Can selenium deficiency in Malawi be alleviated through consumption of agro-biofortified maize flour? Study protocol for a randomised, double-blind, controlled trial

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Abstract

Background Micronutrient deficiencies including selenium (Se) are widespread in Malawi and potentially underlie a substantial disease burden, particularly among poorer and marginalised populations. Concentrations of Se in staple cereal crops can be increased through application of Se fertilisers – a process known as agronomic (agro-) biofortification – and this may contribute to alleviating deficiencies. The Addressing Hidden Hunger with Agronomy (AHHA) trial aims to establish the efficacy of this approach for improving Se status in rural Malawi.

Methods A double-blind, randomised, controlled trial will be conducted in a rural community in Kasungu District, Central Region, Malawi. The hypothesis is that consumption of maize flour agro-biofortified with Se will increase serum Se concentration. We will recruit 180 women of reproductive age (WRA; 20–45 years) and 180 school-aged children (SAC; 5–10 years) randomised in a 1:1 ratio to receive either maize flour enriched through agro-biofortification with Se or a control flour not enriched with Se. Households will receive flour (330 g i capita/i-1 day-1) for 12 weeks. The primary outcome is Se concentration in serum (µg L-1). Serum will be extracted from venous blood samples drawn at baseline (prior to flour distribution) and end-line. Selenium concentration will be measured using Inductively Coupled Plasma Mass Spectrometry (ICPMS).

Discussion Findings will be communicated to policy stakeholders and participating communities and reported in peer-reviewed journals.

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Background

Micronutrient deficiencies are widespread in Malawi [1]. Selenium is an essential micronutrient with important roles in thyroid function and cognitive development and as a component of selenoprotein antioxidants [2,3]. In a recent study of a nationally-representative sample of Malawian women and pre-school children, plasma Se concentrations were below the threshold for optimal activity of the antioxidant enzyme glutathione peroxidase 3 (GPx3) in 62% of women (15-49 years; n=754) and 86% of pre-school children (6-59 months; n=990) [4]. Deficiency of Se occurs due to inadequate dietary Se intakes, and this is driven by low concentrations of plant-available Se in the weathered agricultural soils that are typical of Malawi [5, 6, 7].

There are various potential strategies to alleviate Se deficiencies including dietary diversification, food fortification at processing stage and supplementation. Alternatively, the concentration of bioavailable Se can be increased in the edible portion of staple crops – a process known as biofortification. For some micronutrients, biofortification can be achieved through crop breeding [8], but agro-biofortification (i.e. application of micronutrient fertilisers) is required for Se due to the dominance of environmental over genetic controls of Se concentration in crops [9]. Agro-biofortification of maize can increase concentrations of Se in the grain and may be a cost-effective and equitable strategy in Malawi where maize contributes 60% of dietary energy supply, and more among poorer populations [5, 7].

Agro-biofortification with Se has policy precedent in Finland where incorporation of Se into granular fertilisers has been mandatory since 1984. Mean plasma Se concentrations of adults (n=60) increased from 70.3 μg/L prior to the fertiliser
policy change to 110.5 μg/L in 2010 which is considered optimal status [10].

However, this strategy has not been implemented in low-middle income countries where agriculture, food systems and dietary patterns are substantially different from those in high-income countries.

This study aims to test the efficacy of improving Se status among a rural Malawian population through consumption of maize flour enriched with Se through agro-biofortification. The evidence may inform fertiliser policies in Malawi.

Method and Design

Study setting

The Addressing Hidden Hunger with Agronomy (AHHA) Malawi trial will be conducted in Wimbe Traditional Authority (TA), Kasungu District, Central Region, Malawi. The study area was selected due to the high prevalence of Se deficiency [4]. Most households rely on subsistence farming alongside smallholder and estate tobacco production [11]. To define a study area, two neighbouring Enumeration Areas (EAs) were randomly selected from a total of 51 EAs in Wimbe TA; EAs are the primary sampling unit of the national Demographic and Health Survey (DHS). We conducted a census to create a list of all households in the EA (n=1179) and a roster of all household members.

Study design

The AHHA Malawi trial is a two-arm randomised, double-blind, controlled trial with participants receiving maize flour enriched through agro-biofortification with Se or a control flour that is not enriched with Se (Figure 1).
Prior to baseline assessments, 180 households will be randomly selected from eligible households (see inclusion criteria). An additional 50 households will be pre-selected in case of consent refusals. Households will be visited for recruitment of one non-pregnant woman of reproductive age (WRA; 20-45 years) and one school-aged child (SAC; 5-10 years). Recruitment and baseline questionnaires will be conducted in the household and participants subsequently directed to nearby mobile field clinics set up in large tents where anthropometry and blood sampling will be conducted. Recruitment will be conducted by trained Research Assistants (RAs) hired by Lilongwe University of Agriculture and Natural Resources (LUANAR). The trial aims to recruit 180 WRA and 180 SAC.

After baseline, households will be randomly allocated in a 1:1 ratio to receive Se flour (n=90) or control flour (n=90). The trial statistician will allocate households remotely using a computer-based randomisation programme. Intervention allocation will be concealed from participants, RAs conducting the baseline and end-line surveys, laboratory analysts and those involved in managing or over-seeing the trial. Locally, only the supervisor of the maize flour distribution will know the allocation of households to treatments.
| Week | Cens | Baseline | Allocation | Post-
|------|------|----------|------------|-------|
| -2   |      |          |            |       |
| 0    |      |          |            |       |
| 1    |      | X        |            |       |
| 2    |      |          |            |       |
| 4    |      |          |            |       |
| 6    |      |          |            |       |

**ENROLMENT:**

- Eligibility screen: X X
- Informed consent: X
- Allocation: X

**Flour distribution**

**ASSESSMENTS:**

- Serum selenium: X
- Haematology: X
- Dietary selenium: X
- Morbidity: X
- Anthropometry: X
- Adherence: X X

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**Inclusion criteria**

**Household level:**

Household members include at least one non-pregnant WRA (aged 20-45 years) and at least one SAC (aged 5-10 years) in residence during July-October.
Household typically prepares and consumes meals at home.
Household head agrees that the household will receive and all members consume maize flour in place of their own flour for the 12-week flour distribution period.
Individual level:

One non-pregnant WRA (aged 20-45 years) and one SAC (aged 5-10 years) per household. Pregnancy status is self-reported. Participant is planning to be in residence in the household during July-October. Participant WRA is willing and able to provide consent, and the caretaker of the participant SAC is willing and able to provide assent.

Formative work

The trial delivery team have undertaken substantial preparatory work with the community. Formative work was conducted in July-September 2018 to identify potential concerns among community members about participation in the trial and to develop mitigating strategies. Community engagement activities have been conducted subsequently including briefings with traditional leaders and relevant government officials, question and answer sessions with each village in the study area, and a community visit to the maize production site.

Description of treatment

The flour distribution period will last 12 weeks during July-October 2019. The intervention will begin 1-2 weeks after baseline assessment is complete. End-line blood samples will be taken 1-2 weeks prior to the final flour distribution (Figure 2). Maize flour will be distributed to intervention and control households in multiples of 5 and 10 kg sacks at pre-determined distribution points set up in vicinity to villages. Households will receive sufficient maize flour to meet requirements for the 12-week flour distribution period with distributions at six timepoints. Household requirements will be calculated based on estimated consumption of 330 g capita\(^{-1}\) day\(^{-1}\) for all household members over the age of 1 year (equivalent to 10 kg capita\(^{-1}\) month\(^{-1}\)).
The provision of non-biofortified flour to control households allows the trial to be blinded. However, bags of maize flour will be labelled to ensure households receive the correct treatment, and for monitoring of consumption. The trial statistician will generate a set of unique seven-digit codes that will be used to label the bags of study flour. The statistician will communicate the treatment codes directly to the maize flour distribution team lead. Only the trial statistician and maize distribution team will know how to interpret the code. The rest of the trial team and participants will be blinded to treatment allocation. At distribution points, study flour bags will be stacked in piles according to the bag code. Participants will present their ID cards (Appendix 2i) and the maize distribution team will check their allocated treatment before providing the correct quantity and type of flour. Participants will receive help transporting maize flour to their home if necessary.

Non-participating households in the study area will also receive non-biofortified flour for the duration of the study. Control-equivalent flour will not be labelled with codes and will be distributed separately. The provision of free flour to non-participant households was deemed appropriate to avoid individuals feeling coerced into participating in the trial, to avoid jealousy or stigma within the community, and to reduce the likelihood of sharing flour between households.

The trial maize was grown at LUANAR, Bunda Campus, Malawi. A widely grown hybrid cultivar (DKC80-53) was grown using conventional agronomy. Plants were spaced at 0.25 m within-rows; the distance between rows was 0.75 m. When the crop was approximately 1-1.5 m in height, at the pre-tasselling stage, sodium selenate solution was sprayed continuously along the tops of ridges (stem bases) at 18 g Se ha\(^{-1}\) (elemental basis) applied using knapsack sprayers and an application
volume of 100 L water ha\(^{-1}\) [12]. Maize grain was sampled when the crop was mature, approximately one month prior to harvest, and analysed by Inductively Coupled Plasma Mass Spectrometry (ICPMS). The mean concentration of Se was 0.010 mg kg\(^{-1}\) in control grain and 0.279 mg kg\(^{-1}\) in Se-biofortified grain. In comparison, the median Se concentration in maize grain samples from farmer fields on non-calcareous soil types in Malawi was 0.015 mg kg\(^{-1}\) (n=105) [5, 7,13]. Grain will be milled and packed in labelled sacks. Milling equipment will be thoroughly cleaned between processing the Se-biofortified and control grain.

Maize flour in Malawi comes in three main types: *ufa mgaiwa* (unrefined), *ufa granmill* (partly refined with bran removed only) or *ufa woyera* (fully refined with bran and endosperm removed). We will provide *ufa granmill* during the trial as this was the preferred type of flour among participants in formative research.

**Objectives**

The objective of the study is to test whether Se status of non-pregnant WRA and SAC can be improved through consumption of maize flour enriched with Se through agro-biofortification. We hypothesise that dietary Se intake will be substantially greater among those receiving Se-biofortified maize flour compared to control flour, and that this will translate into increased Se status among those receiving fortified maize measured as serum Se concentration.

Initially, the trial was designed to test the efficacy of alleviating Se and zinc (Zn) deficiency (May 2019; ISRCTN85899451). However, in production of the maize for the study, the target maize Zn concentration was not reached, likely due to a combination of soil factors and heavy rainfall during the 2018/19 production season.
For ethical reasons, the planned Zn arm of the trial was therefore dropped.

Outcome measures

Primary outcome measure

**Serum Se concentration**

Serum Se concentrations will be measured at baseline and end-line. Whole venous blood will be drawn into trace element-free tubes (BD Diagnostics, Switzerland) and after clotting the serum will be separated by centrifugation within 40 min. Serum 0.5 mL aliquots will be transferred into cryovials, transported in mobile freezers and stored at −80°C. Serum samples will be shipped on dry ice to the University of Nottingham, UK, for determination of elemental Se concentration by ICPMS [4]. The unit of measure is concentration of Se in serum (µg/L).

Secondary outcomes

The prevalence of Se deficiency will be compared between treatment groups. Deficiency will be defined using established thresholds for the optimal activity of glutathione peroxidase 3 (GPx3; <84.9 µg L\(^{-1}\)), iodothyronine deiodinase (IDI; <64.8 µg L\(^{-1}\)), and for Keshan Disease (<30 µg L\(^{-1}\)) which is a cardiomyopathy linked to Se status reported in China [14].

Concentration in serum of the markers of inflammation alpha (1)-acid glycoprotein (AGP; mg/L) and C-reactive protein (CRP; mg/L). These will be measured using a sandwich ELISA.

Concentration of haemoglobin in whole venous blood (g/dL). Haemoglobin concentration will be measured in the field by trained nurses using a portable HemoCue Hb201\(^+\) (HemoCue AB, Ängelholm, Sweden).
Prevalence of anaemia will be compared between treatment groups. Anaemia will be defined using standard thresholds [15].

Dietary selenium intakes will be quantified among WRA. Dietary data will be collected among participating WRA using 4-pass interactive 24-hour recall. Two days before the 24-hour recall, participants will be given plates/bowls and asked to consume foods on a plate separate from other family members. In the first pass, participants will be asked to recall all foods and beverages they consumed over the previous day (from midnight to midnight). In the second pass they will be asked details about the foods/beverages and time/place of consumption. In the third pass, they will be asked to estimate the quantity of each food/beverage consumed using an interviewer-assisted method (e.g., food models and diet scales) and the source of each food (i.e., home produced, purchased, the AHHA project, from neighbours/relatives, gifted, food aid/food for work or wild). In the fourth pass, the recalled intake over the day will be reviewed. Dietary data will be collected across all days of the week (population level) to account for any day of the week effect. A repeat 24-hour recall will be collected from a sub-sample of participants (n=60) at least 2 days after the initial recall. Dietary data will be combined with relevant food composition data [7] to determine the baseline intakes of energy and micronutrients. The percentage of women at risk of inadequate intakes of Se will be estimated using the Estimated Average Requirement (EAR) cut-point method [16] after adjustment for intra-subject variability. The percentage of energy from food groups, sub-groups and specific foods will be estimated to examine changes in diet patterns.

Morbidity outcomes will be self-reported at baseline and end-line using standard questions from the Malawi Demographic and Health Survey (DHS). Diarrhoea
incidence, severity and duration, incidence of vomiting and incidence of fever will be recorded for WRA and SAC. Prevalence of pneumonia will be self-reported among SAC only (as per the DHS), i.e. number of days of coughing and fast or difficult breathing (due to a problem in the chest) in the two weeks prior to the survey.

Monitoring of adherence

Participant consumption of flour will be monitored during the course of the intervention. Households will be visited once every 15 days. Research Assistants will observe the number of 10 kg bags of study flour that have not been consumed and will count and collect the number of empty bags of study flour for reconciliation. A household member responsible for meal preparation will be asked what flour was used the previous day for each meal; and what quantity of non-study flour was used by the household over the past two weeks. The same member of the household will be asked how many bags of study flour have been sold or gifted away. Combined, these data will provide a proxy measure of household consumption. Data collected for the monitoring of adherence will not be used to alter the amount of study flour received by participating households at future distribution time points.

Monitoring of participant safety

Consumption of 330 g $\text{capita}^{-1}\text{day}^{-1}$ of the agro-biofortified maize flour with Se concentration of $\sim 0.3 \text{ mg kg}^{-1}$ would supply 99 $\mu$g $\text{Se capita}^{-1}\text{ day}^{-1}$. The adult Recommended Dietary Allowance for Se is 55 $\mu$g $\text{capita}^{-1}\text{ day}^{-1}$ and the Tolerable Upper Level of intake is 400 $\mu$g day$^{-1}$ [17]. The concentration of Se in maize grain depends largely on soil factors, and the concentration in Se-biofortified grain (mean 0.279 mg kg$^{-1}$) produced for this study is within the “natural” range of maize grain Se concentrations sampled from farmers’ fields in Malawi (0.005–0.533 mg kg$^{-1}$) [5].
Comparatively greater Se concentrations occur naturally (i.e. without addition of Se fertiliser) in maize grown on Eutric Vertisol soil types, found in southern Malawi in the Shire Valley and along the shore of Lake Malawi.

Consumption of Se-biofortified flour in the quantities provided in this study is not known or expected to cause adverse events. Procedures will be set up to capture and monitor safety. Non-serious Adverse Events (AEs) will be captured and recorded in electronic monitoring forms by RAs during adherence monitoring visits to participating households (every 15 days). Research Assistants will ask participants and caregivers of children about any AEs including fever, diarrhoea and coughing. These will be compiled and reported to the PI on a monthly basis.

Serious Adverse Events (SAEs) include death, life-threatening illness, hospitalisation, persistent or significant disability, or other such occurrences, and these will be reported to the study coordination centre (LUANAR) through completion of an SAE form and submission within 24 hours of the monitoring team being made aware of the event. Participants will be informed how to contact the study coordination centre; and RAs, Health Surveillance Assistants and Health Volunteers will be trained on how to record and report SAEs. A Medical Doctor will be “on call” and available to visit participants through the duration of the intervention to advise on seriousness and causality. The study principal investigator (PI, Edward Joy) will record the event and its seriousness, causality and expectedness.

The PI will report SAEs to the trial statistician who is aware of random treatment allocation, and who will report to the independent statistician on the TSC to decide about the continuation of the trial. All AEs and SAEs will be compiled by the PI and reported to the Research Ethics Committees (REC) according to their criteria.
Unblinding will only occur at the request of the independent statistician on the TSC. Unblinding would involve revealing the allocation of households to treatment, e.g. to investigate the occurrence of SAEs and their association with treatment. At this point the TSC may request the trial is paused or terminated.

Withdrawal

Any individual will remain free to withdraw at any time from the study. If a participant withdraws consent from further trial participation their data will remain on file and will be included in the final study analysis. If a participant withdraws consent for their data to be used, the data will be destroyed immediately.

Loss to follow up

Households or individuals that move out of the village will leave the trial and no attempt will be made to follow them up. Any data collected from participants who are lost to follow up will be included in the analysis where possible. Continued community engagement will be conducted to minimise loss to follow up. Contact details including mobile phone numbers have been recorded during household enumeration, and participants will be contacted 1–2 days prior to the mobile clinic is set up in their vicinity. Mobile clinics will be located close to participant households, and transport will be provided if participants require. Also, participants will be contacted 1–2 days prior to flour distribution.

Data collection and management

All participants will be assigned a unique numeric ID that will be used in data capture forms and subsequent analyses to maintain anonymity. Questionnaire, anthropometry and adherence data will be collected by trained RAs in the field via
passcode-protected tablets using Open Data Kit (ODK) [18]. Blood samples will be taken by trained and experienced nurses and processed to serum by trained technicians. Sample tubes and subsequent data will be labelled with unique, anonymous codes. Samples will be logged in the field using ODK forms. Completed forms will be uploaded to a secure cloud server daily and will be encrypted for security. Mobile clinic team leads will report daily to the anthropometry and blood collection team supervisors to ensure standard procedures are followed. The Trial Manager and Data Manager will reconcile samples and data forms daily. Recruitment rates and number of eligible individuals will be assessed and compared with numbers enrolled, and completeness of follow-up.

Dietary data will be collected by trained RAs, using a paper-based data collection process. Dietary forms will be labelled with participant IDs. Data collection will be supervised by the diet team supervisor who will observe individual 24-hour recalls and check the data collected each day. The RA, supervisor and co-ordinator will sign off all forms before they are filed for data entry. Quality control check lists (for observations and forms) will be used to ensure standard procedures are followed. At the end of the baseline and end-line surveys, all diet data will be double entered to reduce errors and ensure consistent data entry decisions are made.

The Data Manager will hold the data tables that match participant ID to sample codes, and these will not be available to the Trial Statistician until after statistical analyses are complete. Thus, the trial statistician will be blinded to treatments. Sample analysis will be undertaken by Malawian and UK study collaborators in laboratories in the UK (University of Nottingham and University of Central Lancashire). Good practices in quality control will be followed such as analysis in duplicate and inclusion of sample blanks and reference materials and use of
sufficiently sensitive analytical instrumentation to ensure quantification of the variables of interest.

All data collection and storage will be compliant with the General Data Protection Regulation [19] and the conditions of the REC Approval. Anonymised data will be uploaded to an open data platform following analysis in compliance with open access data requirements. Data will be kept for a minimum of 10 years after completion of the trial.

**Biological sample management**

Serum samples will be transported in cool boxes packed with ice from mobile field clinics to the Community Health Surveillance Unit, Ministry of Health, Area 3, Lilongwe, where they will be stored at -80°C. Cryovials and cryovial racks will be labelled with unique numeric and QR codes. Instrumentation and methodologies are not established in Malawi for ICPMS to measure elemental concentrations in the parts-per-billion range. Samples will be transferred on dry ice to a secure laboratory at the University of Nottingham, UK under a Material Transfer Agreement (MTA) with appropriate licences. Samples will be cross-checked with shipment lists. Following ICPMS analysis, samples will be stored for a period of 5 years following completion of the trial after which they will be destroyed in accordance with licence and safety requirements.

**Statistical analysis**

The trial is designed to have statistical power to detect an increase in Se concentration in serum of 4.9 µg L⁻¹ with 5% significance and 90% power, allowing for 20% drop-out, based on a standard deviation of 9.0 µg Se L⁻¹ (standardised difference of 0.54).
Data on the number of participants (WRA and SAC) randomised (with exclusions and reasons for exclusion), the flow of participants through enrolment, allocation to intervention, follow-up and analysis will be presented in a flow chart. The primary analysis will be carried out on groups as randomised (‘intention to treat’).

Tabulation of demographic data and other characteristics will be done using the intention-to-treat datasets. No significance tests will be performed to test for differences at baseline. Descriptive statistics for continuous variables will include the mean, standard deviation, median, range and the number of observations. Categorical variables will be presented as numbers and percentages.

For the primary outcome, analysis of covariance (adjusting for baseline values) will be used to estimate a mean difference in Se status between the two arms of the trial. The results will be presented with a 95% confidence interval. For secondary outcomes, appropriate models will be used to examine the effect of the relevant intervention. Appropriate measures of effects will be reported with 95% confidence intervals.

Unadjusted and adjusted results will be presented for all analyses. Planned subgroup analyses for WRA will include 10-year age groups (20-29, 30-39, 40-45 years), lactation status (yes – exclusive breastfeeding; yes – breastfeeding and complementary feeding; no), and pregnancy status (self-reported at end-line). All sub-group analyses will be performed by including a variable (or variables, as appropriate) for the sub-group and its interaction with the treatment in the model. Results will be interpreted with due caution. Full details of all analyses, including any additional covariates to be included in the adjusted models, will be set out \textit{a priori} in a Statistical Analysis Plan.
Whilst we have allowed for 20% missing data in the sample size calculations, only a small amount of missing data is expected and it is unlikely that it will have to be accounted for in any analysis. We would consider using multiple imputation if missing data were larger than expected and/or there was differential attrition between the trial arms. We would also attempt to ensure that the reason for the differential attrition was fully understood.

Discussion

The AHHA Malawi trial is a pragmatic, community-based trial that will provide evidence on the efficacy of alleviating Se deficiency through consumption of maize flour enriched with Se through agro-biofortification. Agro-biofortification may be a cost-effective way to alleviate Se deficiency in Malawi, with previous studies estimating a cost-per-alleviated case of ~ US$ 0.36 year\(^{-1}\) [7]. The evidence from the trial may be used to inform future agriculture policy in Malawi and the wider region. The trial findings will be communicated to policy stakeholders and participating communities, and reported in peer-reviewed journals.

Trial Status

Protocol version 4.2

Recruitment started on 26\(^{th}\) June and was completed on 6\(^{th}\) July 2019.

Abbreviations

| Abbreviation | Description                        |
|--------------|------------------------------------|
| AE           | Adverse Event                      |
| AGP          | Alpha (1)-acid glycoprotein        |
| AHHA         | Addressing Hidden Hunger with Agronomy |
Declarations

Ethics approval and consent to participate

The trial is being conducted in accordance with the principles of Good Clinical
Practice [20]. Ethical approval for the formative research was sought and obtained from the London School of Hygiene & Tropical Medicine Observational Research Ethics Committee (reference: 15730) and the Malawi College of Medicine Research Ethics Committee (reference: P.05/18/2393). The trial protocol and amendments have been approved by the London School of Hygiene & Tropical Medicine Interventions Research Ethics Committee (reference: 16181) and the Malawi College of Medicine Research Ethics Committee (reference: P.11/18/2539) and is a registered clinical trial (March 2019; ISCRTN85899451).

Participation in the study is voluntary. Trained RAs will seek written informed consent for WRA and assent for SAC in the presence of an adult caregiver prior to recruitment. Participants have the right to withdraw at any stage.

Trial Steering Committee

The AHHA Malawi trial is overseen by a Trial Steering Committee (TSC), with the following independent members: Professor Penelope Nestel (chair), Dr Chrissie Thakwalakwa (Malawi nutrition expert) and Dr Charles Opondo (statistician). The TSC is responsible for overseeing the safe and ethical conduct of the trial according to the standards set out in the ICH Guidelines for Good Clinical Practice. The AHHA study is considered a low-risk trial due to the nature of the intervention. Furthermore, no interim data will be collected. Thus, there will not be a Data Monitoring Committee; instead, the independent statistician will advise on the continuation/discontinuation of the trial following safety reports from the PI. The Trial Management Group will discuss any potential protocol amendments with the TSC. If the TSC approves the amendment, then an application will be submitted to the RECs. Participant Information Sheets will be updated accordingly.
Consent for publication
Not applicable

Availability of data and material
Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Competing interests
The authors declare that they have no competing interests

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Authors’ contributions
EJMJ, MRB, AAK, PCN, FPP and DG conceived the study. EA advised on trial design and statistics. All authors contributed to the design of the study, and all authors read and approved the final manuscript.

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Figures
CONSORT diagram of the Addressing Hidden Hunger with Agronomy (AHHA) Malawi trial.

**Figure 1**

| Week | -2 | 0 | 1 | 2 | 4 | 6 | 8 | 10 | 12 | 14 |
|------|----|---|---|---|---|---|---|----|----|----|
| **ENROLMENT:** |    |   |   |   |   |   |   |     |     |    |
| Eligibility screen | X  | X |   |   |   |   |   |     |     |    |
| Informed consent   |    | X |   |   |   |   |   |     |     |    |
| Allocation          |    |   |   |   |   |   |   | X   |     |    |
| **Flour distribution** |   |   |   |   |   |   |   |     |     |    |
| **ASSESSMENTS:** |    |   |   |   |   |   |   |     |     |    |
| Serum selenium      | X  |   |   |   |   |   |   | X   |     |    |
| Haematology         | X  |   |   |   |   |   |   | X   |     |    |
| Dietary selenium    | X  |   |   |   |   |   |   | X   |     |    |
| Morbidity           | X  |   |   |   |   |   |   | X   |     |    |
| Anthropometry       | X  |   |   |   |   |   |   | X   |     |    |
| Adherence           |    |   |   |   |   |   |   | X   | X   | X   |

**Figure 2**

Schedule of enrolment, interventions, and assessments for the Addressing Hidden Hunger with Agronomy (AHHA) Malawi trial.
Supplementary Files

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