (*n* 2), gravity (i.e. tap water, *n* 3) and swamp water (*n* 4). The iodine concentrations were analysed by Vestfold Lab Ltd. (Sem, Norway) using the ISO 17294-2 method (level of detection 0.5 μg/L). Iodine was extracted by an aqueous solution of tetramethyl-ammoniumhydroxide (TMAH; 0.2 - 1.0 g sample in 1 mL TMAH and 5 mL H_2O). The extraction was carried out at a temperature of 90°C for 3 hours. After cooling the sample was diluted, the liquid phase was separated, and prepared for the measurement with ICP-MS (including addition of an internal standard).

Statistical analyses

Values are reported as means (95% confidence intervals) or medians with interquartile range (IQR), as appropriate. Differences between the two study groups in concentrations of urine compounds were tested by Mann-Whitney U tests for each time point since the data was not normally distributed. For the secondary analyses, we used mixed models to investigate the

effect of urine iodide on growth and development outcomes in the intervention and control groups separately. The individual child was set as random identifier, time and ICR/UIC as fixed variables, and we adjusted for baseline values (obtained when the children were 6-8 months) of the outcome of interest. To investigate whether the effect of urine iodide on the different outcomes changed between 20-24 and 36 months we included an interaction term "time x UIC" and "time x ICR". The association of iodine with child development and growth was expressed as the regression coefficient with 95% confidence interval and its corresponding *P*-value. The analyses were performed with Stata/SE 14 (StataCorp. 2015, Stata Statistical Software: Release 14. College Station, Stockholm, Sweden) and IBM SPSS Statistics version 22.0 (IBM SPSS Statistics, IBM Corp., Armonk, NY).

Results

Inclusion and characteristics of participants in the follow-up study

Figure 1 shows the study profile inclusion from both the original trial cohort and from the follow-up study cohort. The characteristics of the original trial cohort and of the follow-up study cohort are shown in Table 1. Anthropometry and BSID-III scores are given in Table 2. The intervention group in the follow-up study cohort had significantly fewer cases of stunting than the controls when they were 20-24 months. Moreover, the intervention groups in both the original trial cohort and in the follow-up study cohort had significantly higher BSID-III scores at 20-24 months.

Urine iodide concentrations at 20-24 and at 36 months

According to the criteria proposed by Andersson et al.[13], adequate UICs for small children range from 100 to 199 μg/L (0.79 to 1.60 μmol/L). The median UICs for both study groups at 20-24 and at 36 months were within this range (Table 3). At 20-24 months, 20.8 and 21.8% of the children in the intervention and control group, respectively, had a UIC below 100 μg/L (0.79 μmol/L), whereas 35.1 and 52.6%, respectively, had a UIC above 200 μg/L (1.60 μmol/L). The corresponding values at 36 months were 26.0 and 31.6% with UIC below 100 μg/L (0.79 μmol/L) and 40.3 and 52.6% with UIC above 200 μg/L (1.60 μmol/L) in the intervention and control group, respectively. No child had a severe iodine deficiency defined as UIC < 20 μg/L (0.16 μmol/L) at either time point.

The ICR was nearly twice as high in the intervention compared to the control group at 20-24 months ($P = 0.03$) whereas no significant difference was found at 36 months (Table 3). We did not detect any significant differences in the UIC, neither between the two study groups nor between the two time points of assessment (Table 3).

Associations between iodine status and child development outcomes

In both the original trial and the current follow-up study, we found that the intervention led to better developmental outcomes. We here show that the intervention group had higher ICR at 20-24 months compared with the controls. Therefore, we next examined whether the ICR was associated with any of the development outcomes in the two study groups. Table 4 shows that the ICR was positively associated with BSID-III cognitive scores at 20-24 months in the intervention group, but not with any other developmental outcome in either the intervention or the control group when adjusting for baseline values. None of the ASQ scores were significantly associated with ICR either at 20-24 or at 36 months in the two study groups except that ICR was negatively associated with ASQ gross motor score ($P = 0.020$) among the controls at 20-24 months (Table 4). The effects of ICR on the BSID-III and the ASQ development outcomes did not differ between the two time points (i.e. $P > 0.05$ for the interaction term).

UIC was positively associated with BSID language development scores at 20-24 months in the control group, but negatively associated at 36 months (Table 5). Thus, we found a significant interaction effect between time and UIC on BSID language development in the control group. Furthermore, UIC was positively associated with ASQ fine motor scores at 36 months age in the intervention group, but negatively associated among the controls.

Associations between iodine status and growth outcomes

None of the anthropometrical markers of growth was associated ($P > 0.05$) with ICR at the two time points (Table 6). However, a significant interaction effect of ICR and time for head circumference z-score was found in the intervention group. Moreover, we found no significant associations between UIC and growth outcomes at 20-24 and or at 36 months, except that at 20-24 months the z-scores for head circumference ($P = 0.006$) and weight-for-

height (P = 0.047) were negatively associated with UIC in the control group (Table 7). Furthermore, a significant interaction effect was evident for UIC and time on z-scores for head circumference (P = 0.033).

Iodine concentration in drinking water

Since iodine in drinking water can be an important iodine source in settings similar to ours[30,31] , we measured the iodine concentration in sources of drinking water from randomly selected 10 intervention and 10 control villages. The median (IQR) iodine concentrations were 17.5 (3.0 -28.8) and 11.0 (2.5-30.1) µg/L for the intervention and control sources (P = 0.78), respectively. Hence the children in both study groups drank water with apparently similar iodine content.

Discussion

To our knowledge this study is apparently the first focusing on iodine status and its associations with child development and growth outcomes longitudinally in a rural setting and conducted in a low-income country.

The majority of children in our study cohort had sufficient iodine intake (i.e. UIC > 100 μg/L) both at 20-24 and at 36 months of age. We also found that the median UIC in both study groups were within the recommended range as proposed by Andersson et al.[13]. This could be attributed to the Ugandan policy on the levels of iodine fortification of salt (95% Ugandan households consuming iodized table salt)[14]. However, we found that about 1/5 of the children in both study groups had low UIC at 20-24 months, and this fraction increased to about 1/4 and 1/3 in the intervention and control groups, respectively, after one year (at 36 months). Moreover, we found that the median ICR among the intervention children was higher compared to the control group at 20-24 months, possibly indicating higher iodine consumption among children in the intervention group. In contrast, we did not detect any significant difference in median ICR at 36 months. Collectively our data indicate that the intervention may have led to higher iodine consumption when the children reached the age of 20-24 months, but over time consumption possibly declined.

Interestingly, in our separate analysis of data from the two study groups, we found a positive association between ICR and BSID-III cognitive score in the intervention group at 20-24 months and a negative association between ICR and ASQ gross motor among the controls aged 20-24 months. Although the use of global assessments in our study suggests an association between ICR and certain child development outcomes, Bell et al. in their systematic review identified inconsistencies in the results of the relationship between iodine and child development using similar global assessments[32].

We do not know why the association results for BSID-III language and motor scores were not significant at the two time points as opposed to the cognitive scores. Possibly, cognition has different development pathways compared to language and motor skills at an early age[33]. Our findings are consistent with those from other studies including children, indicating that the main effects of iodine on development may be restricted to children age 3 years and below[3,34,35]. Whereas Robinson et al. found no associations between ICR and executive function outcomes[35], the Ethiopian study focusing on the use of iodized salt, reported similar findings to ours on the association of urine iodide and BSID-III cognitive outcomes[4].

We detected no significant association between ICR and the anthropometrical markers of child growth (HAZ, WAZ, WHZ, head circumference and mid-upper arm circumference) at the 20-24 and 36 months' assessments. Our findings disagree with previous results obtained among Albanian and Moroccan children whose HAZ and WAZ increased after iodine supplementation[17]. Possibly this difference could be related to the fact that the children in the current study were not supplemented with iodine. Notably, in our study the children were using the iodized table salt as part of Uganda's salt iodization programme. However, our findings are comparable to studies from South Africa and Mexico investigating the effects of increased iodine intake which reported no effect on child growth outcomes[36,37]. These latter studies included micronutrient supplementation in their interventions. In contrast, we only provided the education intervention to the mothers, no foods or supplements were provided at any time. To support our findings, results of a recent systematic review reported no clear evidence on the association between UIC and physical development, instead their findings rather identified likely increases in urinary iodine concentration[38].

We did not detect any significant difference in the iodine content in the drinking water obtained from randomly selected water sources in the intervention and control villages. Our

results of the median range for water iodine concentrations are in line with those reported from e.g. Denmark[39], Austria, Spain[40], USA[41], and Australia and New Zealand[42]. Notably, WHO guidelines on recommended values for iodine in drinking water are not yet defined[43].

Several years of follow-up and including iodine measurements in both urine and drinking water samples, constitute major strengths of our study. We also chose to adjust for any effects of dilution or concentration of the collected spot morning urine samples by reporting the urine iodide/creatinine ratio (ICR). Moreover, since the urine creatinine concentrations were similar between the two study groups, there is no reason to believe that the nitrogen intake (and thus protein intake) was different between the intervention and control group. The major limitation of this study was the lack of data on the actual intake of iodine among the children, neither from foods nor from drinking water as well as household table salt as this was not included in the design of the original trial. For the same reason we also lack data of the baseline urine iodine concentration when the children were recruited to the original trial. Furthermore, the size of the follow-up sample was smaller than in the original trial. ASQ being a maternal report could possibly be biased.

In conclusion, the intervention led to a positive association between ICR and child development outcomes at 20-24 (measured as BSID-III cognitive scores). ICR was not associated with any growth outcomes neither at 20-24 nor at 36 months. Our data indicate that iodine is important for child mental development, at least for cognitive skills. Still, there is a need for further studies to establish the associations between iodine intake, child development and growth outcomes, especially in low-resource areas.

References

1. Bougma K, Aboud FE, Harding KB. *et al.* (2013) Iodine and mental development of children 5 years old and under: a systematic review and meta-analysis. *Nutrients* **5**, 1384-1416.
2. Zimmermann MB. (2013) Iodine deficiency and excess in children: worldwide status in 2013. *Endocrine Pract* **19**, 839-846.
3. Aboud FE, Bougma K, Lemma T. *et al.* (2017) Evaluation of the effects of iodized salt on the mental development of preschool-aged children: a cluster randomized trial in northern Ethiopia. *Matern Child Nutr* **13(2)**.
4. Bougma K, Aboud FE, Lemma TM. *et al.* (2018) Introduction of iodised salt benefits infants' mental development in a community-based cluster-randomised effectiveness trial in Ethiopia. *Br J Nutr* **119**, 801-809.
5. Millward DJ. (2017) Nutrition, infection and stunting: the roles of deficiencies of individual nutrients and foods, and of inflammation, as determinants of reduced linear growth of children. *Nutr Res Rev* **30**, 50-72.
6. Farebrother J, Naude CE, Nicol L. *et al.* (2015) Iodised salt and iodine supplements for prenatal and postnatal growth: a rapid scoping of existing systematic reviews. *Nutrition J* **14**, 89.
7. UNICEF. Tracking Progress on Child and Maternal Nutrition. 2009.
8. Iodine Global Network Legislation Database. http://www.ign.org/cm_sata/Salt11X14.png (accessed June 2019).
9. UNICEF State of the World's Children 2015. http://data.unicef.org/corecode/uploads/document6/uploaded_pdfs/corecode/SOWC_2015_Summary_and_Tables_210.pdf (accessed June 2019).

10. Iodine Global Network Global Scorecard 2016: Moving toward Optimal Iodine Status. http://www.ign.org/newsletter/idd_nov16_global_scorecard_2016.pdf (accessed June 2019).
11. World Health Organization Micronutrient Deficiencies: Iodine Deficiency Disorders. http://www.who.int/nutrition/topics/idd/en (accessed June 2019).
12. Harika R, Faber M, Samuel F. *et al.* (2017) Are low intakes and deficiencies in iron, vitamin A, zinc, and iodine of public health concern in Ethiopian, Kenyan, Nigerian, and South African children and adolescents? *Food Nutr Bull* **38**, 405-427.
13. Andersson M, Karumbunathan V, Zimmermann MB. (2012) Global iodine status in 2011 and trends over the past decade. *J Nutr* **142**, 744-750.
14. Food and NutritionTechnical Assistance II Project (FANTA-2). The Analysis of the Nutrition Situation in Uganda 2010. FANTA: Washington, DC, 2010.
15. Ehrenkranz J, Fualal J, Ndizihiwe A. *et al.* (2011) Clarke, I.; Alder, S. Neonatal age and point of care TSH testing in the monitoring of iodine deficiency disorders: findings from western Uganda. *Thyroid* **21**,183-188.
16. Abel MH, Caspersen IH, Meltzer HM. *et al.* (2017) Suboptimal maternal iodine intake is associated with impaired child neurodevelopment at 3 years of age in the Norwegian Mother and Child Cohort Study. *J Nutr* **147**, 1314-1324.
17. Zimmermann MB, Connolly K, Bozo M. *et al.* (2006) Iodine supplementation improves cognition in iodine-deficient schoolchildren in Albania: a randomized, controlled, double-blind study. *Am J Clin Nutr* **83**,108-114.
18. Osei J, Baumgartner J, Rothman M. *et al.* (2017) Iodine status and associations with feeding practices and psychomotor milestone development in six-month-old South African infants. *Matern Child Nutr* **13,** e12408.

19. Muhoozi GKM, Atukunda P, Diep LM. *et al.* (2017) Nutrition, hygiene, and stimulation education to improve growth, cognitive, language, and motor development among infants in Uganda: A cluster-randomized trial. *Matern Child Nutr* **14,** e12527.
20. Peyre H, Charkaluk ML, Forhan A. *et al.* (2017). Do developmental milestones at 4, 8, 12 and 24 months predict IQ at 5-6 years old? Results of the EDEN mother-child cohort. *Eur J Ped Neurol* **21**, 272-279.
21. Campbell MJ, Donner A, Klar N. (2007) Developments in cluster randomized trials and statistics in medicine. *Stat Med* **26**, 2-19.
22. PAHO/WHO. Guiding Priciples for Complementary Feeding of the Breastfed Child. Washington D.C, Division of Health Promotion and Protection; World Health Organisation; 2003.
23. Bandura A. (2001) Social cognitive theory: an agentic perspective. *Ann Rev Psychol* **52**, 1-26.
24. Singla DR, Kumbakumba E, Aboud FE. (2015) Effects of a parenting intervention to address maternal psychological wellbeing and child development and growth in rural Uganda: a community-based, cluster randomised trial. *Lancet Glob Health* **3**, e458-e469.
25. Hornman J, Kerstjens JM, de Winter AF. *et al.* (2013) Validity and internal consistency of the Ages and Stages Questionnaire 60-month version and the effect of three scoring methods. *Early Hum Develop* **89**, 1011-1015.
26. World Health Organization. 2006. WHO Child Growth Standards: Length/height-for-age, Weight-for-age, Weight-forlength, Weight-for-height and Body Mass Index for-age: Methods and Development. http://www. who.int/childgrowth/standards/Technical_report.pdf 2006 (accessed Dec 2018).
27. World Health Organization . WHO Anthro. Geneva: World Health organization. 2005.

28. Ohashi T, Yamaki M, Pandav CS, Karmarkar MG. *et al.* (2000) Simple microplate method for determination of urinary iodine. *Clin Chem* **46**, 529-536.
29. Bath SC, Furmidge-Owen VL, Redman CW. *et al.* Gestational changes in iodine status in a cohort study of pregnant women from the United Kingdom: season as an effect modifier. *Am J Clin Nutr* **101**, 1180-1187.
30. Jooste PL, Weight MJ, Kriek JA. *et al.* (1999). Endemic goitre in the absence of iodine deficiency in schoolchildren of the Northern Cape Province of South Africa. *Eur J Clin Nutr* **53**, 8-12.
31. Barikmo I, Henjum S, Dahl L. *et al* (2011) Environmental implications of iodine in water, milk and other foods used in Saharawi refugee camps in Tindouf, Algeria. *J Food Compost Anal* **24**, 641-647.
32. Bell MA, Ross AP, Goodman G. (2016) Assessing infant cognitive development after prenatal iodine supplementation. *Am J Clin Nutr* **104** (Suppl 3), 928s-934s.
33. Markhus MW, Dahl L, Moe V. *et al* (2018). Maternal iodine status is associated with offspring language skills in infancy and toddlerhood. *Nutrients* **10(9)** E1279.
34. Pereira KR, Valentini NC, Saccani R. (2016) Brazilian infant motor and cognitive development: Longitudinal influence of risk factors. *Ped Int* **58**, 1297-1306.
35. Robinson SM, Crozier SR, Miles EA. *et al.* (2018). Preconception maternal iodine status is positively associated with IQ but not with measures of executive function in childhood. *J Nutr* **148**, 959-966.
36. Van Stuijvenberg ME, Kvalsvig JD, Faber M. *et al.* (1999) Effect of iron-, iodine-, and beta-carotene-fortified biscuits on the micronutrient status of primary school children: a randomized controlled trial. *Am J Clin Nutr* **69**, 497-503.
37. Rivera JA, Gonzalez-Cossio T, Flores M. *et al.* (2001). Multiple micronutrient supplementation increases the growth of Mexican infants. *Am J Clin Nutr* **74**, 657-663.

38. Santos JAR, Christoforou A, Trie K. *et al.* (2019). Iodine fortification of foods and condiments, other than salt, for preventing iodine deficiency disorders. *Cochrane Database Syst Rev* **2**, Cd010734.
39. Pedersen KM, Laurberg P, Nohr S. *et al.* (1999) Iodine in drinking water varies by more than 100-fold in Denmark. Importance for iodine content of infant formulas. *Eur J Endocrinol* **140**, 400-403.
40. European Commission. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Iodine. 2002. http://ec.europa.eu/food/fs/sc/scf/out146 en.pdf (accessed May 2019).
41. U.S Department of Health and HumanServices. Toxicological Profile for Iodine. Public Health Service. Agency for Toxic Substances and Disease Registry (ATSDR). 2014. http://www.atsdr.cdc.gov/ToxProfiles/tp158.pdf (accessed Dec 2018).
42. Australia Nutrition : Iodine Nutrition Fact Sheet. 2010. http://www.nutritionaustralia.org/national/resource/iodine-facts (accessed Dec 2018).
43. World Health Organisation. Iodine in drinking water. World Health Organisation Report No.:WHO/SDE/WSH/03.04/46. Geneva, 2003.
44. Schreiner M. A Simple Poverty Scorecard for Uganda. 2011. http://www.microfinance.com/English/Papers/Scoring_Poverty_Uganda2009_EN.pdf (accessed Dec 2018).

Figure legend

Fig. 1 Flowchart of the inclusion process.

Table 1 Study population characteristics for the original trial cohort and the follow-up study cohort

Characteristics	Original trial cohort (6-8 months)		Follow-up study cohort (20-24 months)	
	Intervention (*n* 263)	Control (*n* 248)	Intervention (*n* 77)	Control (*n* 78)
Children (*n*, %)				
Males	139 (52.9)	123 (49.6)	44 (57.1)	41 (52.6)
Females	124 (47.1)	125 (50.4)	33 (42.9)	37 (47.4)
Mean (SD) child age (months)	7.4 (0.8)	7.3 (0.9)	21.4 (1.0)	21.2 (1.0)
Maternal data (mean (SD))				
Maternal education (years)	4.9 (2.8)	4.9 (2.8)	5.5 (2.5)	5.0 (2.6)
Maternal age (years)	26.1 (5.8)	26.8 (6.3)	26.2 (6.1)	27.4 (6.4)
Number of children per mother	3.4 (2.2)	3.3 (2.2)	3.4 (2.2)	3.3 (2.2)
Household data (mean (SD))				
Household head age (years)	31.3 (7.7)	33.4 (10.7)	30.2 (7.3)	33.1 (10.9)
Household head education (years)	6.4 (3.1)	5.9 (3.1)	6.6 (3.3)	6.5 (3.4)
Household size (n)	5.5 (2.1)	5.5 (2.1)	5.7 (2.2)	5.8 (2.2)
Household poverty score*	47.8 (11.7)	47.6 (11.4)	49.0 (11.6)	46.3 (12.3)
Sanitation composite score	7.2 (1.9)	7.3 (1.9)	7.0 (1.8)	7.1 (1.9)

*Poverty score card[44].

Table 2 Anthropometry and BSID-III scores for the original trial cohort and the follow-up study cohort

	Original trial cohort		Follow-up study cohort	
	Intervention (*n* 240-263)*	Control (*n* 212-248)*	Intervention (*n* 73-77)*	Control (*n* 74-78)*
Child growth at 6-8 months (*n*, %)				
Stunting**	55 (20.9)	70 (28.0)	14 (18.9)	28 (38.4) [#]
Underweight**	25 (9.5)	36 (14.5)	7 (9.5)	8 (11.0)
Wasting**	12 (4.6)	12 (4.8)	3 (3.9)	2 (2.6)
Child growth at 20-24 months (*n*, %)				
Stunting**	142 (49.3)	146 (50.7)	32 (41.6)	46 (59.0)
Underweight**	22 (8.9)	27 (12.1)	6 (7.8)	8 (10.3)
Wasting**	1 (0.4)	2 (0.9)	3 (3.9)	2 (2.6)
BSID-III scores at 6-8 months				
Cognitive	102.1 (12.9)	103.4 (13.8)	101.3 (11.9)	104.4 (14.5)
Language	103.5 (14.4)	100.2 (14.1)	102.4 (16.2)	101.8 (15.2)
Motor	104.9 (13.8)	104.4 (14.7)	108.8 (14.6)	106.6 (15.8)
BSID-III scores at 20-24 months				
Cognitive	114.9 (21.3)	99.3 (17.1)[#]	117.8 (20.9)	101.6 (19.1)[#]
Language	98.3 (14.3)	88.4 (9.1)[#]	100.3 (12.9)	89.0 (9.3)[#]
Motor	113.7 (18.9)	99.1 (14.3)[#]	113.8 (16.1)	100.0 (15.5)[#]

Values are means (SD) unless otherwise stated.
*The variation in n is due to missing data.
**Based on z-score values below 2SD of the median of the reference population.
[#]Significant differences between the intervention and control group.

Table 3 Urine Iodide Concentration (UIC) and Iodide/Creatinine Ratio (ICR) in the two follow-up study groups

Variable	Child age (months)	Intervention (*n* 75-77)*		Control (*n* 73-78)*		*P*-value**
		Median	Interquartile range	Median	Interquartile range	
Iodide (UIC; µmol/L) ***	20-24	1.50	0.20 – 5.50	1.60	0.20 – 5.51	0.11
	36	1.20	0.20 – 5.50	1.60	0.20 – 5.52	0.08
Iodide/creatinine ratio (ICR)	20-24	4.9	1.0 – 9.6	2.7	1.0 – 10.1	0.03
	36	4.2	1.1 – 10	4.6	1.1 – 9.2	0.34
Creatinine (mg/dL)***	20-24	22.4	3.1- 146.2	18.9	3.5 – 158.1	0.69
	36	27.0	2.0 – 121.5	24.4	3.0 – 112.1	0.35

Values are medians (interquartile range). *The variation in *n* was due to some children not completing all the tests. **_P_-value is for the difference between the two study groups at each time point. ***1 µmol/L or UIC corresponds to 127 µg/L whereas 1 mg/dL of creatinine corresponds to 88.4 µmol/L.

Table 4 Associations between the urine Iodide/Creatinine Ratio (ICR) and child developmental scores for the two study groups

		Intervention (*n* 75-77)**				Control (*n* 73-78)**			
BSID-III	**Child age (months)**	**R***	**95% CI**	***P*-value*** **	***P*-value interaction**[#]	**R***	**95% CI**	***P*-value*** **	***P*-value interaction**[#]
Cognitive	20-24	1.76	0.19, 3.33	0.028	0.106	0.51	-0.10, 2.03	0.505	0.765
	36	-0.32	-2.29, 1.65	0.753		0.17	-1.55, 1.88	0.849	
Language	20-24	0.31	-0.81, 1.43	0.584	0.573	-0.0	-0.95, 0.87	0.931	0.897
	36	-0.21	-1.61, 1.19	0.773		0.05	-0.98, 1.07	0.920	
Motor	20-24	0.62	-0.78, 2.01	0.384	0.081	0.32	-1.10, 1.84	0.678	0.369
	36	-1.39	-3.15, 0.37	0.121		1.39	-0.35, 3.12	0.117	
ASQ									
Communication	20-24	-0.19	-1.19, 0 .81	0.711	0.933	0.04	-1.10, 1.27	0.954	0.814
	36	-0.26	-1.52, 1.01	0.688		0.26	-1.10, 1.63	0.704	
Gross motor	20-24	0.36	-0.35, 1.07	0.316	0.647	-1.27	-2.34, 0.21	0.020	0.180
	36	0.09	-0.80, 0.99	0.835		-0.15	-1.32, 1.01	0.795	
Fine motor	20-24	0.41	-0.43, 1.26	0.340	0.858	0.15	-0.10, 1.29	0.801	0.837
	36	0.54	-0.55, 1.62	0.331		0.33	-0.95, 1.61	0.614	
Problem solving	20-24	0.77	-0.11, 1.66	0.087	0.358	0.09	-1.01, 1.18	0.878	0.545
	36	0.10	-1.03, 1.22	0.867		0.60	-0.61, 1.82	0.333	
Personal social	20-24	0.23	-0.61, 1.07	0.586	0.072	-0.30	-1.16, 0.55	0.489	0.927
	36	-1.01	-2.08, 0.05	0.063		-0.24	-1.20, 0.72	0.622	

*Values are regression coefficients (R) adjusted for baseline scores. **This variation in n was due to incomplete data. ***Mixed effects linear regression *P*-values for the association between ICR and BSID-III/ASQ scores. [#]*P*-value is for the interaction difference between the two time points' regression coefficients.

Table 5 Associations between Urine Iodide Concentration (UIC) and child developmental scores for the two study groups

		Intervention (*n* 75-77)**				Control (*n* 73-78)**			
BSID-III	**Child age (months)**	**R***	**95% CI**	***P*-value*****	***P*-value interaction**[#]	**R***	**95% CI**	***P*-value*****	***P*-value interaction**[#]
Cognitive	20-24	-2.68	7.52, 2.16	0.278	0.356	0.58	2.39, 3.57	0.70	0.266
	36	0.31	3.73, 4.36	0.880		-1.74	-4.55, 1.08	0.23	
Language	20-24	1.63	-1.74, 5.01	0.342	0.434	1.77	0.07, 3.46	0.041	0.001
	36	-0.13	-2.96, 2.70	0.928		-2.05	-3.63,-0.46	0.011	
Motor	20-24	0.92	-3.36, 5.19	0.42	0.821	1.59	-1.39, 4.57	0.296	0.037
	36	1.57	-2.03, 5.16	0.85		-2.75	-5.55, 0.05	0.054	
ASQ									
Communication	20-24	-0.08	-3.11, 2.94	0.957	0.548	-0.73	-3.11, 1.66	0.551	0.632
	36	1.34	-1.41, 3.68	0.381		-1.52	-3.75, 0.71	0.183	
Gross motor	20-24	1.41	-0.69, 3.52	0.189	0.879	1.10	-1.04, 3.25	0.312	0.162
	36	-0.63	-2.60, 1.33	0.527		1.32	-0.48, 3.12	0.150	
Fine motor	20-24	0.01	-2.57, 2.59	0.996	0.166	0.16	-2.03, 2.35	0.885	0.087
	36	2.39	0.25, 4.52	0.029		-2.44	-4.49, -0.40	0.019	
Problem solving	20-24	0.54	-2.18, 3.26	0.698	0.557	0.30	-1.83, 2.43	0.784	0.203
	36	1.62	-0.63, 3.89	0.159		-1.60	-3.59, 0.40	0.117	
Personal social	20-24	0.27	-2.32, 2.86	0.837	0.793	-0.69	-2.35, 0.97	0.413	0.518
	36	-0.18	-2.36, 1.99	0.869		-1.45	-3.03, 0.14	0.074	

*Values are regression coefficients (R) adjusted for baseline scores. **This variation in n was due to incomplete data. ***Mixed effects linear regression *P*-values for the association between UIC and BSID-III/ASQ scores. [#]*P*-value is for the interaction difference between the two time points' regression coefficients.

Table 6 Associations between urine Iodide/Creatinine Ratio (ICR) and child growth z-scores for the two study groups

		Intervention (*n* 75-77)**				Control (*n* 73-78)**			
Growth	**Child age (months)**	**R***	**95% CI**	***P*-value*****	***P*-value interaction**[#]	**R***	**95% CI**	***P*-value*****	***P*-value interaction**[#]
Height-for age z scores	20-24	0.03	-0.04, 0.11	0.381	0.196	-0.05	-0.12, 0.02	0.167	0.214
	36	-0.05	-0.14, 0.04	0.338		0.02	-0.06, 0.10	0.625	
Weight-for-age z score	20-24	-0.02	-0.07, 0.03	0.371	0.735	-0.01	-0.06, 0.05	0.829	0.262
	36	-0.01	-0.07, 0.05	0.776		0.04	-0.02, 0.10	0.174	
Weight-for-height z score	20-24	-0.03	-0.09, 0.03	0.324	0.415	0.01	-0.05, 0.08	0.688	0.939
	36	0.01	-0.06, 0.08	0.796		0.01	-0.06, 0.08	0.780	
Head circumference z score	20-24	0.06	-0.00, 0.11	0.051	0.027	-0.02,	-0.09, 0.04	0.503	0.352
	36	-0.05	-0.12, 0.03	0.197		-0.07	-0.14, 0.00	0.055	
Mid-upper-arm z score	20-24	0.01	-0.05, 0.06	0.846	0.772	0.00	-0.06, 0.06	0.905	0.802
	36	-0.01	-0.07, 0.06	0.829		0.02	-0.05, 0.08	0.651	

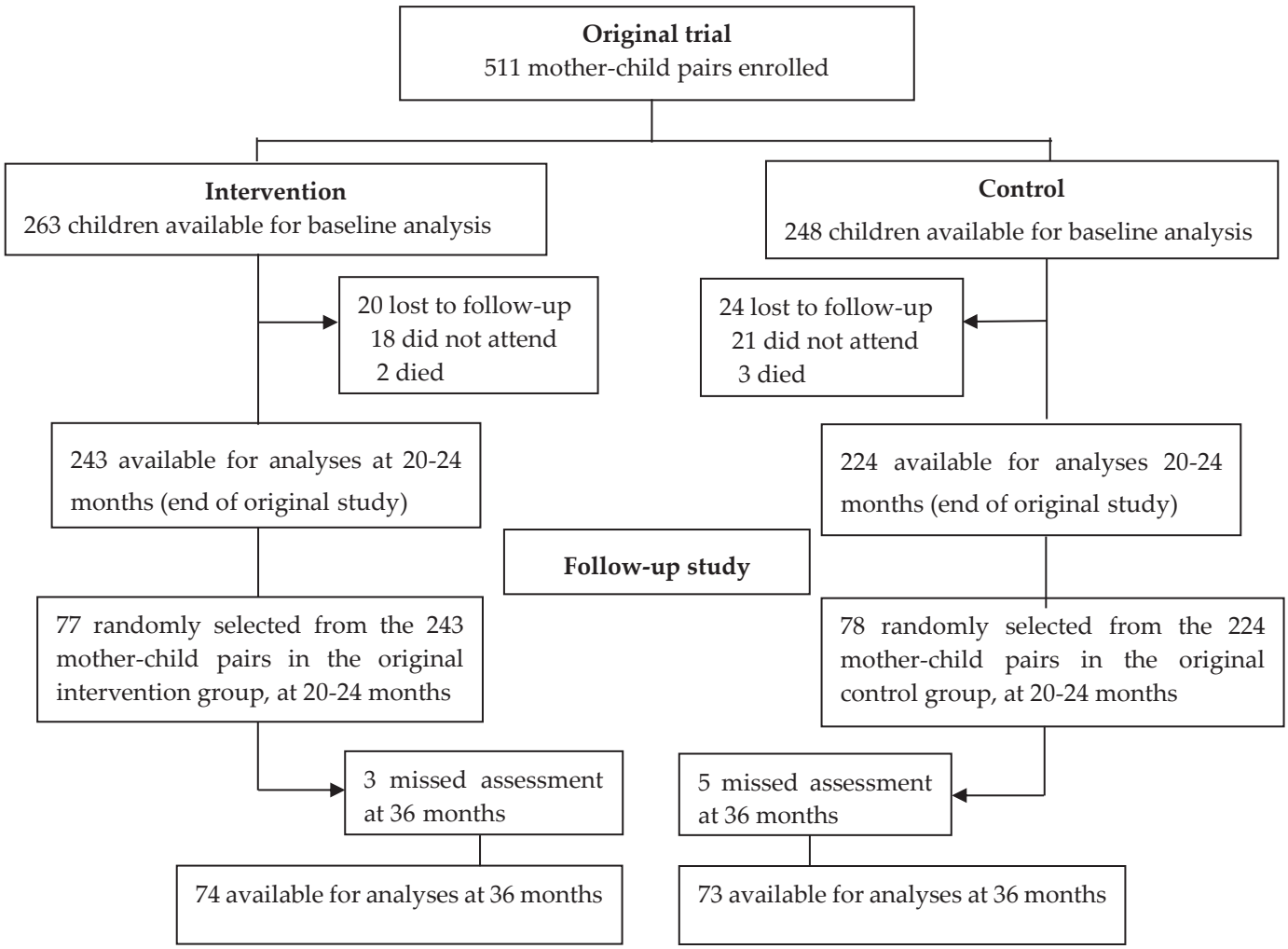
*Values are regression coefficients (R) adjusted for baseline scores. **This variation in n was due to incomplete data. ***Mixed effects linear regression *P*-values for the association between ICR and growth scores. [#]*P*-value is for the interaction difference between the two time points' regression coefficients.

Table 7 Associations between Urine Iodide Concentration (UIC) and child growth z-scores for the two study groups

		Intervention (*n* 75-77)**				Control (*n* 73-78)**			
Growth	**Child age (months)**	**R***	**95% CI**	***P*-value*****	***P*-value interaction**[#]	**R***	**95% CI**	***P*-value*****	***P*-value interaction**[#]
Height-for age z scores	20-24	-0.02	-0.25, 0.21	0.858	0.900	0.02	-0.12, 0.16	0.778	0.450
	36	-0.00	-0.19, 0.89	0.987		-0.05	-0.18, 0.08	0.423	
Weight-for-age z score	20-24	-0.06	-0.21, 0.09	0.424	0.568	-0.07	-0.17, 0.04	0.208	0.816
	36	-0.00	-0.12, 0.12	0.967		-0.05	-0.15, 0.04	0.300	
Weight-for-height z score	20-24	-0.07	-0.25, 0.10	0.419	0.266	-0.12	-0.24,-0.00	0.047	0.251
	36	0.06	-0.08, 0.20	0.399		-0.03	-0.14, 0.09	0.652	
Head circumference z score	20-24	0.06	-0.13, 0.24	0.59	0.734	-0.18,	-0.30, -0.05	0.006	0.033
	36	0.01	-0.14, 0.16	0.18		0.01	-0.11, 0.13	0.876	
Mid-upper-arm z score	20-24	0.09	-0.07, 0.26	0.256	0.232	-0.07	-0.18, 0.05	0.273	0.924
	36	-0.04	-0.17, 0.10	0.588		-0.06	-0.17, 0.05	0.300	

*Values are regression coefficients (R) adjusted for baseline scores. **This variation in n was due to incomplete data. ***Mixed effects linear regression *P*-values for the association between UIC and growth scores. [#]*P*-value is for the interaction difference between the two time points' regression coefficients.

Figure 1


Original trial
511 mother-child pairs enrolled
Intervention
263 children available for baseline analysis
Control
248 children available for baseline analysis
20 lost to follow-up
18 did not attend
2 died
24 lost to follow-up
21 did not attend
3 died
243 available for analyses at 20-24 months (end of original study)
224 available for analyses 20-24 months (end of original study)
Follow-up study
77 randomly selected from the 243 mother-child pairs in the original intervention group, at 20-24 months
78 randomly selected from the 224 mother-child pairs in the original control group, at 20-24 months
3 missed assessment at 36 months
5 missed assessment at 36 months
74 available for analyses at 36 months
73 available for analyses at 36 months

Paper III

nutrients

MDPI

Article

Nutrition, Hygiene and Stimulation Education for Impoverished Mothers in Rural Uganda: Effect on Maternal Depression Symptoms and Their Associations to Child Development Outcomes

Prudence Atukunda [1,*], Grace K. M. Muhoozi [2], Ane C. Westerberg [3] and Per O. Iversen [1,4,5]

1 Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, 0372 Oslo, Norway
2 Department of Human Nutrition and Home Economics, Kyambogo University, P.O. Box 1, 256 Kyambogo, Uganda
3 Institute of Health Sciences, Kristiania University College, Kirkegata 24 Oslo, Norway
4 Department of Hematology, Oslo University Hospital, 0372 Oslo, Norway
5 Division of Human Nutrition, Faculty of Medicine and Health Sciences, Stellenbosch University, 7505 Tygerberg, South Africa
* Correspondence: prudence.atukunda@studmed.uio.no; Tel.: +47-48-64-38-34

Received: 9 May 2019; Accepted: 9 July 2019; Published: 11 July 2019

Abstract: Optimal nutrition improves child development, and impaired development is associated with maternal depression symptoms, in particular in low resource settings. In this follow-up of an open cluster-randomized education trial, we examined its effects among mothers in rural Uganda on their depression symptoms and the association of these symptoms to child development. The education comprised complementary feeding, stimulation, and hygiene. We assessed 77 intervention mothers and 78 controls using Beck Depression Inventory-II (BDI-II) and Center for Epidemiologic Studies Depression Scale (CES-D) scores. Child development was assessed with Bayley Scales of Infant and Toddler Development-III (BSID-III) composite scores for cognitive, language and motor development. Compared to controls, the intervention reduced depression symptoms' scores with mean (95% CI) differences: −8.26 (−11.49 to −1.13, $p = 0.0001$) and −6.54; (−8.69 to −2.99, $p = 0.004$) for BDI II at 20–24 and 36 months, respectively. Similar results were obtained with CES-D. There was a negative association of BDI-II scores and BSID-III cognitive and language scores at 20–24 ($p = 0.01$ and 0.008, respectively) and 36 months ($p = 0.017$ and 0.001, respectively). CES-D associations with BSID-III cognitive and language scores showed similar trends. BSID-III motor scores were associated with depression scores at 36 months for both BDI-II and CES-D ($p = 0.043$ and 0.028, respectively). In conclusion, the group education was associated with reduced maternal depression scores. Moreover, the depression scores were inversely associated with child cognitive and language development outcomes.

Keywords: children; complementary feeding; developmental outcomes; group dynamics theory; maternal depression; nutrition education

1. Introduction

Early childhood is characterized by rapid cognitive and social development changes that require optimal nutrition [1]. Introduction of complementary feeding involves a gradual transition from breastfeeding to eating foods and liquids along with breast milk when breast milk alone is no longer sufficient to meet the nutritional requirements of infants [2]. This promotes adequate nutritional and developmental achievements in infancy [3].

Inadequate infant and young child feeding practices increase the risk of morbidity and mortality, especially in low resource settings. Moreover, childhood morbidity has been associated with maternal depression symptoms in such settings [4]. The prevalence of postnatal maternal depression symptoms in 2015 was 50% in South Africa [5] while in Zimbabwe the prevalence increased from 16 to 34% between 1995 and 2015 [6]. A systematic review from 2019 indicated that maternal depression symptoms contributed to 20% of postpartum deaths [7]. Furthermore, maternal depression symptoms are also common among women with small children [8]. Depression during the time of introduction of complementary foods compromises the mothers' ability to make adequate and nutritious food for the child [9]. In line with this, mothers' depressive symptoms markedly affect their ability to attend to children's feeding practices and cognitive development, henceforth placing their children at risk of delayed developmental outcomes [10]. Consequently, several negative repercussions of maternal depression symptoms on child development domains are reported [11]. Specifically, children of depressed mothers report poor cognitive and socioemotional development as well as higher incidents of disruptive behavior. Moreover, maternal depression symptoms during early child development promote children's maladaptive social cognition resulting in lower social competence in later adulthood [10].

Among mothers with depression symptoms, meta-analysis and observational studies have reported more symptoms, such as negative maternal behaviors, negative maternal affect, and hostile/coercive behaviors as well as disengagement with their infants than among non-depressed mothers [12]. Initiatives for early identification and interventions on maternal depression symptoms during pregnancy and postpartum to improve maternal and child health are underway [9]. However, in low- and middle-income countries, such initiatives are rare despite increasing prevalence of maternal depression symptoms.

Notably, recently a Lancet Series indicated an increasing worldwide burden of mental health disorders including maternal depression symptoms [13]. The Lancet Series' recommendations clearly indicate the urgency and the role mental health has in most, if not all, of the 17 UN Sustainable Development Goals (SDGs) for 2030, such as SDG 2, 3, and 4, which have an in-depth focus on mental health [13].

In a cluster-randomized controlled trial initiated in 2013, we performed a 6-months' intervention comprising nutrition, stimulation and hygiene education among impoverished mothers of children aged 6–8 months in rural districts of Uganda [14]. The intervention consisted of educating mothers aimed at (i) increasing complementary foods' dietary diversity to improve nutrient intake as well as continued breastfeeding, (ii) improving hygiene and sanitation practices, and (iii) enhancing stimulation based on a social-cognitive learning theory to improve development. While this intervention did not alter child growth at the age of 20–24 months, cognitive, language, and motor development improved markedly [14].

We have now examined the possible effects of this intervention on maternal depression symptoms and their associations to child development, among a sub-sample of these mother/child pairs at the child-ages of 12–16, 20–24, and 36 months. In our parental trial, the intervention mothers formed groups that frequently met to practice and share the childcare practices introduced as part of the education intervention. These groups were formed based on the group dynamics theory [15]. According to this theory, groups are known to naturally provide opportunities to develop relationships, help members successfully to accomplish goals, and assist in executing tasks that could not be accomplished individually [15]. Previous studies on small groups indicate that group members enjoy their experiences more when assigned to work in groups rather than by themselves [16].

In this follow-up study, mothers in the intervention group would identify with each other and have shared values, practices and skills on the appropriate complementary feeding taught in the intervention. Moreover, the complementary feeding skills would be learnt through observation, imitation, and positive reinforcement from mother group members. In this follow-up study, we examined if this education intervention (i) would be associated with reduced depression symptoms

among the mothers, and (ii) if there were any association between maternal depression symptoms and child development outcomes.

2. Materials and Methods

2.1. Participants and Approvals

This is a follow-up study of a two-armed, open cluster-randomized education intervention regarding nutrition including focus on complementary feeding, stimulation and hygiene among impoverished mothers of children aged 6–8 months conducted in the Kisoro and Kabale districts of South-Western Uganda [14]. In the current follow-up study, 155 mother/child pairs (77 in the intervention and 78 in the control group) participated. All mothers gave written or thumb-printed, informed consent to participate and could decline an interview or assessment at any time. Exclusion criteria were households with a child having congenital malformation, a physical disorder that would influence assessments and/or nutrient intake, and/or a diagnosis of mental or brain illness, as reported by the mother or a health worker.

The study was approved by The AIDS Support Organisation Research Ethics Committee (no. TASOREC/06/15-UG-REC-009) and by the Uganda National Council for Science and Technology (no. UNCST HS 1809) as well as by the Norwegian Regional Committee for Medical and Health Research Ethics (no. 2013/1833). The parental trial was registered with clinicaltrials.gov (ID no. NCT02098031). We report the data according to the CONSORT guidelines and with intention-to-treat analysis.

2.2. Randomisation of the Parental and follow-up Participants

For the parental trial we used proportionate sampling, 10 sub-counties (i.e., clusters) were obtained (6 out of 19 in Kabale and 4 out of 14 in Kisoro). We used a three-stage procedure to identify eligible households. First, by simple random sampling, three sub-counties in Kabale were allocated to the intervention group and the other three to the control group. Similarly, two sub-counties were allocated to the intervention and the other two to the control group in Kisoro. Second, all the villages in each participating sub-county (intervention or control) were listed alphabetically and assigned numbers in an ascending order. By use of computer-generated random numbers, villages to whose assigned number matched with the random numbers were selected. The intervention villages did not share common geographical boundaries with control villages to minimize contamination of the intervention contents between the two study groups. Third, by complete enumeration, all consenting households with children aged 6–8 months within a participating village were recruited to the study. If a household had more than one eligible child, the youngest was selected, and in the case of twins, we randomly selected one for evaluation. We finally enrolled 511 mother-child pairs in the parental study and they were randomized to the intervention (n = 263) or the control (n = 248) group.

In the current follow up study, the child had to be 12–16 months during the period of January-May 2014 to be included in the this follow-up study. At this stage, group processes of developing relationships among members to successfully accomplish goals would have taken place [14]. Depression data was collected when the children were 12–16, 20–24 months, and at 36 months. Other data was collected when they were 6-8 months (baseline), 12–16, 20–24 months and at 36 months. The data collection teams in the follow-up study were masked to group allocation and never had any interaction with the study team that delivered the education intervention in the parental trial.

2.3. The Intervention in the Parental Trial

Details of intervention are reported elsewhere [14,17]. Briefly, the intervention was conducted by the study team at three group meetings over a period of 6 months to 26 groups of mothers (6–10 mothers per group). It was delivered by a trained education team and included two behavior change techniques: Providing information and prompt practice (i.e., demonstrations of preparing food and stimulation of the children). The nutrition education curriculum was based on the 10 guiding

principles of complementary feeding [2]. Recipes were formulated and cooking demonstrated using locally available foods with emphasis on protein. Moreover, the need to take ill children to hospital for medical attention and to increase the feeding frequency during and after illness was emphasized. Hand washing before feeding as well as use of clean utensils during food preparation and feeding were parts of the hygiene intervention. A novel aspect of this intervention was the focus on oral hygiene, and distribution of toothbrushes to all household members and demonstration of their use. The education team highlighted the importance of play to improve cognitive, language and motor development. The stimulation intervention was based on social-cognitive learning theory [18]. Based on the group dynamics theory, mothers would share their experiences and practice more of the taught skills as well as assigned to work in groups rather than by themselves [16]. In addition to the three group meetings, the women met at monthly intervals to practice what they had learnt, thus ensuring compliance with the intervention.

2.4. Outcomes

The primary outcome of this follow-up study was maternal depression symptoms using Beck Depression Inventory II (BDI-II) scores and Center for Epidemiologic Studies Depression Scale (CES-D) scores. The BDI-II is a self-reported tool for assessing symptoms of depression. It asks mothers to report on a 4-point scale from 0 to 3 with 21 questions, giving a possible range of 0–63 (see: https://www.ismanet.org/doctoryourspirit/pdfs/Beck-Depression-Inventory-BDI.pdf). A score of 10 or above is considered to be indicative of probable depression [19]. Similarly, CES-D asks the mothers to report, on a 4-point scale (0=rarely/none of the time to 3=all of the time), the frequency of symptoms for 20 scale assessment items (see: https://www.outcometracker.org/library/CES-D.pdf). A total score of 16 or higher is considered to indicate depression in the general population [20]. The BDI-II and CES-D have been validated for use in Uganda [21,22]. In addition, CES-D has been used extensively in psychological and epidemiologic studies of postnatal women [23]. Both tools were used because BDI-II has evaluated many different populations in Uganda [19,21], while CES-D has been extensively used in epidemiological studies [22,24]. In the present study, inter-rater reliability for the BDI-II was at least 0.80 across all measurement time points while that for CES-D was at least 0.85. We report scores when the children were 12–16, 20–24 months, and at 36 months. When the parental trial was designed we did not include the BDI-II and the CES-D tools in order not to burden the mothers too much since the education protocol was quite comprehensive and the number of various assessments was large. Consequently, at baseline, we only identified maternal depression symptoms by the following categorized interview question included in our social demographic questionnaire: "How sad did you get with the birth of this child?" Their responses were categorized as not sad (score = 0) or sad (score = 1). After completion of the parental trial, we experienced that the mothers were willing also to undertake a more thorough assessment of their mental health, hence we included the BDI-II and CES-D tools from the 12–16 months' time point and onwards.

Secondary outcomes included an assessment of whether the maternal depression symptoms were associated with child development, as assessed by the Bayley Scales of Infant and Toddler Development-III (BSID-III) score, which is the most comprehensive development measure for children up to 3.5 years and has been adapted and used in Uganda [24]. Inter-observation agreement was good, as indicated by an intra-class correlation coefficient of 0.75.

2.5. Statistical Analysis

In a similar low-resource-setting, Van der Heijden et al. reported a mean difference of about 1.5 SD in BDI-II scores between intervention and control groups [25]. Thus, to calculate the sample size we assumed a difference in BDI-II score of 1.5 SD when the children were 36 months, a power of 0.8 and α of 0.05, hence a minimum of 44 mother/child pairs per group were required. To account for an intra-cluster correlation (ICC) of 0.01 and dropouts, a total of 155 children were included [26]. Among

these 155, we randomly selected 77 mother/child pairs from the parental trial intervention group and 78 children from the parental trial control group.

Maternal depression symptoms and child development outcomes were analyzed using Stata/SE (StataCorp. 2015, Stata Statistical Software: Release 14. College Station, Stockholm, Sweden) and SPSS version 22.0 (IBM SPSS Statistics, IBM Corp., Armonk, NY). Significance level was set at $p < 0.05$. According to the skewness test for normality (in the software programme), our data residuals showed a normal distribution. In addition to this, we generated histogram plots, which showed a bell-shaped curve pointing to a normal distribution. We used a mixed effect linear regression to compare the intervention with the control group and estimated ICC. The sub-county (cluster), village, mother, and mothers within villages were the random intercepts, while the time points and group affiliation (intervention or control) were the random slope and fixed variables in the model. Exchangeable variance-covariance structure was used for the random part at the mother level and the models were fitted via the restricted maximum likelihood method. We fitted data by the maximum likelihood method and used a log likelihood-ratio test to determine the overall effect of the intervention for the entire study period. Differences between the two study groups are given as mean (SD or 95% CI).

For the secondary outcomes (association analyses) we used pooled data from the two study groups (intervention and control) to examine associations between maternal depression symptoms using the BDI-II and CES-D scores and the development outcomes by the BSID-III scores using mixed effects linear regression models to handle three data points per mother. In the association analyses, time, maternal depression symptoms' scores, and interaction were treated as the fixed variables, the sub-county as the cluster and reml as a fitting method. Additionally, these analyses were adjusted for group affiliation.

3. Results

3.1. Characteristics of the Participants

The flow chart in Figure 1 shows the inclusion process of the participants in both the parental trial and in the current follow-up study. Of the 511 participants involved in the parental trial, 155 participants were included in the current follow-up study at 12–16 and 20–24 months. By 36 months, eight of them were lost to follow-up (three in the intervention group and five in the control group).

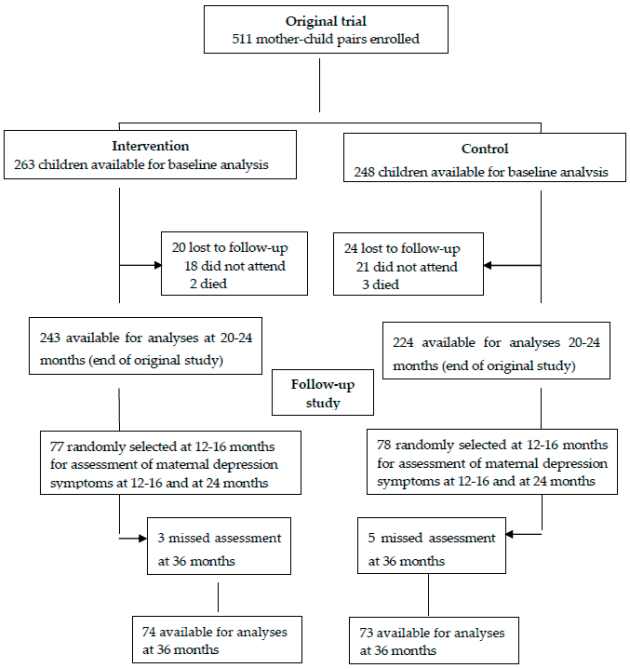


Figure 1. Flow chart of the inclusion process.

There were no significant differences in the characteristics between the parental cohort (data obtained at baseline) and the follow-up cohort (data obtained at 12–16 months; Table 1), thus no adjustments for baseline differences were made in subsequent analyses.

On the question about maternal sadness asked at baseline, the number (proportion) of mothers in the control group who responded as being sad (score 1) was 43 (55%), while the corresponding value for the intervention group was 40 (52%) ($p > 0.05$).

3.2. Effect of the Intervention on Maternal Depression Symptoms

The intervention group mothers had a marked reduction of the depression symptoms at the 20–24 and at the 36 months' assessment, based on both the BDI-II and CES-D scores (Figure 2, Table 2). In the control group, the depression symptoms had increased at the 20–24 months' assessment, whereas there was a reduction of the reported symptoms at 36 months. However, the maternal depression symptom scores among the controls were still higher compared to the intervention group mothers.

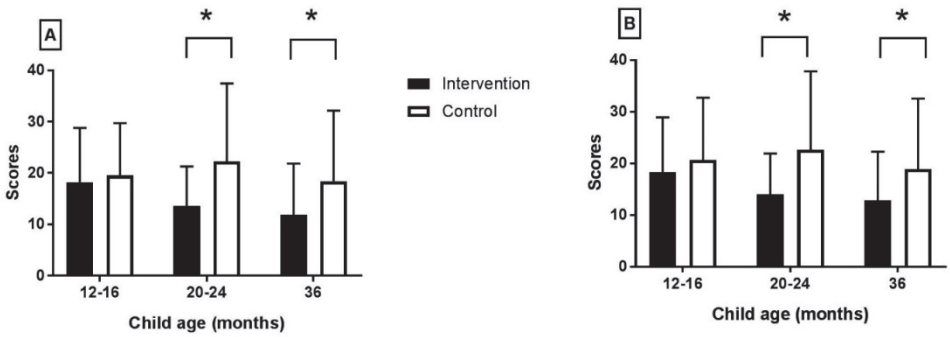


Figure 2. Maternal depression scores derived from the (**A**) BDI-II and (**B**) CES-D questionnaires. Values are means and SD. Asterisk denotes significant difference.

3.3. Associations between Maternal Depression Symptoms and Child Development Outcomes

In the parental trial, we found that the intervention led to better developmental outcomes [14]. We here show that the intervention group had fewer depression symptoms at 20–24 and 36 months based on both the BDI-II and CES-D scores. Therefore, we next examined whether the maternal depression symptoms were associated with the BSID-III child development outcomes. To this end, we pooled the intervention and control groups into one single follow-up cohort (i.e., $n = 155$ mother/children pairs). Notably, the reported depression symptoms assessed by the BDI-II scores were significantly associated with all BSID-III child development outcomes at both 20–24 and 36 months, except the motor child development outcome at 20–24 months ($p = 0.34$; Table 3). At the 36 months' assessment, all BSID-III child development outcomes were significantly associated with the maternal depression symptoms.

Table 4 shows the corresponding data for associations between the maternal depression symptoms assessed as the CES-D scores. Similar to the results obtained with the BDI-II scores, the CES-D scores were significantly associated with all BSID-III child development outcomes at 20–24 months, except the motor child development outcome ($p = 0.59$). At the 36 months assessment, all BSID-III child development outcomes were significantly associated with the maternal depression symptoms.

Table 1. Study population characteristics for the parental trial at baseline and at start of the follow-up study.

Characteristics	Parental Trial (Data Obtained at Baseline)		Follow-up Study (Data Obtained at 12–16 Months)	
	Intervention (n = 263)	Control (n = 248)	Intervention (n = 77)	Control (n = 78)
Children (n, %)				
Males	139 (52.9)	123 (49.6)	44 (57.1)	41 (52.6)
Females	124 (47.1)	125 (50.4)	33 (42.9)	37 (47.4)
Age at inclusion (months)	7.4 (0.8)	7.3 (0.9)	21.4 (1.0)	21.2 (1.0)
Child growth (n, %)				
Stunting *	55 (20.9)	70 (28.0)	32 (41.6)	46 (59.0)
Underweight *	25 (9.5)	36 (14.5)	6 (7.8)	8 (10.3)
Wasting *	12 (4.6)	12 (4.8)	3 (3.9)	2 (2.6)
BSID-III composite score				
Cognitive	114.9 (21.3)	99.3 (17.1)	116.1 (15.6)	105.9 (15.9)
Language	98.3 (14.3)	88.4 (9.1)	106.5 (14.8)	98.9 (12.8)
Motor	113.6 (18.9)	99.1 (14.3)	122.3 (18.7)	113.3 (19.9)
Maternal data				
Maternal education (years)	4.9 (2.8)	4.9 (2.8)	5.5 (2.5)	5.0 (2.6)
Maternal age (years)	26.1 (5.8)	26.8 (6.3)	26.2 (6.1)	27.4 (6.4)
Number of children per mother	3.4 (2.2)	3.3 (2.2)	3.4 (2.2)	3.3 (2.2)
Household data				
Household head age (years)	31.3 (7.7)	32.6 (19.4)	30.2 (7.3)	33.1 (10.9)
Household head education (years)	6.4 (3.1)	5.9 (3.1)	6.6 (3.3)	6.5 (3.4)
Household size (n)	5.5 (2.1)	5.5 (2.1)	5.7 (2.2)	5.8 (2.2)
Household poverty score	47.8 (11.7)	47.6 (11.4)	49.0 (11.6)	46.3 (12.3)
Sanitation composite score	7.2 (1.9)	7.3 (1.9)	7.0 (1.8)	7.1 (1.9)

Values are means (SD) unless otherwise stated. * based on z-score values below 2SD of the median of the reference population. There were no significant differences between the intervention and control groups in either the parental trial or in the follow-up study.

Table 2. Mean maternal depression scores derived from the BDI-II and CES-D scales.

	Intervention *	Control *	Inter-Group Difference *	*p*-Value	Overall *p*-Value
	(*n* = 73–77)	(*n* = 74–78)	(*n* = 147–155)		
Age of Child (months)	**Beck Depression Inventory (BDI-II)**				
12–16	18.23 (10.55)	19.50 (10.22)	−1.27 (−2.50 to −1.00)	0.48	0.0001
20–24	13.58 (7.70)	22.24 (15.20)	−8.26 (−11.49 to −1.13)	0.0001	
36	11.87 (9.99)	18.41 (13.75)	−6.54 (−8.69 to −2.99)	0.004	
Age of Child (months)	**Center for Epidemiological Studies-Depression (CES-D)**				
12–16	18.36 (10.56)	20.67 (12.06)	−2.31 (−4.99 to −1.31)	0.32	0.002
20–24	14.06 (7.85)	22.62 (15.18)	−8.56 (−10.82 to −2.72)	0.0001	
36	12.81 (9.47)	18.90 (13.66)	−6.09 (−9.21 to −3.09)	0.002	

Values are means (SD or 95% CI) of scores and analyzed using linear mixed effect model. * The variation in n was due to missing data because some mothers did not complete all the tests. *p*-value is for the difference between the two study groups at each time point. Overall *p*-value is for the overall effect of intervention obtained from the log likelihood ratio test.

Table 3. Associations between maternal depression (BDI-II scores) and BSID-III child developmental scores for the whole study cohort.

Outcome	Child Age (months)	R *	95% CI	*p*-Value **	*p*-Value Interaction ***
BSID-III scores					
Cognitive development	12–16	−0.25	−0.30 to 0.20	0.70	0.005
	20–24	−0.30	−0.54 to −0.06	0.01	
	36	−0.31	−0.57 to −0.06	0.017	
Language development	12–16	−0.06	−0.32 to 0.20	0.63	0.031
	20–24	−0.01	−0.15 to 0.13	0.008	
	36	−0.20	−0.23 to 0.16	0.001	
Motor development	12–16	−0.02	−0.25 to 0.02	0.82	0.031
	20–24	−0.11	−0.33 to −0.11	0.34	
	36	−0.29	−0.57 to −0.003	0.043	

* Values are regression coefficients (R) adjusted for group affiliation. At 20–24 months we assessed *n* = 155 children whereas at 36 months *n* = 148 children were assessed. This variation in n was due to incomplete data. ** Mixed effects linear regression *p*-values for the association between maternal depression symptoms and BSID-III child development outcomes. *** *p*-value is the interaction difference between the three time points' regression coefficients.

Table 4. Associations between maternal depression (CES-D scores) and BSID-III child developmental scores for the whole study cohort.

Outcome	Child Age (months)	R *	95% CI	*p*-Value **	*p*-Value Interaction ***
BSID-III scores					
Cognitive development	12–16	−0.04	−0.29 to 0.21	0.73	0.026
	20–24	−0.30	−0.04 to −0.001	0.03	
	36	−0.28	−0.53 to −0.04	0.023	
Language development	12–16	−0.05	−0.32 to 0.30	0.52	0.032
	20–24	−0.01	−0.15 to 0.14	0.006	
	36	−0.20	−0.23 to 0.16	0.001	
Motor development	12–16	−0.04	−0.18 to 0.26	0.71	0.025
	20–24	−0.06	−0.27 to 0.15	0.59	
	36	−0.29	−0.55 to 0.03	0.028	

* Values are regression coefficients (R) adjusted for group affiliation. At 20–24 months we assessed $n = 155$ children whereas at 36 months $n = 148$ children were assessed. This variation in n was due to incomplete data. ** Mixed effects linear regression *p*-values for the association between maternal depression symptoms and BSID-III child development outcomes. *** *p*-value is the interaction difference between the three time points' regression coefficients.

4. Discussion

In their recent Cochrane report, Arikbo and colleagues found evidence for effects of education interventions on complementary feeding, but no effects on child growth were found and neither developmental outcomes nor maternal depression were evaluated [27]. Moreover, their report only included a few cluster-randomized trials, and none from sub-Saharan Africa. Thus, our study is probably among the first randomized group education intervention trials with a long-term follow-up focusing on nutrition with focus on complementary feeding, stimulation, and hygiene among impoverished mothers of children aged 6–8 months in this African region.

In the parental trial, the 6-months education intervention led to significant improvements in development outcomes when the children reached 20–24 months, but without affecting growth [14]. The follow-up of a sub-sample of the parental trial at 36 months showed a sustained improvement in the development outcomes using three independent tools. Interestingly, the intervention reduced linear growth faltering at 36 months, but had no effect on gut microbiota composition [17].

In the current follow-up study, we found that the education intervention significantly reduced maternal depressive symptoms among intervention mothers compared with the controls. These findings from our randomized trial are consistent with those of interventions focusing on maternal childcare practices. For example, similar findings were reported by Singla et al., whose manualized, parenting intervention in rural Uganda resulted in mothers in the intervention group reporting significantly fewer depressive symptoms than mothers in the control group [24]. Furthermore, a study in Jamaica examining early childhood stimulation intervention among mothers of undernourished children, reported similar findings of reduced maternal depressive symptoms in the intervention group [28].

How our maternal education intervention led to a decrease in the maternal depression symptoms is unknown. In the parental trial we found that the child diet diversity score improved among children in the intervention group [14], and at 36 months, these children had less growth faltering than the control children [17]. Improved nutritional status and/or growth may possibly then have alleviated a burden to mothers in the intervention group who were depressed at study start, so that they later reported fewer depression symptom scores. It has been demonstrated previously that the association

between maternal depression symptoms and infant developmental outcomes can inform several interventions to improve early childhood health [29]. Moreover, we cannot exclude that the whole process of forming mother groups based on the group dynamics theory [15] and the subsequent empowering of the intervention women in various ways, may have contributed to a beneficial effect on their depression scores.

Our study findings indicated an inverse relationship between maternal depressive symptoms and child cognitive, language and motor development outcomes. These findings agree with those of observational studies that identified persistent declines in the rate of child development upon exposure to late onset of maternal depressive symptoms [30,31]. A meta-analysis from 2017 based on 14 studies confirmed the association between maternal depressive symptoms and lower cognitive scores among children less than 5 years of age [32]. Moreover, a recent review investigating the various mechanisms through which maternal depressive symptoms are associated with early childhood cognitive development, concluded that the association is linked to family processes and parenting practices [33]. In yet another study, maternal depression symptoms in early childhood were associated with impaired verbal skills in later to middle childhood [34]. Similarly, a Canadian longitudinal study found a direct association between maternal depressive symptoms and child receptive language at ages 4 and 5 years [35].

There is vast evidence on the negative effect of maternal depression symptoms on children's cognitive, behavioral, and socio-emotional development [33,36]. Our findings of no association between maternal depression and child development outcomes at 12–16 months are also consistent with previous studies. Still, mixed findings are reported in relation to maternal depression effect on child development outcomes among various child age groups. For example, a recent study from the UK concluded that assessing maternal depression symptoms before a child-aged <2 years, is less likely to identify any association than at later ages [37]. Instead, it is the persistent exposure to maternal depression symptoms at 2 years and beyond that will negatively influence child development outcomes during early childhood. In contrast to this, a 2017 meta-analysis of 14 studies showed a statistically significant relationship between maternal depressive symptoms and child cognitive development among children aged 5 years and below [32].

Our results also showed an inverse relationship between maternal depression symptoms and motor development. In support of these findings, previous research reported that maternal depression during early childhood increased the risk of delayed motor (fine and motor) development at three years [38,39].

Collectively, previous research and our current study highlight the importance of early intervention to address maternal depression in early childhood. Specifically, interventions should possibly be directed at educating the mothers regarding the importance of nutrition, hygiene and sanitation as these measures are likely to improve the health of their children and thereby reduce the many burdens mothers in low-resource settings face every day.

This study has several strengths. For example, we adopted a multidisciplinary approach combining aspects of child care practices of nutrition, child stimulation, hygiene, and two independent and validated psychological research instruments for assessing maternal depression symptoms (BDI-II and CES-D) and one well established tool for assessing child development outcomes (BSID-III). Notably, the children were studied for several years with little loss to follow-up and in a randomized controlled trial based in a rural community setting. The study also has some limitations. The use of self-reported maternal depression symptoms could have compromised reporting of as well as mistakes in presenting the symptoms. Notably, the BDI-II and the CES-D tools only provide scoring of maternal depressive symptoms and not a diagnosis of depression per se, although higher scores are considered to be indicating of a depressive disorder as categorized in the ICD-10 and DSM-IV classifications [40,41]. Additionally, we did not assess maternal depression symptoms using the questionnaires (BDI-II and CES-D) at baseline when children were 6–8 months. Lastly, adherence to complimentary feeding as well as possible individual benefits from the formed groups was not assessed in our study.

5. Conclusions

Our randomized group education trial focusing on complementary feeding, hygiene, and stimulation education among mothers of 6–8 months old children significantly reduced maternal depression symptoms at child-ages of 20–24 and 36 months. We also found inverse associations between maternal depression symptoms and child cognitive and language development outcomes. The reported positive effects from this intervention would call for further studies of similar interventions in other low-income rural settings before consideration of a larger scale-up in the sub-Saharan region and elsewhere.

Author Contributions: Conceptualization, P.A., G.K.M.M., A.C.W and P.O.I.; methodology, P.A. and G.K.M.M.; formal analysis, P.A.; investigation, P.A. and G.K.M.M.; resources, P.A. and G.K.M.M.; writing—original draft preparation, P.A.; writing—review and editing, P.A., G.K.M.M., A.C.W and P.O.I.; supervision, A.C.W. and P.O.I.; project administration, A.C.W. and P.O.I.; funding acquisition, P.O.I.

Funding: This research was funded by the Throne Holst Foundation and the University of Oslo.

Acknowledgments: We thank the mothers and children in the Kisoro and Kabale districts whose valuable time enabled us to complete this study, as well as all field-staff for their dedicated work.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Perez-Escamilla, R.; Moran, V.H. The role of nutrition in integrated early child development in the 21st century: Contribution from the Maternal and Child Nutrition journal. *Matern. Child Nutr.* **2017**, *13*, 3–6. [CrossRef] [PubMed]
2. PAHO/WHO. *Guiding Priciples for Complementary Feeding of the Breastfed Child*; Division of Health Promotion and Protection; World Health Organization: Washington, DC, USA, 2003.
3. Vissers, K.M.; Feskens, E.J.M.; van Goudoever, J.B.; Janse, A.J. The timing of initiating complementary feeding in preterm infants and its effect on overweight: A systematic review. *Ann. Nutr. Metab.* **2018**, *72*, 307–315. [CrossRef] [PubMed]
4. Smith Fawzi, M.C.; Andrews, K.G.; Fink, G.; Danaei, G.; McCoy, D.C.; Sudfeld, C.R.; Peet, E.D.; Cho, J.; Liu, Y.; Finlay, J.E.; et al. Lifetime economic impact of the burden of childhood stunting attributable to maternal psychosocial risk factors in 137 low/middle-income countries. *BMJ Glob. Health* **2019**, *4*, e001144. [CrossRef] [PubMed]
5. Stellenberg, E.L.; Abrahams, J.M. Prevalence of and factors influencing postnatal depression in a rural community in South Africa. *Afr. J. Prim. Health Care Fam. Med.* **2015**, *7*, 874. [CrossRef] [PubMed]
6. January, J.; Chimbari, M.J. Prevalence and factors associated with postnatal depression among women in two rural districts of Manicaland, Zimbabwe. *South Jersey Psychol.* **2018**, *24*, 1176. [CrossRef] [PubMed]
7. Payne, J.L.; Maguire, J. Pathophysiological mechanisms implicated in postpartum depression. *Front. Neuroendocrinol.* **2019**, *52*, 165–180. [CrossRef]
8. Garber, J.; Goodman, S.H.; Brunwasser, S.M.; Frankel, S.A.; Herrington, C.G. The effect of content and tone of maternal evaluative feedback on self-cognitions and affect in young children. *J. Exp. Child Psychol.* **2019**, *182*, 151–165. [CrossRef]
9. Howard, L.M.; Challacombe, F. Effective treatment of postnatal depression is associated with normal child development. *Lancet Psychol.* **2018**, *5*, 95–97. [CrossRef]
10. Wang, Y.; Dix, T. Mothers' early depressive symptoms predict children's low social competence in first grade: Mediation by children's social cognition. *J. Child Psychol. Psychiatry Allied Discip.* **2015**, *56*, 183–192. [CrossRef]
11. Nuttall, A.K.; Froyen, L.C.; Skibbe, L.E.; Bowles, R.P. Maternal and paternal depressive symptoms, home learning environment, and children's early literacy. *Child Psychiatry Hum. Dev.* **2019**, in press. [CrossRef]
12. Conners-Burrow, N.A.; Bokony, P.; Whiteside-Mansell, L.; Jarrett, D.; Kraleti, S.; McKelvey, L.; Kayzer, A. Low-level depressive symptoms reduce maternal support for child cognitive development. *J. Pediatr. Health Care* **2014**, *28*, 404–412. [CrossRef] [PubMed]

13. Patel, V.; Saxena, S.; Lund, C.; Thornicroft, G.; Baingana, F.; Bolton, P.; Chisholm, D.; Collins, P.Y.; Cooper, J.L.; Eaton, J.; et al. The Lancet Commission on global mental health and sustainable development. *Lancet* **2018**, *392*, 1553–1598. [CrossRef]
14. Muhoozi, G.K.M.; Atukunda, P.; Diep, L.M.; Mwadime, R.; Kaaya, A.N.; Skaare, A.B.; Willumsen, T.; Westerberg, A.C.; Iversen, P.O. Nutrition, hygiene, and stimulation education to improve growth, cognitive, language, and motor development among infants in Uganda: A cluster-randomized trial. *Matern. Child Nutr.* **2018**, *14*, e12527. [CrossRef] [PubMed]
15. Forsyth, D.R. *Group Dynamics*, 6th ed.; Wadsworth Publishing Cengage learning: Belmont, CA, USA, 2014.
16. Paulus, P.B.; Poletes, G.; Camacho, L.M. Perception of performance in group brainstorming: The illusion of group productivity. *Personal. Soc. Psychol. Bull.* **1993**, *19*, 78–89.
17. Atukunda, P.; Muhoozi, G.K.M.; van den Broek, T.J.; Kort, R.; Diep, L.M.; Kaaya, A.N.; Iversen, P.O.; Westerberg, A.C. Child development, growth and microbiota: Follow-up of a randomized education trial in Uganda. *J. Glob. Health* **2019**, *9*, 010431. [CrossRef] [PubMed]
18. Bandura, A. Social cognitive theory: An agentic perspective. *Ann. Rev. Psychol.* **2001**, *52*, 1–26. [CrossRef] [PubMed]
19. Ovuga, E.; Boardman, J.; Wasserman, D. Undergraduate student mental health at Makerere University, Uganda. *World Psychiatry* **2006**, *5*, 51–52. [PubMed]
20. Lewinsohn, P.M.; Seeley, J.R.; Roberts, R.E.; Allen, N.B. Center for Epidemiologic Studies Depression Scale (CES-D) as a screening instrument for depression among community-residing older adults. *Psychol. Aging* **1997**, *12*, 277–287. [CrossRef]
21. Ovuga, E.; Boardman, J.; Wasserman, D. The prevalence of depression in two districts of Uganda. *Soc. Psychiatry Psychiatr. Epidemiol.* **2005**, *40*, 439–445. [CrossRef]
22. Natamba, B.K.; Achan, J.; Arbach, A.; Oyok, T.O.; Ghosh, S.; Mehta, S.; Stoltzfus, R.J.; Griffiths, J.K.; Young, S.L. Reliability and validity of the center for epidemiologic studies-depression scale in screening for depression among HIV-infected and -uninfected pregnant women attending antenatal services in northern Uganda: A cross-sectional study. *BMC Psychiatry* **2014**, *14*, 303. [CrossRef]
23. Pietikainen, J.T.; Polo-Kantola, P.; Polkki, P.; Saarenpaa-Heikkila, O.; Paunio, T.; Paavonen, E.J. Sleeping problems during pregnancy-a risk factor for postnatal depressiveness. *Arch. Womens Ment. Health* **2019**, *22*, 327–337. [CrossRef] [PubMed]
24. Singla, D.R.; Kumbakumba, E.; Aboud, F.E. Effects of a parenting intervention to address maternal psychological wellbeing and child development and growth in rural Uganda: A community-based, cluster randomised trial. *Lancet Glob. Health* **2015**, *3*, e458–e469. [CrossRef]
25. Van der Heijden, I.; Abrahams, N.; Sinclair, D. Psychosocial group interventions to improve psychological well-being in adults living with HIV. *Cochrane Database Syst. Rev.* **2017**, *3*, Cd010806. [CrossRef] [PubMed]
26. Campbell, M.J.; Donner, A.; Klar, N. Developments in cluster randomized trials and Statistics in Medicine. *Stat. Med.* **2007**, *26*, 2–19. [CrossRef] [PubMed]
27. Arikpo, D.; Edet, E.S.; Chibuzor, M.T.; Odey, F.; Caldwell, D.M. Educational interventions for improving primary caregiver complementary feeding practices for children aged 24 months and under. *Cochrane Database Syst. Rev.* **2018**, *18*, CD011768. [CrossRef] [PubMed]
28. Baker-Henningham, H.; Powell, C.; Walker, S.; Grantham-McGregor, S. The effect of early stimulation on maternal depression: A cluster randomised controlled trial. *Arch. Dis. Child* **2005**, *90*, 1230–1234. [CrossRef]
29. Miklush, L.; Connelly, C.D. Maternal depression and infant development: Theory and current evidence. *Am. J. Matern. Child Nurs.* **2013**, *38*, 369–374. [CrossRef]
30. Aoyagi, S.S.; Takei, N.; Nishimura, T.; Nomura, Y.; Tsuchiya, K.J. Association of late-onset postpartum depression of mothers with expressive language development during infancy and early childhood: The HBC study. *PeerJ* **2019**, *7*, e6566. [CrossRef]
31. Milgrom, J.; Holt, C.J.; Bleker, L.S.; Holt, C.; Ross, J.; Ericksen, J.; Glover, V.; O'Donnell, K.J.; de Rooij, S.R.; Gemmill, A.W. Maternal antenatal mood and child development: An exploratory study of treatment effects on child outcomes up to 5 years. *J. Dev. Orig. Health Dis.* **2018**, *10*, 221–231. [CrossRef]
32. Liu, Y.; Kaaya, S.; Chai, J.; McCoy, D.C.; Surkan, P.J.; Black, M.M.; Sutter-Dallay, A.L.; Verdoux, H.; Smith-Fawzi, M.C. Maternal depressive symptoms and early childhood cognitive development: A meta-analysis. *Psychol. Med.* **2017**, *47*, 680–689. [CrossRef]

33. Ahun, M.N.; Cote, S.M. Maternal depressive symptoms and early childhood cognitive development: A review of putative environmental mediators. *Arch. Womens Ment. Health* **2019**, *22*, 15–24. [CrossRef] [PubMed]
34. Ahun, M.N.; Geoffroy, M.C.; Herba, C.M.; Brendgen, M.; Seguin, J.R.; Sutter-Dallay, A.L.; Boivin, M.; Tremblay, R.E.; Côté, S.M. Timing and chronicity of maternal depression symptoms and children's verbal abilities. *J. Pediatr.* **2017**, *190*, 251–257. [CrossRef] [PubMed]
35. Letourneau, N.L.; Tramonte, L.; Willms, J.D. Maternal depression, family functioning and children's longitudinal development. *J. Pediatr. Nurs.* **2013**, *28*, 223–234. [CrossRef] [PubMed]
36. Goodman, S.H.; Rouse, M.H.; Connell, A.M.; Broth, M.R.; Hall, C.M.; Heyward, D. Maternal depression and child psychopathology: A meta-analytic review. *Clin. Child Fam. Psychol. Rev.* **2011**, *14*, 1–27. [CrossRef] [PubMed]
37. Netsi, E.; Pearson, R.M.; Murray, L.; Cooper, P.; Craske, M.G.; Stein, A. Association of persistent and severe postnatal depression with child outcomes. *JAMA Psychiatry* **2018**, *75*, 247–253. [CrossRef] [PubMed]
38. Mughal, M.K.; Giallo, R.; Arnold, P.D.; Kehler, H.; Bright, K.; Benzies, K.; Wajid, A.; Kingston, D. Trajectories of maternal distress and risk of child developmental delays: Findings from the All Our Families (AOF) pregnancy cohort. *J. Affect. Disord.* **2019**, *248*, 1–12. [CrossRef] [PubMed]
39. Cornish, A.M.; McMahon, C.A.; Ungerer, J.A.; Barnett, B.; Kowalenko, N.; Tennant, C. Postnatal depression and infant cognitive and motor development in the second postnatal year: The impact of depression chronicity and infant gender. *Infant Behav. Dev.* **2005**, *28*, 407–417. [CrossRef]
40. World Health Organization. International Statistical Classification of Diseases and Related Health Problems (11th Revision). 2018. Available online: https://icd.who.int/browse11/l-m/en (accessed on 8 December 2018).
41. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed.; American Psychiatric Association: Arlington, VA, USA, 2000.