Dietary Intervention Research Proposal

Title: The inflammatory and metabolic effects of a 2-weeks food change between traditional Tanzanian and western-type foods in healthy Tanzanian male individuals

Project Acronym: DIET-study

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Contents

Abbreviations

BMI	Body Mass Index
CANTOS	Canakinumab Anti-inflammatory Thrombosis Outcomes Study
Castor EDC	Castor Electronic Data Capture
ChEBI	Chemical Entities of Biological Interest
CRERC	College Research and Ethical Review Committee
CVDs	Cardiovascular Diseases
DMP	Data Management Plan
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FDR	False Discovery Rate
GCP	Good Clinical Practice
HMDB	Human Metabolome Database
HIV	Human immunodeficiency viruses
IATA	International air transport association
IFN	Interferon
IL	Interleukin
LEfSe	Linear Discriminant Analysis Effect
LIMES	Life & Medical Sciences Institute
IRB	Institutional Review Board
KCMUCo	Kilimanjaro Christian Medical University College
KCRI	Kilimanjaro Clinical Research Institute
KEGG	Kyoto Encyclopedia of Genes and Genomes
NCDs	Non-communicable diseases

NIMR	National Institute for Medical Research
Radboudumc	Radboud university medical centre
SSA	Sub-Saharan Africa
TNF	Tumor necrotic factor

Definition of terms

Western diet: a diet with high content of simple sugar, fat, preservatives, taste enhancers, chemical leaven and with low-fibre. It also includes industrially processed food, packed to be consumed at a later time.

Traditional diet: a diet with low-fat, low simple sugar, high-fibre and unprocessed or locally processed and consumed.

Urbanization: refers to the population shift from rural to urban areas, the decrease in the proportion of people living in rural areas, and how societies adapt to this change.

Gut microbiome: The gut microbiome, as defined by molecular biologist Joshua Lederberg, is the totality of microorganisms, bacteria, viruses, protozoa, and fungi, and their collective genetic material present in the gastrointestinal tract (GIT).

Metabolomics: refers to the systematic identification and quantification of the small molecule metabolic products (the metabolome) of a biological system (cell, tissue, organ, biological fluid eg. plasma, urine CSF etc, or organism) at a specific point in time.

Transcriptomics: is the study of the transcriptome—the complete set of RNA transcripts that are produced by the genome, under specific circumstances or in a specific cell—using high-throughput methods, such as RNASeq or microarray analysis.

Lipidomics: the large-scale study of pathways and networks of cellular lipids in biological systems. Lipidomics research studies the structure and function of the complete set of lipids (the lipidome) in a given cell or organism as well as their interactions with other cellular components.

Revision

- Serum tube (4ml)
- 20 subjects in the mbege arm
- EDTA (6 ml)

Summary

Background

Sub-Saharan Africa (SSA) faces rapid urbanization. This coincides with changes in disease epidemiology with a sharp increase in non-communicable diseases (NCDs), along with the ongoing high burden of infectious diseases. Important gaps remain in our insight into the mechanisms underlying this epidemiologic transition. The production of proinflammatory cytokines by monocytes and monocyte-derived macrophages is pivotal for host defence against infectious diseases and is also a key process in inflammatory processes responsible for the pathophysiology of NCDs. Recent studies, including data generated by our group in healthy Tanzanian individuals, suggest that a switch from an unprocessed or locally processed traditional low-calorie, high-fiber diet to a high-caloric, high-fat, more industrialized processed food 'western-type' diet increases inflammation.

Study objective:

To assess the inflammatory and metabolic effects and changes in gut microbial profiles of a 2-week food change in healthy adult male Tanzanians, in which rural living individuals will be fed a high-fat, high simple-sugar, high calorie intake and low-fibre more industrialised processed food 'western-type' diet and urban living individuals a low-fat, low simple sugar, high-fibre unprocessed or locally processed traditionally rural type diet.

Methods:

The prospective intervention study will be conducted in Northern Tanzania, enrolling adult healthy male individuals between 20 and 40 years of age residing in a rural or urban area in the Moshi district in the Kilimanjaro region. Participants will be followed for 1 week while consuming their usual traditional or western-type diets. Then their diet will be changed for 2 weeks. The diet will be provided. by a cook and intake will be closely supervised by a member of the study team to encourage adherence. Blood and stool samples for analysing circulating inflammatory mediators, whole blood cytokine production, blood transcriptome, plasma metabolome, lipidome, and gut microbiome will be collected before and after the dietary change and four-weeks after participants resuming their usual diet.

Hypothesis:

It is hypothesized that diet change to industrialized processed food with high-fat, high-calorie and low-fibre ‘western-type’ diet increases inflammation and leads to significant modulation in food-derived and endogenous plasma metabolites and lipids and changes in gut microbial profiles. This study is expected to provide important new insight into the mechanisms by which urbanization and the nutrition transition affect disease epidemiology and how efficient the use of natural resources may help to promote the overall health of populations.

1.0 INTRODUCTION

1.1 Background

Increasing urbanization in Sub-Saharan Africa (SSA) is associated with changes in disease epidemiology. This coincides with a sharp increase in non-communicable diseases (NCDs) such as diabetes, hypertension and cardiovascular diseases (CVDs). Urbanization is associated with a nutrition transition, characterized by reduced consumption of traditional staple foods rich in starch and dietary fibre, and plant protein sources like legumes, and increased consumption of energy-dense processed foods (1, 2) To date, important gaps remain in our insight in the impact of this nutrition transition on inflammation and immune responses. The production of proinflammatory cytokines by monocytes and monocytes-derived macrophages is pivotal for host defence against infectious diseases, but also a key process in the pathophysiology of NCDs. Recent literature including data from our group highlights the role of diet in the regulation of inflammation and immune responses via metabolites produced by bacteria in the gut and metabolites directly derived from food (*manuscript under review*).

1.2 Literature Review

Increasing urbanization is among the most important demographic changes of the past century (3). For the first time in human history, the majority of the world population is now living in urban areas. Rural to urban migration coincides with changes in disease epidemiology, which is referred to as the "epidemiological transition." This exhibits a change from a high prevalence of infectious diseases to a high prevalence of NCDs, such as diabetes, hypertension, and cardiovascular diseases (CVD) (4, 5). Populations in many parts of Sub-Saharan Africa are presently suffering from a double burden of infectious diseases and NCDs. The latter has become the most frequent cause of death worldwide, and 80% of these deaths are estimated to occur in low-income and middle-income countries (6). In Tanzania, urban residency is associated with increased risk factors for CVD, such as a higher prevalence of diabetes, obesity, and hypertension (7-11). Also, dietary habits were reported to contribute to the variation in CVD risk factors among rural and urban Tanzanians (12). As mentioned above, urbanization coincides with a nutrition transition.

The mechanisms via which this nutrition transition contributes to changes in disease epidemiology in SSA - and especially the current epidemic of NCDs - are only partly understood. Recent literature highlights the role of diet in the regulation of inflammation and immune responses via metabolites produced by bacteria in the gut and metabolites directly derived from food (13-15).

Not only the traditional staples food of Tanzanians contains high levels of flavonoids but also the traditional drinks. Banana beer commonly known as ‘Mbege’ is an alcoholic beverage traditionally made at home and consumed by many people of the Kilimanjaro region. It is produced mostly by mixing ripened-boiled and fermented banana (*Musa spp.*), porridge of germinated finger millet (*Eleusine*

Coracana) and water. Banana and millet are the most important crops used as a traditional staple food in many parts of Tanzania especially in northern Tanzania. 'Mbege' has a sweet wine like aroma and taste sweet, especially in the early hours of its production. The ethanol content of 'Mbege' varies from 1% after mixing banana juice and cooled porridge of germinated finger millet to 3.2% after 12 hours fermentation (19). Fig. 2 summarizes the process of brewing banana beer. Germinated finger millet has high levels of flavonoids including apigenin which plays a crucial role as an anti-inflammatory, anti-cancer and anti-oxidants.

Moreover, data from our group (*unpublished*) show that 'Mbege' contain high levels of trehalose. Trehalose is a naturally occurring disaccharide containing two glucose molecules bound in an α, α-1,1 linkage found naturally as an energy source, and stabilizing agent (20). Trehalose is consumed as part of a normal diet in food produced using brewer's and baker's yeast such as *Saccharomyces cerevisiae*, and other foods such as mushrooms, honey, lobsters and shrimp, and also used as sweetener, stabilizer, thicker and flavor enhancer in different kind of foods. The role of trehalose produced by yeast during fermentation on the inflammation and immune response is partially understood. In recent years, much attention has been drawn to a possible anti-inflammatory and anti-cancer role of trehalose. Several animal studies have demonstrated the immune-modulatory effect of trehalose in the experimental model of septic shock (21), in mouse peritoneal macrophages (22) and atherosclerotic animal model (23). Besides, our recent data in healthy individuals in the Kilimanjaro region show that anti-inflammatory cytokine production of the rural-living individuals is higher compared to the urban living individuals, and we identified among others significant abundant of the plasma food-derived metabolites including trehalose in the rural-living individuals (Temba et al., *manuscript under review*).

1.3 Problem Statement

Many areas in Sub-Saharan Africa, including Tanzania, face epidemiological transition and a related sharp increase in NCDs, such as diabetes, hypertension and cardiovascular diseases. Inflammation is a key process in the pathogenesis of these NCDs and dietary interventions are an attractive intervention to curb the epidemic of these NCDs. Currently, important gaps remain in our understanding of how diet change due to the epidemiological transition affects inflammation and host metabolism in healthy individuals in SSA.

1.4 Rationale

The production of inflammatory cytokines by monocytes and monocyte-derived macrophages is pivotal for host defence against infectious diseases, but also a key process in the pathophysiology of NCDs (16). This was recently affirmed by the results of the CANTOS trial, which showed that inhibition of the interleukin (IL)-1β pathway reduces cardiovascular death (17, 18). Recent studies highlight the important role of diet in the regulation of inflammation and immune responses via metabolites produced by bacteria

in the gut and metabolites directly derived from food (13). Moreover, we recently found in a cross-sectional study that people living in a rural area in Tanzania had lower inflammation than those living in a nearby urban area in the Kilimanjaro region, which was mediated, at least in part, by food-derived metabolites (Temba et al., *manuscript under review*). Among the metabolites which were significantly abundant in the plasma of rural-living individuals were flavonoids especially flavone apigenin. Apigenin has anti-inflammatory, anti-cancer and antioxidant properties. This suggests that unprocessed or locally processed traditional, rural-style diets may reduce inflammation and thus the incidence of NCDs. These findings enticed us to perform the proposed study to provide further evidence for the effect of diet on inflammation. Insight in the effects of dietary change on the blood transcriptome, plasma metabolome, lipidome, and gut microbiome, and how these interact with immune responses and health in Tanzanian individuals will provide important information of how urbanization and the nutrition transition affect disease epidemiology and how the use of natural resources may help to promote the health of populations.

1.5 Research hypothesis

Members of our research group recently showed in animal models that a western-type diet induces systemic inflammation and a long-term epigenetic reprogramming of innate immune cells (24). Besides, our recent data in healthy individuals in the Kilimanjaro region show that pro-inflammatory cytokine production of urban-living Tanzanians is higher compared to rural-living Tanzanians, and we identified different food-derived and endogenous metabolites explaining this difference (Temba et al., *manuscript under review*). Traditional foods are locally harvested: people eat what the local fields provide depending on the seasons and most of the traditional food staples such as cereals are consumed unprocessed or locally processed. Previous studies have shown cereals including those traditionally consumed in Tanzania contain high levels of flavonoids. Interestingly, Smuda, S.S et al., have shown that milling by-products of cereals including cons, wheat and rice contain high levels of total flavonoids (25). Therefore, we speculate that industrial processing of cereals removes most of the important bioactive compounds including flavonoids which play a crucial role as an anti-inflammatory, anti-cancer and anti-oxidants. On the contrary, a western diet contains more industrialized processed food that often includes a high content of simple sugar, fat, preservatives, taste enhancers, chemical leaven etc. and is packed to be consumed at another time and place.

The hypothesis underlying the current study proposal is that the change from unprocessed or locally processed traditional Tanzanian food to an industrially processed, energy-dense, high-fat, high-calorie food, increases inflammation and leads to significant modulation in food-derived and endogenous plasma metabolites, lipids and the gut microbiome changes.

2.0 Research objectives

2.1 General objective

To assess the inflammatory and metabolic effects and changes in gut microbial profiles of 2-weeks food change in healthy adult male Tanzanians in which rural living individuals will be fed a high-fat, high simple-sugar, high calories intake and low-fibre more industrialized processed food 'western-type' diet and urban living individuals a low-fat, low simple sugar, high-fibre unprocessed or locally processed traditionally rural type diet.

2.2 Specific objectives

1. To assess the role of diet change between traditional and western-type diets on circulating inflammatory mediators and cytokine production capacity of the circulating immune cells in Tanzanian healthy male individuals.
2. To assess the role of diet change between traditional and western-type diets on changes in blood transcriptome, plasma metabolome and lipidome, and gut microbiome profiles in healthy Tanzanian male individuals.
3. To assess the effect of consuming local brew 'Mbege' (banana beer) on circulating inflammatory mediators, cytokine production capacity of circulating immune cells and changes in blood transcriptome, plasma metabolome, and gut microbiome profiles in healthy Tanzanian male individuals.
4. To interrogate the effect of the changes in transcriptome, metabolome, and lipidome on innate immune responses in healthy male Tanzanians.

3.0.0 Materials and Methodology

3.1.1 Study design

The study is designed as a prospective intervention study. A total of 66 healthy male individuals aged 20-40 years living in either urban area (n=38) or a rural area in Moshi districts (n=28) will be enrolled. The intervention study will comprise of two parts.

In part one: Participants consuming a traditional high-fibre, low-simple sugar, low-fat traditional Tanzanian type diet (rural area) or a low-fibre, high-simple sugar, high-fat western-type diet (urban area) will be selected using a dietary recall history of the previous week. First, dietary habits in the home environment will be studied for one week with participants taking their usual diet. We will provide participants with a diet diary/forms in which participants will be asked to record the type of food they consumed in the past 24 hours for 3 non-consecutive days including at least one festival or weekend day. They will also be required to describe whether the food was prepared from unprocessed or processed products (e.g. unprocessed maize flour 'Dona' Vs. processed maize flour 'Sembe'. Blood and stool samples will be taken after this week. Per group, participants will be randomized for the dietary intervention for 2 weeks (n=23 per group) or remain on their usual diet (n=5 per group).

To ensure compliance with dietary changes and to better estimate the actual food and nutrients intake, the intervention diet will be prepared for the entire group by a cook in a central location and food intake will be given under close supervision and will be recorded. A similar portion of food will be given for all meals and in case of leftovers, this will be recorded and considered during the caloric and nutrients intake calculations. Participants will be given a special coupon, with this coupon, participants can access our cooking facility and have breakfast, lunch, and dinner including drinks. One-bottle of commercially available beer (~350mL) will be provided to the rural participants every day after dinner. Participants will be encouraged not to eat (except water) outside the intervention site and in case they do, will be asked to record what they ate or drank using a special sheet and pictures of various food and drinks provided. It is the common practice in the study area for the people even in the rural areas to have three meals daily. The choice for the type, amount and time for the drinks based on the common practice where many people go for drinks in the evening after work both in the rural and urban areas. Blood and stool samples will be taken after these 2-weeks of diet change and 4-weeks after participants resume their usual diet. A period of two week is considered sufficient to observe a dietary effect on both the immune response and gut microbiota as previously shown (26). The advantage of this type of study design is that each participant will serve as his/her control. This type of study design in short dietary changes has been previously described (26).

In part two: Banana brew (mbege) is commonly consumed in rural areas. Mbege is made from fermented banana to which millet is added. Mbege contains yeast (*Saccharomyces cerevisiae*) and is a rich source

of fibers, flavonoids (millet) and the disaccharide trehalose. Both flavonoids and trehalose exhibit anti-inflammatory properties. To test the immune-modulatory effect of consuming banana brew, 10 volunteers from the urban area who consume alcohol, but not banana beer will be randomized for a banana beer intervention for 1 week. Participants will receive one-litre of a local brew 'Mbege' daily while consuming their usual diet. The effect of other factors will be controlled by taking measurements from the same participants before and after the intervention. Blood and stool samples will be taken before and after the intervention period of 1 week. Stool samples will also be taken weekly for a period of four weeks post-intervention in order to study how long the acquired microbiota will remain in the gut. Food samples from the brewing process will be stored for later analysis of the presence of yeast and food-derived metabolites. These will include unfermented banana juice, fermented banana juice, millet porridge and the final banana brew product.

3. 1.2 Study area

This study will be performed in Moshi in the Kilimanjaro region in Northern Tanzania. Moshi town is the administrative, commercial and educational centre of the Kilimanjaro region with over 200,000 inhabitants. Due to this regional function, there is significant diversity in ethnic groups, economic status, and lifestyle, whereby most inhabitants have adopted a western lifestyle. People residing in the Moshi municipality (urban zone) have access to good sanitation with universal coverage of chlorinated tap water and toilet facilities. In contrast, people we designated as living in rural conditions represent the rural population of Tanzania. In general, people live within large family units, whereby the economy depends mainly on subsistence farming and animal husbandry. Most individuals in the rural area belong to the Chagga tribe and follow a traditional lifestyle whereby consumption of starch and vegetable diet is common. They also have access to good sanitation and water primarily from the slope of Mount Kilimanjaro. We will enrol individuals from both urban and rural zones. 1) Urban participants will be recruited from KCMC community located at Longuo street. 2) For the rural zone, participants will be recruited from a selected village in Moshi rural area. We will rent a cooking facility located close to the home of the participants.

3.1.3 Study population and recruitment

We will select two groups of age, and body mass index (BMI) matched healthy male volunteers: one group consisting of individuals living in Moshi rural area consuming predominantly a traditional Tanzanian diet and one group living in Moshi town (in KCMC community located at Longuo street) who consume predominantly a western-style diet. Information about the study will be advertised through leaflets for the urban population and local government leaders and church leaders and announcements during mass gatherings in churches for the rural population. Dietary habits will be screened in interested participants by a member of the study team using a guided dietary habit questionnaire to verify that participants

consume a traditional or western-type diet. Written informed consent will be obtained from individuals fulfilling the inclusion criteria. All volunteers will be screened for Human immunodeficiency virus (HIV) and malaria infections using rapid diagnostic tests. Fasting blood sugar, blood pressure, and body weight and height measurements will also be taken. A standardized questionnaire will be used to record metadata, lifestyle, health information and daily activities including the use of tobacco, level of physical activities, disease history and use of medication and vaccination history.

3.1.4 Inclusion criteria:

- Tanzanian healthy male individuals aged 20-40 years and BMI range of 18-25kg/m^2.
- Living either in rural or in urban areas in the Moshi district for more than a month before participation and consuming either a traditional Tanzanian or a western-type diet.
- Use alcohol either local brew 'Mbege' or commercially available beer.
- Can stay in the study area throughout the intervention period.

Justification: Age, gender and BMI are known to influence immune responses including inflammation, and they also affect the composition of gut microbiota. Elderly individuals have declined immune function, a phenomenon referred to as immunosenescence, while higher BMI is associated with increased inflammation. On the other hand, females have varying hormonal levels linked to menstrual cycle and use of contraceptives.The choice of age categories, BMI range, and sex are done to limit the well know confounding effects of these factors on inflammation and on gut microbiota composition among individuals.

3.1.5 Exclusion criteria:

- HIV seropositive
- Malaria seropositive
- Blood pressure outside the defined range (≤60mmHg diastolic or ≥140mmHg systolic)
- Fasting blood sugar (>6.0 mmol/L)
- BMI outside the defined range (18-25kg/m^2)
- Food allergies
- Acute (febrile) illness in the previous month
- Use of any medication as well as the use of antibiotics in the past three months or vaccination
- Hospital admission in the past year
- A Known chronic condition such as active malignancy, liver or kidney disease, tuberculosis infection, chronic hepatitis B or C infection
- History of hypertension, diabetes, cardiovascular diseases

- Failure to consent.
- Female sex
- None alcohol users or reported alcoholism
- Participation in another clinical trial at the same time or within the last 30 days

3.1.6 Sample size estimation

A total sample size of 66 participants (28 and 38 from rural and urban population respectively), assuming equal group sizes, to achieve a power of 80% and a level of significance of 5% (two-sided), for detecting a true difference in means between the test and the reference group of 269 (i.e. 679 - 410) units. The sample size calculation is based on the previous data (Temba et al., *manuscript under review*) from the same community on mean cytokine responses between urban vs rural and the pooled standard deviation for both groups.

3.1.7 Sampling techniques and sampling tools

Volunteers will be actively recruited using flyers and through advertising during the mass gatherings. Eligible volunteers who consented to participate will first receive a 24 hours food recall questionnaire to record food intake for 3-non-consecutive days while taking their usual diet. Based on this screening procedure participants will be defined as either rural (having traditional dietary habits) or urban (having western-type dietary habits)

At day 7, blood samples consisting of heparin tube (6 ml), EDTA tube (6ml), citrate tube (3ml) and paxgene tube (2.5ml); and stool sample will be collected from all enrolled participants. For the rural group, samples will be collected at the field site and for urban group samples will be collected at the KCRI research unit (*baseline data*).

After the baseline sampling, the dietary intervention will begin.

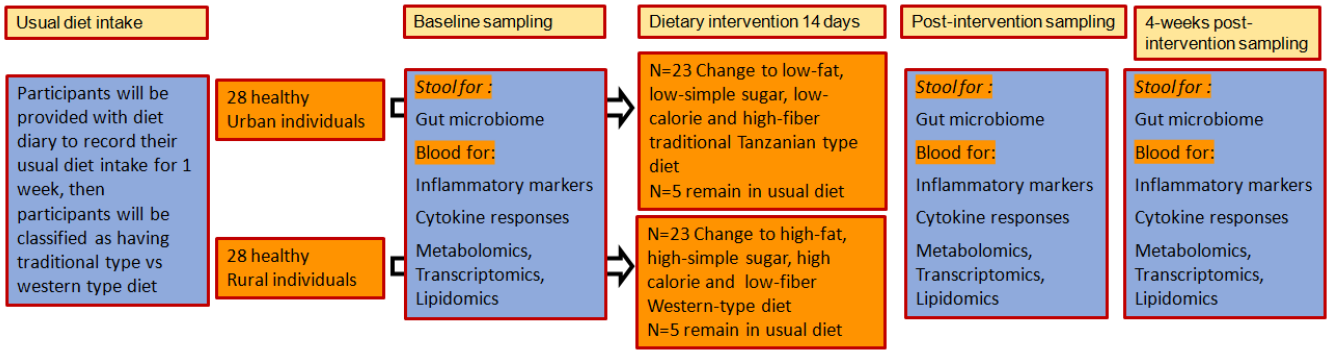
In part one: rural participants will receive the western-type diet and selected urban participants will receive a traditional diet (*intervention phase*). Intervention diet will be prepared at the same place with a hired cook and a daily dietary information record will be taken. Bodyweight, blood pressure and fasting blood sugar will be measured on day 0, 7 and 14. At day 14 post-intervention, a second sampling of blood and stool will be performed as described above (*effect of the intervention*). After the 2-weeks of diet change participants will resume their usual diet and 4-weeks later the third sampling of stool for microbiome analysis and blood for omics analysis (depending on available budget) will be performed.

In part two: Ten participants from the urban area will receive the local brew ‘Mbege’ for 1-week. Samples will be taken before and after the intervention. In addition, stool samples will be taken on weekly bases for up to four weeks post-intervention.

F**low chart 1 and table 1** summarizes the selection, inclusion and sampling procedures.

Flow chart 1: Intervention Scheme

PART ONE



PART TWO

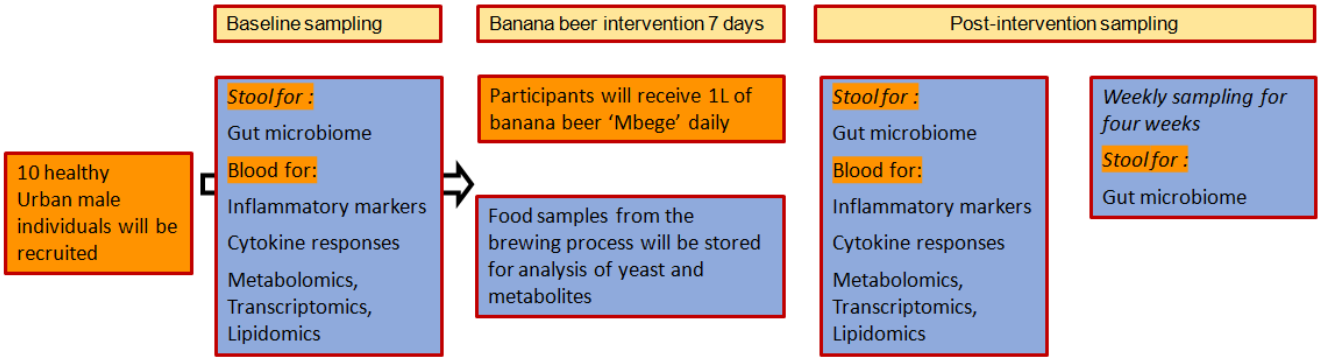


Table 1: Schedule and sampling

PART ONE					
	Usual diet intake*		Dietary intervention#		Post-intervention
Day	0-6	7	0-13	14	4-weeks later
Dietary observation	xxx	x	xxxxxxxxxxxx	x	
Blood and stool samples		x_b		x_i	x_p
PART TWO					
	Baseline		Banana beer intervention		Post-intervention
Day	0		1-7		8
Dietary observation	x		xxxxxxx		
Blood and stool samples	x_b				x_p

X corresponds to dietary evaluation. It indicates the frequency of dietary recording; *7 days during the usual diet intake recorded by the participants using a diet diary. #14 days when consuming the intervention diet recorded by a study team member.
x_b, $x_{i \text{ and } Xp}$ indicate the baseline, after the intervention and post-intervention sampling respectively.

Study Outcomes

The primary endpoint(s):

- Changes in circulating inflammation-related human protein biomarkers
- Changes in the capacity of the circulating immune cells to produce inflammatory cytokines in *ex vivo* whole blood stimulation
- Changes in plasma lipids (lipidome) and metabolites (metabolome) and blood transcriptome

Secondary endpoint:

- Changes in the gut microbiome composition.

3.2.0 LABORATORY PROCEDURES

3.2.1 Measurement of the circulating inflammatory and coagulation proteins

The circulating inflammatory and coagulation proteins will be quantified using a high-throughput, multiplex immunoassay enabling analysis of 92 inflammation-related protein biomarkers across 96 samples simultaneously. The protein expression levels will be compared between the two groups before and after the intervention. This technique is currently not available at KCRI and therefore will be performed through the Radboud university medical centre (Radboudumc), The Netherlands.

3.2.2 Whole blood stimulation assays and cytokine quantification

Ex-vivo cytokine stimulation experiments will be performed at the Biotechnology Laboratory facility available at KCRI in Moshi Tanzania. Briefly, 100µl of heparinized whole blood will be stimulated with 400µl of either bacterial or fungal or toll-like receptor (TLR)-3 or TLR4 agonists for 48 hours at 37^{o}C and 5% CO_2. After 48 hours supernatants will be collected and the concentrations of cytokines interleukin (IL)-6, IL-1β, interferon (IFN)-γ, tumour necrosis factor (TNF)-α and IL-10 will be measured in the stored supernatants using enzyme-linked immunosorbent assay (ELISA).

3.2.3 Plasma metabolome

We will apply the untargeted metabolomics workflow to measure plasma metabolites from EDTA plasma samples. We will use a high throughput mass spectrometry technique time-of-flight mass spectrometer (Agilent, Santa Clara, CA) equipped with an electrospray source operated in negative and positive mode. A list of putative metabolites will be annotated with a series of analysis strategies including deisotoping, decluttering, adduct detection, and library matching in KEGG, HMDB, and CHEBI databases. This technique is currently not available at KCRI and therefore will be performed through the Radboudumc.

3.2.4 Blood Transcriptome and lipidome

These assays will be performed at Radboudumc affiliated reference laboratories as these techniques are currently not available at KCRI nor Radboudumc. The Radboudumc affiliated laboratory for this type of essay is The Life & Medical Sciences Institute (LIMES), Bonn University, Germany: who will analyze blood transcriptome and lipidome. Material and data transfer agreement will be signed by both parties and submitted to the National ethics clearance board for review and approval.

3.2.5 Gut Microbiome and Immune-phenotyping analysis

Evaluation of gut microbiome and mycobiome composition from stool: DNA will be isolated with the AllPrep DNA/RNA Mini Kit (Qiagen; cat. #80204) with the addition of mechanical lysis. Whole-genome shotgun sequencing libraries will be prepared using the Nextera XT DNA Library Preparation kit (Illumina). Libraries will be pooled using a Labcyte Echo 550 liquid handler, and the concentrations and insert size ranges for each pooled library will be checked using an Agilent Bioanalyzer DNA 1000 kit

(Agilent Technologies). Libraries will be subsequently sequenced on the Illumina HiSeq 2000 platform in paired-end mode (2x101bp) targeting ~2.5Gb of sequences per sample. Details on these techniques have recently been published in an HFGP study (27). This test will be performed at the University of Florence, Italy.

In addition, we will perform two immune-phenotypic analysis to study the adaptive immunity to gut microbiome. First, we will determine the percentages of commensal bacteria bound by immunoglobulins A, M and G. Secondly, we will sort those commensals into IgA positive and IgA negative fraction and perform 16S rRNA analysis of both fractions to identify which microbes are bound by IgA. Immune-phenotyping will be performed at the Centre of immunology and infectious diseases (CIMI Paris), Paris, France.

3.2.6 Analysis of food samples

Food samples from the brewing including unfermented banana juice, fermented banana juice, millet porridge and the final banana brew product will be stored at -80℃ for analysis of the presence of yeast and food metabolites.

3.2.7 Sample storage and shipping

For tests that will be performed at Radboudumc, The Netherlands; Bonn University, Germany; University of Florence, Italy; and Centre of immunology and infectious diseases (CIMI Paris), Paris, France as specified under specific tests above, samples will be stored at -80 °C and later on shipped to the above-mentioned centres for analysis. Samples will be shipped under cold chain using dry ice as guided by the International air transport Association (IATA) regulations for shipping dangerous goods. Material and data agreement forms (MTA and DTA) will be signed between KCMC and collaborating institutions before shipping of samples. MTA and DTA will be submitted to NIMR for ethical approval.

3.2.8 Data management and Data Analysis

All the data generated in this work package will be entered in Castor EDC, which is a data management software compatible with good clinical practice (GCP) guidelines, and will include an audit trail. The data generated in this work package will be available for further research directly related to this study and will be accessible for verification. For durable archiving, data will be stored in a publicly available repository.

Analysis plan of the primary endpoints

For the demographic characteristics, frequencies and percentages will be used to summarize the data across all participants. While for the *ex vivo* cytokines, circulating inflammatory mediators and metabolomes, mean and standard deviations will be used to summarize the data that are normally distributed and median and interquartile range will be used to summarize the skewed data.

Paired T-test (for normally distributed) and the Wilcoxon signed ranks test (for skewed data) will be used to test the null hypothesis that there was no difference in means of cytokines production, circulating inflammatory mediators and the plasma metabolomes before and after the intervention. The differences will be considered statistically significant if they have a p-value of < 0.05 after correction for multiple testing using FDR/Bonferroni. All statistical analyses will be performed in SPSS and using packages available in R software.

Analysis of the secondary endpoints:

Bioinformatics tools will be applied to characterize the gut microbiota profiles among subjects according to the different dietary interventions. Linear discriminant analysis effect size (LEfSe) analysis (28) will be performed to evaluate enriched taxa and to define metagenomics markers of the different diets. Alpha and beta diversity will be estimated. A non-parametric statistical test (Kruskal Wallis test and pairwise Wilcoxon rank-sum test) will be performed to compare relative abundances of microbial/fungal taxa, at the different taxonomic levels, between groups by different diets (p values will be corrected for multiple testing controlling FDR).

4.0 Ethical Considerations

4.1 Ethical statement

Ethical clearance will be sought from the CRERC at the KCMU College, Moshi and the National Institute of Medical Research. The study will be performed according to the principles of Good Clinical Practice (GCP), and to the principles expressed in the Declaration of Helsinki.

4.2 Assessment of risks, benefits, and burden

Risks associated with short dietary intervention are minimal. This may include mild gastrointestinal symptoms that are easy to resolve in a few days, such as diarrhoea, bloating or constipation, due to sudden dietary changes. Regarding sample collection, procedures used have only minimal risk e.g local hematoma related to venous puncture may occur. Volunteers will also have no direct benefit from the intervention. However, volunteers who consented to participate will receive free health check-ups for HIV, malaria, blood sugar and blood pressure. Eligible participants will be provided with meals 3 times daily for two weeks under the cost of the project. Food will be prepared by an experienced cook using stringent hygienic measures and served at the place of preparation. The cost of transport to the KCRI research centre for blood and stool sampling will be reimbursed to the participants. The only burden overseen for participation in this study is that participants will be required to visit the cooking facility to take meals 3 times a day for 2 weeks and to visit the KCRI Clinical Trial Unit for sampling at the beginning and the end

of the intervention. However, the cooking facility for the rural participants will be placed close to the sampling area so that participants can access it easily.

4.3 Informed consent and confidentiality

All volunteers will provide written informed consent after having received verbal and written information. Data will be handled confidentially. Each participant will receive a study identification number. The key to the code will be safeguarded by the principal investigators and the study coordinator. This identification number will be used for the labelling of blood tubes, forms, and storage of data. Data will be handled confidentially and will only be accessible for investigators or trail monitors directly involved in this study. Study forms signed informed consent, and reports of possible adverse events will be archived for at least 10 years. Blood will be stored for a maximum of 10 years for possible future measurements directly related to this study.

Compliance, withdrawal, and replacement of subjects

The study is a short dietary intervention, with minimal risk. Volunteers who consent to participate will be screened to ensure enrolled participants are in good health. Intervention food will be given at the cooking facility under close supervision. This will minimize the risk of compliance issues. Theoretically, participants may discontinue the dietary intervention for several reasons (compliance, illness, emergency antibiotic treatment, gastrointestinal disease or voluntary dis-consent). Because compliance with the two different diets is critical to the success of the dietary intervention, we anticipated a dropout rate of 2 volunteers per intervention group. If individuals that are enrolled withdraw or drop-out of the study before the start of the intervention diet (i.e. before the dietary change), this individual will be replaced by another individual fulfilling inclusion criteria but, if an individual drops-out after the dietary change, he or she will not be replaced. We have added into our sample size 3 participants in each group to compensate for dropouts. In our pretest-posttest design, participants that are lost to follow-up will not be included in the analysis (per-protocol analysis)

Stopping rules

Given the absence of important safety issues with such a short dietary intervention, only a few stopping criteria are defined: For individual participant: i) upon their request; ii) need to start emergency antibiotics; iii) upon a decision by the responsible study physician when the continuation of the diet is considered a health risk for the participant (e.g. severe diarrhoea); iv) when participants miss their intervention diet for more than five days in total.

Dissemination of Results

The results will be disseminated in the form of written reports. Significant results will be communicated to the research community via publications in scientific journals and through social media. The permission to submit manuscripts for publication will be sought from NIMR as per regulations and the published articles will also be shared with NIMR. The presentation of the findings will be organized for the following groups; first, to the CRERC and to the clinicians at KCMC to raise awareness on the impact of urbanization particularly dietary change on the epidemic of non-communicable diseases in our setting. Second, to the public to create awareness and provide education.

The results will also be presented in different national and an international conference organized on non-communicable diseases in which the results of this project will be shared with the NCDs stakeholders and the community at large.

5.0 Quality assurance and safety reporting

5.1 Quality assurance

The trial unit in KCRI is a well-equipped GCP-compliant clinical trial research facility with frequent international monitoring visits. Therefore, no special monitoring visit to the trial site will be conducted for a short dietary intervention study.

5.2 Safety reporting

Risks involved in participating in this study are minimal. Some minor abdominal complaints due to the change in diet can be expected, which will be monitored daily and recorded. In the unlikely event that an adverse event occurs, it will be notified to the Principal Investigator and the accredited Ethical Committees using standard adverse event reporting procedures.

6.0 Budget

Description	Input Description	Quantity	Unit of Measure	Total Quantity	Cost Per Unit (EUR)	TOTAL AMOUNT IN EURO
Personnel cost						
PI	100% post-doc	1	month	5	800.00	4,000
Co-PI	Allowance	1	month	5	150.00	750
Study coordinator/technologist	100% researcher	1	month	5	600.00	3,000
Research Nurse	50% FTE	1	month	2	300.00	600
Nutritionist	50% FTE	1	month	5	300.00	1,500
Cooking team	100% FTE	1	month	2	600.00	1,200
Subtotal						**11,050**
Study Materials						
Laboratory consumables	Laboratory consumables		month	5	300	1,500
Screening Kits	HIV		Kit	3	65.00	195
Screening Kits	Malaria		Kit	3	24.00	72
Screening Kits	Blood sugar		Kit	2	24.00	48
Food	Intervention food (breakfast, lunch and dinner)	56 subjects for 14 days	months	1	7,000.00	7,000
Drinks	Intervention drinks	66 subjects for 14 days	month	1	1,400.00	1,400
Cooking facility	Rent the cooking places, kitchen lump sum and study site		month	2	2,000.00	2,000
Subtotal						**12,215**
Laboratory running costs						
Laboratory accessibility and utilization	Lab accessibility and utilization		month	2	880.35	1,760.70
Laboratory storage	Lab. Storage		month	2	151.85	303.70
Internet Usage	ICT	2	month	5	45.00	450.00
Office space	Office space	2	month	5	11.25	112.50
Subtotal						**2,626.90**
Subject recruitment						
Volunteers transport reimbursement	Volunteers (part 1:part 2)	(56:10)	(month:week)	(1:1)	(15:12)	960
Subtotal						**960**
Ethical clearance fees						
Ethical clearance CRERC and renewal	KCMC Ethics	(458+93)	months	24	551	551

Ethical clearance CRERC and renewal	NIMR	(548+186)	months	24	734	734
Local authorities					100	100
Subtotal						**1,385**
Travel/Transport						
Site visits	350km per month x 5month x 0.60€/km		month	5	1,050.00	1,050
Sample shipping	From KCRI to Nijmegen, NL	-	-	-		7,000
Subtotal						**8,050**
Postage/office consumables						
Postage/office consumables	Posts and Stationaries		month	5	450.00	450.00
Communication			month	3	70.00	210.00
Subtotal						**660.00**
Total						**36,946.90**
12% overhead						4,433.63
Total at KCRI						**41,380.52**
Laboratory Measurements at RadboudUMC and affiliated laboratories						
Cytokines	Cytokine measurements	100	-	-	30	3,000.00
Metabolome	Metabolome measurements	100	-	-	50	5,000.00
Proteome	Measurement of inflammatory proteins	100	-	-	70	7,000.00
Transcriptome	Measurement of mRNA expression	100	-	-	70	7,000.00
Lipidome	Measurement of lipid profile	100	-	-	70	7,000.00
Microbiome	Gut microbiome	100			100	10,000.00
Subtotal						**39,000.00**
Grand Total						**80,380.52**

6.1 Budget Justification and Team Responsibilities

- **Personnel cost budget;** A total of **€11,050.00** has been budgeted for the research staff for the period of the study. This includes a) **€9,250.00** for the cost of key personnel consisting of the local

PI, co-PI, Post-doc, study coordinator and nutritionist; and b) **€1,800.00** for the cost of supportive staff consisting of study nurse and cook team.

Responsibility of the local key study team

1. **Dr. Vesla Kullaya, Molecular immunologist- Principle investigator/Post-doc.** She is a laboratory scientist and expert in the field of molecular biology and immunology. She will be engaged as a full-time post-doc under the mentorship of Prof. Blandina Mmbaga to ensure proper implementation of the project in all aspects. She will work hand in hand with the study coordinator in conducting field activities, setting and optimization of laboratory assays and training the study team. She will also lead the communication with stakeholders and collaborators be involved in data analysis and report writing.
2. **Prof. Blandina Mmbaga - Pediatrician, Co-Investigator-** She will be involved in proposal development, supervise the implementation of the project and provide mentorship to the Post-doc in project management and report writing.
3. **Prof. Reginald Kavishe, a Professor of Biochemistry and Molecular biology, co-investigator.** He will assist in the implementation and coordination of laboratory activities. He will also provide mentorship in project management, analysis and report writing.
4. **Mr. Godfrey Temba, a Biomedical Scientist- Study Coordinator and lab. technologist.** He is a biomedical scientist and currently working as a PhD researcher on the role of urbanization and nutrition transition on the emerging epidemic of non-communicable diseases in Tanzania under the supervision of Dr. Quirijn de Mast and Prof. Andre van der Ven the foreign PI and Co-PI of the project and Prof. Blandina Mmbaga who is the local Co-PI. Godfrey will coordinate the overall activities of the study. He will work on proposal development, and together with the nutritionist and Dr Kullaya, he will be responsible for setting up the study, performing laboratory analysis, data analysis and report writing.
5. **Mary Mosha, Nutritionist.** She is a key advisor of the project in all aspects of nutrition. She is responsible for developing dietary habit questionnaire, intervention menus and supervise the collection of data for the 24- hours dietary recall. The nutritionist will work for hand in hand with the cook to ensure food is prepared under a stringent hygienic condition and to supervise the food intake by the participants to encourage adherence. She will also be involved in data analysis and report writing.

Responsibility of other supportive staff

6. **Study nurse.** She/he will be involved in data and sample collection during the three-time point in both study arms.

7. **Cook.** She will prepare food for all study participants under the supervision of the nutritionist. She will also be involved in purchasing foodstuff, weighing, and documentation of food intake. She will supervise and give instructions to her cooking team.

- Funds (a total of €12,215) have also been budgeted for **Study Materials**, including laboratory consumables, screening kits, intervention menus (food and drinks) and for renting cooking facilities and temporary study site.
- We have also budgeted **laboratory running cost** a total of €2,626.90 to support laboratory accessibility and utilization, laboratory storage space for 2 months and office space for 2 people and internet use for 5 months.
- We also budgeted for **Volunteer Incentives** a total of €960 to support for volunteer travel to the site of recruitment and sample collection.
- We have also allocated funds (€1,385) for **Ethical clearance fees** as follows**;** €551 for local ethical submission (KCMUCo-CRERC), €734 for National (NIMR) including initial submission and one renew and €100 for local authorities in the village where the study will take place.
- We have budgeted for **local travel** a total of €1,050.00 to support for the site visit during participants recruitment and data and sample collection.
- We also budgeted for **Sample shipment abroad** a total of € 7,000 to cover sample shipment from KCRI-KCMC Moshi-Tanzania to RadboudUMC-Nijmegen, the Netherland.
- **Office supplies/postage and communication;** A total of €660 has been budgeted to cover costs for office supplies, postage for 5 months (€450.00) and communication €210.00).
- **Overhead costs** are budgeted at 12% **(€4,433.63)** of the total eligible cost which is **€36,946.90.**
- We also budgeted a total of €39,000 to support **laboratory measurements at Radboudumc and its affiliated laboratories.** These techniques are currently not available at KCRI-biotechnology laboratory and thus will be performed at Radboudumc and its affiliated laboratories. These techniques include transcriptome, lipidome, metabolome, microbiome and cytokine quantifications.

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