Research Protocol

Official Tittle: Trial to establish causal linkage between mycotoxin exposure and child stunting

Date of last Revision: September 18, 2019

Project Summary

In 2018, an estimated 150.8 million children under 5 were stunted, with greatest burden in Southern Asia and Eastern Africa (1). While the number of stunted children has fallen globally, it continues to increase only in Africa. Stunting has been estimated to contribute to 14-17% of child deaths under 5 years of age and is a risk factor for poor cognitive and motor development and educational outcomes (2-6). Inadequate dietary intake and disease are the immediate causes of undernutrition and stunting, along with multiple proximate and distal factors, including access to health care and maternal education (7).

Improving infant diets through complementary feeding interventions has been shown to only modestly reduce stunting, improving length-for-age Z scores by 0.10, thus additional factors are likely contributing to the burden of stunting (8). Multiple observational studies show an association between fetal and post-natal aflatoxin exposure and linear growth (9-11). However, the effects of confounding factors such as socio-economic status, food insecurity and nutrient deficiencies due to monotonous diets have not been ruled out. This trial will quantify the causal role of infant aflatoxin ingestion on post-natal growth by performing a cluster randomized trial in children 6-18 months of age in the Dodoma Region of Tanzania.

All health facilities in one district in Dodoma will be randomized to the control or intervention arm. Infants will be recruited into the study over one year to account for seasonal variation in AF exposure. Both arms will receive infant and young child feeding education, a thermos flask and plastic measuring scoops. The intervention arm will receive a low-aflatoxin pre-blended porridge flour containing maize and groundnut (ratio 4:1 respectively) and low-aflatoxin groundnut flour, whereas in the control arm the same porridge mix will be promoted through education, but acquired by the household. The primary outcome is length-for-age Z-score at 18 months.

I. General Information

Project title: Trial to establish causal linkage between mycotoxin exposure and child stunting
Funded by: The Bill and Melinda Gates Foundation (BMGF)

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II. Rationale and Background Information

Mycotoxins are naturally occurring secondary metabolites produced by fungi that frequently colonize crops. There are 300-400 identified mycotoxins, 12 of which are believed to be predominantly contribute to animal and human mycotoxicosis. Of these, aflatoxin (AF) and fumonisin (FUM) are of particular concern. AF is a carcinogen, known to cause hepatocellular carcinoma (HCC, liver cancer) and synergize with the hepatitis B virus to increase the risk of HCC 30-fold, compared with either exposure alone (12, 13). Consumption of FUM has been associated with renal tumors, esophageal cancer and increased risk for neural tube defects (14). AF is produced by *Aspergillus* mold strains and is commonly found in staple foods such as maize and groundnut. FUM is produced by *Fusarium* mold strains and is commonly and predominantly found in maize.

Approximately 0.5 billion people, mainly living in developing countries, are at risk of being chronically exposed to dietary AF at high levels (15). Exposure to AF in low-income populations, especially those in rural sub-Saharan Africa, is particularly problematic because the diet is monotonous, largely based on highly AF-prone crops, grown and stored under conditions that predispose them to contamination. While the consequences of intermittent outbreaks of extremely high levels of AF consumption and acute toxicosis are well understood, the effects of chronic AF exposure at moderate to high levels, especially during critical periods of fetal and early-life development, are not. Exposure to both these mycotoxins is of concern, however current evidence more strongly implicates AF as the primary mycotoxin of concern for stunting. This trial will therefore focus on AF but we will assess FUM contamination and AF/FUM co-exposures throughout the course of the trial.

Two critical knowledge gaps hinder our understanding of the effects of AF and motivation to mitigate AF from food supplies in sub-Saharan Africa. These include lack of causal evidence for the effect of AF on post-natal growth and lack of context-adapted strategies to mitigate AF consumption in low-resource settings.

1. Lack of causal evidence for the effect of aflatoxins on post-natal growth: The weight of evidence linking aflatoxin exposure to intrauterine growth restriction and child stunting has increased over the years but is far from definitive, coming from animal or observational studies. The effects of confounding factors such as socio-economic status, food insecurity and nutrient deficiencies due to monotonous maize-based diet have not been ruled out in the evidence to date. Serum aflatoxin albumin (AF-alb) concentration was associated with stunting among young children in rural Benin and Togo, as demonstrated by significant dose-response relationship with length-for-age (LAZ) and weight-for-age Z scores (WAZ) (9). In a subsequent longitudinal study in rural Benin, infants in the highest quartile of AF- alb, compared to the lowest quartile were 1.7 cm shorter after 8 months (10). A longitudinal survey of maternal AF exposure during pregnancy and subsequent infant growth from birth to 52 weeks conducted in The Gambia, also revealed a highly significant inverse relationship between maternal AF exposure and infant growth (11). Early infant exposure (prior to week 16) additionally adversely contributed to the effects of maternal AF exposure on growth.

In an observational study in Tanzania the effects of AF and FUM exposure on attained LAZ at 12 months was assessed (16). Urinary FUM was negatively associated with LAZ ($p=0.014$), with

children in the highest quartile of exposure 1.8 cm shorter than children in the lowest quartile of exposure; the negative association between AF-alb and LAZ did not reach statistical significance, however AF-alb levels were lower compared to studies in West Africa described above. The MAL-ED study in Nepal also did not find an association between AF consumption and stunting, but it too was conducted in an area of lower exposure and lower prevalence of stunting compared to West Africa (17).

The MAICE Trial in Kenya was the first RCT to assess an intervention to reduce AF consumption on child growth through household level sorting and community stocking interventions to reduce AF consumption (18). There was not an improvement in LAZ at 18 months (endpoint) in the intervention group, despite a 27% decrease in AF-lys (19). However, there was a significant difference in LAZ at midpoint, even though AF-lys did not differ between groups at midpoint. However, taking into account the relatively high loss to follow-up, incomplete data and the low percent of AF-lys samples analyzed (65%), these results are difficult to interpret.

2. Lack of context-adapted strategies to mitigate aflatoxin. Mycotoxin contamination of foods in low-input and fragmented food systems is a complex problem most effectively managed through multiple mitigation strategies(20). Yet there are a limited number of effective mitigation interventions that are practical and feasible for small-scale rural farmers and communities, where populations are most at risk for mycotoxin exposure (14, 21). A recent IARC Working Group concluded that there are four general intervention strategies considered to have sufficient evidence for implementation. Only two of these strategies are relevant to the sub-Saharan rural context: 1) sorting and 2) a package of post-harvest storage and handling practices (22).

There is much more global experience with these two intervention strategies for groundnuts than maize. Sorting of groundnuts to remove AF-contaminated kernels is simpler than sorting maize because visual and tactile inspection of pods is a good indicator of food safety: if the pod is average size and intact, the risk of AF is low (23). By contrast, internal colonization of maize kernels can lead to cryptic toxicity: healthy-looking kernels can carry substantial levels of both AF and FUM, requiring additional techniques to sort contaminated grain. Therefore, innovation is urgently needed to develop successful maize sorting techniques relevant to low-input foods systems and could be an evidence-based recommendation to policy makers, complimenting the results of our trial.

III. References

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IV. Study Goals and Objectives

The goal of this research is to provide experimental evidence about the effect of aflatoxin ingestion on linear growth in infants and young children between 6 and 18 months of age.

Primary objective: To measure the effect of a reduced-AF diet on length-for-age Z scores at 18 months in central Tanzania.

Secondary objectives:

1. To determine the effect of an intervention to reduce AF on LAZ scores at 12 months and stunting at 12 and 18 months
2. To determine the effect of an intervention to reduce AF on weight for age Z scores (WAZ) at 12 months and 18 months and underweight at 12 and 18 months
3. To examine the effect of an intervention to reduce AF on mid-upper arm circumference and head circumference at 12 and 18 months
4. To assess the effect of an intervention to reduce AF on the blood biomarker AF-alb at 12 and 18 months
5. To assess the effect of an intervention to reduce AF on the urinary biomarker AFM1 in a cohort of 600 infants at 9, 12, 15 and 18 months
6. To assess the effect of an intervention to reduce AF on the urinary biomarker FUM in a cohort of 600 infants at 9, 12, 15 and 18 months
7. To describe the relationship between recent consumption AF in food and AFM1 in urine in a cohort of infants at 12 months
8. To determine the relationship between FUM in food and urinary FUM biomarkers in a cohort of infants at 12 months
9. To determine how demographics, geography and climate modify spatial measures of food consumption and food contamination with AF and FUM
10. To determine how pre- and post-harvest practices influence contamination of food with AF and FUM
11. To use blood and urinary biomarkers to hypothesize mechanisms of AF-induced stunting at 12 and 18 months, including a threshold of exposure
12. To identify early life food and cultural behaviors that may influence food choices and AF exposure
13. To investigate role AF and FUM co-exposure on stunting, LAZ, underweight and WAZ at 12 and 18 months
14. To determine the effect of an intervention to reduce AF on inflammatory markers (CRP and TNFa) at 18 months of age

V. Study Design

This study is a cluster randomized design. The trial is designed to test the hypothesis that provision of a complementary food porridge flour and separate groundnut flour, both with very low levels of aflatoxin (TZ regulations for complementary foods is AF≤5ppb), will be associated with better linear growth in infants compared to the consumption of complementary porridge and foods made with similar ingredients and groundnut flour, but acquired by the household.

We have designed an IYCF educational intervention to improve IYCF practices and infant diets for all trial participants, which will take place in Kongwa District, Dodoma Region, an area with low dietary diversity and high rates of food insecurity (1, 2). The experimental group will be provided with infant feeding education and provision of pre-blended porridge flour made of maize and groundnut flour (ratio 4:1). They will also be provided separate groundnut flour because infants eat groundnut flour in family foods. The control group will be provided with the same infant feeding education and promotion of the same pre-blended porridge flour and groundnut flours, but these will be acquired by the household. The control group will receive gifts related to baby care, including skin lotion and diaper covers. Both groups will be given a thermos flask to safely store the porridge and a plastic scoop to measure porridge flour.

Inclusion and exclusion criteria

Inclusion criteria:

1. Babies >6 weeks old and <5 months old, who seek Expanded Program on Immunization (EPI) from a randomized health facility and reside in Kongwa District

Exclusion criteria, assessed at recruitment and again at the 6-month visit:

1. Babies with disabilities that preclude normal feeding and swallowing
2. Refusal to consent to assigned intervention
3. An infant who has shown signs of a potential groundnut allergy (assessed the first time mother reports groundnut consumption)
4. An infant who is a twin
5. If the mother plans to travel for more than 2 months at or after the randomized intervention begins
6. If the mother is below 16 years of age

VI. Methodology

In the intervention group, the study will provide low-aflatoxin infant porridge flour. In the control group we will recommend mothers feed a porridge made from similar flours. Complementary feeding porridge flours are culturally familiar in Tanzania (called *lishe*) and mothers' awareness of them is high. We will educate and advise all mothers to provide porridge to their infants, with a composition, consistency and frequency that is consistent with World Health Organization infant feeding best practices.

Critical to causal inference is the intervention's ability to create a contrast of AF consumption between the control and intervention groups, without creating differential macro- or micro-nutrient intake or differences in feeding and care practices that could affect stunting between arms. To reduce the risk of introducing these biases, we designed the intervention to include: 1) education to improve infant feeding and care practices, using Tanzania Food and Nutrition Center's materials, and 2) behavior change communication on the use of blended infant porridge (Table 1).

Table 1. IYCF Intervention Activities

Intervention Group	Control Group
Infant feeding education: ● Breastfeeding ● Dietary diversity ● Feeding frequency ● Hand washing	Infant feeding education: ● Breastfeeding ● Dietary diversity ● Feeding frequency ● Hand washing
Behavior change communication on use on use of lishe ● Timing of introduction, frequency of feeding, density and composition of lishe ● **Provision** of 4:1 ratio of maize meal to groundnut powder ● GN flour	Behavior change communication on use of lishe ● Timing of introduction, frequency of feeding, density and composition of lishe ● **Promotion** of 4:1 ratio of maize meal to groundnut powder ● GN flour

All participants will be asked to respond to a survey at four time points. The first survey, the recruitment survey, will take place when the index infant is between 1.5-4 months of age. The second survey will take place at 6 months of age, the third at 12 months and the fourth and final survey at 18 months of age. We will also include a cohort of 600 infants (300 per arm) who will be visited at 9 and 15 months, in addition to the standard visits. Therefore, children will be in the study from the time they are recruited, between 1.5-4 months, to the time they turn 18 months of age. However, the randomized intervention of providing pre-blended porridge flour and groundnut flour will start from 6 months of age onward, so as not to disrupt exclusive breastfeeding.

The surveys will assess socio, demographic and economics of the household, infant feeding practices, access to health-care, food procurement practices, and water, sanitation and hygiene of the household and community health worker contact (Table 2). At the 6, 12, and 18 month survey points we will perform anthropometric assessment of weight, height, middle upper arm circumference (MUAC) and head circumference in all infants. Surveys and anthropometric data collection will be performed by data collectors who are hired and trained by the project

We will collect blood at three time points (6, 12 and 18 months) in all infants in the study. We will collect urine only from the cohort of 600 infants at five time points (6, 9, 12, 15 and 18 months). A certified nurse or laboratory technician will be hired to perform urine and blood collection, consistent with Tanzanian regulations and hired in collaboration with the Kongwa District Medical Officer. In the full cohort we will collect infant food samples and assess adherence to the intervention and porridge-specific feeding practices. We will perform 24-hour recalls in 200 cohort household households at 12 months.

In the lab, samples will be cataloged, prepared and analyzed by lab technicians. Disposal of biohazard material and sharps will be performed according to Tanzanian regulations and in partnership with the Kongwa District Hospital and NM-AIST, where research labs are already established.

Table 2 - Summary of Interventions, Measurement and Surveys by Randomized Treatment Arm

	Control Arm: Standard of Care Infant feeding and lishe education only+ distribution of lotion and baby gifts		**Intervention Arm: Lishe Provision** Infant feeding and lishe education+ distribution of AF-free lishe	
Age in months	**Measurement**	**Surveys**	**Measurement**	**Surveys**
42 day (6 week) EPI visit up to 4 months	None (recruitment)	Informed Consent Recruitment survey (including screening, infant health, socio demographic modules and antenatal care)	None (recruitment)	Informed Consent Recruitment survey (including screening, infant health, socio demographic modules and antenatal care)
6	AF-alb AFM1*, FUM* Anthropometry (weight, height, MUAC, head circumference)	Baseline survey: food security, health and WASH, SES and assets, livelihood/shock, and intervention related questions, and health seeking behavior WHO IYCF indicators survey Lishe-specific feeding module	AF-alb AFM1*, FUM* Anthropometry (weight, height, MUAC, head circumference)	Baseline survey: food security, health and WASH, SES and assets, livelihood/shock, and intervention related questions and health seeking behavior WHO IYCF indicators survey Lishe-specific feeding module
9*	AFM1	WHO IYCF indicators survey Lishe-specific feeding module	AFM1	WHO IYCF indicators survey Lishe-specific feeding module; Adherence to feeding AF-free lishe module
12	AF-alb AFM1* Anthropometry (weight, height, MUAC, head circumference)	Midpoint survey (select matching modules to baseline) WHO IYCF indicators survey Lishe-specific feeding module Food testing* 24-hour recalls (100 HH/arm)	AF-alb AFM1* Anthropometry (weight, height, MUAC, head circumference)	Midpoint survey (select matching modules to baseline) WHO IYCF indicators survey Lishe-specific feeding module Food testing* 24-hour recalls (100 HH/arm) Adherence to feeding AF-free lishe module
15*	AFM1	WHO IYCF indicators survey Lishe-specific feeding module	AFM1	WHO IYCF indicators survey Lishe-specific feeding module Adherence to feeding AF-free lishe module
18	AF-alb Multiple mycotoxin panel, FUM and AFM1* Anthropometry (weight, height, MUAC, head circumference)	Final survey (select matching modules to midpoint and baseline) WHO IYCF indicators survey Lishe-specific feeding module	AF-alb Multiple mycotoxin panel or FUM and AFM1* Anthropometry (weight, height, MUAC, head circumference)	Final survey (select matching modules to midpoint and baseline) WHO IYCF indicators survey Lishe-specific feeding module Adherence to feeding AF-free lishe module

Notes: * indicates visit or measurement for longitudinal cohort only (n=600)

Randomization Protocol

The unit of randomization is government-run health facilities (health centers, dispensaries and hospital, 52 clusters in total) in the Kongwa District. Mothers and infants will be recruited into the trial based on 42 day EPI visit attendance, which has very high (>95%) coverage in Kongwa District. We will recruitment infants for one complete calendar year to capture variability in exposure by season.

The health facilities in Kongwa serve populations of different sizes, such that the annual attendance between August 2017 and July 2018 for the 6-week vaccination visit at the 50 health facilities operating during that time ranged from 14 to 427 visits. In 44 health facilities, more than 60 babies attend the 6-week vaccination visit in one year, and in 48 facilities more than 40 attend the 6-week visit annually. Only two have less than 30 children attend this visit annually and two new dispensaries have opened in 2019. We will randomize all 52 health facilities to capture full representation of the district.

We want to ensure close balance between the two groups on two important characteristics:

- Altitude, which is associated with mycotoxin risk in the altitude range of the district.
- Health facility size/attendance, which is a proxy for population density and access to resources such as roads and markets.

To do this, we:

1. Sorted the health facilities by low v. high altitude. We began by using the cutoff of 1200 meters. This altitude is near the median for the district, and 1000-1200 meters is considered the threshold for drier and colder conditions in which toxin-producing fungi are less likely to thrive.
2. Sorted the health facilities by size within each altitude category, using 6-week immunization visit attendance data from the most recent year of data (August 2017-July 2018).
3. Grouped health facilities into successive pairs from the top to the bottom of each list. We randomly chose half of the pairs within a list. For the chosen pairs, the first health facility in each pair was assigned to the experimental group and the other health facility assigned to the control group. For the pairs not chosen, the assignments will be reversed: the first health facility in each pair was be assigned to the control group and the other health facility assigned to the experimental group.

There is no blinding in this study, as all parties will be able to see what is received by the participant – pre-blended flour and groundnut flour or lotion and baby-care gifts.

VII. Safety Considerations

The safety of the research participants is critical. Although we do not expect any severe events caused from our intervention, we can expect potential non-serious adverse events from blood draws. As required by GCP and the Cornell IRB, we will report all unexpected and adverse events in the timeframe required and outlines below (Table 3).

Table 3 - Classification and Reporting of Adverse Events

	Classification of event	Example	Reporting to IRB	Means of tracking/documenting events
1	**Attrition** Drop out and loss to follow-up	● Mothers drop out of study due to displeasure with the study, perceived risk, lack of time, etc. ● Higher than expected 20% loss to follow up at 6, 9 12, 15 and 18 months	Not reported to IRB	Review study data for trends - monthly Data Collector Weekly Report
2	**Participant complaints:** not associated with potential for increased risk profile or changes to research	● Caregiver complains child has an illness unrelated to infant food consumption/trial intervention (malaria, cough/upper respiratory infection, fever, etc.)	Not reported to IRB	Review study data for morbidity trends – monthly Report of complaint to research team member, likely data collector; DC will file a report and submit to Field Supervisor Report of complaint to non-research team member (maternal, health facility staff, CHW, etc.); Trained to contact Intervention Officer or Field Supervisor to report event
	Participant complaints: associated with potential for increased risk profile or changes to research	● Serious event – Hospitalization or death from any cause ● Non-serious event - Caregiver complains child has diarrhea or other illness believe to be associated with consumption of provided infant food	Report serious events (death or serious injury/illness) within 24 hours of discovery Report non-serious events within 14 days of discovery	Review study data for morbidity trends – monthly; Compare incidence between groups at 6, 9, 12, 15 and 18 months Report of complaint to research team member, likely data collector; DC will file a report and submit to Field Supervisor Report of complaint to non-research team member (maternal, health facility staff, CHW, etc.); Trained to contact Intervention Officer or Field Supervisor to report event

3	**Protocol deviations:** not associated with potential for increased risk profile or changes to research	● Data collector skips survey section or fails to take all measurements ● Project food runs out and is not available for participants on schedule ● Lab technician fails to process samples according to the SOP	Not reported to IRB	Review study data for trends – weekly (missing questions, sections, excess refusal or DKs, etc.) DC and lab reports – review for trends weekly Field team meetings Active supervision of data collectors and lab staff Gift logistician and RCH weekly and monthly gift reports
	Protocol deviations: associated with potential for increased risk profile or changes to research	● Breach of confidentiality or data privacy (non-serious) ● Urine collection or blood draw that does not follow protocol (non-serious)	Report serious events (death or serious injury/illness) within 24 hours of discovery Report non-serious events within 14 days of discovery	DC reports – review for trends weekly Field team meetings Active supervision of data collectors and lab staff IT staff QC weekly checks of data system
4	**Expected or unexpected clinical complications:** not related to any research procedures	● Infant is found to have signs of clinical malnutrition or severe stunting or wasting based on anthropometric assessment by data collector ● Infant has signs of severe illness, such as rapid, shallow breathing during evaluation by data collector	Not reported to IRB Reported to caregiver as soon as possible and referred to health facility using referral form	DC referral report submitted to Field Supervisor Review study data for morbidity trends – monthly

5	**Expected serious events:** related to the research	• None expected	Report serious events (death or serious injury/illness) within 24 hours of discovery	Report of event to research team member, likely data collector; DC will file a report and submit to Field Supervisor Report of complaint to non-research team member (maternal, health facility staff, CHW, etc.); Trained to contact Intervention Officer or Field Supervisor to report event
	Expected non-serious events: related to the research	• Soreness/bruising from blood draw • Fainting from blood draw • Infant crying while being evaluated or taking of samples	Not reported to IRB	Study data - review for trends monthly
	Unexpected events: related to the research	• Severe diarrhea reported by multiple mothers receiving project flour, leading to hospitalization (serious) • Chronic diarrhea reported by multiple mothers receiving project flour (non-serious) • Increase in growth faltering in the intervention group compared to control group at 12 or 18 months (non-serious)	Report serious events (death or serious injury/illness) within 24 hours of discovery Report non-serious events within 14 days of discovery	Study data – review for morbidity trends monthly; compare morbidity between groups monthly; Compare LAZ between groups at 12 and 18 months, following collection of 6 months of LAZ data Report of event to research team member, likely data collector; DC will file a report and submit to Field Supervisor Report of complaint to non-research team member (maternal, health facility staff, CHW, etc.); Trained to contact Intervention Officer or Field Supervisor to report event
6	**Unexpected events:** not related to the research	• Car accident • Broken limb	Not reported to IRB	Report of event to research team member, likely data collector; DC will file a report and submit to Field Supervisor Report of complaint to non-research team member (maternal, health facility staff, CHW, etc.); Trained to contact Intervention Officer or Field Supervisor to report event

7	**Unanticipated problem:** unexpected, related to the research & associated with increased risk profile	N/A (since unanticipated)	Report serious events (death or serious injury/illness) within 24 hours of discovery Report non-serious events within 14 days of discovery	Study data – review for trends monthly Field team meetings Report of event to research team member, likely data collector; DC will file a report and submit to Field Supervisor Report of complaint to non-research team member (maternal, health facility staff, CHW, etc.); Trained to contact INTERVENTION Officer or Field Supervisor to report event

Summary of non-reportable events:

- Participant complaints not associated with potential for increased risk profile or changes to research
- Protocol deviations not associated with potential for increased risk profile or changes to research
- Clinical complications not related to research procedures
- Non-serious expected events that are related to the research
- Unexpected events that not related to the research

All complaints, protocol deviations, events and problems will be documented by the research team member who receives the complaint. This will likely be the data collector, but could also be the Data Collector Supervisor, Data Quality Supervisor, Intervention Officer, Field Supervisor or Lab Supervisor. All documentation of these events will be submitted to the Research and Field Supervisor who will pass them on to N.K. and E.P.

All weekly and monthly reports from the data will be generated by the biostatistician (E.M.) and sent to Neema Kassim, Edna Makule, Erica Phillips, Francis Ngure for review

VIII. Follow-up

Participation in the study will end when infants turn 18 months of age. Following the 18 month visit (conducted prior to 19 months), there will be no follow-up unless there is an adverse event or abnormal laboratory result (see below) that requires further resolution. As there are no anticipated serious events from this research and few non-serious events, we will evaluate these on a case-by-case basis and work with the IRB and local health officials to resolve these problems.

IV. Data Management and Statistical Analysis

Data Collection, Management and Supervision

Data will be collected electronically, using hand-held devices (Samsung Galaxy 7, KoBoToolbox Platform), with paper copies provided as back up in case of electronic failure. The primary outcome of length will be recorded on the tablet and by hand.

We will hire up to 22 data collectors at the peak of the trial. For collection of biological samples, hiring of certified nurses will be performed in collaboration with the Kongwa District Medical Officer. For collection of non-biological samples, non-nurses will be considered. Data collectors will be supervised by the Data Collector Supervisor, who will travel with the data collectors on a daily basis (Figure 1).

Before leaving the field at the end of each workday, the Data Collector Supervisor, Assistant Data Collector Supervisor and/or Data QC Supervisor will review all surveys for completeness and quality. Additionally, data quality checks will be built into the tablet version of the survey, for example limiting ranges of responses/measurements to those that are within a credible range and consistency checks between questions. Once the data quality is satisfactory the Data QC Supervisor will upload data on a daily basis. This will be encrypted and transferred to Arusha or the KoboToolbox cloud for backup storage.

The Lab Scientist in Kongwa will receive blood and urine samples and enter these samples into a lab archiving system that will be shared between research trial laboratory at Kongwa District Hospital and NM-AIST, Arusha. He/she will also be hired in collaboration with the Kongwa District Medical Officer.

Data quality will be monitored in multiple ways and at all levels of management, performed jointly between representatives from NM-AIST and Cornell. In the field, adherence to all protocols and data quality will be full time responsibilities of the Data Collector Supervisor, Assistant Data Collector Supervisor and Data QC Supervisor. Both of these positions will be supervised by the Field and Research Coordinator, who is in turn supervised by the PI and leadership team at Nelson Mandela. The IT Data Manager (A.M.) based at NM-AIST in Arusha will be in frequent communication with the Data QC Supervisor to assure safe management and transfer of the data and will therefore help to supervise the Data QC Supervisor. He will also stay in contact with the Data Collector Supervisor in case of any electronic problems/issues that might arise.

Data monitoring will be performed on a weekly basis by Arusha-based IT Data Supervisor and

Biostatistician. The IT Team will produce a weekly report of key data, including enrollment numbers, rejection/non-qualifier numbers, drop-out rates, completed visit rates, anthropometry and biological sample collection success rates. Every other week to one month the IT Team will produce a report to show confirmation of receipt of biological sample by the Kongwa and Arusha labs and their condition. These reports will be shared with the core investigator team (N.K., E.E.M., E.P., F.N.), in addition to the event reporting described above. This data monitoring system will be discussed and approved by the DSMB, along with key "red flags" that will warrant rapid adjustment to protocols. The full data set will be shared through secure and IRB-approved means and will be stored in at least two sites (e.g. Arusha/KoBoToolbox Cloud and Ithaca).

If need arises to correct data, the corrected file will be kept in a separate database with appropriate documentation for changes made. No data in the original database will be changed at any time. Access to edit data will be restricted to E.P., F.N. and N.K. However any edits to data will be performed after reaching consensus by E.A.M., E.E.M., N.K, F.N and E.P.

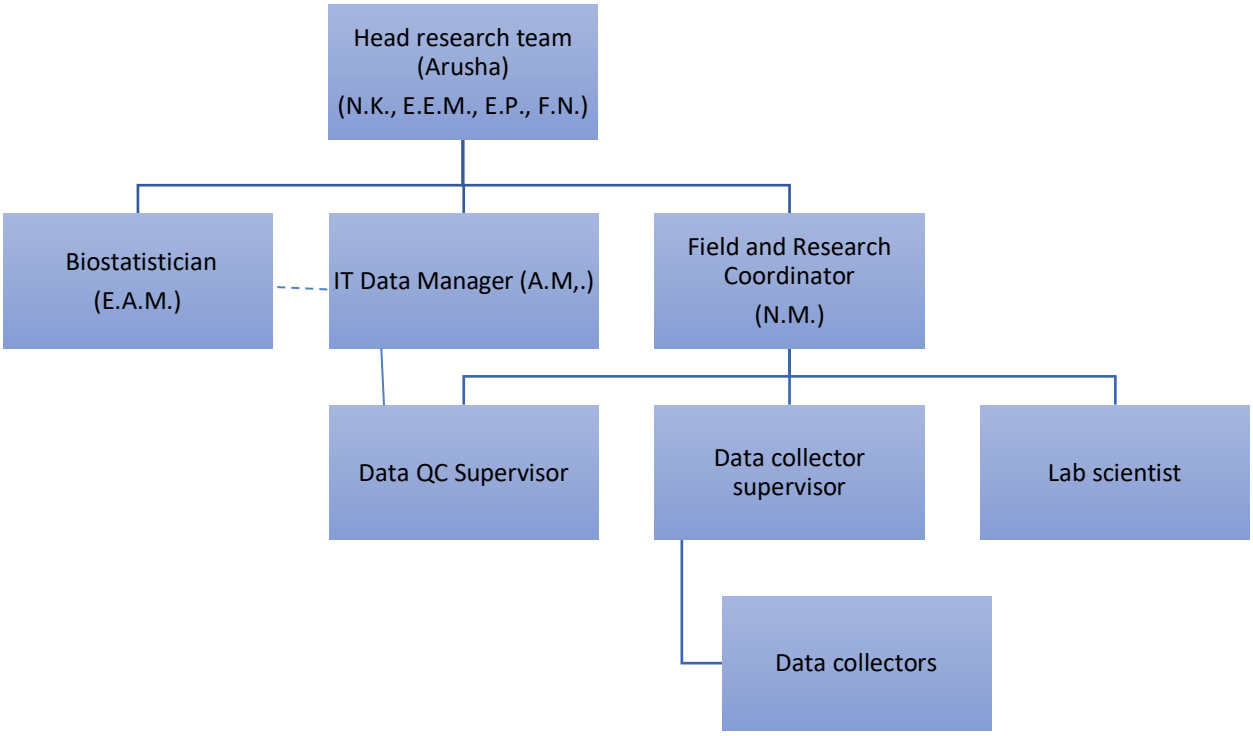
Identifiers will be removed from the data set and stored separately from other data and linked by a participant ID number.

Plan for potential breaches

Although we will only use IRB-approved and encrypted means of sending data, there remains a possibility of a data breach if somehow one of the servers storing the data was compromised or if a laptop or computer was stolen or compromised.

To protect against a data breach, all field staff, including lab staff, will be trained and closely supervised on following the protocol of safe storage of laptops and hardcopies of forms and sending data only through approved means. As stated above, identifiers will be kept separately from other data, to help protect privacy and confidentiality. The IT Data Manager will be responsible for checking the integrity of the data on a weekly basis. If ever data was found to be compromised, he will alert the PIs immediately. If any identifiers have been compromised, this will be reported to the IRB within 24 hours and the affected participants as soon as possible. If data that does not include identifiers have been somehow obtained, we will report this to the IRB within 48 hours, but will not inform participants, as their privacy has not been compromised.

Figure 1 – Field Research Team and Data Organization



Data sharing plan

Our funder, the Bill and Melinda Gates Foundation, has an Open Access Policy. De-identified participant data that underlie the results related to the primary outcome will be made publicly available in a public data repository following the publication of the primary outcome paper in an open-access journal, ideally 12 months following the end of data collection. Data. that underlie the results for all secondary outcomes will also be placed in a public repository following the publication of these papers.

Sample size calculation and cluster selection

The sample size calculation was estimated to detect a difference of length-for-age Z score between the intervention and control groups using the STATA (version 15.1) command for two independent sample means in a CRT. We based on our sample on the ability to detect a difference of 0.2 LAZ, which is believed to be clinically significant and would therefore motivate public health decision-making.

Using a one-sided test of independent sample means, with a standard deviation of 1.2 Z, type I error of 0.05, and power of 0.90, design effect of 2.0 (justified below) and randomizing all 52 health facilities (26 per arm), our total sample size is calculated to be 2,322 (1,161 infants per cluster). This also assumes a CV of .144 for varying cluster size, based on previous year's data for EPI attendance at 42 days. If we conservatively estimate a 20% loss to follow-up and infant mortality, we will need a total of 2,787 infants, or 54 infants recruited per health cluster

annually or 4.5 infants per cluster per month. We therefore will round up to 5 infants per cluster each month for a total of 3,120 potential infants, recognizing that in approximately 6 of the health facilities, it is unlikely that we will be able to recruit up to 60 infants because of the size of the population served by the facility.

To estimate our design effect, we identified two cluster-randomized trials with educational and/or food or LiNS provision interventions delivered through community health workers with LAZ as an outcome[1,2]. Based on these studies, we used an ICC of 0.02 to reach our design effect of 2.0 (rounding up slightly). This is in line with the estimated design effects for stunting using population-based surveys in three African countries calculated by Katz[3].

Statistical Analysis and data analysis plan

All outcomes will be presented using descriptive statistics; normally distributed data by the mean and standard deviation (SD) and skewed distributions by the median and interquartile range (IQR). AF biomarkers and food contamination, usually skewed, will additionally be presented as geometric means and 95% Cis, medians and IQRs. Binary and categorical variables will be presented using counts and percentages. These outcomes will additionally be presented by study arm and season.

The primary analysis will compare LAZ at 18 months between the control and intervention groups and will be analyzed using statistical approaches (such as Stata GEE regression procedure) to account for with-in cluster variance. The primary analyses will be intention-to-treat.

Secondary analyses will include:

- Intention-to-treat analyses of human mycotoxin biomarker outcomes
- Analyses of mediation of mycotoxin biomarkers on the primary outcomes, using path analysis and/or regression approaches
- Adjusted estimates of the randomized treatment effects on primary and secondary outcomes, using multiple regression equations to adjust for imbalances between randomized groups, and potentially increase precision of the effect estimate. For this analysis we will include covariates that differ substantially between randomized groups and/or that modify the intervention effect size by at least 10% when included in the model.
- Per-protocol analysis of those with the highest contact with CHWs responsible for delivering the intervention and those who report high adherence to the intervention. In these analyses, we will consider the CHW contact and adherence to the intervention as ordinal variables defined by tertile or quartile of the continuous variables.

Trial results will be reported in accordance with the extended CONSORT guidance for cluster randomized trials.

Specified subgroups

- SES Quartile
- Male/female
- Elevation of residence
- Quartiles of LAZ at 6 months

Missing Data

We will make our best effort to minimize missing data. In the main analyses we will include all relevant continuous covariates with less than 10% missing. For categorical variables, we will create a dummy variable for missing. For variables that have substantial missing we will conduct sensitivity analyses with multiple imputation to compare to the main analyses.

X. Quality Assurance

A DSMB will be established, consisting of a biostatistician, a pediatrician and a nutritionist. They will convene (virtually) once around the time of the study's launch, half way through the 12 month data collection (estimated January 2020), when the 12 month data collection is complete (Estimated June 2020) and at the end of the trial.

All researchers and staff working on this trial will be trained in GCP every three years. Those with a strong internet connection and English comprehension will pass either the Society for Behavioral Medicine/NIH GCP or CITI Social and Behavioral Research e-course. Those with limited internet and/or limited English ability, can choose from two options: a) the Johns Hopkins Good Clinical Practice for Social and Behavioral Research Field Guide in English or b) the condensed Kiswahili version of this guide (also by Johns Hopkins). Record of passing the training will be kept R.N. and E.P.

As mentioned in Section VII, we have designed an Unexpected Events Reporting plan and will train all staff in this plan to ensure appropriate and timely reporting to the Cornell IRB and NIMR. This plan is proactive and will review data on a weekly, every other week, and monthly time interval to look for trends that might warrant further safety review.

XI. Expected Outcomes of the Study

There is evidence from multiple observational studies in sub-Saharan Africa of an inverse relationship between AF exposure and longitudinal child growth (the more exposure, the less linear growth). Additionally, there appears to be a minimum threshold of exposure above which growth faulting takes place, although most observational studies have been small with varying assessment and analytic methods used between them, making this effect statistically difficult to analyze and compare between studies. The only clinical trial conducted to assess the effect of AF on growth did not find an association between AF-lys and LAZ at 22months, but did at 13 months, albeit with a relatively high rate of loss to follow-up. The current trial would strengthen the evidence base by generating evidence in a well-powered trial conducted in a site where 37% of children under 5 are stunted (<-2LAZ) and AF exposure is similar to those sites in West Africa where the association between AF and growth faltering was first described.

XII. Dissemination of Results and Publication Policy

In accordance with the recommendations of the International Committee of Medical Journal Editors (ICMJE), authorship of any paper that is a result of data collection efforts from this trial will be based on 1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published. Any listed authors will all meet conditions 1, 2, and 3.

Accepting co-authorship carries a level of accountability and authors should have the ability to discuss and publicly defend what is being presented as well as have confidence in the integrity of the contributions of their co-authors.

Analysis, presentation, and publication of data

All investigators, staff, partners and students are encouraged to initiate and contribute to dissemination projects. All manuscripts and materials containing data from the MMT must be reviewed and approved by the Principle Investigators, Rebecca Stoltzfus, Rebecca J Nelson and Neema Kassim before submission. The data collected during this study may only be analyzed, presented, and/or published with the written consent of the PI's. No data or study information relating to the study should be used or shared without formal review and agreement. A proposal should be submitted in writing to the PIs for review. Reviews will receive one of the three designations: 1) approved; 2) approved with requested modifications; or 3) not approved. Such requests will be considered in light of the integrity of the overall program objectives.

Non-author contributors

Note that non-author contributors are those who meet fewer than all of the above criteria for authorship and should not be listed as authors, but they should be acknowledged. Those whose contributions do not justify authorship may be acknowledged individually or together as a group under a single heading, and their contributions should be specified (e.g., "served as scientific advisors," "critically reviewed the study proposal," "collected data, "participated in writing or technical editing of the manuscript", etc.). Common roles that meet the criteria for other contributions under the acknowledgement section include technical and supportive assistance, writing assistance, general support and scientific advisement, financial support, and critical review of the manuscript.

The Bill and Melinda Gates Foundation, the funder of this trial, requires an open access data policy. Therefore all manuscripts from this funded work to be published in open access journals with the data underlying the published research results. More on this policy can be found: https://www.gatesfoundation.org/how-we-work/general-information/open-access-policy

We will present our results with local (Kongwa) government officials, as well as a final report to NIMR and COSTECH. We are not authorized to share any results with the participants or communities directly but can request the local government to share only summary results (not results by health facility) to assure confidentiality is upheld. We will also share our results with

the national Steering Committee on Mycotoxins, possibly presenting our data to the committee in person.

XIII. Duration of the Project

We expect recruitment to begin in April of 2019. All participants will be in the study until 18 months of age and we will recruit over one year's time. If we meet our recruitment target, the trial should end in July 2021.

XIV. Problems Anticipated

Potential problems include:

1. *Mothers' reluctance to participate in the trial, especially those in the control group who will not receive flours.* This could slow recruitment and/or results in a non-random group of participants. We will monitor recruitment and attrition closely to reduce potential bias. In an effort to educate mothers and communities about the trial, we conducted a series of informational meetings with community leaders across the district prior to recruitment.
2. *Difficulty in producing infant flours to meet Tanzanian food safety regulations.* We have been extremely proactive in setting up contacts with food traders who can source clean groundnuts and can provide strong QC systems to ensure that we can produce a clean product at a reasonable cost without further concentrating AF in the food supply.
3. *Provision of flours increases caloric intake in the experimental group compared to the control group.* The porridge flour we provide is based on the median composition of pre-blended flours in select villages in Kongwa. Our education intervention will promote the same amount of porridge composition, consistency and frequency of feeding in both groups. To check for any potential differences, we will perform 24-hour dietary recalls to measure intake in 100 infants per arm at 12 months.

XV. Project Management

- Rebecca Stoltzfus – Project PI, responsible for all project oversight
- Rebecca Nelson – Project co-PI, responsible for leading the maize sorting innovation
- Neema Kassim – PI of Nelson Mandela African Institution of Science and Technology, responsible for oversight of trial design and implementation in Tanzania
- Edna Makule – Responsible for production of trial foods to meet legal safety standards
- Erica Phillips – Responsible for providing technical leadership and support for trial design, implementation and analysis
- Francis Ngure – Responsible for providing technical leadership on production of trial foods to meet legal safety standards; food surveys and analysis of food samples. Provides technical support on trial design, implementation and data analysis.
- Laura Smith – Provide technical support and oversight for urinary and blood biomarker collection and laboratory and statistical analysis

- Paul Turner - Provide technical support and oversight for urinary and blood biomarker collection and laboratory and statistical analysis
- Adam Mawenya – Responsible for electronic data management and database and IT support
- Emmanuel Mpolya – Biostatistician responsible for co-data analysis
- Nyabasi Makore – Research and Field Supervisor to oversee all field operations

XVI. Ethics

While planning for this trial, our team performed a literature review on ethical principles of trials with similar exposures and drafted a working paper on the ethical considerations of conducting such a trial. Additionally, we communicated with the PIs of the MAICE trial to build from their knowledge and experience. The current design is a result of consideration of multiple study and intervention designs and reporting options.

Our first consideration was if a randomized trial was an ethical approach to testing this hypothesis, given that the exposure is AF consumption. Considering the multitude of observational studies already performed analyzing this hypothesis, another observational study would not be conclusive enough to motivate decision makers. Furthermore, we concluded that a randomized trial would meet the criteria for clinical equipoise because of lack of strong causal evidence about the effects of low to moderate chronic AF exposure in infancy. To build strong causal evidence, this trial will collect multiple AF biomarkers in the full sample at three time points and in the cohort at five time points.

Next, we considered multiple design options for this trial. We considered implementing a stepped-wedge design, in which all participants would eventually receive the experimental package of interventions, as an alternative to a cluster-randomized design. However, since we believe the trial does meet the criteria for equipoise, a stepped-wedge design does not increase the ethical value of the study, but would slow the time to inference and make inference more complicated.

The intervention was designed to meet multiple objectives 1) provide strong causal evidence, 2) promote a nutritionally diverse and adequate diet for all infants, 3) minimize dietary differences between intervention arms, and 4) keep all recommendations for foods potentially contaminated with AF in line with current practices/local recommendations, so as not to increase AF consumption. In Kongwa, both the District Nutrition Officer and the former USAID project called “Mwanzo Bora” promote(d) the use of lishe with a composition of 3:1 or 4:1 maize meal to groundnut powder. We therefore consider these lishe recipes to be the regional standard of care. Our formative research confirmed that 66% of infants are fed groundnuts in a variety of foods, including porridges and sauces, as it is one of the few protein sources fed to infants in this area.

Our IYCF education will include messages on dietary diversity and the importance of continued breastfeeding through 24 months, using education materials approved by the Tanzanian Food and Nutrition Center. We will monitor IYCF and breastfeeding practices during the trial to assure the promotion and provision of lishe do not reduce dietary diversity or breast milk

intake.

A final major consideration is if and how to respond to biomarker or food samples with results that could be considered "high" or are outliers. To report these results to participants or relevant authorities we would need a). clearly established cut-off points, b) the ability to report results in a timeframe that could be clinically useful to the participant, and c) advice to prevent future contamination. Each of these proves challenging given current knowledge and available technologies for locations similar to Kongwa.

First, AF biomarkers are not clinical measures with determined cut-offs to distinguish between a safe or potentially unsafe level. In the case of no established clinical cut-offs, there is no clear scientific or health justification to flag or report results except at the levels observed in cases of acute aflatoxicosis. Outside of the range of acute illness, there will undoubtedly be results that are outliers and/or in the upper ranges compared to other research subjects or other reported data, prompting questions about the ethics of reporting to the participant's guardian or the relevant government institutions in these cases.

Secondly, there is no available technology that will allow rapid analysis of blood or urine samples in the field. Currently there is no capacity to analyze AF-alb in Tanzania, so samples will be exported for analysis, guaranteeing a long delay between sample collection and obtaining results. Because the dynamics of AFM1 and AF in food are highly variable, by the time sample outliers would be identified, the properties of food consumed by the individual would undoubtedly differ from those consumed when the sample was taken. The results of the analyzed sample might not be relevant by the time it could be reported.

Finally, once a sample was determined to be an outlier, what useful advice could be given to the parent or caretaker of the infant? Proven interventions to reduce AF exist, but few are practical and feasible in resource-poor settings such as Kongwa. Give these complexities, we have decided to take the following actions to prevent contamination and report our findings.

Education to Prevent Contamination

We will teach mothers how to sort groundnuts to reduce AF contamination as part of our blanket IYCF education after 12 months of age, when infants consume more groundnut. Manual groundnut sorting has shown to be effective to reduce AF, although the evidence is limited for household sorting in low-resource settings. It is not effective to sort maize manually because contaminated kernels can't be determined visually.

Blood and Urinary Biomarker Reporting

We will take the following precautions for AF-alb results, even with the understanding that there will be a significant time delay between taking the sample and analysis:

1. We will report the percent of samples above 1000pg/mg to the Kongwa District Council. We have subjectively chosen this cut-off because these are these are levels usually found in the highest quintile in other high-risk countries. One limited case-control study of aflatoxicosis from Kenya reported cases (n=10) had higher AF-alb versus controls (n=50) with geometric means of 3500pg/mg vs 150pg/mg (Azziz-Baumgartner et al., 2004). The ages of these individuals was not clearly defined.

Several control individuals had AF-alb level between 1000-5000pg/mg. We feel that 1000pg/mg cut point represents our best estimate to identify this as a putative reporting concern.

2. If an infant is found to have an AF-alb value > 1000 pg/ml, we will make a home visit to the family to assess the health of the infant and understand more the food security and food quality issues in the household, particularly as they pertain to the infant. Based on the findings, research staff will provide customized advice to the caregivers of the child to protect the infant from continued high exposures, with possible referral to health and agricultural authorities.

For AFM1, there is not sufficient evidence to intervene at any level.

Food Sample Reporting

Unlike biomarkers, there are stated safe limits to AF contamination found in food; in Tanzania the upper legal limit for commercial baby food is 5 ppb. Given the documented AF problem in Dodoma and our formative research, we expect to find at least 30% of samples above this limit, which is the motivation for doing the trial here. Food samples will be analyzed in Arusha and values will be known more rapidly than for AF-alb. We have decided upon the following responses to exceptionally high levels of AF in for food samples (defined as >1000 ppb in maize or >5000 ppb in ground nut):

1. We will report the percent of maize samples above 1000ppb and groundnut samples >5000ppb to the Kongwa District Council. We have subjectively chosen these cut-offs because these are these are levels usually found in the highest quintile in other high-risk countries. Additionally, we estimate that maize is consumed 5 times as much as groundnut, the reason we set the limit 5 times higher for groundnut than maize.

2. If a household sample of food reported to be fed to the study child exceeds these thresholds, we will return to the household to resample that food. This is to account for the high sampling variability in foods.

3. If the second sample confirms high level of toxin, we will make a home visit to the family to assess the health of the infant and understand more the food security and food quality issues in the household, particularly as they pertain to the infant. Based on the findings, research staff will provide customized advice to the caregivers of the child to protect the infant from continued high exposures, with possible referral to health and agricultural authorities.

Finally, because of the limited number of interventions that are feasible in low-resource settings, part of our overall project, but outside the scope of the trial, includes developing a maize and groundnut sorting innovation for use in such settings. This is under development simultaneous to the trial. If effective, we hope it will be rolled it out across Kongwa (and beyond).

XVII. Informed Consent Forms

Written informed consent will be asked and obtained by all participants in Kiswahili. Our

informed consent forms have been written specifically for a population with limited research experience and research knowledge and contains multiple questions to check for comprehension of the study and the participant's rights.

All forms have been translated from English to Kiswahili. Attached are the forms, adapted by randomized group.

XVIII. Budget

The budget for this research is provided solely from the BMGF. Additional funding is being sought for future analysis of samples.

XIX. References Cited

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Rockville; 2016.
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