Research Protocol

Reducing short and long-term consequences of early stunted growth.

Study acronym: MAGNUS 2 – Milk affecting growth, cognition and the gut in child stunting

Study ID number: S407

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1 Administrative information

Title	Reducing short and long-term consequences of early stunted growth (MAGNUS-2).
Version	V03_20 Dec 2024
Study site	Busoga sub-region, Eastern Uganda
Funding	The Novo Nordisk Foundation
Study design	Follow-up Study of the MAGNUS (1) trial
Study sponsor	Department of Nutrition, Exercise and Sports, University of Copenhagen, Denmark
Sponsor representative	Benedikte Grenov, Associate professor, PhD, Department of Nutrition, Exercise and Sports, University of Copenhagen, Denmark
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Conflict of interest	The principal investigators declare that they have no financial interest in the results of this study. The Novo Nordisk Foundation will have no role in study design, data collection, analysis, decision to publish, or preparation of manuscript.

2 Study investigators and agreements

2.1 Signatures and agreement with protocol

We, the undersigned, acknowledge that we have read this protocol. We agree to conduct this study in accordance with the study protocol, the current version of the Declaration of Helsinki, and with any additional local laws and regulations.

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4 Abbreviations

AAT	Alpha-1-antitrypsin
AGP	Alpha-1-acid glycoprotein
BAZ	Body mass index-for-age Z-score
BIA	Bioelectrical impedance assessment
CDI	Child development index
CRF	Case report form
CRP	C-reactive protein
DFE	Dietary Folate Equivalent
e-CRF	Electronic case report form
ELISA	Enzyme-linked immunosorbent assay
FFM	Fat free mass
FFMI	Fat free mass index
FM	Fat mass
FMI	Fat mass index
GCP	Good Clinical Practice
HAZ	Height-for-age Z-score
Hb	Haemoglobin
HbA1C	Haemoglobin A1C
HDL	High density lipoprotein
HIV	Human immunodeficiency virus
HTS-DNA	High throughput DNA sequencing
HSP	Human Subject Protection
IGF-1	Insulin-like Growth Factor-1
IRB	Institutional Review Board
ITC	Inpatient clinic – for the treatment of complicated SAM
LDL	Low density lipoprotein
LNS	Lipid based nutrient supplement
MAGNUS	Milk affecting growth, cognition and the gut in child stunting
MAM	Moderate acute malnutrition
MMA	Methyl malonic acid

MUAC	Mid-upper-arm circumference
MoH	Ministry of health
MPO	Myeloperoxidase
NEO	Neopterin
OTC	Outpatient clinic - for the treatment of uncomplicated SAM
PCR	Polymerase chain reaction
PD	Portable device
QA	Quality assurance
QC	Quality control
RBP	Retinol-binding protein
RDT	Rapid diagnostic test
SAM	Severe acute malnutrition
Fe	Ferritin
SOP	Standard operating procedures
sTfR	Soluble transferrin receptor
UNCST	The Ugandan National Council of Science and Technology
VHTs	Village health teams
WHZ	Weight-for-height z-score

5 Definitions of terms

Cardiometabolic risk markers	Factors that can increase the risk of cardiovascular diseases (CVD) and diabetes. These include hyperglycaemia (elevated blood glucose, haemoglobin A1C (HbA1C), insulin and C-peptide), dyslipidemia (raised serum triglycerides and total cholesterol, reduced high density lipoprotein (HDL) cholesterol and raised low density lipoprotein (LDL) cholesterol), high blood pressure, obesity and central adiposity (elevated waist circumference).
Lipid based nutrient supplements (LNS):	A generic term for fortified, lipid-based ready-to-use supplementary or therapeutic products which are modified in their energy density, protein, fat or micronutrient composition to help meet the nutritional requirements of specific populations. They are currently in use in the treatment or prevention of child malnutrition.
Stunting	Also known as linear growth faltering or short-for-age. Defined as height-for-age Z-score < -2.

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7 Summary of protocol

Title of Study: Reducing short and long-term consequences of early stunted growth

Acronym: MAGNUS 2

MAGNUS 2 – Milk affecting growth, cognition and the gut in child stunting

Study Code: S407

Funding:

The Novo Nordisk Foundation

Sponsor:

University of Copenhagen, Department of Nutrition, Exercise and Sports

Sponsor representative:

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Study Operations:

Study Site: Site operations facilitated from two health centres in Jinja, Busoga sub-region of Eastern Uganda. The sites used in MAGNUS 1 trial are maintained. Central office will be in Jinja town.

Planned Study Period:

First patient in: May 2025, Last patient out: December 2025

Objectives:

The main objective is to assess long-term effects of supplementation with large quantity LNS on child health.

The primary objectives are i) to assess the long term effects of 3-mo supplementation with large-quantity LNS among 1-5-year old Ugandan stunted children on cardiometabolic risk

markers at age 6-10 years; and ii) to assess the association between stunting and cardiometabolic risk among 6-10-year-old Ugandan children.

Secondary objectives are to assess long-term effects of 3-mo supplementation with large-quantity LNS among 1-5-year-old Ugandan stunted children on growth, body composition, child development, haemoglobin and micronutrient status, organ size, gut microbiota and function at age 6-10 years; to assess predictors of cardiometabolic risk markers among 6-10-year-old previously stunted Ugandan children; and to assess the associations between stunting and growth, body composition, child development, haemoglobin, micronutrient status, organ size, gut microbiota and function among 6-10-year-old previously stunted Ugandan children.

Measurable Outcomes:

Primary outcome: cardiometabolic risk markers (blood glucose, insulin and HbA1C, blood pressure, lipid profile and c-peptide).

Secondary outcomes: weight, height, knee-heel length, BMI-for-age z scores (BAZ), height-for-age z scores (HAZ), body composition (bioelectrical impedance analysis (BIA), organ size (liver, kidneys and spleen), skin folds: triceps, subscapularis, waist circumference, mid-upper arm circumference (MUAC)), haemoglobin (Hb) and child development including a cognition test, school achievement, fine and gross motor markers finger tapping and broad jumping).

Tertiary outcomes: Blood hormone markers (Insulin-like growth factor-1 (IGF-1)); markers of systemic inflammation: C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP); blood markers of micronutrient status: Iron (serum ferritin and soluble transferrin receptor (sTfR), vitamin B12 (cobalamin and methyl malonic acid (MMA)), folate and vitamin A (retinol binding protein); gut microbiota (diversity and composition); gut function: serum citrulline, faecal myeloperoxidase (MPO), neopterin (NEO) and alpha-1-antitrypsin (AAT)) and morbidity.

Study participants:

Up to 750 children aged 6 -10 years with earlier stunting who were enrolled in MAGNUS 1 trial and 200 age- and sex-matched non-stunted children recruited from the neighborhoods as a reference group.

Study design:

Follow-up study

Study site and recruitment:

Study clinics are run from selected community health centres in Jinja district, Busoga sub-region of Eastern Uganda. Recruitment will take place in the same study sites as in MAGNUS 1 trial.

Participant inclusion:

If both inclusion and exclusion criteria are fulfilled and informed consent is given, children are enrolled.

Criteria for inclusion:

- Former participation in MAGNUS-1* or age-sex-matched children with HAZ >-1#
- Living within the catchment area, according to the SOP.
- Written informed consent given by parent/caregiver

** Confirmed by birth certificate, identity card, or similar documents and/or other confirmation of previous participation in the MAGNUS 1 trial (e.g. informed consent form)*
#Age-sex-matched according to recruitment SOP

Criteria for exclusion:

- SAM; measured as BMI-for-age <-3 OR bipedal pitting oedema.
- Medical complications requiring hospitalization$.
- Disability that makes length/height assessment problematic.
- Participation in another study which impacts on this study.

$*Will initially be excluded until they are treated and stable.*

Statistical Methods:

Descriptive statistics will be used to summarize population baseline and demographic characteristics. Comparative analysis; we shall use T-test to compare continuous variables and Chi-square tests for categorical variables. Linear mixed regression models will be used for continuous variables and logistic regression models for categorical variables to assess associations between exposure and outcomes

8 Introduction

8.1 Background

Stunting is a form of chronic malnutrition affecting one hundred and forty million children globally (World Health Organization, 2020). Stunting is a public health concern, especially in East Africa, where absolute numbers of children with stunting are on the rise, as a result of population growth (De Onis et al., 2013). Stunting is defined as height-for-age (HAZ) below -2 standard deviations (SD) from the median of the WHO Child Growth Standards. Stunting has short and long-term consequences; poor cognition, education performance, impaired immune function and increased risk of mortality. Stunted children are also predisposed to adverse cardiometabolic outcomes such as dyslipidemia and hypertension especially in children with excess weight gain later in life. These are precursors for non-communicable diseases (NCDs), increasing concern about the triple burden of malnutrition and NCDs in low and middle income countries (LMIC) (Chopra et al., 2023). Stunting is also associated with low adult wages, lost productivity and hence has the ability to influence socio-economic development in a nation (Humphrey and Prendergast, 2017) .

In Uganda, 26 % of children below 5 years are stunted and 23% for Busoga sub-region (UBOS, 2022). Busoga sub-region is reported to enter a crisis phase of food insecurity (IPC Technical working group, 2017). In addition, prevalence of anemia (mild to severe) in this region is above 40%, which is above the national average levels by 10% (UBOS, 2016).

Some of the most important factors that contribute to stunting during infancy and childhood include poor nutrition, infections and poor water, sanitation and hygiene (WASH) (Figure 1). Most research has therefore aimed at preventing stunting through either improved breastfeeding practices and complementary foods to infants, or improved WASH. The nutrition trials have only had small effects. A recent meta-analysis found that nutrition interventions only increased height or length-for-age (HAZ) by 0.08 (Panjwani and Heidkamp, 2017), a magnitude that is negligible. Results of WASH trials have also been disappointing as they had effects similar to nutritional interventions i.e., around HAZ 0.08 (Dangour et al., 2013). Since neither nutrition nor

WASH interventions had more than negligible effects, three very large proof-of-concept trials were done to test if combining nutrition and WASH interventions would work, i.e. the WASH trials in Bangladesh and Kenya, and the SHINE trial in Zimbabwe (Pickering et al., 2019) Unfortunately, the combinations had only very minor effects on linear growth, which were attributable to no to small effects of the nutritional interventions, and no effect of the WASH interventions.

Therefore, the MAGNUS 1 trial was conducted in 2020 among children aged 1-5 years in Uganda to assess the role of milk protein and whey permeate in large quantity LNS on the growth and development of children that were already stunted [Mbabazi, 2023 #439]. Findings from this study showed that supplementation with LNS supports linear catch-up growth and accretion of fat free mass (FFM), but not fat mass (FM) in children with stunting [Mbabazi, 2023 #439]. In contrast, among children that were not supplemented stunting worsened and they gained fat at the expense of fat-free mass. Additionally, a secondary analysis showed that large-quantity LNS supplementation increased Haemoglobin (Hb) and improved specific micronutrient markers. While the intervention was only for a short period of time (3 months), it is possible that it may have longer lasting effects.

This follow up study (MAGNUS 2) therefore, aims to assess the long-term effects of LNS supplementation on growth among 6-10-year-old Ugandan children from the MAGNUS 1 trial. This study will explore; the association between stunting and early signs of cardiometabolic risk markers and if these are affected by supplementation with LNS. Additionally, the long-term effects of supplementation with LNS on growth, body composition, child development, haemoglobin and micronutrient status will also be examined. We also intend to assess the associations between stunting and organ size (liver, kidney, spleen), and whether earlier supplementation with LNS modifies these associations. We therefore also recruit age- and sex-matched children from the neighborhood without stunting and compare with stunted children. The design of this part of the study is cross-sectional. The findings from this study will increase knowledge on effect of supplementation of large-quantity LNS in treatment and prevention of stunting.

8.1.1 Problem statement

Stunting affects more than 20% of children below 5 years (World Health Organization 2023 & 2014). It often starts *in utero*, is aggravated in early childhood and may have life-long consequences (Prendergast & Humphrey 2014). Stunting is associated with delayed cognitive development and increased morbidity and mortality in childhood. In adulthood, stunting tracks into reduced educational achievement, working capacity and economic productivity. Furthermore, it may increase the risk of obesity, cardiovascular diseases and diabetes, especially in the context of nutrition transition. (Prendergast & Humphrey, 2014, Wells et al, 2020).

We conducted a randomized, controlled trial among children with stunting in 2020. The results of the study showed that LNS formulations with milk ingredients had no additional effect on height or body composition compared to soy-based LNS formulations. However, LNS had overall positive effects on both linear growth and body composition. Unsupplemented children became more stunted and gained fat mass, whereas children that received LNS gained 0.17 Z-score in height compared to the unsupplemented children and gained predominantly fat-free mass (Mbabazi et al, 2023).

Our finding that a food supplement can revert a stunting trajectory which involves accretion of fat and loss of lean mass, and result in catch-up of linear growth and fat-free mass, may translate into improved cardiometabolic health in later life.

Rationale

The MAGNUS 1 trial found beneficial effects of LNS on linear catch-up growth, lean mass accretion, haemoglobin and micronutrient markers among 1-5 year-old children with stunting. The MAGNUS 2 study will assess the long-term effects of LNS. Additionally, a reference group of non-stunted children will be enrolled to assess associations of stunting per se with cardiometabolic risk markers, growth and development.

8.2 Study location and capacity building

The study is located in the Busoga sub-region of Eastern Uganda and will be implemented at community health clinics in collaboration with the Department of Paediatrics and Child Health, Makerere University, Kampala, Uganda. We shall use the same study sites as in MAGNUS 1 trial. Decisions concerning the location of MAGNUS 1 trial were made in collaboration with the District Health Office and the officer responsible for the relevant community health clinics.

One Ugandan PhD student is included as a co-investigator on the team. The study also involves qualified local nurses, nutritionists, child development officers, lab technicians, village health workers (VHTs), and where possible, masters students.

9 Objectives

9.1 Main objective

To assess long-term effects of supplementation with large quantity LNS on child health

9.2 Primary objectives

1. To assess the long term effects of 3-months supplementation with large-quantity LNS among 1-5-year old Ugandan stunted children on cardiometabolic risk markers at age 6-10 years.
2. To assess the association between stunting and cardiometabolic risk among 6-10-year-old Ugandan children.

9.3 Secondary objectives

1. To assess the long-term effects of 3-months supplementation with large-quantity LNS among 1-5-year-old Ugandan stunted children on growth, body composition, child development, haemoglobin, micronutrient status and organ size (liver, kidney, spleen) at age 6-10 years.
2. To assess the long-term effects of 3-months supplementation with large-quantity LNS among 1-5-year-old Ugandan stunted children on gut microbiota and function at age 6-10 years.
3. To assess predictors of cardiometabolic risk markers among 6-10-year-old previously stunted Ugandan children.

4. To assess the associations between stunting and growth, body composition, child development, haemoglobin, micronutrient status and organ size (liver, kidney, spleen) among 6-10-year-old previously stunted Ugandan children.
5. To assess the associations between stunting and gut microbiota and function among 6-10-year-old previously stunted Ugandan children.

The main hypotheses of MAGNUS 2 are:

i) Fat mass among 1-5-year-old stunted children is associated with later unhealthy cardiometabolic risk markers

ii) Large quantity LNS supplied to 1-5-year-old children with stunting positively affects cardio metabolic risk markers 5 years after supplementation, and these effects are mediated by effects previously shown on FFM in MAGNUS 1.

iii) Stunted children compared to their non-stunted counterparts have increased risk of poor child development, accumulation of fat mass, elevated cardiometabolic risk markers, poor gut microbiota and function, and the negative effects of stunting on these outcomes increase with age.

10 Study design

10.1 Overall study design and duration

This is a follow-up study of children aged 6-10 years who were enrolled in the MAGNUS 1 trial. In addition, 200 age- and sex-matched non-stunted children will be included from the neighborhood as a reference group. The comparison between stunted and non-stunted children to assess the association between stunting and various outcomes is based on a cross-sectional design. More studies may follow at regular time intervals as the children reach adolescence and early adulthood (Figure 1).

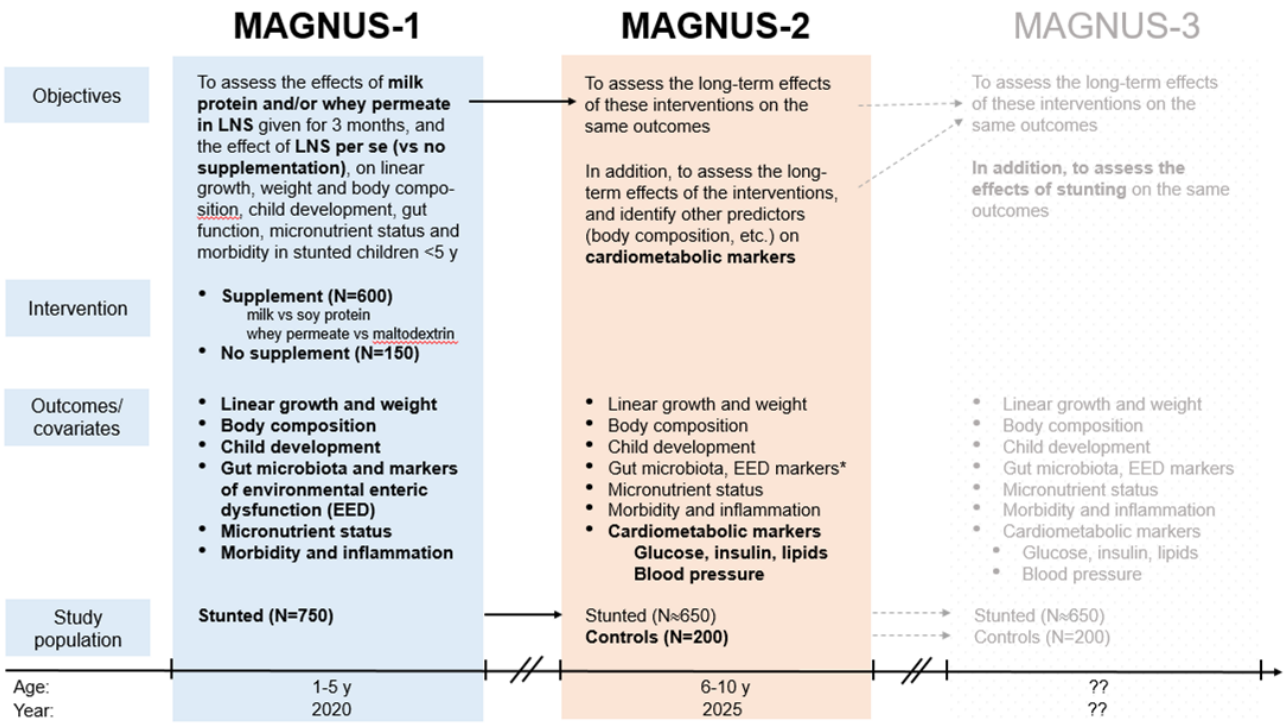

Figure 1. Overview of the main MAGNUS trial (MAGNUS 1), follow-up (MAGNUS 2) and indication of potential future follow-up studies (MAGNUS $_X$). New items in each study are marked with bold.

10.2 Description of study flow and processes

10.2.1Community mobilization

Prior to recruitment within a particular community, community leaders will be informed about the study to strengthen community engagement in support of pre-screening for the study.

10.2.2 Pre-screening and referral

Village Health Teams (VHTs) will mobilise communities to participate in the study. Working closely with local authorities, the VHTs will conduct house to house visits to identify and refer children who were previously enrolled in MAGNUS 1 trial for screening at the study site. For the reference group of non-stunted children, VHTs will identify children aged 6-10 years within the same locality who were not part of MAGNUS 1 trial and refer them for pre-screening. The pre-screening team will screen children for stunting and severe acute malnutrition (SAM). Those identified as not stunted are matched for age and sex, and referred according to the standard operating procedures (SOP). Children identified as having

SAM are referred to outpatient care (OTC) or inpatient care (ITC) for relevant treatment. The VHTs will be remunerated for their work in the study.

10.2.3 Screening

Screening is carried out at the study site.

Children previously enrolled in MAGNUS 1 trial:

Screening will involve checking/verification of biodata of parents and children, conducting short interviews with the caregivers and VHTs, according to the SOP.

Reference group

Study staff who are trained in history taking and measurement of anthropometrics will assess the child for history of stunting, measure the child's weight, height and check for oedema. If the child is classified as being non-stunted without SAM, qualified clinical study staff will assess the child according to the remaining inclusion and exclusion criteria. If all criteria are met, the caregiver is taken through the informed consent process. If informed consent is given, the child is included in the study.

10.2.4 Inclusion

Upon inclusion, children are allocated/re-allocated a unique study ID number and are taken through a registration process according to the relevant SOP.

10.2.5 Measurements

Following inclusion, the following measurements are taken/assessed according to the relevant SOPs: questionnaires of medical history, demographics and dietary information, clinical assessment is conducted including ultrasound scans and blood pressure assessment, blood and stool samples are collected, anthropometrics of the mother (if available) and child are measured, child bioelectrical impedance and child development are assessed. A full description of the data collected at inclusion is provided in section 19.

10.2.6 Nutrition counselling and training

All caregivers receive locally adapted nutrition counselling, in line with the ministry of health (MoH) maternal, infant, young child and adolescent nutrition guidelines (2021). If stool

sample collections are not possible on the day of inclusion, the caregiver is given instructions and a kit for stool collection at home. Refer to the SOP for further detail.

10.2.7 Home visits and WASH

On the day of inclusion (or as close as possible to inclusion date), study staff/s will follow each caregiver home, make an assessment of water sanitation and hygiene (WASH) characteristics and record this in the CRF. The WASH assessment is further described in the related SOP. During the home visit, the staff will also map the GPS coordinates of the houses of those included in the reference group. The GPS coordinates of the new houses of families of children who were included in MAGNUS 1 trial (in case of migrations) will also be mapped. Home visits will be carried out by well-trained study staff.

10.2.8 Phone follow-up

In case of delays in delivery of stool samples among caregivers who are given a kit for stool collection at home, phone follow-up will be attempted in order to encourage them to bring the samples at the study sites.

11 Study setting and participants

11.1 Study setting

The study will be conducted in Jinja city and district in the Busoga sub-region, Eastern Uganda. Here, the prevalence of child stunting is 23%, which is close to the 26% national average, (UBOS, 2022), and both are still above the acceptable international standard of <20% (SPHERE standards, 2018). The MAGNUS 1 study clinics sites will be maintained and these are set up at Walukuba HCIV in Masese division of Jinja city and Buwenge HCIV along Kamuli road. The study office housing the field laboratory among other operations will be based in Jinja city.

11.2 Study participants

Children who participated in the MAGNUS 1 trial who meet the eligibility criteria will be invited to participate in the study. These children will be aged 6-10 years when MAGNUS 2 study is conducted in 2025. A further 200 children without known history of stunting will be recruited as a reference. These will be matched for age, sex, and neighborhood.

11.3 Eligibility criteria

11.3.1 Inclusion criteria

Children who participated in MAGNUS 1 trial

- Child having participated in MAGNUS 1 trial*
- Living within the catchment area, according to the SOP.
- Written informed consent given by parent/caregiver.

**Confirmed by birth certificate, identity card, or similar documents and/or other confirmation of previous participation in the MAGNUS 1 trial (e.g. informed consent form)*

Reference group

- Age 6-10 years
- Height-age-z-scores >-1 according to the WHO growth standards
- Living within the catchment area of MAGNUS 1 trial participants
- Written informed consent given by parent/caregiver

11.3.2 Exclusion criteria

Children who participated in MAGNUS 1 trial and Reference group

- SAM; measured as BMI-for-age z-score < -3 OR bilateral pitting oedema
- Medical complications requiring hospitalization
- Disability that makes height assessment problematic
- Participation in another study or program which impacts on this study

12 Referral

Referral of children identified with acute malnutrition will follow the national guidelines for integrated management of acute malnutrition (2024). Children identified as having SAM (BMI-for-age z-score Z <-3 OR bipedal pitting oedema) prior to inclusion without other complications are referred for outpatient treatment of SAM (OTC). If the child also has complications, they are referred to inpatient care (ITC). Those identified as having MAM but who are younger than 5 years will be linked to the host facility for further assessment and management. All participants requiring hospital attention will be referred for treatment.

13 Concomitant care for study participants

Participants will not be provided with any medical treatment by the study. Wherever treatment is needed, children are referred for the necessary medical attention with linkage to the host facility or to higher facility as necessary.

14 Outcomes

All outcomes are measured at inclusion in the study.

14.1 Primary outcome

- Cardiometabolic risk markers
    - Glucose i.e., plasma glucose, mmol/L and HbA1c, mmol/mol
    - Insulin, pmol/L
    - Lipids (Total cholesterol, mmol/L, High density lipoprotein (HDL) cholesterol, mmol/L and Low density lipoprotein (LDL) cholesterol, mmol/L and Triglycerides, mmol/L)
    - Blood pressure, mmHg
    - C-peptide, ng/mL

14.2 Secondary outcomes

- Weight, kg
- Total height, cm
- Knee-heel length, mm
- BMI-for-age z-scores, (BAZ)
- Height-for-age z-scores (HAZ)
- Child development
    - Cognition test
    - School achievement
    - Fine motor (grib strength)
    - Gross motor (broad jump)
- Haemoglobin, g/dL
- Body composition
    - Bioelectrical impedance: FM, kg, FFM, kg, FMI, kg/m^2, FFMI, kg/m^2

- Organ size (liver, kidneys and spleen)
- Skin folds: triceps, subscapularis, mm
- Waist circumference, cm
- Abdominal fat (ultrasound scan)
- MUAC, cm

14.3 Tertiary outcomes

- Blood hormone markers
  - Insulin-like Growth Factor-1 (IGF-1), ng/mL
- Blood markers of systemic inflammation
  - C-reactive protein (CRP), mg/L
  - Alpha-1-acid glycoprotein (AGP), µg/mL
- Blood markers of micronutrients status
  - Iron: serum ferritin, µg/L and soluble transferrin receptor, mg/L
  - Vitamin B12: cobalamin, pmol/L and methyl malonic acid (MMA), µmol/L
  - Folate, nmol/L
  - Vitamin A (retinol binding protein), µmol/L
- Gut microbiota
- Gut function (E.g. plasma citrulline, faecal myeloperoxidase, faecal neopterin, faecal alpha-1-antritrypsin)
- Morbidity

14.4 Other assessments

- Demographics, medical history
- Physical examination
- Dietary intake assessment
- WASH assessment taken during home visit after inclusion in the study

15 Sample size

All the 750 children that participated in the MAGNUS 1 trial who meet the eligibility criteria will be invited to participate in the study. As the drop-outs in MAGNUS 1 trial comprised below 2% and people generally do not move a lot in these rural areas, we expect a retention

of more than 85%, giving a sample size of about 650 children (Figure 1, above). We will compare with a new reference group of 200 non-stunted children aged 6-10 years. With the 650 children followed up, we will have 80% power at 5% significance level to detect a difference of 0.28 SD or greater between the 520 previously receiving LNS and the 130 unsupplemented. Likewise, we will have 80% power at 5% significance level to detect a difference of 0.23 SD or greater between the 650 children followed up with the 200 newly recruited non-stunted children.

16 Pilot phase

A pilot will be conducted prior to the study start. This is done in order to identify/locate MAGNUS 1 trial participants, test different ways of identifying age- and sex-matched children without stunting as a reference group, test the study procedures, staff roles and test if Case Report Forms (CRFs) and SOPs are operational and clear to all who will use them. During the pilot phase of the study, a maximum of 30 children will be enrolled. Importantly, there is an exception to following the protocol during the pilot: no blood samples will be collected during the pilot phase. Data collected during this period will be stored separately and will not be used as part of the study data.

17 Schedule of activities, assessments and measurements

The schedule of activities and assessments that will take place during screening and enrolment is shown in Figure 2 below.

ACTIVITY AND ASSESSMENT	SCREENING & ELIGIBILITY	ENROLLMENT
Informed Consent		X
Nutrition counselling		X
Demographics	X	X
Clinical examination including ultra sound scans		X
Anthropometry	X	X
Bioelectrical impedance		X
Child development		X
Blood sample collection		X
Stool sample collection		X

Dietary intake assessment		X
Maternal anthropometry		X
WASH assessment in the home		X

Figure 2: MAGNUS 2 schedule of activities, assessments and measurements

18 Data collection and measurements

18.1 Age and birth weight

For MAGNUS 1 trial, this information is already available in the database. However, for the non-stunted reference group, caregivers will be asked to present child health cards that provide a record of date of birth and birth weight. In the event that the child health card is not present, we shall use a calendar of events to estimate the child's age.

18.2 Sociodemographic and medical questionnaire

Interviewer-administered questionnaires will be used to collect information on participant socio- demographic and medical history.

18.3 Clinical examination

Physical examinations will be conducted for all participants and this will include; assessment for vital signs such as pulse, blood pressure, respiratory rate, and ultrasound scans of the liver, kidneys, spleen and visceral fat. Additionally, blood samples will be taken for rapid tests for malaria and Human immunodeficiency virus (HIV) and cardio-metabolic risk markers using standard laboratory techniques.

18.4 Anthropometrics

Trained study staff will take anthropometrics. Weight, height, MUAC, waist circumference and skin fold thickness will be taken in triplicate while knee-heel-length is repeated 5 times (quintuple) to improve precision. The median will be used for analysis. All equipment used will be calibrated regularly to ensure proper function and a record of this field.

18.4.1Child anthropometry

Measurements of weight (kg), height (cm), MUAC (cm), knee-heel length (mm), skinfold thickness (triceps and subscapular, mm), and mid-upper arm and waist circumference (cm)

will be taken and recorded. Weight measurements are taken using a digital scale to the nearest 100 g. Height is measured to the nearest millimetre using a wall mounted retractable stadiometer. MUAC measurements are taken using non-elastic MUAC tapes. Indicators of HAZ and BAZ will determine nutrition status. Waist circumference will be measured using a standardized non-elastic measuring tape. Measurements of the triceps and subscapular skinfolds will be taken using the Harpenden skinfold calliper. The knee-heel length will be measured with a hand-held knemometer. The details on taking child anthropometry are described in a related SOP.

18.4.2 Maternal anthropometry

Weight and height measurements of the mother where applicable, will be taken using digital weighing scales and a wall-mounted retractable stadiometer according to the SOPs. Maternal anthropometrics have been associated with the growth of their offspring and it is therefore of interest to measure maternal BMI in this study (Subramanian, Ackerson and Smith 2010).

18.5 Assessment of nutrition intake

We shall administer a 24-hour dietary food recall questionnaire to assess for dietary intake of the child to assess compliance with minimum dietary diversity and minimum acceptable diet. Assessment of nutritional intake also includes a short food insecurity questionnaire - Household Food Insecurity Access Scale (Coates et al., 2007). Trained staff will conduct the assessment.

18.6 Body composition

18.6.1Bioelectrical impedance

Trained staff will measure FM and FFM using Bioelectrical impedance analysis (BIA). This simple, non-invasive technique determines body composition using flow of current. The child is asked to rest for approximately 10-15 minutes and measurements are taken in a recumbent position, with legs and arms spread out. Electrodes are attached on the dorsal surface of the right hand and foot and the impedance, resistance, reactance and phase angle are measured. Impedance is converted to FFM using a population-specific algorithm. FM is

calculated as total weight – FFM, and the height-adjusted indexes fat mass index (FMI) and fat-free mass index (FFMI) are calculated by dividing FM and FFM with height squared.

18.6.2 Ultrasound

Ultrasound scans will be performed according to the SOP to determine the size of the liver, kidneys and spleen and to measure visceral fat. These variables are expected to be associated with cardiometabolic risk (Wells and Shirley, 2016).

18.7 Child development

Trained study staff members will conduct child development assessments according to the SOP.

18.8 Mortality

We intend to systematically collect data on mortality among the children who were enrolled in MAGNUS 1 trial. We shall also conduct verbal autopsies if any children have died.

18.9 Sample collection and storage

18.9.1Stool

We shall collect stool samples to assess markers of gut function and microbiota at a later stage. Each caregiver will receive a stool collection kit and instructions on how to use it onsite. Approximately 5-10g of stool is collected from each participant. See figure 3 below for stool sample flow; storage, transportation and temperature.

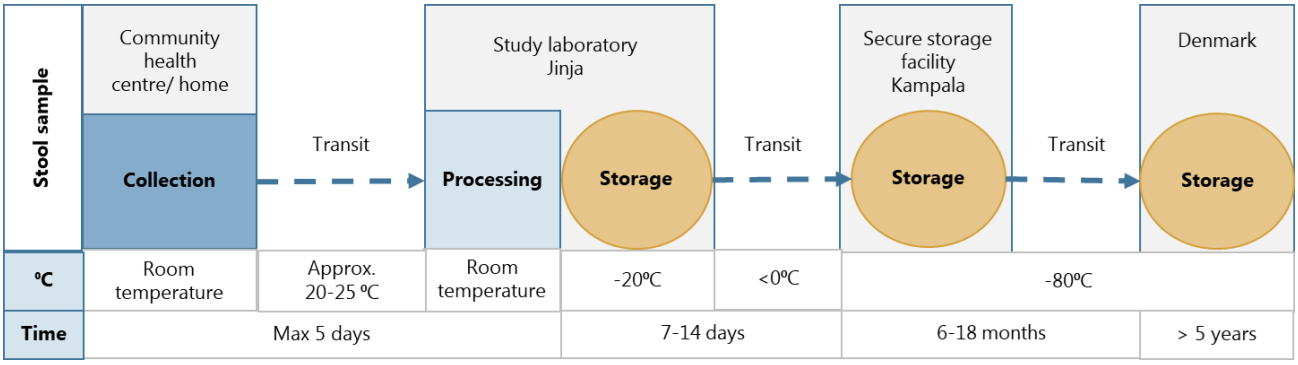

Figure 3: Stool sample flow, including approximate periods and approximate temperatures

18.9.2 Blood

Blood samples will be collected to a maximum volume of 8-10 ml per participant by trained study nurses/phlebotomists, and stored at -20°C at the site laboratory in Jinja. This volume is within the range reported in a review of existing guidelines on allowable blood sample volumes in children involved in research, which recommend limits ranging from 1% to 5% of total blood volume in a single draw (Howie, 2011).

Whole blood haemoglobin, glucose and HbA1c status will be measured using Haemocue. We shall conduct Rapid diagnostic test (RDT) tests for malaria and HIV for all participants and referral to community health facilities for HIV counselling where applicable. More details of sample collection are provided in the SOP. The blood sample flow; storage, transportation and temperature are shown in figure 4 below. As some of these markers change considerably after feeding, children will be requested to come fasted in the morning. After initial assessments and blood sample collection, all children will receive breakfast before continuing with other study-related procedures.

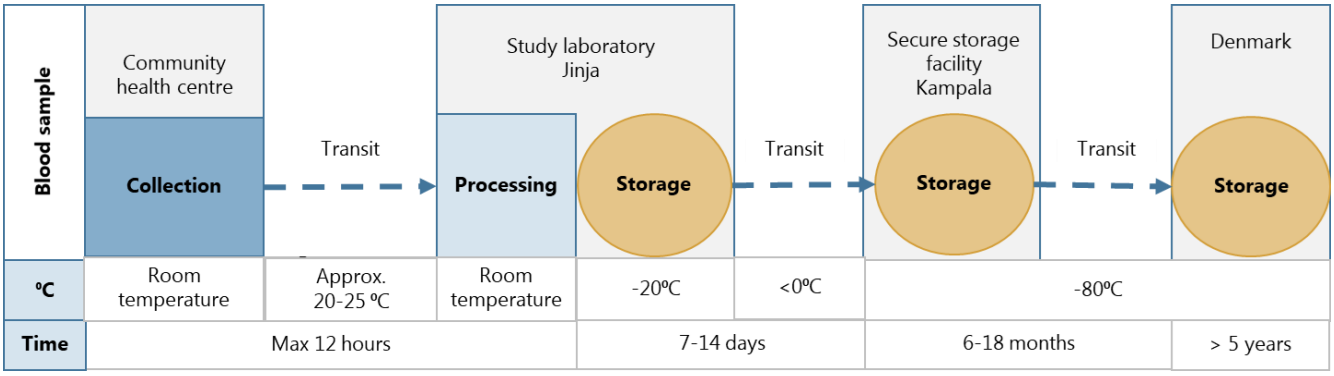

Figure 4: Blood sample flow, including approximate time frames and approximate temperatures

18.9.3 Transportation and shipment of samples

Blood and stool samples are transported approx. once a week from the site laboratory in Jinja to Kampala where they will be stored at -80°C, in a secure laboratory prior to international shipment to Denmark/Europe for later analysis. Samples shall be stored for a minimum of 5 years after study completion. Prior to shipment of samples, all caregivers would have given consent. In addition, only study investigators will have access to identifying information associated with samples being shipped.

18.9.4 Sample collection and analysis plan

Blood and stool samples are collected and analysis is done a summarized in table 1 below.

Table 1: Sample collection and analysis plan

Analysis				
Sample Collected	Test	Type of sample	Suggested method of analysis	Site of analysis
Blood sample, 10 ml	HIV status	Whole blood, drop	RDT Antibody (all ages)	Uganda – MoH HIV clinics
	Malaria	Whole blood, drop	RDT	
	Hb	Whole blood, drop	Hemocue RDT	
	HbA1c, glucose	Whole blood, drop	Hemocue RDT	
	IGF-1, insulin	Serum	Cobas immunoassay analyzer	Shipment abroad after study completion
	CRP, AGP	Serum	ELISA	
	Iron status (Fe, sTfR), vit A	Serum	ELISA	
	Citrulline	Plasma/serum	LC/MS/MS	
	Folate and cobalamin	Plasma	Advia Centaur CP Immunoassay System (Siemens)	
	MMA	Plasma	Liquid chromatography-tandem mass spectrometry	

Analysis				
Sample Collected	Test	Type of sample	Suggested method of analysis	Site of analysis
	Lipid profile (total cholesterol, HDL, LDL, triglycerides) C-peptide	Serum/plasma	Pentra 400 analyzer	
Stool sample, approx.1-5 g	MPO	Stool, 0.5-1.0g	ELISA	Shipment abroad after study completion
	NEO	Stool, 0.5-1.0g	ELISA	
	AAT	Stool, 0.5-1.0g	ELISA	
	Gut microbiota	Stool, 0.5-1.0g	HTS-DNA PCR	

RDT: Rapid diagnostic test; PCR: Polymerase chain reaction; ELISA: Enzyme-linked immunosorbent assay; LC/MS/MS: Liquid chromatography tandom mass spectrometry; Hb: Haemoglobin; IGF-1: Insulin Growth Factor-1; CRP: C-reactive protein; AGP: Alpha-1-acid-glycoprotein; Fe: Ferritin; sTfR: Soluble transferrin receptor; MMA: Methyl malonic acid; MPO: Myeloperoxidase, NEO: Neopterin; AAT: Alpha-1-antitrypsin; HTS-DNA: High throughput DNA sequencing

19 Quality assurance

Study personnel will be trained in study procedures before they can participate in data collection or study conduct. Relevant staff will receive training on the following SOPs: recruitment, screening and inclusion, consenting, stool and blood collection, storage and analysis, anthropometry, BIA, ultra sound scans, child development, clinical assessment, data entry and management. A post-training written test will be done after every SOP/module to assess their understanding. There will be regular monitoring to ensure adherence to SOPs. The study supervisor will conduct quality assurance and quality control (QA/QC) checks to ensure complete data entry.

20 Data management

20.1 Data entry

All participant data (with the exception of the informed consent forms and person identifiers) are collected in an electronic case report form (e-CRF) using the secure data collection platform RedCAP (Open Source Vanderbelt University) and a hard copy version

of the CRF. Data is collected and directly entered in 'offline mode' into the e-CRF using a portable device (PD). As such, the data gathered into the PD is considered the main source data for the study. Data should not be written down elsewhere unless stipulated. In case of any technical issues during the data collection process, hard copy versions of the CRF are used for data collection. The data are then entered into RedCAP by a trained staff member. All study staff are trained extensively in how to fill in the applicable sections of the e-CRF, as well as the paper version of the entire CRF as back-up. Certain study staff members are responsible for the PD to ensure that the data is completed and that each PD is available for uploading and charging, according the delegation log.

20.2 Coding

Data integrity is enforced with inbuilt value checks, data ranges and consistency checks. Immediate checks and prompts are enforced to ensure correct data input. Automatic notifications prompt for checks of incomplete participant data and to follow-up on defaulters.

20.3 Data Security

All electronic participant data is encrypted and is kept confidential with pseudo anonymity. Personal identifiers are not visible in the RedCAP platform. Any piece of data is only visible to those given access. Access can only be given by the system administrators in Denmark. All staff user access and data entry is regulated by the privileges associated with his/her unique user identification code and password. Furthermore, any entry and modifications made to the data are logged in the RedCAP system along with the user's identification code. If data is changed on a paper CRF, it must be dated and signed by the respective staff member. That way, all changes to data can be tracked After a participant has been discharged from the study, both the informed consent form and hard copy of personal identifiers are kept in a secure and safe location away from the rest of the data. Any hard copies of CRFs are source data and are to be stored in numerical order according to the ID number in a secure but accessible place. All source data is kept securely on file for a minimum period of 5 years after completion of the study. The RedCAP system is centralised at the University of Copenhagen on secure online servers which are monitored and backed up regularly. The database does not contain personal identifying information of any participant to ensure their anonymity.

20.4. Data storage

At the end of each day or when an internet connection becomes available, the data on each PD is uploaded to a secure site for storage. This task is carried out by a delegated trained study staff member to ensure that data is not lost.

21 Statistical methods

Descriptive statistics will be used to summarize population baseline and demographic characteristics. Comparative analysis; we shall use T-test to compare continuous variables and Chi-square tests for categorical variables. Linear mixed regression models will be used for continuous variables and logistic regression models for categorical variables to assess associations between exposure and outcomes.

22 Monitoring

The study intends to have internal on-site monitoring. Monitoring is carried out according to the monitoring plan. Remote monitoring will be conducted from Denmark.

23 Ethics and dissemination

23.1 Assessment of anticipated benefits and risks

Clinical benefits: It is anticipated that all children participating in this study will benefit from undergoing a medical examination, nutritional assessment and blood sugar evaluation. Nutrition counselling and referrals for appropriate treatment will also be conducted.

Community benefits: It is anticipated that the training of VHTs and clinical staff within the community will build capacity and enhance their awareness on the monitoring for child malnutrition. This will encourage them to screen and monitor child malnutrition at community clinic sites in the future.

Procedural risks: There are very few procedural risks in this study. Blood samples are collected by trained nurses. Some temporary discomfort is experienced by the children when blood samples are taken, but no other risk is expected. Ultrasound scans and BIA procedures are safe and cannot be felt by the children.

23.2 Discontinuation of participation in the study

Parents or caregivers are allowed to withdraw their child from the investigation at any time for whatever reason. The Investigator also has the right to discontinue participant investigations at any time if they deem that it is in the best interest of the participant. A child may be premature withdrawn from a study if a child's safety and well-being is compromised by further participation.

23.3 Research ethics approval

The study is conducted in accordance with the ethical principles set forth in the current version of the Declaration of Helsinki and all applicable local regulatory requirements. The rights, safety and well-being of the children involved in the study will prevail over science and society.

The study has been submitted for approval to the School of Medicine Research Ethics Committee (SOMREC) at Makerere University. Also, administrative clearance at district and health facility level will be obtained. Upon approval, the study will be registered by the Ugandan National Council of Science and Technology (UNCST). The study has also been submitted for consultative approval from the Danish National Committee on Biomedical Research Ethics. The study will only be initiated after all authorities concerned have approved the study.

This clinical study follows Ugandan laws of data protection. All sensitive information collected during the course of this study is to be kept strictly confidential. Study participants are identified by participant ID number and any identifying information is archived with study documents. Each participant remains anonymous during data analysis.

Should the study require review, relevant regulatory authorities and ethics committees are allowed to access all relevant information for audit and inspection purposes.

23.4 Training in good clinical practice (GCP) or human subject protection (HSP)

The principal investigator, co-investigator and all study staff who are directly involved in the collection of participant data will undertake a course in GCP or HSP.

23.5 Protocol amendments

Substantial amendments to this protocol can only be made after the IRB have given approval for the changes. Amendments to the protocol are regarded as substantial if they have significant impact on:

- The safety, physical health and mental integrity of the study participants
- The scientific value of the study
- The conduct or the management of the study

Any amendments to this protocol will be signed by the signatories included in section 2. If any event occurs related to the conduct of the study which may affect the safety of the children, the investigator may take appropriate measures to protect the participants against immediate hazards without notifying the IRB first. The IRB will be informed as soon as possible thereafter.

23.6 Protocol deviations and violations

Protocol deviations are unplanned instances of non-compliance to the protocol. These are noted during the study, recorded and evaluated as major or minor. Any major protocol violations are reported to SOMREC within seven (7) calendar days of becoming aware of the event (in accordance with UNCST guidelines). All minor deviations from SOPs or the protocol will be reported in "Notes to file" and submitted with the annual progress reports to SOMREC, e.g. if a participating child does not provide a stool sample on time. Participants with major protocol deviations or violations are excluded from Per-Protocol-statistical analysis.

23.7 Participant informed consent

Written, informed consent is obtained from all study participants prior to entry into the study. A parent/caregiver will consent on behalf of the participant, according to Ugandan laws and guidelines. Emancipated minors (mothers under 18 years old) can also consent on behalf of the child in their care. Assent to participate in research shall be obtained from all children eight (8) years of age and above. A child's assent is obtained after parental/guardian's consent. The child's assent or dissent takes precedence over the parent's or guardian's consent. Participants are screened for eligibility to the study and then approached for possible participation in the study. See section 11.3 for more detail. Before a

child can be included, the delegated study personnel will explain verbally to parents/caregivers, in relevant local language; the objectives, nature, risks and implications of the study. The same information will be described in the study participant information sheet. All information material will be translated to Lusoga and Luganda. To ensure that caregivers understand the information provided during the informed consent process, they will be asked a short follow-up questionnaire (informed consent questionnaire) by a host facility staff or study staff member who did not carry out the informed consent process with the caregiver.

In particular, the participants will be informed about the following:

- The possibility of withdrawing from the study at any time
- How the sensitive and health-related data will be collected and used during the study
- Future follow-up studies

The participants are given time to discuss any concerns or questions they may have and make a decision regarding the participation of the child in their care. If the parents/caregivers are illiterate, a fingerprint will be accepted instead of a signature. In this case, a literate witness is required to be present during the reading of the consent form and to sign the consent form in addition to the caregiver's fingerprint.

All parents/caregivers receive a copy of the participant information sheet and the signed consent form. The original is retained by the investigator. The informed consent form includes contact information of the study PI and the Chairperson of SOMREC should the caregiver have further questions.

Information about the participants is kept confidential and not disclosed to anyone outside the study team and clinical staff, unless explicit permission is obtained from the caretaker. When study assessments identify an abnormal condition requiring treatment, relevant data may be provided to clinical staff so that actions may be taken to treat the condition.

23.8 Photos and video permissions

On the day of inclusion, caregivers (who have given consent for study inclusion) will be asked if they give permission for photos and videos to be taken of them and their participating child

for use as a complement to research aims. To be shared in situations such as conference or publication settings. The caregiver may also consent for a photo to be taken of them and their child for purposes of identification during the study. All photo permissions are completely voluntary and do not impact on whether a participant will be included or not. The caregiver can opt to withdraw consent for photos/videos at any time.

23.9 Permissions to contact for later follow-up

On the day of inclusion, caregivers will be asked if they give permission for the study team to contact them again after study completion for one or more follow-up study/studies. The follow-up studies may take place after approx. 5 years and later. Permissions are completely voluntary and do not impact on whether a participant will be included or not. The caregiver can opt to withdraw consent for us to contact them at any time.

23.10 Confidentiality and data handling

All study-related information is stored securely; either in a database or on site with secure access. To maintain participant confidentiality, all laboratory specimens, reports, data collected and administrative forms are identified by an ID code only. All records that contain names or other personal identifiers, such as locator forms and informed consent forms, are stored separately and securely from study records and remain in Uganda. All local databases are secured with password-protected access systems.

The study includes researchers from Uganda and Denmark. Results of the assessments and results of analysis of blood and stool samples will be shared between the researchers. Data handling in Uganda is performed under the Ugandan Data Protection and Privacy Act. When data and samples are handled in Denmark, researchers work under the EU General Data Protection Regulation and data are processed according to Article 9, (2), (a) of the General Data Protection Regulation. When data are returned to Uganda, data handling is no longer within the scope of EU regulation.

23.11 Declaration of interests

The investigators declare that they do not have any financial interest in the results of the study and no affiliation with or financial interests in Novo Nordisk A/S or the Novo Nordisk Foundation.

23.12 Compensation/Reimbursement

23.12.1 Compensation for participating in the study

Participation in the study is free of charge. Caregivers will receive compensation fee of 20,000UGX for the time spent participating in this study. Also, caregivers will be reimbursed for their transportation costs ; a maximum of 10,000UGX will be provided per return trip, up to a maximum of 2 trips (trip 1= inclusion, trip 2=deliver stool sample to the study clinic).

23.12.2 Compensation in case of injuries

There are no or minimal risks associated with participating in this study. Thus, we do not expect any injuries related to participation in the study, and no compensation will be paid in this regard.

23.13 Community engagement plan

Prior to study implementation, we shall map key stakeholders at district, village and community levels i.e., DHO, VHTs, parents and caregivers of potential participants. We shall conduct study inception meetings with the community. At these meetings, information and feedback will be given and received.

A study staff member will be delegated the role of “community engagement coordinator”, and will be responsible for coordinating all community engagement activities. In addition, we intend to leverage on the VHT system and local leaders to mobilize mothers/caregivers of children targeted for this study. VHTs are community members who are committed and work on voluntary basis. They are also key agents through which the community can provide input to ensure that the research addresses their concerns. Therefore, we shall train them on screening processes and mobilization.

For those parents/caregivers of children who do not fit the eligibility criteria, they will be given clear explanations as to why they will not be included in the study.

Prior to study initiation, we shall disseminate findings from the MAGNUS 1 trial. During these meetings, we shall also collect participants’ views about the study processes for MAGNUS 2. We shall then use this information together with information from the pilot phase to refine the study processes. In addition, findings from this study will be disseminated

to community members through appropriate channels. Further details on community engagement are described in Appendix 2.

23.14 Risk aversion plan

Uganda still faces challenges of both COVID-19 and MPOX. Although measures such as vaccinations against COVID-19 are ongoing, the risk for spread continues due to community transmission, emerging variants and limited vaccine (MOH, 2024). This situation has been further exacerbated by the emerging MPOX.

We shall put in place measures to ensure safe conduct of this study while mitigating risks associated with the ongoing COVID-19 and MPOX outbreaks. We intend to conduct a rapid needs assessment in the study communities to identify potential hotspots for COVID-19 and MPOX. We shall ensure that all study staff are trained on; infection prevention and control (IPC), and screening and referral of COVID-19 and MPOX. All study staff will have access to personal protective equipment and will maintain preventive measures such as social distance, safe sample collection, storage and transportation. Additionally, we shall maintain close contact and open communication with the District Health Team and local authorities about any emerging cases. Further details are available in Appendix 3.

23.15 Dissemination policy

After completion of the study, the results will be published in abstracts, posters, magazines, web pages or scientific articles or presented at conferences, irrespective of positive, negative or inconclusive data. In all cases participant identity will remain confidential. Publication of scientific articles will follow the Vancouver rules of publication. Data from the investigation is considered confidential until it is published.

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25 Appendices

Appendix 1: Study plan and Timeline

MAGNUS-2 STUDY	**2024**		**2025**											
Activities	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
IRB submission														
Protocol, Informed consent forms and CRFs development														
Payment														
Prepare other documents for submission file (Application form, CVs and necessary documents)														
Submission for initial review														
Ethics + UNCST approval														
Prepare for study initiation														
Procure study supplies/Purchases														
Set up Office														

Develop SOPs														
Set up and trial REDCap														
Hire study Staff														
Training of study staff														
Establish sites (equipment etc)														
Pilot study														
Amendment if any; Protocol, CRF, Consents														
Recruitment														
Data Analysis and report														

Appendix 2: Community engagement plan

Appendix 3: Risk aversion plan