RESEARCH METHODOLOGY/STUDY DESIGN

CURRENT DEVELOPMENTS IN NUTRITION

Maternal and Pediatric Nutrition

The Role of Milk Protein and Whey Permeate in Lipid-based Nutrient Supplements on the Growth and Development of Stunted Children in Uganda: A Randomized Trial Protocol (MAGNUS)

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ABSTRACT

Stunting is associated with cognitive impairment and later chronic disease. Previous trials to prevent stunting have had little effect, and no trials seem to have provided larger amounts of energy and high-quality proteins to already stunted children. We aimed to assess the effects of milk protein (MP) and whey permeate (WP) in large-quantity lipid-based nutrient supplements (LNS-LQ), among stunted children, on linear growth and child development. This was a randomized, double-blind, 2-by-2 factorial trial. Stunted children aged 12–59 mo from eastern Uganda ($n = 750$) were randomly assigned to receive 100 g LNS-LQ with or without MP and WP ($n = 4 \times 150$) or no supplement ($n = 150$) for 3 mo. The primary outcomes were change in knee-heel and total length. Secondary outcomes included child development, body composition, anthropometry, and hemoglobin. Micronutrient status, intestinal function, and microbiota were also assessed. Our findings will contribute to an understanding of the role of milk ingredients and LNS in linear catch-up growth. This trial was registered at www.isrctn.com as ISRCTN13093195. *Curr Dev Nutr* 2021;5:nzab067.

Keywords: stunting, linear growth, lipid-based nutrient supplement (LNS), milk protein, whey permeate, child development, body composition, gut

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Abbreviations used: DIAAS, Digestible Indispensable Amino Acid Score; EED, environmental enteric dysfunction; FFM, fat-free mass; FM, fat mass; HAZ, height-for-age *z* score; IGF-I, insulin-like growth factor I; LNS, lipid-based nutrient supplement(s); LNS-LQ, large-quantity lipid-based nutrient supplement(s); LNS-SQ, small-quantity lipid-based nutrient supplement(s); MAGNUS, Milk Affecting Growth, Cognition, and the Gut in Child Stunting; MDAT, Malawi Development Assessment Tool; MP, milk protein; MPI, milk protein isolate; MUAC, midupper arm circumference; SAM, severe acute malnutrition; WASH, water, sanitation, and hygiene; WAZ, weight-for-age *z* score; WHZ, weight-for-height *z* score; WP, whey permeate.

Introduction

Globally, 144 million children under the age of 5 y are classified as stunted, having a length- or height-for-age *z* score (HAZ) of less than −2 (1). Stunting is associated with adverse short- and long-term health outcomes. It is associated with delayed cognitive development, increased morbidity and mortality (2), poor schooling performance (3, 4), and later with reduced economic productivity (4) and risk of chronic disease (5, 6). Stunting also contributes to an intergenerational cycle of malnutrition and poverty, whereby a child born to a stunted mother is more likely to be stunted themselves (7).

In the east African region, close to 1 in 3 children under the age of 5 are stunted (1). High stunting prevalence is experienced in many low- and middle-income countries and is indicative of exposure to environments of inadequate care, suboptimal nutrition, and recurrent infections (3, 5, 6). The majority of growth faltering occurs from 3 to 24 mo of age (8). Nutrition interventions to reduce the risk of stunting have therefore focused on prevention through optimizing maternal and early infant nutrition (9, 10). These interventions, however, have had little impact on linear growth. This was summarized in a recent meta-analysis, whereby complementary feeding interventions, in food-insecure settings, improved HAZ by a mere 0.08 overall (10). The lack of effect on linear growth has been attributed, at least in part, to environmental enteric dysfunction (EED) (11, 12). The premise is that frequent exposure to pathogens in environments with unsafe water and inadequate sanitation and hygiene [water, sanitation, and hygiene (WASH)] encourages a

state of systemic and intestinal inflammation, as well as morphological and functional changes to the intestine, which can, in turn, exacerbate nutrient deficiencies. However, large trials combining comprehensive WASH interventions with small-quantity lipid-based nutrient supplements (LNS-SQ) reported no effects from the WASH interventions and only minimal effects from the LNS-SQ (13–17).

While there have been many studies aiming to prevent stunting in young children, or to improve linear growth in wasted children, this is to our knowledge, the first trial which provided large-quantity LNS (LNS-LQ) to children recruited on the basis of stunting. There have been concerns that supplementation in stunted children will lead to excessive accretion of fat rather than lean tissue, and therefore increase the subsequent risk of chronic disease. These concerns, however, are not substantiated by the evidence. Recent supplementation studies among children with moderate (18) and severe acute (19) malnutrition have shown that even those who are also stunted predominantly gain fat-free mass (20). There is a gap in the evidence, however, as to the extent that an LNS-LQ or one containing milk protein (MP) will encourage catch-up growth in already stunted children, and to what extent this impacts body composition (21–23), mitigates vulnerability to illness, and improves child development and other functional outcomes (23, 24). However, it is possible that nutritional support to stunted children could have beneficial effects even in the absence of linear catch-up growth. We now know that the co-existence of wasting (low weight-for-height) and linear growth faltering increases a child's risk of morbidity and mortality (25, 26). Moreover, new evidence from a large 40-y cohort study in The Gambia suggests that stunting not only develops as a chronic condition but also develops interactively with episodes of wasting as a short-term adaptation (27, 28).

Previous nutrition interventions may have been limited by an inadequate supply of energy and high-quality proteins. Considering this, and the recent evidence demonstrating that even short children with wasting predominantly gain fat-free mass (20), there is sufficient justification to assess the effects of an LNS-LQ among stunted children.

Milk intake has long been associated with linear growth (29, 30) and is suggested to have a stronger effect in low-income compared with high-income countries (29). However, a new review based on studies from predominantly high-income countries was not able to confirm an effect of milk intake on linear growth (31). Several studies have shown that the addition of milk in supplements to treat acute malnutrition has had positive effects on body composition, weight gain, recovery, and anemia (32–37), but limited (18) or no effect in encouraging linear catch-up growth (32, 35, 38). In studies from low- and high-income countries, milk intake in children has been associated with improved lean mass deposition (31), bone-mineral composition (39), and cognitive function (40, 41), benefits that may be experienced to a greater extent in children exposed to growth-deficient environments (31, 42). Furthermore, the different components of milk may provide unique health benefits (43). MPs have a complete amino acid profile (31) and are thought to promote growth by stimulating the growth factors insulin-like growth factor-I (IGF-I) and insulin (44). On the other hand, whey permeate (WP) is predominantly composed of lactose and bioavailable minerals, which may have prebiotic effects (45) as well as a role in bone mineralization and fat-free mass accretion (46).

In this study, we aimed to assess the individual and combined effects of MP and WP, provided as part of an LNS-LQ, using a 2 × 2 factorial design, among stunted children. The primary outcome was linear growth. Secondary outcomes were child development, body composition, HAZ, weight-for-age (WAZ), and weight-for-height *z* scores (WHZ), weight, midupper arm circumference (MUAC), head circumference, and hemoglobin. In addition, we assessed the main effect of LNS on these outcomes, irrespective of milk ingredients, as well as the role of the gut as mediator or modifier of effects.

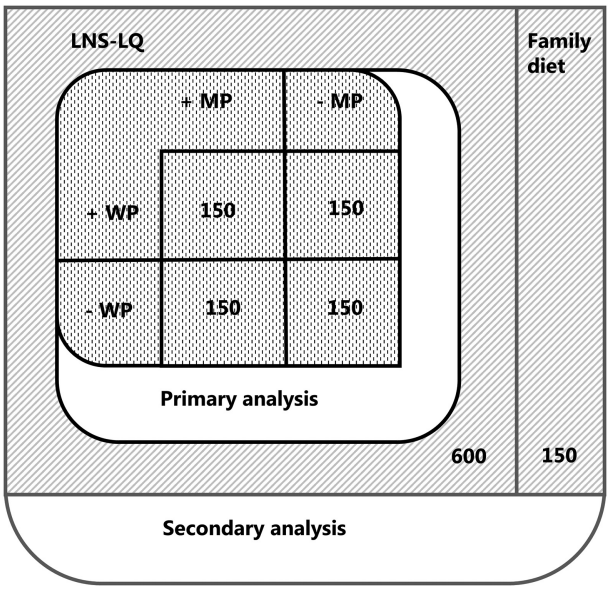

FIGURE 1 The primary analysis will compare LNS-LQ with and without MP and WP in a 2-by-2 factorial design with 150 participants in each given combination. Secondary analysis will compare all LNS-LQ interventions ($n = 600$) with the reference group (family diet, $n = 150$). LNS-LQ, large-quantity lipid-based nutrient supplements; MP, milk protein; WP, whey permeate.

Methods

The reporting of this protocol followed the Standard Protocol Items: Recommendations for International Trials (SPIRIT) 2013 checklist.

Trial overview and design

The MAGNUS study (Milk Affecting Growth, Cognition, and the Gut in Child Stunting) was a randomized, double-blind, 2-by-2 factorial trial testing the effects of MP and WP in LNS-LQ. An unsupplemented group was included as a reference. For a 12-wk period between February and December 2020, 750 Ugandan children classified as stunted received 1 of 4 formulations of LNS-LQ as a daily supplement ($n = 4 \times 150$) or continued with the family diet ($n = 150$) (see **Figure 1**). All caregivers received individual nutrition counseling at baseline. All participants were followed up at the same intervals throughout the intervention period (see **Figure 2**). This design will allow us to assess the individual and combined effects of MP and WP among the 600 children allocated to LNS, based on the factorial design: If the effects are independent, then we can compare the 300 given LNS with MP to the 300 given LNS without milk. And likewise, we can compare the 300 given LNS with WP to

			Time-point (weeks)							
Activity			**S**	**T_0**	**T_2**	**T_4**	**T_6**	**T_8**	**T_{10}**	**T_{12}**
Referral		Screening for referral	▪							
Enrollment		Screening for eligibility		▪						
		Informed consent		▪						
		Nutrition counselling		▪						
		Allocation		▪						
Data collection	Primary outcomes	Knee-heel length		▪	▪	▪		▪		▪
		Height	▪	▪	▪	▪		▪		▪
	Secondary outcomes	Weight	▪	▪	▪	▪		▪		▪
		Mid-upper arm circumference	▪	▪	▪	▪		▪		▪
		Head circumference		▪						▪
		Family care indicators		▪						
		Child development assessment		▪						▪
		Bioimpedance		▪						▪
		Skinfolds		▪	▪	▪		▪		▪
	Tertiary outcomes	Blood sample collection		▪						▪
		Stool sample collection		▪						▪
		Clinical examination		▪						
		Clinical review			▪	▪		▪		▪
	Baseline	Demographics		▪						
		WASH assessment		▪						
		Dietary intake assessment		▪						
		Maternal anthropometrics		▪						
Intervention		Supplement provision		▪	▪	▪	▪	▪	▪	
		Reference group gift allocation		▪	▪	▪	▪	▪	▪	
Adherence		Empty sachet collection			▪	▪	▪	▪	▪	▪
		Phone follow-up		▪	—	—	—	—	—	▪
		Home visit		▪	—	—	—	—	—	▪

FIGURE 2 MAGNUS data collection time points and visits. Timeline and visit overview for participants enrolled in the study. Phone follow-up and home visits were carried out as required and thus were unfixed time points. All participants were invited to the same follow-up visits. Time points were considered valid if taken within ±7 d of baseline, week 2 and week 4, and ±14 d from all other time points. Hemoglobin was a secondary outcome. LNS-LQ, large-quantity lipid-based nutrient supplement; MAGNUS, Milk Affecting Growth, Cognition, and the Gut in Child Stunting; MUAC, midupper arm circumference; S, screening in village for referral; T, week of visit from baseline (T0) to discharge (T12); WASH, water, sanitation, and hygiene.

the 300 given LNS without WP. If the effects are not independent, then we will compare each of the 4 combinations pairwise. In addition, we will be able to assess the effect of LNS by comparing the 600 given LNS, irrespective of milk ingredients, to the 150 given no supplements.

The intervention

LNS are fortified lipid-based pastes that are well adapted for use in resource-limited settings; they are produced to a high safety standard, do not require refrigeration or preparation, and are packaged in standard portion sizes.

Our LNS-LQ supplements, manufactured by Nutriset (Malaunay, France), varied with respect to the incorporation of WP and MP isolate (MPI). The MPI contained casein and whey proteins in the same proportions as milk; the lactose and mineral components were removed so that the MPI was close to 90% protein by weight with a Digestible Indispensable Amino Acid Score (DIAAS) of 120 (47). As a comparator to MPI, soy protein isolate, a high-quality plant protein with a DIAAS of 84, was used (47). WP contained 80–85% lactose and minerals (potassium, phosphorus, magnesium, calcium, sodium, and to a lesser extent zinc). As a comparator to WP, maltodextrin, a standard ingredient used in LNS products was used. All formulations were standardized to contain similar proportions of energy, protein, and carbohydrates. The supplements contained a mineral and vitamin mix to improve micronutrient content, and in 2 of the formulations the milk minerals provided by WP were in addition to the standard amount provided in all formulations (**Table 1**).

The 600 participants randomly assigned to LNS-LQ received one 100-g sachet (530–535 kcal)/d for 12 wk, distributed every 14 d. Those randomly assigned to the family diet received laundry soap at each visit.

Participant recruitment and enrollment

The study was conducted from 2 local community health centers in Walukuba and Buwenge. All participants were recruited from within the surrounding district of Jinja, in the Busoga Subregion, eastern Uganda. Here, the prevalence of child stunting is estimated to be 29%, similar

TABLE 1 Nutrient composition for 4 formulations of LNS-LQ supplied to 1- to 5-y-old stunted children[1]

	Milk protein and whey permeate LNS-LQ	Milk protein and no whey permeate LNS-LQ	Soy protein and whey permeate LNS-LQ	Soy protein and no whey permeate LNS-LQ
Macronutrients (components per 100 g)				
Energy, kcal	531	535	530	534
Carbohydrates, g	42	43	42	43
Lactose, g	15.7	0.4	15.3	0
Proteins, g	13.9	13.5	13.9	13.5
Milk proteins, g	7.15	6.75	0.40	0
Vegetable proteins, g	6.75	6.75	13.50	13.50
Lipids, g	33.7	33.7	33.7	33.7
Linoleic acid (C18:2), g	3.0	3.0	3.0	3.0
Linolenic acid (C18:3), g	0.5	0.5	0.5	0.5
Minerals				
Calcium, mg	691	594	691	594
Copper, mg	1.65	1.65	1.65	1.65
Iron, mg	12	12	12	12
Iodine, μg	127	113	127	113
Magnesium, mg	199.2	175.8	199.2	175.8
Manganese, mg	1.8	1.8	1.8	1.8
Phosphorus, mg	661	539	661	539
Potassium, mg	1315	985	1315	985
Sodium,[2] mg	84	7	156	79
Selenium, μg	30	30	30	30
Zinc, mg	12.5	12.5	12.5	12.5
Vitamins				
Vitamin A, mg	619	619	619	619
Vitamin B-1, mg	1.2	1.1	1.2	1.1
Vitamin B-12, μg	3.2	3.0	3.2	3.0
Vitamin B-2, mg	3.1	2.8	2.7	2.4
Niacin, mg	14.9	14.6	14.9	14.6
Pantothenic acid, mg	5.7	4.5	5.7	4.5
Vitamin B-6, mg	2.1	2.0	2.1	2.0
Biotin, μg	74.1	67.6	74.1	67.6
Folic acid, μg	223	223	223	223
Vitamin C, mg	67.9	67.6	67.9	67.6
Vitamin D, μg	16.9	16.9	16.9	16.9
Vitamin E, mg	18	18	18	18
Vitamin K, μg	30	30	30	30

[1]The same amount of micronutrient premix was used in all formulations. The additional micronutrients provided are from the other ingredients used. LNS-LQ, large-quantity lipid-based nutrient supplement.
[2]Soy protein isolate and whey permeate contribute additional sodium.

to the national average (48). To identify stunted children, communities within the district of Jinja were mobilized by Village Health Teams for an initial screening for referral. Study staff screened children in the community for age, stunting, and severe acute malnutrition (SAM). All children identified as having SAM were referred for appropriate treatment; others who met the inclusion criteria for stunting and age were invited to one of the study sites for eligibility screening.

At the study sites, children were considered eligible if they were aged between 12 and 59 mo and had an HAZ of less than −2, according to the WHO growth standards (49). Children <12 mo old were not eligible to avoid interfering with breastfeeding. Caregivers had to be living in the catchment area and willing to return for follow-up visits, and able to provide written informed consent and agree to both phone follow-up (if a phone contact was available) and home visits. Children were excluded if they were identified with SAM according to the WHO classification (50), had medical complications requiring hospitalization, a history of allergy to peanuts or milk, obvious disability that impeded eating capacity, or a disability that impeded the measurement of length or height. Children were also excluded if they were participating in another study, if the family planned to move away from the catchment area within 6 mo, if previously enrolled in the MAGNUS study, or if another child from the same household was already included.

Informed consent

If all eligibility criteria were met, trained staff took the caregiver through the informed-consent information individually, using the most appropriate of 3 commonly spoken languages in the region (English, Lusoga, or Luganda). The same information was given verbally and in writing. Caregivers were also taken through a short verbal questionnaire to ensure that the information provided was adequately understood. After necessary clarifications were given, the caregiver consented on behalf of the participant. If illiterate, a literate witness was present dur-

ing the informed-consent process. Consenting caregivers were asked for permission to store 1–2 mL of blood and stool samples from the participant for future use; this was independent of trial consent. The caregiver could opt to withdraw consent at any time.

Blinding, randomization, and assignment of interventions

The sachets of LNS-LQ were labeled with a unique 3-letter code that corresponded to the different formulations. Two unique codes were given to each of the 4 formulations and a further 2 codes were created for the reference group so that 10 unique codes were used in the allocation sequence list. Only the manufacturer (Nutriset) had access to the blinding code. Two allocation sequence lists, one for each study site, were computer generated using R (R Foundation for Statistical Computing). These were generated and sealed by a member of staff at the University of Copenhagen, Denmark, who was otherwise not involved in the study. Site-stratified, block randomization, with variable block sizes of 10 and 20 were used to allocate the sequential list of ID numbers to the 10 unique codes.

Upon inclusion, administrative staff allocated a unique ID from a sequentially ordered list. After completion of baseline activities, the study pharmacist allocated the intervention according to a hard-copy random allocation list. Only the pharmacist had access to the allocation list, which was checked for each participant, at each visit. Using QR codes, the pharmacist recorded the code of what was distributed in a spreadsheet, which was regularly monitored by an independent assessor in Copenhagen. Hard copies of the allocation lists were kept securely in sealed envelopes at the University of Copenhagen.

Outcome assessors and data analysts were blinded both with respect to the allocation of the intervention and to the type of ingredients contained in differently coded LNS sachets. Caregivers were blinded with respect to the type of LNS allocated, since the taste, smell, and appearance of all 4 products were indistinguishable. Caregivers were not, however, blinded with respect to receiving LNS or not. Only the Data Safety Monitoring Board, which operated independently of the study, could choose to break the blinding in order to monitor safety parameters.

Adherence

The LNS was distributed in packs of 14 sachets. To counteract the likelihood of sharing, an additional pack of the same LNS product code was distributed every 2 wk to caregivers with other children aged between 6 and 59 mo living in the same household. The additional stock provided to the household increased the likelihood that the participating child had access to the required daily quota. When collecting new sachets, the caregiver was requested to return any empty and unused sachets from the previous 2-wk supply, including those from the additional pack.

Outcomes

Primary outcomes.

The primary outcomes were changes in knee-heel length (mm) and total length/height (cm) from baseline to 12 wk.

Secondary outcomes.

All secondary outcomes were measured over time from baseline to 12 wk. Child development was assessed at baseline and at discharge using a locally adapted version of the Malawi Development Assessment Tool (MDAT). Anthropometric indices HAZ, WAZ, and WHZ were assessed as well as weight (g), MUAC (cm), and head circumference (mm). Body composition was assessed using bioimpedance and the triceps and subscapular skinfold thicknesses (mm). The raw data from bioimpedance were used to calculate the fat mass (FM) (kg), fat-free mass (FFM) (kg), fat mass index (kg/m^2), and fat-free mass index (kg/m^2). Hemoglobin concentration was assessed from blood samples collected at baseline and 12 wk.

Tertiary outcomes

Biological samples.

Blood and stool samples were collected at baseline and at week 12. Blood samples will be analyzed for growth factors (IGF-I and insulin), markers of micronutrient status [i.e., iron (ferritin, soluble transferrin receptor), folate (serum folate), vitamin B-12 (cobalamin, methylmalonic acid), and vitamin A (retinol binding protein)], markers of systemic inflammation [C-reactive protein and α1-acid glycoprotein (AGP)], and markers of intestinal function (citrulline), together with other amino acids. Stool samples will be analyzed for markers of intestinal inflammation [myeloperoxidase (MPO), neopterin (NEO)] and function (α1-antitrypsin (AAT)] and the gut microbiota.

Safety, morbidity, and loss to follow-up.

Data will be reported on the proportion of children who, during the intervention period, deteriorated to moderate acute malnutrition or SAM according to the WHO classifications (49). The proportion of participants who died during the study period will be reported, as well as the number of morbidity episodes including the duration and severity of the illness. Finally, the number of children who were lost to follow-up will be reported. Caregivers were called with reminders to attend upcoming or missed appointments. Loss to follow-up was defined as those who had not returned for the 12-wk follow-up visit by 14 wk post-inclusion.

Baseline participant characteristics.

Additional information collected at baseline included demographics, a dietary intake assessment, and a WASH assessment taken at the initial home visit.

Measurements

Time points for each measurement are shown in Figure 2.

Anthropometrics.

Knee-heel length was measured using a digital caliper with a resolution of 0.01 mm (Mitutoyo) mounted with knee and heel caps, cast in hard plastic. The distance between the knee (from the lateral condyle) and the heel (calcaneus) was measured 5 times consecutively on the left leg while the child was seated with both legs hanging over the edge of a table or the caregiver's lap. All other anthropometric measurements were repeated in triplicate. Participant length and height measurements were taken using a wooden Shorrboard (Weight and Measure), ensuring 4 points of contact with repositioning between measurements. Maternal height was measured using a fixed wall stadiometer (SECA 206). The weights of the mother and participant were measured using an electronic double-weighing scale (SECA 876). Head circumference, MUAC, and skinfold thickness were measured using a windowed, nonelastic head cir-

cumference tape (SECA 212); a nonelastic MUAC tape (UNICEF SD); and a Harpenden skinfold caliper (Baty International), respectively. Height or length, weight, MUAC, and head circumference were measured according to accepted international standards for anthropometric measurement (51). Skinfold thicknesses were measured on the left side, according to the manufacturer's instructions. For referral and inclusion, *z* scores were calculated using the WHO field growth charts. The WHO Anthro program will be used to calculate *z* scores for data analysis (52).

Bioimpedance.
Bioimpedance was measured using the Bodystat 500 (50 kHz) and in accordance with the manufacturer's instructions (Bodystat Ltd.). Measurements were taken while the child was lying on his/her back, with limbs spread apart, preferably at rest and with removal of wet or soiled diapers. A measure was repeated a minimum of 2 times but up to 3 times if the child's positioning or movement rated poorly. Measurements for impedance, resistance, reactance, and phase angle were recorded. Using an equation, the raw data will be used to calculate FM and FFM.

Child development.
Child development officers, trained in use of the MDAT (Manual V06, March 2018), took the participant through a series of activities adapted for the Ugandan context. The activities were related to 4 domains of development: gross motor, fine motor, language, and social development (53). The participant was graded as to whether or not he/she could complete each task successfully. The assessment continued until the child had failed to complete 6 tasks consecutively. At baseline, an interviewer-administered questionnaire was also used to gather information from the caregiver about household and family indicators for the support of child development (54).

Clinical assessment.
A thorough clinical examination was carried out at baseline. It included rapid tests for HIV and malaria, a thorough medical history with questions related to signs and symptoms of wasting or hospitalization due to SAM, and assessment of vital signs (pulse, blood pressure, and respiratory rate). At follow-up visits, a short review was conducted, assessing the most recent medical history, milk intake, and where applicable, monitoring of adverse events. To maintain blinding, the pharmacist distributing LNS inquired about adherence and if the caregiver had experienced problems with the LNS.

Biological sample collection.
Stool samples were collected at 2 time points and stored for later analysis of markers of gut function and microbiota. If not collected on site, a sample collection kit was given to caregivers along with specific instruction on stool sample collection at home. Collection vials contained StayRNA (A&A Biotechnology), allowing samples to be stored at room temperature for up to 5 d after collection. A maximum of 6.0 mL of venous blood was collected on site at 2 time points. A small amount was used for rapid tests: HIV status, malaria, and hemoglobin status. The remaining sample was processed and stored within hours for later analysis of selected markers. All biological samples were stored at −20°C until delivery to the main storage site in Kampala where they were stored at −80°C until they were shipped on dry ice to the University of Copenhagen, Denmark, for additional analyses.

Other measurements, baseline questionnaires, and WASH assessment.
At baseline, the child's age and birth weight were recorded, wherever possible, using a birth information card. Information on sociodemographic characteristics, breastfeeding status, food frequency, and diet diversity was collected via interviewer-administered questionnaires. At the baseline home visit, GPS coordinates of the home site location were collected to facilitate later follow-up. In addition, trained staff conducted a short assessment of observed household WASH characteristics, including water source, access to basic sanitation, and the use of soap. To minimize response bias, local study staff with a good knowledge of the language and the culture were trained in asking questions to get as clear and precise answers as possible.

Participant retention, reimbursement, referral, and withdrawal

If visits were missed and phone contact was unsuccessful, attempts were made to visit the caregiver's home. To facilitate attendance, a travel reimbursement was provided at each visit to cover the cost of return transport and food while at the clinic visit. Any participants requiring hospital attention were referred for treatment. If a caregiver requested for their child to stop receiving LNS, this was permitted; however, all included participants continued to be followed up for the remainder of the 12-wk intervention period. In case of participant withdrawal, all available data up to the point of withdrawal were used in data analysis.

Data management

Participant data were collected in a paper case report form and were double entered using Epidata software (https://www.epidata.dk/) with inbuilt range checks. The secure electronic data collection platform REDCap (Open Source; Vanderbilt University) was used to monitor participant registration and visits but not for primary data collection. All source data will be kept securely on file for a minimum of 5 y after completion of the study. Adverse and serious adverse events occurring during the intervention period were recorded and reported to the sponsor and the institutional review board. Events occurring after a subject was discontinued from the study were not reported unless the investigator suspected that the event was related to the LNS-LQ intervention.

Sample size calculation

To detect a 0.35-SD or greater difference between any 2 groups, with 5% significance and 80% power, 129 children were required in each group. To allow for 10% loss to follow-up, 150 children were included in each group, based on the 4 combinations of MP and WP. If there were no interactions between the 2 experimental interventions, 2 groups of 300 children could be compared, enabling differences of 0.24 SD to be detected. In the Treatfood trial (18), the SD of knee-heel length at baseline was 18.1 mm (18), so that a 0.24-SD difference corresponded to 4.3 mm. In secondary analysis, to assess the effect of LNS, 600 supplemented children were compared with 150 unsupplemented children, with the ability to detect a 0.27-SD difference, corresponding to 4.9 mm.

Statistical methods

Primary and secondary outcomes will be analyzed using linear mixed models that account for the correlation between repeated measurements from the same participant, whereas tertiary outcomes will be analyzed using ordinary ANCOVA models. In all of these ANCOVA models, the baseline value will be included as a covariate. Additional covariates may be included as appropriate. Results will be reported as estimated differences with corresponding 95% CIs and *P* values. A statistical analysis plan was prepared before unblinding of the trial and uploaded to the ISRCTN registry.

This is an effectiveness trial. Therefore, the primary statistical analysis will be carried out as intention to treat. In subsequent per-protocol statistical analysis, participants with major protocol deviations or violations are excluded.

Ethics approval and consent to participate

The study was 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 study was approved by the School of Medicine Research Ethics Committee at Makerere University and The Ugandan National Council of Science and Technology. The study also received consultative approval from the Danish National Committee on Biomedical Research Ethics. The study was initiated only after approval was given by all aforementioned authorities. Written informed consent was obtained from all caregivers who consented to study participation of the child in their care. The rights, safety, and well-being of the children involved in the study prevailed over science and society. Before participant recruitment, the study was registered at www.isrctn.com as ISRCTN13093195.

Discussion

The findings from the MAGNUS trial will help to clarify to what extent MP and WP, given in LNS, or the LNS per se, play a role in linear catch-up growth and benefit functional outcomes such as cognition and the gut. In this, we will explore to what extent functional benefits are possible with or without effects on linear growth. Our results will also contribute to current knowledge on whether stunted children will predominantly gain lean mass when supplemented with LNS-LQ. Since our study population is aged between 12 and 59 mo, we will also be exploring the potential for catch-up growth in children beyond 2 y of age.

The gut is thought to play a role in the pathogenesis of stunting, but studies aiming to minimize environmental pathogen exposures, and so reduce the risk of EED, have not seen improvements in linear growth. It may be that, once damaged, the gut requires larger quantities of essential nutrients in order to repair and facilitate nutrient absorption. We will explore whether milk components provided in LNS-LQ can improve reparation of the gut in already stunted children and to what extent gut function and inflammation act as mediators and effect modifiers of the effect of LNS-LQ on linear growth.

The high lactose content in WP may have positive effects on the microbiota and growth. If this is demonstrated in our study, it may have implications on the future development of LNS, since WP has the potential to be used as a nutritious substitute for maltodextrin or sugar.

This is the first randomized controlled trial we are aware of that explores the effects of LNS-LQ supplementation in already stunted children. The strengths of this study are the randomized 2 × 2 factorial design, which allows us to assess both the individual and combined effects of the milk ingredients, as well as the unsupplemented reference group, allowing us to assess the main effects of LNS per se. It is also a strength that the study includes several secondary functional outcomes alongside anthropometrics and body composition, as well as tertiary mechanistic outcomes. It is a limitation that we are unable to include a longer follow-up period. A follow-up study of the cohort would be of great benefit to measure the benefits and chronic disease risks associated with the 12-wk LNS-LQ supplementation.

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