Dietary Intervention Research Proposal

Title: The effect of diet on immune and vaccine responses in people living with obesity in transitioning communities.

Project Acronym: TransInf

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1 contents

Abbreviations

BMI	Body Mass Index
COVID-19	Coronavirus disease 2019
CRF	Case Report Forms
CRERC	College Research and Ethical Review Committee
DNA	deoxyribonucleic acid
DSMB	Data Safety Monitoring Board
GCP	Good Clinical Practice
IgA	Immunoglobulin A
IL	Interleukin
KCMC	Kilimanjaro Christian Medical Centre
KCMUCo	Kilimanjaro Christian Medical University College
KCRI	Kilimanjaro Clinical Research Institute
NCD	Non-Communicable Disease
NIMR	National Institute for Medical Research
PCV	Pneumococcal Vaccine
PBMCs	Peripheral Blood Mononuclear Cells
Radboudumc	Radboud university medical centre
RBG	Random Blood Glucose
RNA	Ribonucleic acid
SAE	Severe Adverse Event
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SOP	Standard Operating Procedures
SUSAR	Suspected Unexpected Severe Adverse Reaction
TL	Telomere Length
TNF	Tumour necrotic factor

Definition of terms

Gut micro and mycobiome: Composition of bacteria and fungi, and their collective genetic material present in the gastrointestinal tract (GIT)**.**

Multi-omics technologies: an integrative biological approach where the data sets from the different *omic* platforms are analysed together.

Obesity: abnormal or excessive fat accumulation that presents a health risk.

Polyphenols: organic compounds found abundantly in plants,

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

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**.**

Westernized globalized diet: a diet with high content of simple sugar, saturated fat, fried food, or food with preservatives, taste enhancers, chemical leaven, refined grains and with low-fiber. It also includes industrially processed food, packed to be consumed at a later time.

Keywords: Diet, obesity, immune system, vaccines, polyphenols, fermented food, omics

Summary

Introduction

The prevalence of overweight and obesity is rapidly increasing globally. Obesity is associated with immune dysfunction leading to an increased risk of severe infectious illnesses and diminished vaccine effectiveness. Our previous studies in the Kilimanjaro region have shown that diet has a pronounced effect on the function of the immune system in healthy individuals. The traditional high plant-based fiber and polyphenol diet and a locally consumed fermented banana brew were anti-inflammatory. However, it is still unclear to what extent dietary variation directly or indirectly influences the host immune defense in individuals who live with obesity and whether certain dietary interventions may enhance immune responses and improve vaccine efficacy. The current study ('TransInf') aims to fill these gaps by establishing the cause-and-effect relationship between specific nutritional factors and immune responses to common viral and bacterial infections, as well as the response to vaccines, in people living with obesity in a transitioning societal environment in Tanzania.

Study Objectives

General objective:

To examine and compare the immuno-metabolic and gut microbiome profiles between individuals with overweight/obesity and those with normal weight in a transitioning community in Tanzania, and to analyze the impact of a brief dietary intervention involving either a traditional diet or fermented banana beverage on these profiles as well as vaccine responses in individuals who are overweight or obese.

The specific objectives are:

1. To compare the immune-metabolic profiles between normal-weight and overweight/obese adults including cytokine responses to pathogens, gene transcription, inflammatory and cardiometabolic proteome, and plasma metabolome.
2. To investigate the influence of a short dietary intervention with a traditional plant-based diet or fermented banana beverage on the immune-metabolic profiles and vaccine responses in overweight/obese adults.
3. To identify dietary components and food-related metabolites associated with these changes in immune and vaccine responses in people living with obesity.
4. To determine the effect of diet on intestinal B-cell homeostasis and immunoglobulin responses to the microbiome and the mycobiome and how they affect systemic vaccine responses.

5. To gain a mechanistic understanding of how diet influences immune and vaccine responses in obese people by defining the relationship between gene and epigenetic signatures, including telomere length, and immune and vaccine responses.

Methodology

The study will be conducted in Moshi in the Kilimanjaro region. The study combines a cross-sectional design with a randomized, open-label, proof-of-concept nutritional intervention study (Fig. 1). Tanzanians (n=100) aged 35 to 60 years with a BMI >27 Kg/M^2(Obese) at screening will be recruited, together with age and sex-matched Tanzanians (n=50) with a BMI 18.5–24.9 Kg/M^2 (normal weight controls). Ninety of the overweight participants will be randomly allocated to one of the following 3 arms: i) high plant-based fiber and polyphenol diet (n=30); ii) daily intake of fermented banana beverage (n=30); iii) continuation of usual diet (obese controls; n=30). The dietary intervention will take place for six weeks. Participants who consented for the study will be invited for an initial screening visit (visit 1) and those who meet the inclusion criteria will be enrolled in the study. At visit 2, blood and stool samples will be collected for functional immune assays (ex vivo whole blood stimulation) and a set of omics technologies (baseline sampling, 100 overweight participants, and 50 lean participants). At week 4 (visit 3), blood and stool samples will be collected from 90 participants in the intervention arms (post interventional sampling).To assess the effects of the dietary intervention on T-cell dependent and T-cell independent vaccine responses, participants will receive a conjugated pneumococcal vaccine (Prevenar13, Pfizer) and tetanus toxoid vaccine (Biological E Limited, India) at week 4 and continue with their diets for another 2-weeks. The effect of diet on vaccine response will be assessed by measuring antibody titers 4 weeks after vaccine administartion (visit 4). The following measurements will be performed on the collected biomaterials: metagenomics (changes in the gut microbiome), plasma proteomics (changes in the inflammatory and cardio-metabolic proteins expression), *ex vivo* whole blood stimulation (to assess the capacity of the circulating immune cells to produce cytokines to different microbial ligands), immunoglobulin binding to the gut microbiome, vaccines responses, immune cell telomere length, and whole blood transcriptome.

Hypothesis

Obese individuals can exhibit a dysregulated immune response, which, on the one hand, can increase their susceptibility to severe infections and, on the other hand, can impair their immune response to vaccinations. Moreover, obesity-associated gut dysbiosis compromises intestinal B-cell homeostasis and lowers colonic secretory Ig concentrations, which raises intestinal permeability and encourages systemic and intestinal inflammation. We hypothesize that traditional plant-based diets as well as traditionally

fermented foods have immunomodulatory effects that can lower inflammation and improve vaccine response in people living with obesity. We anticipate demonstrating the causal-effect relationship between diet and immune function in obese individuals, which can lead to the discovery of beneficial foods and/or food-derived metabolites with immunomodulatory effects that can improve the immune and vaccine response in people living with obesity.

Data Analysis

i. Analysis plan of the primary endpoints

For the primary objectives, the study has a pre-post design. Immune responses to pathogens and vaccine responses will be analyzed using paired T-tests (for normally distributed data) and the Wilcoxon signed ranks test (for skewed data) and for the adjusted analysis linear mixed models will be used. ANOVA with posthoc test will be used for the comparison of the immune and vaccine responses across the arms. The secondary outcome parameters will be tested using mixed models in collaboration with our partners.

ii. Multi-omics analysis of the data

This will be done in R using relevant packages. We will also employ multi-omics factors analysis (MOFA) to assess effects of individual features that contribute to this variation and the interaction between them

iii. Modelling "metabolic-related chronic inflammatory burden" to predict immune response

In collaboration with our partners, we will adapt a deep neural network that highlights the most relevant biological information related to the anthropometric, lifestyle, dietary, metabolic, microbiome and genomic data generated in this project to predict immune responses to microbes (i.e. cytokine responses) and vaccines (humoral responses).

Dissemination of findings

After completion of the study we will publish our findings in reputable journals and disseminate the key findings in relevant scientific conferences.

Introduction

1.1 Introduction

Over one-third of the global adult population is currently overweight and about a third of them are obese(1). While it is well known that obesity is strongly associated with non-communicable diseases such as atherosclerotic cardiovascular disease (CVD), type 2 diabetes, and several types of cancer, the recent COVID-19 pandemic has made it very clear that obesity is also an important risk factor for severity of infections. A meta-analysis of 20 cohort studies showed that patients with obesity have nearly twice the risk for unfavourable outcomes related to COVID-19 and a 50% increased risk of death(2,3). Individuals with obesity are also more susceptible to other viral infections such as influenza and common bacterial infections, including pneumococcal pneumonia and postoperative, nosocomial, skin and soft tissue infections(4).

Obesity is also associated with impaired vaccine efficacy with lower immune responses to both influenza and COVID-19 vaccines(5,6). People with obesity have multiple impairments in the host immune response, including but not limited to; impairments in the activity of immune cells, cytokine production and antibody responses(7,8). This is associated with a concurrent low-grade chronic inflammation associated with higher circulating concentrations of pro-inflammatory cytokines such as IL-6 and TNF, as well as adipokines such as leptin. These impaired immune responses result in increased susceptibility to infections and reduced efficacy of vaccinations(5), whereas the pro-inflammatory state may predispose to an excessive and uncontrolled inflammatory response during infection resulting in aberrant immune responses. Hyperinflammation is a key pathogenic factor and a main therapeutic target in severe COVID-19 and other severe infections. Recent evidence also highlights the importance of changes in the intestinal adaptive immune system and its interactions with the gut microbiota and innate immune system in obesity. Obesity with gut dysbiosis impairs intestinal B-cell homeostasis and reduces secretory IgA concentrations in the colon, which increases intestinal permeability and promotes intestinal and systemic inflammation(9). Previous data also suggested that intestinal B-cell homeostasis correlates with systemic B-cell responses(10–13).

The ongoing rapid urbanization in Tanzania has led to a concurrent increase in overweight/obesity rates as a result of lifestyle changes, including dietary habits. This transitioning community offers a unique opportunity to investigate early immune changes related to obesity. Tanzania, like any other country in sub-Saharan Africa, is experiencing a double disease burden, as witnessed by the sharp increase in non-communicable diseases on top of the existing burden of infectious diseases. Although Streptococcus pneumoniae is a leading cause of community-acquired pneumonia globally, the introduction of the 13-

valent pneumococcal conjugated vaccine (PCV13) has substantially reduced severe cases of the disease(14). PCV13 elicits both T cell-dependent and independent antibody responses and is effective in reducing severe pneumococcal pneumonia even in immunocompromised populations such as the elderly and HIV-infected individuals(15). Conversely, the tetanus toxoid vaccine induces a T cell-dependent production of neutralizing antibodies(16). Both vaccines target diseases of public health importance in Tanzania. Given the impaired B and T cell responses described in obesity, this study aims to investigate the aberrant vaccine responses in this population.

Importantly, the devastating health, social, and economic consequences of COVID-19 have highlighted the importance of immune dysfunction as a major public health liability and raised the need for interventions to improve immune function at the population level. Plant-based as well as traditional fermented diets are attractive interventions from a public health perspective. The objective of such an intervention would be to improve immune responses and vaccine efficacy, while at the same time reversing the chronic inflammatory state. For instance, dietary fibre intake has been associated with a more effective humoral response to the influenza vaccine(17). Another recent dietary intervention study showed that fermented foods reduced inflammation even more potently than a high-fiber diet(18). However, whereas it is generally accepted that severe nutritional deficiencies lead to immune defects, the magnitude of the impact of normal dietary variation on immune diversity is only partially known. This includes the effects of diet on immune responses to viruses (e.g., SARS-Cov-2, influenza) and bacteria and whether these effects are different in people living with obesity compared to those with a healthy weight. In addition, more insight is needed into how diet modulates the host defence – for example directly through food-derived metabolites, or indirectly through modulation of body weight or gut microbiome – and the immunomodulatory effects of specific dietary components.

In the present study we will investigate the effect of a plant based diet and a local fermented banana beverage on modulating immune and vaccine responses in overweight/obese adults. Plant based diets particularly those rich in polyphenols have been shown to positive modulate immune responses by reducing inflammatory mediators and pathways in addition to having anti-inflammatory and anti cancer properties (19–22). Fermented foods have also long been utilized in most communitites as a way to preserve food as well as to boost the nutritional value and improve nutrient availability during digestion (23). Additionally fermented foods have been linked to benefits not only to the gut microbiome but are also noted to enhance functionality of the immune system including mitigating metabolic diseases (24). However controversy still exists owing mostly to the wide variation in types of fermented foods and the bioactive components contained within them warranting more research in locally available fermented foods in different communities (25). Immune dysfunction in obesity as described earlier plays a central

role in development of cardiometabolic diseases hence exploring the benefits of diet in mitigating these effects is warranted.

1.2 Problem Statement

Obesity and its related conditions are associated with immune dysfunction and are an important risk factor for severe infectious diseases. Reducing the consumption of ultra-processed foods and promoting healthy eating are important measures to tackle obesity and may as such improve immune function including vaccine efficacy. However, durable implementation and public adherence to these recommendations remain a challenge. It also remains unclear to what degree the daily variation in the diet directly affects immune responses, or whether this is primarily mediated by indirect effects such as changes in body weight or the composition of the gut microbiota. TransInf aims to fill these gaps by establishing the cause-and-effect relationship between a specific food and or food-derived metabolites and immune responses to common viral and bacterial infections and vaccine efficacy in people living with obesity in rapidly urbanizing societies in Tanzania.

1.3 Rationale

The transitioning societies in many areas in Africa including Tanzania offer a unique opportunity to investigate the 'diet-host defence-infectious diseases' axis in obesity for the following reasons: a) differences in diet ('westernized' vs traditional) and lifestyle (urban vs rural) are more extreme than in populations in the global north; b) the traditional African diet is rich in plant-based fibres and immunomodulatory molecules (such as polyphenols), but also fermented foods (fermeneted banana beverage (26) rich in environmental microbes, which have been lost in the industrialized world and which are increasingly recognized to positively influence host defence mechanisms(27); c) we have shown that a high burden of infections has favoured the selection of genotypes mediating a robust immune response against infections(28). This phenotype may not be suitable anymore for the current environment with declining infections and adoption of Western lifestyles and provides novel insights into the mechanisms needed for a finely tuned control of the immune system in a rapidly changing society. For instance, a mismatch between the immune response against infectious threats with a failure to swiftly downregulate this response as soon as the threat is under control may contribute to persistent immune activation with impaired adaptive immune responses and pathological hyperinflammation during infections. Our approach with a combination of state-of-the-art functional immune assays and different *omics* platforms such as metabolomics, proteomics, transcriptomics and metagenomics in this transitioning population will therefore enable us to identify the dietary compounds and food-derived metabolites that may improve immune function in obesity. The cause-and-effect relationship will further be strengthened by *exvivo* laboratory validations of identified metabolites and by performing proof-of-principle intervention studies.

TransInf will also contribute to the development of innovative food solutions. Important components of traditional African diets, such as plants rich in specific polyphenols or fermented foods/beverages have historically been part of many diets around the world, but have been largely eliminated decades ago due to the industrialization and processing of food. TransInf will not only identify cause-and-effect relationships but will perform proof-of-principle studies that may contribute to the reintroduction of these components as a key component of an immunomodulatory diet in people with obesity. Moreover, the identification and characterization of immunomodulatory metabolites that restore immune function in obesity may contribute to their further development as possible food supplements.

Research questions

1.4 General research question

What are the immuno-metabolic and gut microbiome profiles of people living with overweight/obese in a transitioning community in Tanzania and could a short dietary intervention with either a traditional diet or fermented banana beverage alter their immune-metabolic profiles and vaccine responses?

1.5 Specific research questions

1. Are there differences in the immune-metabolic profiles (cytokine responses to pathogens, gene transcription, inflammatory and cardiometabolic proteome, and plasma metabolome) between normal-weight and overweight/obese adults?
2. Could a short dietary intervention with either a traditional plant-based diet or fermented banana beverage modulate the immune-metabolic profiles and vaccine responses in overweight/obese adults?
3. What are the dietary components and food-related metabolites associated with these changes in immune and vaccine responses in people living with obesity?
4. Does diet impact intestinal B-cell homeostasis and immunoglobulin responses to the microbiome and the mycobiome and could this affect systemic vaccine responses in overweight/obese adults?
5. Mechanistically how does diet influence immune and vaccine responses, particularly the relationship between gene and epigenetic signatures, including telomere length, immune and vaccine responses in obese people?

Research Objectives

1.6 General objective

To examine and compare the immuno-metabolic and gut microbiome profiles between individuals with overweight/obesity and those with normal weight in a transitioning community in Tanzania, and

to analyze the impact of a brief dietary intervention involving either a traditional diet or fermented banana beverage on these profiles as well as vaccine responses in individuals who are overweight or obese.

1.7 Specific objectives

1. To compare the immune-metabolic profiles between normal-weight and overweight/obese adults including cytokine responses to pathogens, gene transcription, inflammatory and cardiometabolic proteome, and plasma metabolome.
2. To investigate the influence of a short dietary intervention with a traditional plant-based diet or fermented banana beverage on the immune-metabolic profiles and vaccine responses in overweight/obese adults.
3. To identify dietary components and food-related metabolites associated with these changes in immune and vaccine responses in people living with obesity.
4. To determine the effect of diet on intestinal B-cell homeostasis and immunoglobulin responses to the microbiome and the mycobiome and how they affect systemic vaccine responses.
5. To gain a mechanistic understanding of how diet influences immune and vaccine responses in obese people by defining the relationship between gene and epigenetic signatures, including telomere length, and immune and vaccine responses.

Materials and Methodology

1.8 Study design and duration

Figure 1 summarizes the study design. The study will be conducted in two phases. Phase one will be a cross-sectional study followed by a second phase involving a randomized proof of concept nutritional intervention study. During phase one of the study we will enrol 100 overweight/obese (BMI > 27Kg/M^2) participants and 50 healthy controls (BMI 18.5–24.9 Kg/M^2) participants and assess baseline immune and vaccine responses (obese vs non-obese). This will be followed by the dietary intervention phase involving only the overweight/obese participants. Assuming a 10% attrition rate (we estimate to be left with 90 overweight/obese participants). These 90 participants will be randomized (using block randomization) to one of the following three arms: i) high plant-based fibre and polyphenol diet (n=30); ii) daily intake of fermented banana beverage (n=30), iii) control, who will remain on their usual diet (n = 30). The intervention will start directly after the baseline visit.

Figure 1 depicts the study design, which consists of two phases: a cross-sectional study (phase one) followed by a randomized proof-of-concept nutritional intervention study (phase two). In phase one, we will recruit 100 overweight or obese participants (BMI > 27 kg/m2) and 50 healthy controls (BMI 18.5–

24.9 kg/m2) to assess baseline immune responses in obese versus non-obese individuals. The second phase will involve only the overweight or obese participants, who will undergo a dietary intervention. Only 90 of the overweight or obese participants will be randomized (using block randomization) to one of three arms: i) a high plant-based fiber and polyphenol diet (n = 30); ii) daily intake of fermented banana beverage (n = 30); or iii) a control group that will maintain their usual diet (n = 30). The intervention will commence immediately following the baseline visit.

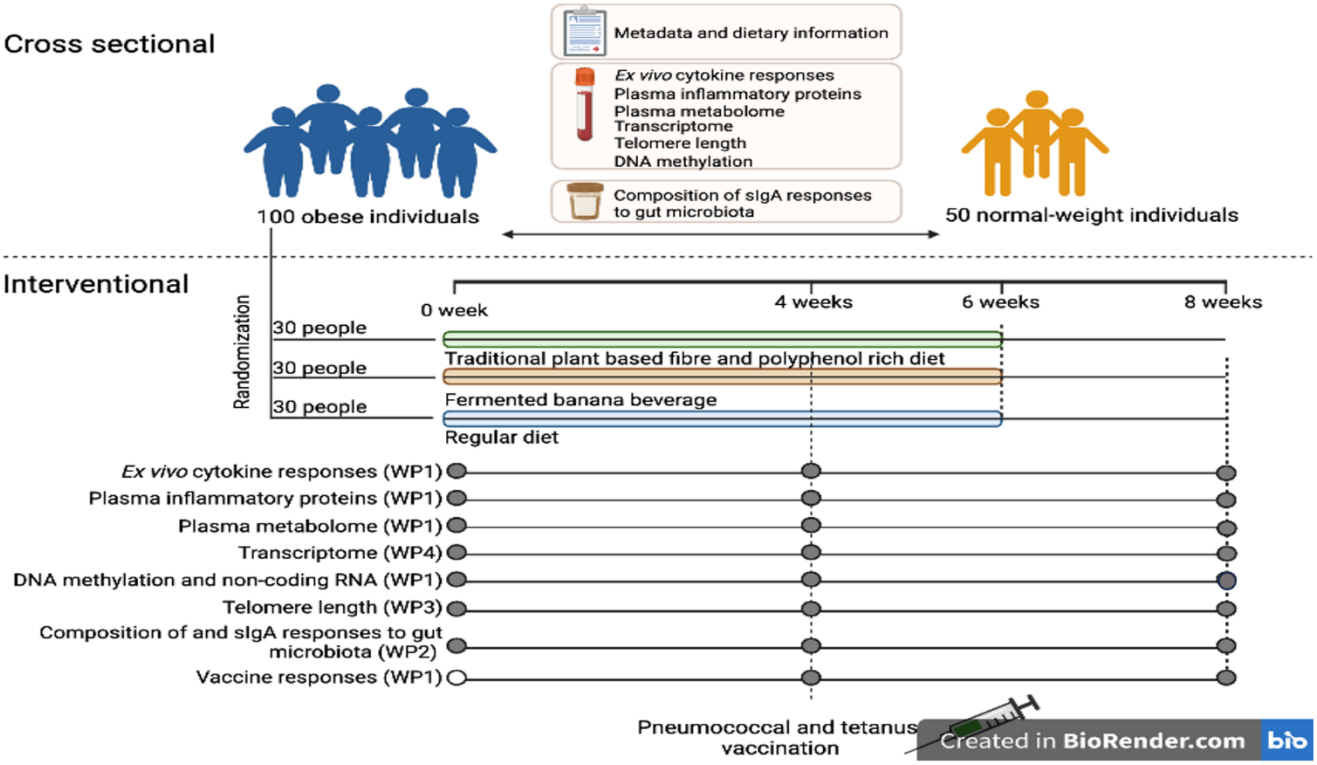
Participants will further be grouped into A, B and C groups, each consisting of 10 participants from each arm of the intervention which will start the intervention two weeks apart. The reason for dividing participants into three groups is that logistics do not allow 90 participants to start their intervention at the same time. The dietary intervention phase will take six weeks. A longer duration of the intervention will be logistically challenging and may come at the expense of compliance with the intervention. Additionally, at week 4 participants will receive a conjugated pneumococcal vaccine (Prevenar 13, 0.5mL) and tetanus toxoid vaccine (≥ 40 IU of tetanus toxoid in 0.5mL) and continue their diets for another 2 weeks. Blood and stool will be taken at baseline, at week 4 and 8 after the start of the intervention. Each participant will be followed up for eight weeks.

Participants will be divided into three groups (A, B, and C), each comprising 10 participants from each arm of the intervention. This division is necessary due to logistical constraints preventing 90 participants from starting the intervention at the same time. The dietary intervention phase will last for six weeks, as a longer duration could compromise compliance with the intervention. Additionally, at week 4, participants will receive Prevenar 13, a conjugated pneumococcal vaccine, and a tetanus toxoid vaccine, and continue with their diets for an additional 2 weeks. Blood and stool samples will be collected at baseline, week 4, and week 8 following the start of the intervention. Each participant will be followed up for eight weeks.

1.9 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.

Figure 1. Overview of the Design



1.10 Study population, inclusion criteria/exclusion criteria:

We will enrol a cohort of Tanzanians living with overweight/obesity together with age-and sex-matched normal weight controls. For logistical reasons (providing food during the intervention), participants will be recruited among the staffs of KCMC/KCMUCo/KCRI or the nearby area. A total of 100 participants with a BMI >27 kg/m2 (overweight/obesity) and 50 participants with a BMI of 18.5–24.9 kg/m2 at screening (normal weight controls) aged between 35 and 60 years will be recruited. These BMI categories are chosen to compare the data with the Dutch 300-Obesity (300-OB) cohort(29). We expect that approximately 70% of participants will be female, as obesity is more common in females than in males in urban Tanzania. We will aim to enrol a similar proportion of women in both groups. The age category is chosen because the prevalence of obesity increases rapidly in individuals aged 30 and above(3). Our study population, therefore, represents the target population well, i.e., individuals living with obesity in urbanized societies. Participants who are screened and found to have abnormal vital signs, such as elevated blood pressure or diseases such as malaria or HIV, will firstly be counseled and informed of their results prior to being referred to the nearest health facility for further medical management.

Inclusion Criteria

- Age between 35 and 60 years·
- BMI > 27 kg/m^2 at screening ('overweight/obesity group') or 18.5–24.9 kg/m^2 at screening ('normal weight controls') ·
- Westernized-style diet as a regular diet, as assessed one week prior to recruitment while participants were taking their usual diets (using a 24-hour dietary recall on three non-consecutive days, including one weekend)·
- Taking alcoholic drinks but not fermented banana beverage (Consuming a fermented banana beverage less than twice a week).
- For female: practiving highly effective birth control method: this means one of the two:
  1. currently using hormonal contraceptive·
  2. or providing a consent to oblige to a "double-barrier method" (i.e. 1 physical barrier method plus the use of a chemical barrier, for example, male condom plus spermicide, Types of barrier methods include condoms, diaphragms, cervical caps, and the contraceptive sponge) to prevent conception during the time of the study·

Exclusion criteria

- Positive HIV or malaria rapid test·
- BMI> 40 kg/m^2 (morbid obesity is associated with severe immune dysregulation and will be outside the scope of this study).
- Vital signs outside an acceptable range at screening (i.e., BP >160/100 or BP <90/60, fever)·
- Known diabetes or RBG > 11.1 mmol/L·
- Acute febrile illness in the previous month.
  An acute febrile illness is defined as any illness accompanied by a fever (≥ 38.0°C) within the 30 days prior to the participant's recruitment into the study.
- A known chronic inflammatory condition
  Chronic infectious diseases, rheumatic diseases (excluding osteoarthritis), inflammatory bowel disease (IBD), chronic kidney disease, chronic liver disease, schistosomiasis, and active malignancies.
- Use of anti-inflammatory drugs·
  Non-steroidal anti-inflammatory drugs (NSAIDS), steroids, chemotherapy and any other immunosuppressive drugs.
- Antibiotic therapy 3 weeks prior to study entry·
- Women who are pregnant or lactating·
- Women who are in menopause (12 consecutive months without a menstrual period without an alternative medical cause) ·

- Mental illness·
  Active depression, delusional states, schizophrenia, or those taking any psychiatric drugs
- Alcohol abuse, medication and/or drug abuse (BTMG-liable or other psychotropic substances)·
  Alcohol abuse will be assessed using an alcohol use disorders identification test.
- Regular intake of supplements (especially n-3 fatty acids, vitamin E, magnesium)·
- Participation in a weight loss program·
- Participation in another clinical trial concurrently or within the last 30 days·
- Any known history of liver disease or symptoms of liver disease (jaundice, ALT levels more than twice the upper limit of normal).

1.11 Sample size estimation

This study is an exploratory proof-of-concept study, and formal sample size calculations are challenging. Based on our previous TransMic study(30), the enrolment of 28 participants per arm was sufficient to show a statistically significant difference in cytokine responses to pathogens. To determine an appropriate sample size in the present study we have inferred the following assumptions based on data from this study (https://www.isrctn.com/ISRCTN15619939). When assessing differences in cytokine profiles between urban and rural males following whole blood stimulation with Staphylococcus aureus, differences in levels of TNF-α had the highest level of significance. Based on these data:

Mean difference in TNF-α levels between the groups was 146 units,
Standard deviation was 216,
Assuming an alpha value of 0.05,
Power of 0.8,
Drop out rate of 0.1,
Accounting for multiple comparison with Bonferroni correction,
Then: n= 27.

Anticipating 10% of participants per arm being either lost to follow-up or non-compliant, we will enroll 30 participants per arm.

1.12 Participant Recruitment and sampling technique

Participants will be recruited among the staffs of the KCMC/KCMUCo/KCRI and the surrounding areas. Information about the study will be provided through leaflets and information gatherings. Interested individuals will be invited to one of the participants' information meetings during which the background, objectives and study procedures will be explained. Volunteers willing to participate will be invited to a screening visit during which a short checklist with in-and exclusion criteria is completed and anthropometric measurements will be taken. Blood for random blood glucose, HIV and malaria RDT will be collected by fingerprick. Furthermore, we will collect 2mls of heparinized blood to screen for liver

diseases by measuring Alanine aminotransferase levels. at baseline Participants fulfilling the inclusion criteria will be enrolled in the study after providing written informed consent. Participants will be provided with a food log sheet to assess their food and beverage intake the week before starting the intervention (a 24-hour dietary recall on three non-consecutive days, including one weekend).

1.13 Study procedures and Data collection Scope

Screening visit:

Potential participants will be invited to the study center, where they will fill out a pre-screening questionnaire and have baseline assessments conducted so as to assess their eligibility to participate in the study based on inclusion and exclusion criteria. Eligible candidates will then be counseled, if compliant they will be requested to give written consent prior to enrolment into the study.

Allocation of participants to the arms:

The 100 participants living with overweight/obesity will be allocated to any of the 3 arms using block randomization. Using block randomization 90 participants from the overweight/obese group will be randomized into 3 intervention arms; plant-based diet arm, fermented banana beverage arm, and control arm (no change in diet). For ease of follow-up and sample processing, and to control for any other influencing factors (eg seasonality), participants from each intervention group will be further allocated to subgroups A, B, and C, such that each subgroup has members from all three intervention groups (n=30, 10 participants from each arm). The 3 subgroups (A,B, & C) will be scheduled for baseline visits that are 2 weeks apart (Flow chart 1).

Dietary interventions:

Participants in the ‘high plant-based fiber and polyphenol diet’ arm will be provided breakfast and lunch at their offices Monday to Friday except on Saturday in which the breakfast, lunch, and dinner packages will be provided on Friday and participants will prepare at their homes. In addition, they will receive a family-size food package for dinner each weekday along with detailed instructions on how to prepare the food at home. Participants will be asked to take pictures of dinner meals consumed at home and will provide them to the study team. The aim is to have participants adhere to the dietary intervention at least five times a week. The duration of the intervention is 6 weeks while the duration of the follow-up is 8 weeks. Participants in the ‘fermented banana brew’ arm will receive at the same facility 1 litre of fermented banana beverage (‘mbege’) every evening after work (Monday to Friday), and be cautioned not to operate a motor vehicle after consuming the beverage. One liter was selected based on our previous study, where this volume was sufficient to induce significant changes to cytokine profiles in study participants (https://www.isrctn.com/ISRCTN15619939). The participants will be required to consume 1L of the

fermented banana beverage per day for atleast 5 days. All participants will be required to complete a daily food log on paper of all the foods/ volume of banana beverage consumed each day.

Baseline visit and the start of dietary intervention:

Participants will be invited to the research site. During this visit, participants will complete a questionnaire (evaluating dietary practices, lifestyle, health status, etc.) and undergo a physical examination (including anthropometric measurements). A total of 43mL of blood and approx. 6 grams of stool samples will be collected to investigate the study's objectives. During the course of the study participants will receive individual counseling sessions with a qualified nutritionist (at the onset of the diet and halfway through the intervention). These sessions serve to educate and reinforce the importance of following the intervention menu. Members of the study team will also maintain regular communication with the study participants.

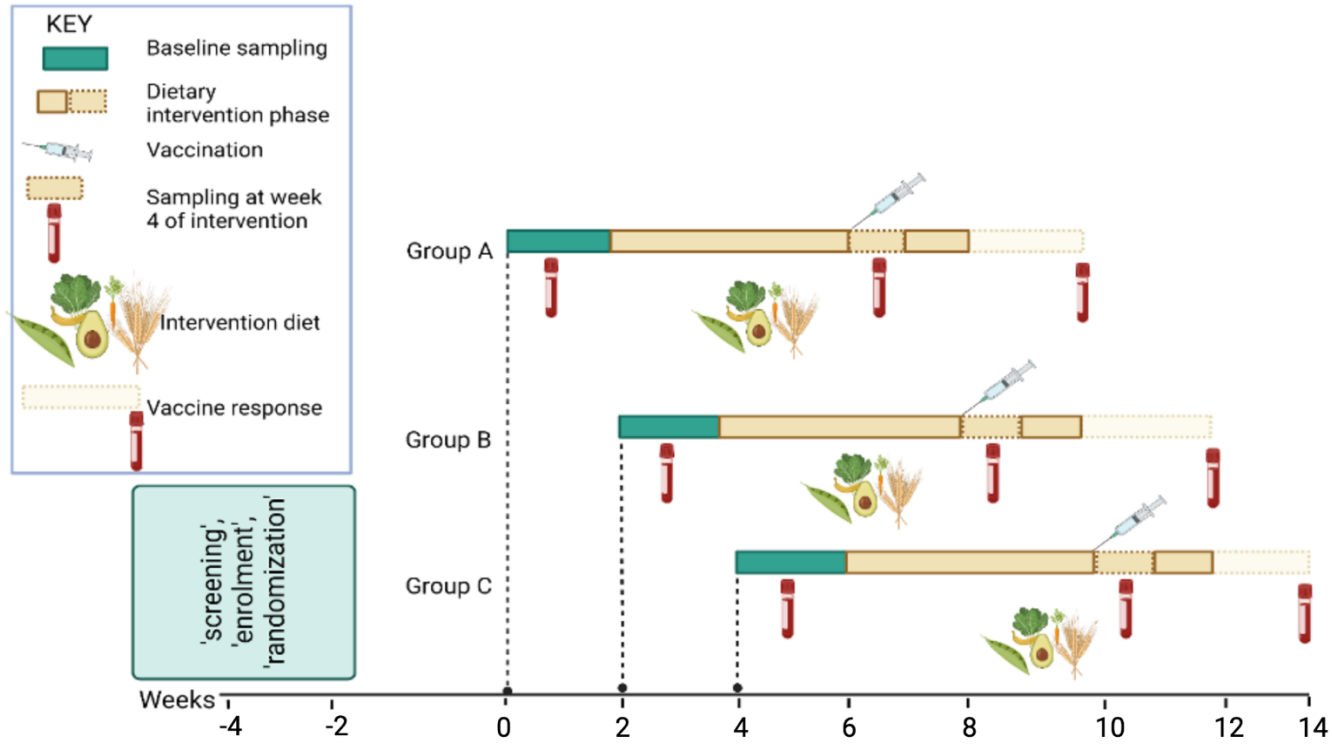
Week 4 visit:

At the week 4 visit, participants are invited to the study site. Here, a physical examination (including anthropometric measurements) will be performed. Similar to the baseline visit, 43mL of blood and approx. 6 grams of stool samples will be collected to assess the effect of diet on immune responses (post-dietary interventional sampling). Additionally, we will collect serum for quantifying the baseline antibody responses to Clostridium tetani and Streptococcus pneumoniae as we plan to investigate vaccine responses to these two bacteria in the next phase of the study. Next, participants will be administered a Conjugated pneumococcal vaccine (Prevenar 13, Pfizer, USA) and a tetanus toxoid vaccine (Biological E Limited, India) intramuscularly. The rationale for these two vaccines is that both are part of the Tanzanian essential medicine list and are thus readily available and capture responses to a T-cell-dependent peptide antigen. Participants will continue with their dietary intervention for another 2 weeks.

Week 8 visit:

Participants from group A will be invited to visit the study centre for the last time (Assessment of vaccine response). A physical examination will be performed and 43mls of blood will be sampled for the effect of diet on vaccine responses using omics technologies including plasma inflammatory proteins, transcriptomes, plasma metabolome, and epigenetics. As our study participants will have resumed their regular diets 2 weeks prior to this time point, we will assess whether the effects of the diet intervention have been sustained after resuming their regular diets.

Flow chart 1: Intervention Scheme



1.14 Study Outcomes

The primary endpoint(s):

1. Changes from baseline in innate and adaptive cytokine responses to microbial ligands at week 4 within and between the intervention arms.
2. Differences in anti-pneumococcal and anti-tetanus antibody responses between the intervention arms at week 8.

Secondary endpoint:

1. Changes from baseline in inflammatory and cardiometabolic plasma proteome at week 4 across the 3 study arms.
2. Changes from baseline in whole blood transcriptome and immune gene regulation by long non-coding RNAs at week 4 across the 3 study arms.
3. Changes from baseline in untargeted plasma metabolome across the 3 arms.
4. Change from baseline in tetanus and pneumococcal specific T-cell responses.
5. Changes from baseline in the composition of the gut microbial, sIgA-binding of gut commensals ex vivo and systemic antibody levels to gut commensals.
6. Changes from baseline in telomere length at week 4 across the 3 arms.

7. Changes from baseline in DNA methylation of peripheral blood mononuclear cells (PBMCs) at 4 weeks across the 3 arms.
8. Differences in vaccine-induces changes in whole blood transcriptome between the 3 arms.

The rationale for primary endpoints

The aim of the project is to study the diet-immune system-infectious diseases axis. Cytokine responses to *exvivo* pathogens are broadly representative of vulnerability to infectious diseases(31) and may predict infection-associated hyperinflammation. These cytokine responses are therefore a relevant outcome measure of this proof-of-concept intervention study, also because assessment of the incidence of severe infectious diseases as an outcome measure is not feasible. The other primary outcome measure is the post-vaccination serotype-specific pneumococcal immunoglobulin G (IgG) concentrations to the pneumococcal serotypes present in the Prevenar13 and tetanus toxoid IgG responses.

1.15 Laboratory procedures

Isolation of PBMC and freezing for further omics analysis

For the measurement of telomere length (TL) and single-cell transcriptome (by scRNA-seq) we will isolate peripheral blood mononuclear cells (PBMC) from whole blood as follow: blood will be diluted with PBS buffer and layerd on top of Ficoll and centrifuged to achieve density gradient separation of the PBMC fraction from the other components of the blood. PBMC will be washed several times with culture media and will be then frozen in freezing media containing 10% DMSO, aliqouted and stored in liquid nitrogen to allow long term preservation.

Whole blood stimulation assays and cytokine quantification

Exvivo cytokine stimulation experiments will be performed at the Biotechnology Laboratory facility available at KCRI in Moshi Tanzania. Immune responses to relevant pathogens will be assessed by measuring whole blood cytokine (IL-6, TNF-α, IFN-γ, IL-1β, IL-1Ra and IL-10) responses to *ex vivo* stimulation with the following microbial or synthetic ligands: inactivated SARS-Cov-2, inactivated influenza, lipopolysaccharide (LPS), *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Salmonella typhi*, *Salmonella entiritidis*, *Mycobacterium tuberculosis*, and *Candida albicans*. In addition, SARS-Cov-2 serology will be performed in all participants.

Antibody responses:

Serotype-specific responses to the 13 pneumococcal serotypes are measured using an in-house 23-plex multiplex immunoassay (Radboudumc); Tetanus toxoid antibodies will be measured using a

commercially available ELISA. The primary outcome measure for the Prevenar 13 vaccine responses will be seroconversion after vaccination. The outcome of antibody responses will be assessed 2 fold; firstly quantitatively (differences from baseline and across the intervention arms) and secondly qualitatively (seroconversion). Consensus on the correlates of protection is lacking. The World Health Organization (WHO) recommends a cutoff value of 0.35 µg/mL to define seroconversion post vaccination with Prevenar 13. However, we will use a more conservative correlate of protection, based on the definition of the American Academy of Allergy, Asthma and Immunology (AAAAI), which as a postimmunization antibody concentration of ≥0.13 µg/mL for ≥70% of all measured serotypes (32). For the tetanus toxoid IgG response, a cutoff value of 0.1 IU/ml will be used (33).

Inflammatory and cardiometabolic plasma proteome (Olink platform) and untargeted plasma metabolome

Plasma proteome will be measured at Radboudumc using proximity extension analysis (PEA) technology. Untargeted plasma metabolome will be measured by General Metabolomics. Briefly, the circulating inflammatory and cardiometabolic protein markers will be quantified using a high-throughput multiplex immunoassay, allowing simultaneous analysis of 92 inflammation-related protein biomarkers and 92 cardiometabolic-related protein biomarkers across 96 samples. The protein expression levels before and after the intervention will be compared. In addition, we will quantify plasma metabolites from EDTA plasma samples using a high-throughput mass spectrometry approach time-of-flight mass spectrometer (Agilent, Santa Clara, CA) equipped with an electrospray source operating in negative and positive mode. A list of potential metabolites will be constructed and annotated using a range of analysis methodologies, such as deisotoping, decluttering, adduct detection, and library matching in the KEGG, HMDB, and CHEBI databases. These technologies are not currently available in the KCMC laboratories, thus, samples will be measured at the Radboudumc laboratory and its affiliated laboratories. Before sharing the samples, the material transfer agreement (MTA), and data transfer agreement (DTA) will be drafted and approved by Tanzania National Institute for Medical Research (NIMR).

Composition of the gut microbial ecosystem

Microbiota composition will be analysed for the Tanzanian cross-sectional obesity/non-obesity cohort (n=150) and the dietary interventional study (n=90 with 2-time points). All samples will be analysed by sequencing of the V3–V4 region of 16S rRNA gene as well as the internal transcribed spacer (ITS) region,

which enables phylogenetic identification of bacteria and fungi, respectively. Raw data will be analysed with a common bioinformatics pipeline applied to all microbiota work within the present project.

sIgA-binding of gut commensals ex vivo

Purified microbes will be stained with anti-IgA FITC antibody followed by anti-FITC MACS bead enrichment. Then positive and negative fractions are collected and analysed for microbial composition using a DNA extraction protocol adapted to low microbial biomass samples and bioinformatic processing. Of note, only 16S rRNA gene analysis will be performed as previous data have demonstrated that fungi are so infrequent that ITS gene analysis is unreliable for low biomass samples. Finally, paired metagenomic profiling will be performed, which allows us to evaluate the specificity of IgA binding at the taxonomic unit level for each stool sample.

Systemic antibody concentrations to gut commensals

Systemic antibody titters to a panel of *in vitro* cultured gut commensals as well as *Saccharomyces* (from fermented banana brew), *Clostridium tetani* and *Streptococcus pneumoniae* will be measured using an in-house developed protocol based on flow cytometry technology of live bacteria with native surface antigens(34). Serum samples from the interventional study will be analysed for IgA, IgM and IgG antibody responses to selected microbes. The commensal microbes tested here will be selected based on prior knowledge of IgA-bound gut microbes(35), such as *Escherichia coli*, *Faecalibacterium prausnitzii*, *Akkermansia muciniphila*, *Lactobacillus* and *Bifidobacterium*. The commensals will be obtained from the consortium partners except for *Clostridium tetani* and *Streptococcus pneumoniae*, which are purchased to ensure they match the vaccines employed in the study.

Telomere length on PBMC

We will analyze telomere length in PBMCs in samples from the cross-sectional cohort (n=150) and dietary intervention cohort (n=90; 2-time points), as previously described REF Fali JCI Insight 2018; Fali JI 2019. Briefly, genomic DNA (gDNA) will be extracted from PBMCs with the QIAamp DNA Mini Kit (QIAGEN). Relative telomere length will be measured as the ratio of standard DNA quantities for telomere template (T) over single copy gene 36B4 (S), determined using a quantitative real-time PCR method adapted from Cawthon.

Telomere length on immune cell subsets (CD34 / T cells/ B cells)

From the dietary intervention cohort (n=90; 2-time points), we will analyze telomere length on sorted cells using BD FACS Aria II (Becton Dickinson). Panels of directly conjugated Abs to identify subsets of hematopoietic progenitors (CD34, CD117, CD10, CD45RA, lineage), naive & memory CD8+ T cells

(CD3, CD4, CD8, CD45RA, CD27, CD57), and B cells (CD19, CD27, CD38, IgD, IgM) are already set up.

Inflammation, oxidative stress & Telomere Length

Microbial translocation (MT) is a feature in obese people and is associated with immune activation. MT is thought to be associated with loss of mucosal barrier function, persistent viral replication in the gut and increased intestinal permeability resulting from immune deregulation and alterations in the intestinal microbiome. To address this source of inflammation, we will measure by Elisa CD14s, CD163s, iFABP2 & endotoxin from sera and corroborate their levels with neopterin (Tecan), a biomarker of systemic immune activation. Oxidative stress has been reported to lead to the inhibition of cell proliferation and to produce a negative impact on the maintenance of TL by directly repressing telomerase activity and promoting telomeric erosion(35). In contrast, increased telomerase activity and decreased telomere shortening have been documented after antioxidant treatment(36) and after a short-chain fatty acids diet(37). To follow oxidative stress, we will measure reactive oxygen species (ROS) production by flow cytometry (CellROX green reagent, Thermofisher).

Generation and analysis of whole blood transcriptome and single-cell blood transcriptome

Blood will be collected via the PaxGene Blood RNA System, which allows the stabilization of intracellular RNA generating high-quality data. According to protocols established in the laboratory at Bonn together with the Platform for SinglE Cell GenomIcS and Epigenomics (PRECISE) platform at DZNE, RNA will be depleted for globin and ribosomal RNA, and Poly-A RNA will be utilized for producing sequencing libraries. Sequencing for poly-A RNA species will be performed on an Illumina NovaSeq 6000 Sequencing System (paired-ended for 50 bp) utilizing strand information to allow for strand-specific downstream analysis. Raw sequencing data will be demultiplexed, and aligned to the human composition of the gut microbial ecosystem.

For single cell RNAseq (scRNAseq), aprox. 2 million PBMC will be isolated from whole blood and frozen as specified above.For the generation of single cell transcriptome, frozen PBMC will be thawed and single cell transcriptome will be produced using the BD Rhapsody Single-Cell Analysis System (BD, Biosciences). Cells from each sample will labeled with sample tags (BD Human Single-Cell Multiplexing Kit), to allow multiplexing of samples to yield a pool sample that will be then loaded onto a BD Rhapsody cartridge, on which cells are distributed among micro-wells. Several buffers and microbeads are then injected to the cartridge that induce cell lysis and the capture of single cell mRNA molecules on single beads. cDNA libraries will be prepared using the BD Rhapsody Whole Transcriptome Analysis Amplification Kit following the BD Rhapsody System mRNA Whole Transcriptome Analysis (WTA) and

Sample Tag Library Preparation Protocol (BD Biosciences). Sequencing will be performed in PRECISE (Bonn) in paired-end mode (2∗75 cycles) on a NovaSeq 6000.

Sample storage and shipping

For tests that will be conducted at Radboudumc, The Netherlands; Bonn University, Germany; and the Centre for Immunology and Infectious Diseases (CIMI Paris), Paris, France, as specified under specific tests above, samples will be stored at -80 °C and then transported to the aforementioned centres for analysis. Samples will be shipped via 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 KCMUCo and collaborating institutions before shipping samples. MTA and DTA will be submitted to NIMR for approval.

1.16 Data Management and Analysis

Data Management

We will generate data in the form of numeric data and tables that contain the measurements of immune responses and the results of the -omics technologies. Furthermore, a multi-omics analysis pipeline will be produced that can be reused by other people in the field. Numerical data will be saved in a Castor EDC database in a comma-delimited text file (.csv) suitable for import into statistical programs such as R studio. All raw and processed data sets collected from this project will be stored and managed under the Digital Research Environment (DRE), a cloud-based, globally available research environment where data is stored and organized securely and researchers can quickly generate workspaces to collaborate in and use the applications. The DRE facilitates users' collaboration on research projects in a safe yet flexible computing and storage environment. The architecture of the DRE allows researchers to use a solution within the boundaries of data management rules and regulations. The DRE offers tools to more easily control and monitor which activities take place within the projects. The study PIs will be the only members of the study team with access to non-anonymized data and will be the custodian of the data, having full access to the DRE platform available at the Radboudumc and can manage data accessibility and usability.

Data Analysis

Analysis plan of the primary endpoints

Data will be analysed according to both an intention to treat protocol. For the primary objectives, the study has a pre-post design. Immune responses to pathogens and vaccine responses will be analyzed using paired T-tests (for normally distributed data) and the Wilcoxon signed ranks test (for skewed data) and for the adjusted analysis linear mixed models will be used. ANOVA with posthoc test will be used for the comparison of the immune and vaccine responses across the arms. The secondary outcome parameters will be tested using mixed models as well. In collaboration with CytoReason Company in Israel, a model to predict immune response will be constructed.

Analysis and modelling of the interaction of diet and food metabolites with immune and vaccination responses

Several approaches will be utilized to reveal immunologically relevant metabolites and/or dietary patterns: i) Simple/multiple linear models to estimate the immune response variance (dependent variable), considering immune response as *ex vivo* cytokine production in response to relevant pathogens and humoral responses to vaccines in the intervention cohort. We will estimate the contribution of metabolites (independent variable) to immune variance while including additional immune-metabolic parameters measured in laboratory analyses (model covariates, i.e., TL, microbiome and DNA methylation). Using multiple regression models, we will be able to estimate the degree of each of the measured parameters and its relationship with the immune response. Another approach will be subgrouping, based on similarity analysis of metabolic profile (calculated by Euclidean distance or correlation analysis) followed by hierarchical clustering and subsequent comparison of the immune response as we previously performed in the TransMic project(30,38). Alternatively, we will perform the subgrouping by adjusting an in-house correlation network work frame, CoCena(30,39) that was used to identify unique features that distinguish severe COVID patients. Data from Cohort and proof-of-principle dietary intervention studies in people living with obesity in a transitioning population will be integrated with the data sets obtained from our previous studies; obesity cohort (300-OB), BCG cohort (300-BCG), Tanzania Human Functional Genomic Project (300-TZ-FG) and the proof-of-principle Tanzania dietary intervention cohorts for validation of results and translation of results to European population. We previously showed the importance of age, sex, and ethnicity(28–30), and in all subsequent analyses, these variables will be included as covariates.

Multi-omics analysis of the data

Gencode reference genome using alignment tool STAR (Spliced Transcripts Alignment to a Reference(40)), and further analyzed in R using the DESeq2 package(41) for normalization and

downstream variance-stabilizing transformation (VST) to allow exploratory data analysis (including principal components analysis), differential expression analysis and co-expression network analysis (CoCena2)(39). Additionally, to reveal and account for underlying compositional differences in cell types and states in our bulk sample, we will utilize deconvolution algorithms, such as CIBERSORTx53 and Cell Population Mapping (CPM)(42), respectively.

To expose the contribution of each biological data layer collected to the whole biological variance and highlight specific features that contribute to this variation and the interaction between them, we will utilize multi-omics approaches, mainly multi-omics factors analysis (MOFA)(43). The MOFA analysis reveals a set of latent factors that capture sources of variance, related to biological and technical factors.

Modelling "metabolic-related chronic inflammatory burden" to predict immune response

In collaboration with the Israel company CytoReason (collaborator Shen-Orr), we will construct a metric for a metabolic-related chronic inflammatory burden that will summarize an individual's inflammatory burden, in a similar manner to previous work done on aging(44) We will adapt a deep neural network that highlights the most relevant biological information related to the anthropometric, lifestyle, dietary, metabolic, microbiome and genomic data generated in this project to predict immune responses to microbes (i.e. cytokine responses) and vaccines (humoral responses).

Ethical Considerations

1.17 Ethical statement

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

1.18 Assessment of risks, benefits, and burden

Risks associated with the intervention are negligible. The alcohol percentage of freshly brewed fermented banana beverage is low, between 1 and 3 percent (45). To ensure quality and safety, we have identified a local producer that we have worked with during our previous study (https://www.isrctn.com/ISRCTN15619939). The production process including purchasing the ingredients will be supervised by our team to ensure the production of the fermented banana beverage will be done using high-quality ingredients and maintain strict hygiene during the brewing process. The production details and alcohol percentage of the fermented banana beverage have been previously described (26). Participants receiving the banana beverage intervention will be advised not to drive or operate any motor vehicle after consuming the beverage.

Furthermore, Prevenar 13 and tetanus toxoid are among the most commonly administered vaccines worldwide with an excellent safety profile. The advantages of participation includes health screening and health education regarding the effects of excess weight, importance of consuming a healthy diet and how it benefits the immune system. In addition, the participants on dietary intervention groups will receive health benefits related to their respective diets. They will receive vaccines against Clostridium tetani and Steptococcus pneumoniae which are common in Tanzania. To ensure the quality of the vaccines, we will follow strict protocols and guidelines set forth by the Tanzania Drug and Food Authority, including adhering to guidelines for storage, transportation, and quality control of the vaccines. The vaccine will be obtained from the KCMC GSF pharmacy, and the cold chain will be maintained at 2-8^{0}C and monitored using a refrigerator temperature chart until the administration of the vaccine. The batch number of the vaccine will be recorded on the CRF forms for each participant.

The intervention presents minimal risk to the participants. The fermented banana beverage contains low levels of alcohol, typically ranging between 1 and 3 percent. Prevenar 13 and tetanus toxoid vaccines are widely administered and have a well-established safety profile(15,16). The study offers several benefits to participants, including free health screening and education on how diet impacts immune responses and the health importance of consuming a plant-based diet. Additionally, those in the dietary intervention groups will potentially reap the health benefits associated with their specific diets. Lastly, participants will receive booster vaccines against Clostridium tetani and Streptococcus pneumoniae, which are infections of public health significance in Tanzania.

1.19 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 investigator. Only collaborator Temba, the data monitoring unit at KCMUCo and partner de Mast will have access to non-anonymized data, whereas all other partners will only have access to the anonymized donor ID. The CRF and signed informed consent form will be archived for maximum of 15 years. All data obtained in TransInf will be handled confidentially.

1.20 Compliance, withdrawal, and replacement of subjects

The dietary intervention is not expected to have important side effects, and we assume that compliance will be satisfactory. Compliance will be stimulated through different measures: i) Participants will receive free food or the fermented banana beverage, which will encourage adherence because the cost of regular foods is high. Lunch and breakfast will be consumed at the offices; ii) The study team will emphasize the importance of adherence and counsel participants on the benefits of a healthy diet on a regular basis. iii) Compliance will further be promoted by the food logs. For the same reasons, we anticipate the loss-to-

follow-up rate to be low. This is also based on our experience in the intervention study in TransMic. In our pretest-posttest design, participants that are lost to follow-up will not be included in the analysis (per-protocol analysis).

1.21 Stopping rules

Given the absence of important safety issues with such a short dietary intervention, only a few stopping criteria are defined. Individual participants: i) at their request; ii) if emergency antibiotics are required; iii) at the discretion of the responsible study doctor; and iv) if participants miss their intervention diet for more than four weeks. For the whole study: upon the decision of the study data or safety monitor.

1.22 Dissemination communication of the expected project results

The results will be disseminated in the form of written reports. Significant results will be communicated to the research community via publications in peer-reviewed 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, the public to create awareness and provide education.

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

Quality assurance and safety reporting

1.23 Quality assurance and Data monitoring

The trial unit in KCMUCo/KCRI is a well-equipped GCP-compliant clinical trial research facility with frequent international monitoring visits. Therefore, no special international monitoring visit to the trial site will be conducted for the dietary intervention study. From a Tanzanian institution other than KCMUCo/KCRI, an independent certified data monitor will be appointed. The data monitor will have experience in Clinical Research. There will be two monitoring visits: one at the halfway point during the trial and one at the end of the trial. The following things will be monitored if >25% of the participants are enrolled: completeness of the investigator file; verification of the source data; evaluation of any possible missed severe adverse event (SAE) and Suspected Unexpected Serious Adverse Reaction (SUSARs); accuracy of reporting; availability of SOPs for research procedures; and verification of the proper labelling

and storage of blood samples. The principal investigator and accredited Medical Research Ethics will receive a report in writing that the data monitor generates.

1.24 Safety reporting

For safety monitoring in this study, we have appointed Prof Kajiru (MD, MMED Internal medicine) as our independent study safety monitor instead of a Data and Safety Monitoring Board (DSMB) as this is a small, early-phase study of short duration with well-known vaccines with good safety records. The risks involved in participating in this study are minimal. A change in bowel movement and minor abdominal complaints may occur, which will be monitored and recorded. The vaccines have been in use for decades and have a very good safety profile. The Independent Safety Monitor is a licensed clinician with the necessary knowledge, and their main duty is to deliver timely independent safety monitoring. This is achieved by evaluating adverse events as soon as they happen and then monitoring them until they are resolved or stabilized. Additionally, we have appointed a study doctor, Dr. Norman (MD, MMED Internal medicine resident), who will monitor and ensure participant safety during the entirety of the study. He will work closely with the independent study safety monitor to assess any safety issues that may arise during the course of the study. In the unlikely event that a severe adverse event (SAE) occurs, it will be notified to the Principal Investigator and the accredited Medical Research Ethics Committees (METCs) as well as to the Tanzania Drug and Medicines Agency using standard SAE reporting procedures.

TENTATIVE Budget details

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	36	1000	36,000
Co-Investihator	10% FTE	1	mnth	36	100	3,600
Study coordinator/PhD student	100% reseacher	1	month	36	900	32,400
Lab technologist	100% FTE	3	month	12	600	21,600
Research Nurse	100% FTE	2	month	12	600	14400
Nutritionist	50% FTE	1	month	12	300	3,600
Cooking team	100% FTE	1	month	3	600	1,800
Subtotal						**113,400**
Study Materials						
Laboratory reagents and consumables	Including reagents for PBMC isolation, HIV,malaria and RBG screening kits and blood pressure machine etc		month	12	10,000	10,000
Food	Intervention food (breakfast, lunch and dinner)	30 subjects for 36 days	months	2	14,748.00	14,748
Drinks	Intervention drinks	30 subjects for 30 days	month	2	4,200.00	4,200

Cooking facility	Rent the cooking place, lunch boxes and other kitchen lump sum		month	2	2,500.00	2,500
Subtotal						**31,448**
Laboratory running costs						
Laboratory accessibility and utilization	Lab accessibility and utilization		month	6	880.35	5,281.95
Laboratory storage	Lab. Storage		month	12	200	2,400
Internet 4G bundle	ICT	2	month	36	20	1,140
Office space	Office space	2	month	6	30	360
Full blood count	330 samples		samples	330	8	2,640
Subtotal						**9,181.95**
Subject recruitment						
Volunteers transport and time reimbursement	Volunteers (cross-sectional: Intervention)	150 participants (screening and baseline sampling) and 90 participants for 2 subsequent visits (at 4 and 8 weeks respectively)	visits	4 visits	20.8	12,499
Subtotal						**12,499**
Ethical clearance fees						
Ethical clearance CRERC and renewal	KCMC Ethics	(500 first sub.+600 renew 3years)	months	36	1,100	1,100
Ethical clearance CRERC and renewal	NIMR	(600 first submission +600 renew 3 years)	months	36	1,200	1,200

Subtotal						**2,300**
Travel/Transport						
Home visit to inspect adherence	150km per month x 4month x 0.80€/km		month	4	480.00	480
Sample shipping	From KCMUCo to Nijmegen, NL	-	-	-		20,000
Subtotal						**20,480**
Postage/office consumables						
Postage/office consumables including labels	Posts and Stationaries including 5 rollers of labels and ink		month	12	3,200	3,200
Communication	Follow up participants ; reminder to take meals etc		month	6	100	600
Subtotal						**3,800**
Total						**193,108.95**
12% overhead						23,173.07
Total at KCMUCo						**216,282.02**
Laboratory Measurements at RadboudUMC and affiliated laboratories						
Cytokines	Cytokine measurements	240			30	7,200.00
Metabolome	Metabolome measurements	240			70	16,800.00
Proteome	Measurement of inflammatory proteins	240			150	36,000.00

Antibody response vaccine	Measurements of antibodies following vaccine administartion	180			58	10,440.00
DNA methylation	Epigenetic measurements	180			150	27,000.00
Subtotal						**97,440.00**
Grand Total						**313,722.02**

1.25 Budget Justification and Team Responsibilities

Personnel cost budget; Personnel cost budget; A total of **€113,400.00** has been budgeted for the research staff for the period of the study. This includes a) **€75,600.00** for the cost of key personnel consisting of the local PI/Post-doc, co-investigator, study coordinator/PhD student and nutritionist; and b) **€37,800.00** for the cost of supportive staff consisting of study nurse, laboratory scientists and cooking team.

Responsibility of the local key study team

1. **Dr Godfrey Temba - Principal investigator/post-doc.** He is an immunologist specializing in understanding the role of diet on immune response using multi-omics and systems biology approaches. He will be a full-time post-doc under the guidance of Dr. Quirijn de Mast and Prof. Reginald Kavishe to ensure the project is properly implemented. He will collaborate with the study coordinator on-field activities, optimize laboratory assays, and train the study team. Additionally, he will take the lead in communicating with stakeholders and collaborators, as well as analyzing data and writing reports.
2. **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.

3. **Dr. Eka-Patricia Kisali - Study Coordinator and PhD student.** Dr. Eka-Patricia is a medical doctor with specialized training as an immunologist at the master's level. She will join the TransInf project as a PhD student. In her role, she will oversee the study's activities, working closely with the nutritionist and study principal investigator (PI) to develop the proposal. Her responsibilities include setting up the study, conducting laboratory analyses, analyzing data, and writing manuscripts. Dr. Eka-Patricia will be responsible for the study's daily operations and will provide regular updates on its progress.

4. **Dr. Mary Mosha, Nutritionist.** She plays a crucial advisory role in the project, providing expertise in all nutrition-related aspects. Her responsibilities include developing questionnaires on dietary habits, designing intervention menus, and supervising the collection of data for the 24-hour dietary recall. The nutritionist will closely monitor the food and beverage preparation according to the SOP, monitor food and beverage intake by the study participants, and provide updates on adherence during project meetings. Additionally, she will contribute to data analysis and manuscript writing.

Responsibility of other supportive staff

5. **Study nurses.** They will be involved in data and sample collection during the four-time point in both study arms.

6. **Laboratory technologists.** They will be responsible for sample collection, processing, archiving and performing laboratory experiments as per standard operating procedures

Appendices

1. Investigators' CVs
2. Participants information sheet and informed concent – English Version
3. Pre-Screening form – English Version
4. Lifestyle questionnaire – English Version
5. Dietary questionnaire – English Version
6. Intervention recipes – English Version

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26. Kubo R, Kilasara M. Brewing Technique of Mbege, a Banana Beer Produced in Northeastern Tanzania. Beverages. 2016 Aug 3;2(3):21.

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28. Boahen CK, Temba GS, Kullaya VI, Matzaraki V, Joosten LAB, Kibiki G, et al. A functional genomics approach in Tanzanian population identifies distinct genetic regulators of cytokine production compared to European population. The American Journal of Human Genetics. 2022 Mar;109(3):471–85.

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45. Kubo R, Kilasara M. Brewing Technique of Mbege, a Banana Beer Produced in Northeastern Tanzania. Beverages. 2016 Aug 3;2(3):21.

Appendix 1: Curriculum Vitae Of Investigators

INTERNAL INVESTIGATORS

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MSc Biomedical Sciences, Radboud University, Nijmegen, The Netherland, Year 2016
BSc Biotechnology and Laboratory Sciences, Sokoine University, Morogoro, Year 2011.

3. *Recent Publications (past 5 years)*

3.1 ***Temba GS****, Kullaya V, Pecht T, Mmbaga BT, Aschenbrenner AC, Ulas T, Kibiki G, Lyamuya F, Boahen CK, Kumar V, Joosten LA. Urban living in healthy Tanzanians is associated with an inflammatory status driven by dietary and metabolic changes. Nature Immunology. 2021 Mar;22(3):287-300.*

3.2 Stražar M, ***Temba GS****, Vlamakis H, Kullaya VI, Lyamuya F, Mmbaga BT, Joosten LA, van der Ven AJ, Netea MG, de Mast Q, Xavier RJ. Gut microbiome-mediated metabolism effects on immunity in rural and urban African populations. Nature communications. 2021 Aug 11;12(1):1-5.*

3.3 ***Temba, G.S.,*** *Vadaq, N., Wan, J., Kullaya, V., Huskens, D., Pecht, T., Jaeger, M., Boahen, C.K., Matzaraki, V., Broeders, W. and Joosten, L.A., 2022. Differences in thrombin and plasmin generation potential between East African and Western European adults: The role of genetic and non-genetic factors. Journal of Thrombosis and Haemostasis.*

3.4 Boahen, C.K., ***Temba, G.S.,*** *Kullaya, V.I., Matzaraki, V., Joosten, L.A., Kibiki, G., Mmbaga, B.T., van der Ven, A., de Mast, Q., Netea, M.G. and Kumar, V., 2022. A functional genomics approach in the Tanzanian population identifies distinct genetic regulators of cytokine production compared to the European population. The American Journal of Human Genetics, 109(3), pp.471-485.*

4. *Current Research Engagement*

4.1 PI TransInf Study (2023-2026). Funding HDHL-INTIMIC (ZonMW). The effect of diet on immune and vaccine responses in people living with obesity in transitioning communities. Partners Radboudumc, CIM institute in Paris and Limes Institute Bonn.

4.2 Work Package leader: SCCOPET Project(2021-2024): Strengthening the capacity of (Covid-19) disease surveillance, diagnostics, vaccination programs and promoting the mental health of frontline

health care workers/professionals. Partners: Kilimanjaro Christian Medical University College (KCMUCo), State University of Zanzibar (SUZA), Jimma University (JU), University of Copenhagen (UCPH), Technical University of Denmark (DTU), University of Oslo (UiO)

4.3 TransMic Project-WP1&4 (2017-2023). Funding HDHL-INTIMIC (ZonMW). The transition from a traditional to a Western lifestyle and its effect on the interrelation between diet, gut microbiome and health. WP1(Human Functional Genomics Project) involved a study of 323 healthy volunteers recruited from Rural and urban areas in the Kilimanjaro region of Tanzania (my Ph.D. work). WP4 involved a study of dietary intervention (traditional vs. western-type diet) in Tanzania of which I coordinate the WP4 activities. Partners Radboudumc, University of Florence and Limes Institute Bonn.

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2. *Academic Qualifications (University degrees)*
PhD, Radboud University, Netherland, Year 2010
MSc Biochemistry and Mol. Biology, Tumaini University, Year 2005
BScED, University of Dar es Salaam, Year 1999.

3. *Recent Publications (past 5 years)*

3.1Nancy A Kassam, Daniel Laswai, Neema Kulaya, Robert D Kaaya, Debora C Kajeguka, Christentze Schmiegelow, Christian W Wang, Michael Alifrangis, **Reginald A Kavishe***; Human IgG responses to Aedes mosquito salivary peptide Nterm-34kDa and its comparison to Anopheles salivary antigen (gSG6-P1) IgG responses measured among individuals living in Lower Moshi, Tanzania; Plos one: 17(10);e0276437, 2022*

3.2 Robert Diotrephes Kaaya, Johnson Matowo, Debora Kajeguka, Filemoni Tenu, Boniface Shirima, Franklin Mosha, **Reginald Kavishe***, The Impact of Submicroscopic Parasitemia on Malaria Rapid Diagnosis in Northeastern Tanzania, an Area with Diverse Transmission Patterns, Infectious Disease Reports, 14 (6) 798-809, 2022.*

3.3 Kaaya, Robert D; **Kavishe, Reginald A***; Tenu, Filemon F; Matowo, Johnson J; Mosha, Franklin W; Drakeley, Chris; Sutherland, Colin J; Beshir, Khalid B; Deletions of the Plasmodium falciparum histidine-rich protein 2/3 genes are common in field isolates from north-eastern Tanzania, Scientific Reports, 12 (1) 2022*

3.4 Godfrey Temba, Nadira Vadaq, Vesla Kullaya, Tal Pecht, Paolo Lionetti, Duccio Cavalieri, Joachim L Schultze, Reginald Kavishe, Leo Ab Joosten, Andre J Van Der Ven, Blandina T Mmbaga, Mihai G Netea, Quirijn De Mastinflammatory Phenotype Differs In East Africa And Western Europe Populations: The Role Of Dietary Metabolites, Tanzania Journal Of Health Research, 23, 2022.

3.5 Robert D Kaaya, Caroline Amour, Johnson J Matowo, Franklin W Mosha, **Reginald A Kavishe***, Khalid B Beshir, Genetic Sequence Variation in the Plasmodium falciparum Histidine-Rich Protein 2 Gene from Field Isolates in Tanzania: Impact on Malaria Rapid Diagnosis, Genes, 13(9) 1642, 2022*

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MSc Immunosciences and Infection, University of Bonn, Germany, Year 2022

Medical Doctor, Muhimbili University of Health and Allied Sciences, Tanzania, Year 2017

3. *Recent Publications (past 5 years)*

   3.1 *Kisali EP*, *Iversen PO, Makani J. Low vitamin B12 blood levels in sickle cell disease: Data from a large cohort study in Tanzania.Br J Haematol. 2023;00:1–7. https://doi.org/10.1111/bjh.19265 (Accepted manuscript)*

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2. Academic Qualifications (University degrees)

PhD, Kilimanjaro Christian Medical University College
MSc, Nutrition for Global Health, London School of Hygiene and Tropical Medicine
MPH, Kilimanjaro Christian Medical University College
BSc, Home Economics and Human Nutrition, Sokoine University of Agriculture

3. Recent Publications (past 5 years)

3.1 Mary Vincent Mosha, Heavenlight A Paulo, Sia E. Msuya, Heiner Grosskurth, Suzanne Filteau (2022). Lack of an association between dietary patterns and adiposity among primary school children in Kilimanjaro Tanzania (BMC Nutrition)

3.2 Mosha, M., Msuya, S., Kasagama, E., Ayieko, P., Todd, J., Grosskurth, H., and Filteau, S (2021). Prevalence and correlates of overweight and obesity among primary school children in Kilimanjaro, Tanzania. Plos One

3.3 Mosha, M., Kasagama, E., Ayieko, P., Todd, J., Msuya, S., Grosskurth, H., and Filteau, S. Description and comparison of physical activity from self-reports and accelerometry among primary school children in Kilimanjaro, Tanzania: a pilot study. African Academy for Sciences Open research

3.4 Public engagement by early career researchers in East Africa during the Covid 19 pandemic: case studies from East Africa (in review)

EXTERNAL INVESTIGATORS

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PhD, Radboud University, Nijmegen, The Netherland, Year 2011
Clinical Fellowship Infectious Diseases, Radboudumc, Nijmegen, Year 2010
Residency training in internal medicine, Radboudumc, Nijmegen, Year 2010
Residency training in internal medicine, Jeroen Bosch Hospital's-Hertogenbosch, Year 2005.
Medical School, Year 2000.

3. *Recent Publications (past 5 years)*

3.1 Wan J, Vadaq N, Konings J, Jaeger M, Kumar V, de Laat B, Joosten L, Netea MG, van der Ven AJ, de Groot PG, de Mast Q, Roest M. Kallikrein augments the anticoagulant function of the protein C system in thrombin generation. J Thromb Haemost. 2021 Sep 17. doi: 10.1111/jth.15530. Epub ahead of print. PMID: 34532976.

3.2 Stražar M, Temba GS, Vlamakis H, Kullaya VI, Lyamuya F, Mmbaga BT, Joosten LAB, van der Ven AJAM, Netea MG, de Mast Q, Xavier RJ. Publisher Correction: Gut microbiome-mediated metabolism effects on immunity in rural and urban African populations. Nat Commun. 2021 Aug 24;12(1):5154. doi:10.1038/s41467-021-25472-z. Erratum for: Nat Commun. 2021 Aug 11;12(1):4845.PMID: 34429426; PMCID: PMC8384881.

3.3 van der Heijden WA, van Deuren RC, van de Wijer L, van den Munckhof ICL,Steehouwer M, Riksen NP, Netea MG, de Mast Q, Vandekerckhove L, de Voer RM, van der Ven AJ, Hoischen A. Clonal hematopoiesis is associated with low CD4 nadir and increased residual HIV transcriptional activity in virally suppressed individuals with HIV. J Infect Dis. 2021 Aug 21:jiab419. doi:10.1093/infdis/jiab419. Epub ahead of print. PMID: 34417800.

3.4 Stražar M, Temba GS, Vlamakis H, Kullaya VI, Lyamuya F, Mmbaga BT, Joosten LAB, van der Ven AJAM, Netea MG, de Mast Q, Xavier RJ. Gut microbiome-mediated metabolism effects on immunity in rural and urban African populations. Nat Commun. 2021 Aug 11;12(1):4845. doi: 10.1038/s41467-021-25213-2. Erratum in: Nat Commun. 2021 Aug 24;12(1):5154. Erratum in: Nat Commun. 2021 Sep 29;12(1):5818. PMID: 34381036; PMCID: PMC8357928.

4. *Current Research Engagement*

4.1 PI 2000HIV project. Funding ViiV Healthcare.

4.2 Project leader TransMic study (2018-2021). Funding HDHL-INTIMIC (ZonMW). The transition from a traditional to a Western lifestyle and its effect on the interrelation between diet, gut microbiome and health. Partners University of Florence and Llimes Institute Bonn. Project involved studies in Tanzania and Burkina Faso

4.3 Off-Road grant ZonMW (2017). Treating dengue with an influenza drug. TOTO trial.

4.4 Palubac study (Study in Burkina Faso on the diagnostic performance of a novel Sysmex haematology analyser in diagnosing malaria and co-infections)

4.5 Galilei Personalized Medicine Grant Radboudumc. The neuropsychiatric side effects of efavirenz. PhD 4 years.

4.6 PI RAPID-study. Switch from an NNRTI or PI-based regimen to a RAltegravir- based regimen in virologically suppressed HIV-infected patients: effects on Platelet reactivity, platelet-monocyte aggregation and the Inflammatory and thrombotic state of monocytes.

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Medical Doctor (MD) University of Medicine and Pharmacy Cluj-Napoca, Romania
PhD Radboud University Nijmegen, the Netherlands

3. Recent Publications (past 5 years)

3.1 Giamarellos-Bourboulis EJ, Tsilika M, Moorlag S, Antonakos N, Kotsaki A, Domínguez-Andrés J, Kyriazopoulou E, Gkavogianni T, Adami ME, Damoraki G, Koufargyris P, Karageorgos A, Bolanou A, Koenen H, van Crevel R, Droggiti DI, Renieris G, Papadopoulos A, Netea MG. Activate: Randomized Clinical Trial of BCG Vaccination against Infection in the Elderly. Cell. 2020 Oct 15;183(2):315-323.e9

5.2 Temba GS, Kullaya V, Pecht T, Mmbaga BT, Aschenbrenner AC, Ulas T, Kibiki G, Lyamuya F, Boahen CK, Kumar V, Joosten LAB, Schultze JL, van der Ven AJ, Netea MG, de Mast Q. Urban living in healthy Tanzanians is associated with an inflammatory status driven by dietary and metabolic changes. Nat Immunol. 2021 Mar;22(3):287-300

5.3 de Bree LCJ, Mourits VP, Koeken VA, Moorlag SJ, Janssen R, Folkman L, Barreca D, Krausgruber T, Fife- Gernedl V, Novakovic B, Arts RJ, Dijkstra H, Lemmers H, Bock C, Joosten LA, van Crevel R, Benn CS, Netea MG. Circadian rhythm influences induction of trained immunity by BCG vaccination. J Clin Invest. 2020 Oct 1;130(10):5603-5617

5.4 Bekkering S, Arts RJW, … , Stunnenberg H, Riksen NP, Netea MG. Metabolic Induction of Trained Immunity through the Mevalonate Pathway. Cell. 2018; 172:135-146

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Ph.D in 1999, Radboud University, Nijmegen, The Netherlands
Postdoc, University of Colorado, Denver, USA (2012-2015)
Full Professor in 2014, Radboud University, Nijmegen, The Netherlands

3. Recent Publications (past 5 years)

3.1 Netea MG, **Joosten LAB**. *J Clin Invest. 2023 Jan 17;133(2): e166467. PMID: 36647822*

3.2 Merriman TR, **Joosten LAB**. *Blood. 2022 Sep 8;140(10):1054-1056. PMID: 36074537*

3.3 Klück V, .. , **Joosten LAB**. *Lancet Rheumatol. 2020 May;2(5): e270-e280. PMID: 33005902*

3.4 Klück V, .. , **Joosten LAB**. *Ann Rheum Dis. 2020 Apr;79(4):536-544. PMID: 32114511*

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Ph.D in biology (2004); Institute of Molecular and Structural Biology, University of Aarhus, Denmark.

Doctor of Science (Habilitation à diriger les recherches), Life Sciences (2016) ; Sorbonne University, Paris. France.

3. *Recent Publications (past 5 years)*

3.1 *Lahmar, O., Salhi, M., Kaabachi, W., Berraies, A., Ammar, J., Hussain Soomro, M., **Larsen, M**., AnnesiMaesano, I., Hamzaoui, K., Hamzaoui, A. Association Between Vitamin D Metabolism Gene Polymorphisms and Risk of Tunisian Adults' Asthma. Lung 2018; doi.org/10.1007/s00408-018-0101-2*

3.2 *Fadlallah, J., El Kafsi, H., Sterlin, D., Juste, C., Parizot, C., Dorgham, K., Autaa, G., Gouas, D., Almeida, M., Lepage, P., Pons, N., Le Chatelier, E., Levenez, F., Kennedy, S., Galleron, N., Pais de Barros, J-P., Malphettes, M., Galicier, L., Boutboul, D., Mathian, A., Miyara, M., Oksenhendler, E., Amoura, Z., Doré, J., Fieschi, C., Ehrlich, S.D., **Larsen, M.*** and Gorochov, G.* Microbial ecology perturbation in human IgA deficiency. Science Translational Medicine 2018 May; 10(439):eaan1217; DOI:10.1126/scitranslmed.aan1217. (*Jointly directed the work)*

3.3 *Sterlin, D., Fieschi, C., Malphettes, M., **Larsen, M.,** Gorochov, G. and Fadlallah, J. Immune/microbial interface perturbation in human IgA deficiency. Gut Microbes 2018 Nov; DOI:10.1080/19490976.2018.1546520 (REVIEW)*

3.4 *Fadlallah, J., Sterlin, D., Fieschi, C., Parizot, C., Dorgham, K., El Kafsi, H., Autaa, G., Ghillani-Dalbin, P., Juste, C., Lepage, P., Malphettes, M., Galicier, L., Boutboul, D., Clément, K., André, S., Marquet, F., Tresallet, C., Mathian, A., Miyara, M., Oksenhendler, E., Amoura, Z., Yssel, H., **Larsen, M.*** and Gorochov, G.* Synergistic convergence of microbiota-specific systemic IgG and secretory IgA. JACI, 2019 Apr; 143(4):1575-1585 (*Jointly directed the work)*

3.5 *Fastenackels, S., Bayard, C., **Larsen, M**., Magnier, P., Bonnafous, P., Seddiki, N., Appay, V., Gautheret-Dejean, A. and Sauce, D. Phenotypic and functional differences between HHV-6 and HCMV specific T-cells. J Virol. 2019 Apr 17. pii: JVI.02321-18*

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Doctor of Science (Habilitation à diriger les recherches), Life Sciences (2015) ; Sorbonne University, Paris. France.

3. *Recent Publications (past 5 years)*

*5.1 Fali T, Vallet H, **Sauce D**[#]. Impact of stress on aged immune system compartments: Overview from fundamental to clinical data. Exp Gerontol. 2018 May;105:19-26*

*5.2 Fali T, Fabre-Mersseman V, Yamamoto T, Bayard C, Papagno L, Fastenackels S, Zoorab R, Koup RA, Boddaert J, **Sauce D***[#], Appay V*[#]. Elderly human hematopoietic progenitor cells express cellular senescence markers and are more susceptible to pyroptosis. JCI Insight. 2018 Jul 12;3(13):95319.*

*5.3 Vallet H, Fali T, **Sauce D**[#].. Aging of the immune system: From fundamental to clinical data. Rev Med Interne. 2019 Feb;40(2):105-111.*

*5.4 Fali T, Papagno L, Bayard C, Mouloud Y, Boddaert J, **Sauce D***[#], Appay V*[#]. New Insights into Lymphocyte Differentiation and Aging from Telomere Length and Telomerase Activity Measurements. J Immunol. 2019 Apr 1; 202 (7):1962-1969*

*5.5 Fastenackels S, Bayard C, Larsen M, Magnier P, Bonnafous P, Seddiki N, Appay V, Gautheret-Dejean A, **Sauce D**[#].. Phenotypic and Functional Differences between Human Herpesvirus 6- and Human Cytomegalovirus-Specific T Cells. J Virol. 2019 Jun 14;93(13):e02321-18.*

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B.Sc. biomedical sciences Tel-Aviv University, Israel
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3 Recent Publications (past 5 years)

* Publish under the name Tal Pecht

5.1: Kretschmer L, Pecht T, Knapp K, Bennstein SB. Community perspective: The importance of in-person meetings for young immunologists. Eur J Immunol. 2022 Dec;52(12):1876-1879. doi: 10.1002/eji.202250209. Epub 2022 Nov 28. PMID: 36330579.

5.2: Frishberg A, Kooistra E, Nuesch-Germano M, Pecht T, Milman N, Reusch N, Warnat-Herresthal S, Bruse N, Händler K, Theis H, Kraut M, van Rijssen E, van Cranenbroek B, Koenen HJ, Heesakkers H, van den Boogaard M, Zegers M, Pickkers P, Becker M, Aschenbrenner AC, Ulas T, Theis FJ, Shen-Orr SS, Schultze JL, Kox M. Mature neutrophils and a NF-κB-to-IFN transition determine the unifying disease recovery dynamics in COVID-19. Cell Rep Med. 2022 Jun 21;3(6):100652. doi: 10.1016/j.xcrm.2022.100652. Epub 2022 May 17. PMID: 35675822; PMCID: PMC9110324.

5.3: Casari S, Di Paola M, Banci E, Diallo S, Scarallo L, Renzo S, Gori A, Renzi S, Paci M, de Mast Q, Pecht T, Derra K, Kaboré B, Tinto H, Cavalieri D, Lionetti P. Changing Dietary Habits: The Impact of Urbanization and Rising Socio-Economic Status in Families from Burkina Faso in Sub-Saharan Africa. Nutrients. 2022 Apr 24;14(9):1782. doi: 10.3390/nu14091782. PMID: 35565752; PMCID: PMC9104313.

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3. Recent Publications (past 5 years)

3.1 Alpert A, Pickman Y, Leipold M, Rosenberg-Hasson Y, Ji X, Gaujoux R, Rabani H, Starosvetsky E, Kveler K, Schaffert S, Furman D, Caspi O, Rosenschein U, Khatri P, Dekker CL, Maecker HT, Davis MM, ***Shen-Orr SS****. A clinically meaningful metric of immune age derived from high-dimensional longitudinal monitoring. Nat Med. 2019 Mar;25(3):487-495. doi: 10.1038/s41591-019-0381-y. Epub 2019 Mar 6. PMID: 30842675; PMCID: PMC6686855.*

3.2 Frishberg A, Kooistra E, Nuesch-Germano M, Pecht T, Milman N, Reusch N, Warnat-Herresthal S, Bruse N, Händler K, Theis H, Kraut M, van Rijssen E, van Cranenbroek B, Koenen HJ, Heesakkers H, van den Boogaard M, Zegers M, Pickkers P, Becker M, Aschenbrenner AC, Ulas T, Theis FJ, ***Shen-Orr SS****, Schultze JL, Kox M. Mature neutrophils and a NF-κB-to-IFN transition determine the unifying disease recovery dynamics in COVID-19. Cell Rep Med. 2022 Jun 21;3(6):100652. doi: 10.1016/j.xcrm.2022.100652. Epub 2022 May 17. PMID: 35675822; PMCID: PMC9110324.*

3.3 Alpert A, Nahman O, Starosvetsky E, Hayun M, Curiel TJ, Ofran Y, ***Shen-Orr SS****. Alignment of single-cell trajectories by tuMap enables high-resolution quantitative comparison of cancer samples. Cell Syst. 2022 Jan 19;13(1):71-82.e8. doi: 10.1016/j.cels.2021.09.003. Epub 2021 Oct 7. PMID: 34624253; PMCID: PMC8776581.*

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PhD, New York University School of Medicine, Year 2003
MSc New York University School of Medicine, Year 1998

3. *Recent Publications (past 5 years)*

5.1 Savulescu A.F., Brackin R., Bouilhol E., Dartigues B., Warrell J.H., Pimentel M et. al., and Nikolski M., **Mhlanga M. M***. (2021) Interrogating RNA and protein spatial subcellular distribution in smFISH data with DypFISH, Cell Reports Methods, Volume 1, Issue 5. doi: 10.1016/j.crmeth.2021.100068*

5.2 Fanucchi S, Domínguez-Andrés J, Joosten LAB, Netea MG, **Mhlanga MM***. The Intersection of Epigenetics and Metabolism in Trained Immunity. Immunity. 2021 Jan 12;54(1):32-43. doi: 10.1016/j.immuni.2020.10.011. Epub 2020 Nov 20. PMID: 33220235.*

5.3 Regev A, Teichmann SA, Lander ES, Amit I, Benoist C, Birney E, Bodenmiller B, Campbell P, Carninci P, Clatworthy M, Clevers H, Deplancke B, Dunham I, Eberwine J, Eils R, Enard W, Farmer A, Fugger L, Göttgens B, Hacohen N, Haniffa M, Hemberg M, Kim S, Klenerman P, Kriegstein A, Lein E, Linnarsson S, Lundberg E, Lundeberg J, Majumder P, Marioni JC, Merad M, **Mhlanga M***, Nawijn M, Netea M, Nolan G, Pe'er D, Phillipakis A, Ponting CP, Quake S, Reik W, Rozenblatt-Rosen O, Sanes J, Satija R, Schumacher TN, Shalek A, Shapiro E, Sharma P, Shin JW, Stegle O, Stratton M, Stubbington MJT, Theis FJ, Uhlen M, van Oudenaarden A, Wagner A, Watt F, Weissman J, Wold B, Xavier R, Yosef N; Human Cell Atlas Meeting Participants. The Human Cell Atlas. Elife. 2017 Dec 5;6:e27041. doi: 10.7554/eLife.27041. PMID: 29206104; PMCID: PMC5762154.*

Appendix 2: Participant Information Sheet And Informed Consent Form.

KILIMANJARO CHRISTIAN MEDICAL UNIVERSITY COLLEGE

(A Constituent College of Tumaini University Makumira)

TITLE OF THE STUDY: *The Effect of Diet on Immune and Vaccine Responses in People Living with Obesity in Transitioning Communities*

COMPOSITION OF THE RESEARCH TEAM

The proposed study will be led by the following team of researchers from KCMUCo: Dr. Godfrey Temba, MSc Biomedical Sciences, PhD (PI), Prof. Reginald Kavishe, MSc Biochemistry, PhD (co-investigator), Dr. Eka-Patricia Kisali, MD, MSc Immunology, PhD student (study coordinator), and Dr. Mary Mosha, MPH, MSc Nutrition for Global Health, PhD (nutritionist). The team of researchers at KCMUCo will collaborate with the following external partners to realize the proposed study: Dr. Quirijn de Mast, MD, PhD (external PI), Prof. Mihai Netea, MD, PhD, Prof. Leo Joosten, MD, PhD, and Prof. Musa Mhlanga, PhD, from Radboudumc, The Netherlands; Dr. Martin Larsen, PhD, and Dr. Delphine Sauce, PhD, from Cimi-Paris; Dr. Tal Pacht, PhD, from LIMES Institute in Bonn; and Prof. Shai Shen-Orr, PhD, from CytoReason in Israel.

WHAT IS THE PURPOSE OF THIS STUDY

The purpose of this study is to understand how overweight and obesity impair the function of the immune system, including the response to vaccination, and whether dietary intervention with a plant-based high fiber diet also rich in polyphenols as well as fermented foods may improve immune function and the vaccine response in overweight and obese individuals. The prevalence of overweight and obesity is rapidly increasing globally. Obesity is associated with immune dysfunction leading to an increased risk of severe infectious diseases and decreased response to vaccination. Our previous studies in the Kilimanjaro region have shown that diet has a pronounced effect on the function of the immune system in healthy individuals. The traditional high plant-based fiber and polyphenol diet and a locally consumed fermented banana brew were anti-inflammatory. However, it is still unclear to what extent dietary variation directly or indirectly influences the host immune defense in individuals who live with obesity and whether certain dietary interventions may enhance immune responses and improve vaccine efficacy. The current study aims to fill these gaps by establishing the cause-and-effect relationship between specific nutritional factors and immune responses to common viral and bacterial infections, as well as the response to vaccines, in people living with obesity.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

The study will enroll a total of 150 participants aged 35 to 60 years. One hundred (n=100) participants with a BMI >27 Kg/M^2(Obese) and the remaining fifty (n=50) will be age and sex-matched controls with a BMI between 18.5–24.9 Kg/M^2 (normal weight controls).

WHAT IS INVOLVED IN THE STUDY?

Participation in this study is entirely voluntary. If you refuse to participate or withdraw from the study, it will not affect any present or future relationships you may have with your medical care team. If you voluntarily agree to be in this study, you will be asked to sign and date this consent form.

After fulfilling the inclusion criteria you will be asked questions to assess your readiness to enrol in the study and if eligible you will be enrolled into the study after providing written informed consent,. Initial assessment will include screening for malaria, HIV, hypertension, and blood glucose levels. In premenopausal women, a pregnancy test will be performed. Your weight and height measurements will also be taken. Based on these anthropometric measurements 100 participants aged 35 to 60 years with a BMI >27 Kg/M2 (Obese) at screening will be recruited, as well as 50 age and gender-matched participants with a BMI between 18.5-24.9 Kg/M2 at screening (normal weight controls). You will not incur any cost for the screening tests. You will be provided with a food log sheet to record your food and beverage intake the week before starting the intervention (a 24-hour dietary recall on three non-consecutive days, that is two weekdays and one day in the weekend/public holiday). You 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"). These food logs will be assessed by the nutritionist to determine your average consumption. The next step will be filling out two questionnaires one collecting information on the general lifestyle including health status and the other one collecting information on frequency intake of different food items (food frequency questionnaire, FFQ) to describe the dietary habits.

At the start of the intervention, the first consecutive ninety of the 100 overweight volunteers will be randomly assigned to one of three arms: i) plant-based high fiber and polyphenol diet (n=30); ii) daily consumption of fermented banana beverage (n=30); iii) remain on their regular diet (obese controls; n=30). Participants in the 'high plant-based fiber and polyphenol diet' arm will be invited for breakfast and lunch every day at a restaurant on the hospital premises during which they will receive a meal. In addition, they will receive a family-size food package for dinner on weekdays as well as a package for all three meals on Saturday (breakfast, lunch and dinner). Participants in the 'fermented banana brew' arm will receive 1 litre of fermented banana beverage ('mbege') every evening after work (Monday to Friday) from the same facility. The dietary intervention will last for six weeks.

The flow of activities during the study is detailed in figure 1 below. Individuals who agreed to participate in the study will be invited for an initial screening visit (visit 1), and those who match the inclusion criteria will be enrolled. Blood (37.5ml) and handed a container for a stool sample (about 2 g) samples will be taken on visit 2 for immune assays and a suite of omics technologies (baseline sampling, 100 overweight participants, and 50 lean participants). Blood (37.5ml) and stool (about 2 g) samples will be taken from 90 participants in the intervention arms at week 4 (visit 3). (post-interventional sampling). Participants will receive a conjugated pneumococcal vaccine (Prevenar13) and tetanus toxoid vaccine at week 4 and continue with their diets for another 2 weeks to examine the impact of the dietary intervention on

vaccination responses. The effect of diet on the immune system and the vaccine response will be evaluated four weeks following vaccination (visit 4), blood (37.5 ml) and stool (about 2 g) samples will also be collected to investigate how long the health benefits of the plant-based diet and fermented banana beverage on immune function and vaccine responses can last. The measurements for the different samples will be conducted in part at the Kilimanjaro clinical research institute (KCRI) and the rest conducted in laboratories outside the country in institutions collaborating on this project. These laboratories will handle the samples following strict protocols and confidentiality measures. The collected blood samples will be used for measuring immune responses to different micro-organisms including different bacteria, viruses and fungi of clinical relevance. They will also be used to assess vaccine responses, changes in plasma proteins and other changes to immune cells following the diet intervention. The stool samples will be used to assess the changes in the microbiome which plays a crucial role in immune regulation and gut health.

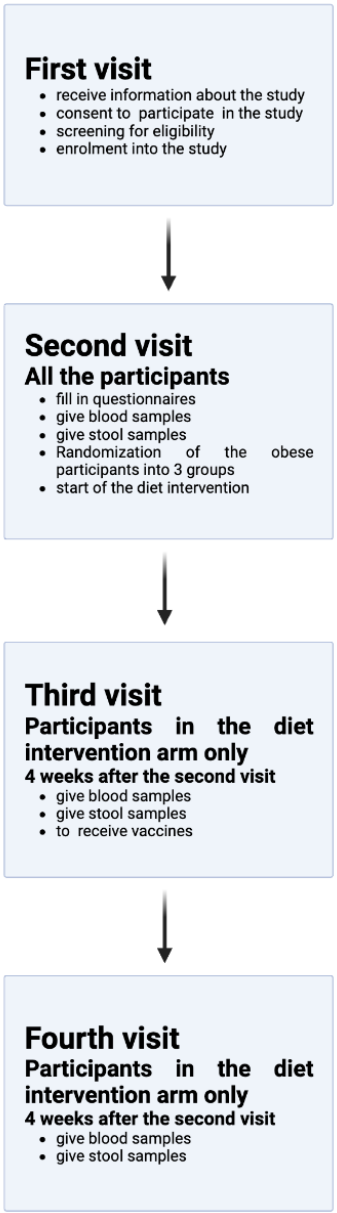

Figure 1: Flow chart showing the events scheduled at each visit during the 2 months of the study duration.

CONFIDENTIALITY OF THE 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 investigator. Only collaborator Temba, the data monitoring unit at KCMUCo, and partner de Mast will have access to non-anonymized data, whereas all other partners will only have access to the anonymized donor ID. The case report forms (CRF) and signed informed consent forms will be archived for a maximum of 15 years. All data obtained in TransInf will be handled confidentially.

FUTURE USE OF MATERIAL

Stored material, including samples and associated data, may be used for additional research questions in the future, provided that the new research is approved by the relevant ethical committees and complies with all applicable legal requirements. The samples will be stored for a maximum period of 10 years.

THE RISK AND BENEFITS OF PARTICIPATING IN THIS STUDY

Risks associated with the intervention are negligible. The alcohol percentage of the fermented banana beverage is low averaging between 1 and 3 percent, and is unlikely to induce liver damage. Prior to your recruitment to the study we will assess your alcohol consumption as well as screen you for any liver diseases by measuring a liver enzyme in your blood so as to establish that you are healthy and able to consume the beverage safely.

The pneumococcal (Prevenar 13) and tetanus toxoid vaccines are among the most commonly administered vaccines worldwide with an excellent safety profile. Some minor adverse effects may occur after receiving a vaccine, including redness, swelling, pain, or tenderness where the shot is given, fever, loss of appetite, feeling tired, headache, muscle aches, joint pain, and chills. These symptoms usually last for a few days, however, if they persist or increase in severity then the participant should report to the nearest health facility and also inform the research team.

Participants will be reimbursed transportation costs for the four visits to be made to the study site(figure 1). During other days, since the study participants will be coming into work, there will be no reimbursement of transport costs. The advantages of participation will be receving free screening for diseases including common infections like HIV and non communicable diseases like diabetes mellitus and hypertension. Participants will also receive nutritional health information and nutritional health advice. Additionally, participants will receive vaccinations for key public health diseases i.e pneumococcal and tetanus toxoid vaccines.

RIGHTS TO WITHDRAW FROM THE STUDY

You may choose not to be in the study, or, if you agree to be in the study, you may withdraw from the study at any time. If you withdraw from the study, no new data about you will be collected for study purposes. Your decision not to participate or to withdraw from the study will not involve any penalty or loss of benefits

to which you are entitled, and will not affect your access to health care in the future. If you do decide to withdraw, we ask that you contact the research team.

WHAT ABOUT COMPENSATION?

Participants will be financially supported to come to the study site during the four sampling periods.

WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

For questions about the study or a research-related injury, or if you have complaints, concerns, or suggestions about the research, contact Dr. Godfrey Temba (PI) at the Kilimanjaro Christian Medical University College Tel. +255 753584878 or Dr. Eka-Patricia Kisali (study coordinator) at the Kilimanjaro Christian Medical University College Tel. +255 687500303.

Either if you need to report or obtain more information related to ethical misconduct in this study please contact the ethics office via phone number 0272753909, or email: kcmc.rec@kcmuco.ac.tz

CONSENT FORM

I have read the information for this study and my questions have been fully answered by a member of the research team

I agree to participate in the study.

Name …… Signature…….. Date……..

I have read and explained the study information to this person and have answered all question raised
Name …… Signature…….. Date……..

Appendix 3: Pre-Screening Form.

KILIMANJARO CHRISTIAN MEDICAL UNIVERSITY COLLEGE

(A Constituent College of Tumaini University Makumira)

Participants Study ID ☐☐☐☐☐☐

Participants initials ☐☐☐

Date of recruitment (dd/mm/yyyy) ☐☐/☐☐/☐☐☐☐

Place of recruitment :----------

Questionnaire filled by:

Name: _____ Initials: _____

This questionnaire will be used to collect information that will determine your eligibility to participate in the study. Filling this form will take about 5 minutes.

	YES	NO
1. Is your age between 35 to 60 years?	O	O
2. What is your weight?.....kg		
3. What is your height? ……cm		
BMI= kg/m^2 = _____ is BMI between 18.5 kg/m^2-39.9kg/ m^2	O	O
4. Do you take commercially produced alcohol (beer, wine, spirits)?	O	O
5. Do you take locally produced fermented banana beverage (mbege)? …… days a week.	O	O
6. Have you used antibiotics within the last 3 weeks?	O	O
7. Have you had any signs of fever within the past 1 month? Do you feel feverish at the moment?	O	O
8. Have you been admited to hospital in the last 3 months?	O when:…….	O
9. Do you have a history of cancer of any type?	O	O
10. Do you have a history of kidney diseases?	O	O
11. Have you been diagnosed with Tuberculosis in the past one year?	O	O
12. Do you have a history of liver diseases?	O	O
13. Do you have a history of hypertension or any other cardiovascular diseases?	O	O
14. Do you have a history of stroke?		
15. Have you used any antipain medication in the past one month? If yes which ones: …….		
16. Have you used any antibiotic medication in the past 3 weeks?		
17. Are you currently receiving treatment for any mental illness, such as depression, biopolar disorder or schitzophrenia?		

18. Do you use cannabis or any other illicit drug?		
19. Are you currently using any of the following supplements? 'n-3 fatty acids', vitamini E au 'Magnesium'?		
20. Are you currently on or do you plan on joining a weight loss program in the next 8 weeks?		
21. Are you currently on or have you participated in any trial in the past 30 days?		
22. Are you diabetic?		
23. Have you ever being diagnosed with HIV?	O	O
24. Have you ever worked in mines or rice farms?	O	O
25. Do you have any activity that will require you to move from your current location in the next two months?	O	O
26. Do you think you can manage to change your diet completely without any health disturbances?	O	O
27. Do you commit to follow the new diet program as guided by the study protocol without cheating?	O	O
28. Do you have any known allergies? If yes which ones: ……	O	O
29. Have you ever had a reaction after receiving either the Tetanus toxoid or Pneumococcal conjugate vaccine?	O	O
30. Do you have any bleeding disorder?	O	O

31. Mention the type of foods that you do not consume, or that you are allergic to

_____

32. Where do you live at the moment?.....

33. Since when do you live at this location?.....

For women

34. When was your last menstral period? .....

35. Are you currently pregnant?

36. Are you currently lacting?

37. Are you currently on any contraceptive method? If yes please list which ones

.....

.....

.....

.....

.....

THANK YOU FOR ANSWERING THE QUESTIONS ABOVE

- You are eligible to participate in this study. You will be asked to visit our centre at .....Day ..... Date..... Time.....
- You are not eligible to participate in the study for the following reasons.
    - Health
    - Age
    - Occupation/residence
    - Diet
    - Other…….

Appendix 4: Lifestyle Questionnaire

KILIMANJARO CHRISTIAN MEDICAL UNIVERSITY COLLEGE

(A Constituent College of Tumaini University Makumira)

Participants Study ID □□□□□□

Participants initials □□□

Date of recruitment (dd/mm/yyyy) □□/□□/□□□□

Place of recruitment :_____

Questionnaire filled by:

Name: _____ Initials: _____

The questionnaire includes questions that have to do with your lifestyle, health information and daily activities and is part of the DIET-Study. Completing the questionnaire will take approximately 30-45 minutes.

SECTION A: Demographic data

In this section, we want to ask you to **describe yourself** based on the following questions. All the information that we receive from your side will be carefully handled and will remain confidential.

DEM 1 What is your age? _ _ _ _ _ _ years

DEM 2 What is your Gender?

- o Male
- o Female

DEM 3 What is your date of birth _ _- _ _ -_ _ _ _ (dd-mm-yyyy)

DEM 4 Whats is your current measured height? _ _ _ meters

DEM 5 Whats is your current measured weight? _ _ _ kg

DEM 6 Whats is your current measured waist circumference _ _ _ centrimeters

DEM 7 What is your current marital status? (Please select the option that best describes your current situation)

- o Single
- o Married
- o Divorced
- o Widowed

DEM 8 How many children do you have ? _ _ _ children

DEM 9 Whom do you live with?

- o I live alone

- I live together with family or friends
- I live in community (student house, nursing home, etc…)

DEM 10 How would you define the area where you live?

- Urban. Name of place _____
- Rural. Village name _____

DEM 11 Which tribe do you belong to?

- Chaga
- Pare
- Maasai/Meru
- Other, specify……

DEM 12 When did you start living in your current residence?

- Since birth
- Moved in past 3 months
- Moved in within last year
- Moved in more than a year

DEM 13 What is your place of birth?

- Urban. Name of place _____
- Rural. Village name _____

DEM 14 Where did you live for most of your life, indicate the years

- Name of place
  - Urban. Name of place _____
  - Rural. Village name _____
- Years _____

DEM 15 What is the highest level of education that you have achieved so far?

(Note: if you are going to school, pick the last bullet)

- Primary education
- O-level secondary education
- A-level secondary education
- College education (Non-degree)
- University education (degree)
- I'm still in training (Specific: _____)

DEM 16 Current work status

- Employed (teacher, doctor, nurse etc.)
- Service workers (market and shops salespersons motorcycle drivers etc.)
- Elementary Occupations (e.g. unskilled farmhands and street vendors etc.)
- In school
- Other, specify: ……..

DEM 17 Are or were you working night shifts?

- No
- Yes (specify; how many nights per week_____)

DEM 18 Considering your current residence and work place

- I live in the village and work/study in the village
- I live in the village and work/study in town and come back to village in the evening
- I live in the village and work/study in town and come back to village in the weekend
- I live in town and work/study in town
- I live in town and work/study in the village and come back to town

SECTION B1: Health status

In this section, we ask questions to evaluate your **general health status**. All the information we receive from you will remain confidential and will be treated as such.

GEZ 1 How do you describe your physical health?

- Excellent
- Very good
- Good
- Discreet
- Poor

GEZ 2 Did you receive any vaccinations the past year?

- Yes (please specify which vaccination_____)
- No

GEZ 3 Did you receive the following vaccinations?

- Bacille Calmette-Guérin (BCG): Yes □ No □
- Inactivated polio vaccine: Yes □ No □
- diphtheria - tetanus - pertussis: Yes □ No □
- Hepatitis B Vaccine (HBV): Yes □ No □
- Haemophilus Influenzae: Yes □ No □
- measles - mumps - rubella - Vzv: Yes □ No □
- Pneumococcus: Yes □ No □
- Meningococcus B: Yes □ No □
- Rotavirus: Yes □ No □
- Human Papilloma Virus (HPV): Yes □ No □
- Yellow fever: Yes □ No □
- COVID-19 vaccine Yes □ No □
- Other……

GEZ 4 Regarding your birth, were you born through:

- Vaginal delivery
- Cesarean section
- I don't know

GEZ 5 As baby, did you get:

- Breastfeeding
- Formula feeding
- Mixed
- I don't know

GEZ 6 Have you taken antibiotics within the last year?

- No
- Yes

GEZ 7 Have you taken antibiotics within the last 3 months?

- No
- Yes

GEZ 8 Have you taken antibiotics within the last 1-3 month(s)?

- No
- Yes

GEZ 9 Have you had fever in the past month?

- No
- Yes

GEZ 10 Have you had fever in the past 72h?

- No
- Yes

GEZ 11 Have you been hospitalized in the last month?

- No
- Yes (what was the diagnosis: _____)

GEZ 12 Have you ever undergone surgery?

- No
- Yes (specify:_____ age:_ _ _ years)

GEZ 13 During the past month, have you had diarrhea? How often?

- No
- Yes, ≤ one day at month
- Yes, 2-4 days at month
- Yes, 5-10 days at month
- Yes, >10 days at month

GEZ 14 Do you take medication regularly?

- No
- Yes , please specify_____

GEZ 15 Is there a member of your family who is obese? (please consider as member of your family-1° degree: mother, father, brother, sister 2° degree: grandparents; 3° degree: uncles, aunts, cousins)

- No
- Yes, speficy_____
- I don't know

GEZ 16 Does anyone in your family suffer from non-communicable diseases (please consider member of your family-1° degree: mother, father, brother, sister 2° degree: grandparents; 3° degree: uncles, aunts, cousins)

Condition	No	Yes	Relative degree diagnosed with condition	Details
Autoimmune diseases	☐	☐	☐ 1° ☐ 2° ☐ 3°	
Allergy	☐	☐	☐ 1° ☐ 2° ☐ 3°	
Eczema	☐	☐	☐ 1° ☐ 2° ☐ 3°	
High blood pressure (hypertension)	☐	☐	☐ 1° ☐ 2° ☐ 3°	
Myocardial infarction (Heart attack)	☐	☐	☐ 1° ☐ 2° ☐ 3°	
Stroke	☐	☐	☐ 1° ☐ 2° ☐ 3°	
Chronic obstructive pulmonary disease (COPD)	☐	☐	☐ 1° ☐ 2° ☐ 3°	
Asthma	☐	☐	☐ 1° ☐ 2° ☐ 3°	
Pulmonary hypertension	☐	☐	☐ 1° ☐ 2° ☐ 3°	
Cancer	☐	☐	☐ 1° ☐ 2° ☐ 3°	
Diabetes (Type 1/Childhood)	☐	☐	☐ 1° ☐ 2° ☐ 3°	
Diabetes (Type 2)	☐	☐	☐ 1° ☐ 2° ☐ 3°	
Gestational diabetes (during pregnancy)	☐	☐	☐ 1° ☐ 2° ☐ 3°	

Pre-diabetes (insulin resistence)	□	□	□ 1° □ 2° □ 3°	
inflammatory bowel disease (IBD)	□	□	□ 1° □ 2° □ 3°	
Crohn's disease	□	□	□ 1° □ 2° □ 3°	
Clotting/bleeding disorders	□	□	□ 1° □ 2° □ 3°	
Chronic kidney disease	□	□	□ 1° □ 2° □ 3°	
Gout	□	□	□ 1° □ 2°□ 3°	
Rheumatic Joint Disease	□	□	□ 1° □ 2°□ 3°	

GEZ 17 Have you ever tested PCR positive for COVID-19?

- No
- Yes (specify month / year :_ _ /_ _ _ _)

GEZ 18 Have you ever been suspected of having COVID-19?

- No
- Yes (specify month / year: _ _ /_ _ _ _)

GEZ 19 Have you ever been diagnosed with Tetanus disease?

- No
- Yes (specify month / year : _ _ /_ _ _ _)

GEZ 20 Do you have any allergies?

- No
- Yes, specify_____

GEZ 21 Do you suffer or have you suffered from asthma?

- No
- Yes

GEZ 22 Do you suffer or have you suffered from eczema?

- No

- Yes

SECTION B2: Health status

In this section, we ask questions to evaluate **cardiovascular disease risk factors**. All the information we receive from you will remain confidential and will be treated as such.

GEZ 23 Have you ever had a heart condition?

- No
- Yes , please specify which one and month/year of diagnosis
  - myocardial infarction (month/Year _ _ /_ _ _ _)
  - cardiomyopathy, (month/Year _ _ /_ _ _ _)
  - rheumatic heart disease , (month/Year _ _ /_ _ _ _)
  - other: _____(month/Year _ _ /_ _ _ _)
  - I don't know

GEZ 24 Have you ever had a stroke?

- No
- Yes (specify: month/Year _ _ /_ _ _ _)

GEZ 25 Have you ever been diagnosed with high blood pressure?

- No
- Yes (specify last measurement: month/Year _ _ /_ _ _ _)

GEZ 26 Have they ever diagnosed with high cholesterol levels?

- Never been tested
- No
- Yes

GEZ 27 Are you currently smoking or have you ever smoked in the past?

- I have never smoked
- I currently smoke
  - Specify at what age you started smoking: _ _ _ years
  - How many smoking products (e.g. cigarettes) etc are consumed on average per day _ _
- I used to smoke in the past

Specify how many years ago smoking was stopped : _ _ _ years

SECTION C: Physical activity and Daily habits

In this section, we ask questions to evaluate your **physical activity and daily habits**. All the information we receive from you will remain confidential and will be treated as such.

At Work

POH 1 Does your work involve physical activity?

- No
- Yes
  - **Vigorous-intensity activity** : causes large increases in breathing or heart rate like [carrying or lifting heavy loads, digging or construction work] for at least 10 minutes continuously.
  - **Moderate-intensity activity:** causes small increases in breathing or heart rate such as brisk walking [or carrying light loads] for at least 10 minutes continuously

Free Time

POH 2 Do you practice physical activity/sport during your free time?

- Yes(specify: _____)
- No

POH 2:1 (If POH1 = yes)

In a typical week, on how many days do you do sports/ fitness activities?

Number of days _ _ _

POH 3 How much time do you spend practicing sport on a typical active day?

Type of activity	**Vigorous-intensity sports** large increases in breathing or heart rate like [*running or football,]*	**Moderate-intensity sports** a small increase in breathing or heart rate such as brisk

		walking,(*cycling, swimming, volleyball*
Time spent each active day	○ < 1hr ○ 1 hr ○ 2 hr ○ >2 hr	○ < 1hr ○ 1 hr ○ 2 hr ○ >2 hr

Commuting

POH 4 To reach home/ school/ work, do you use?

- I walk, time each day: _ _ _
- Bicycle, time each day: _ _ _
- Motorbike
- Automobile (private car)
- Public transport (daladala,tuktuk)
- other (specify…….....)

POH 5 Considering all your movements on foot, how long do you think you are walking per day?

- < 30 minutes
- From 30 to 60 minutes
- > 60 minutes

Sedentary Behaviour

POH 6 How much time do you usually spend sitting or reclining (lying back) on a typical day? (Do not include time spent sleeping)

- < 1 hours
- 1-2 hours
- 3-4 hours
- 4-6 hours
- 6-8 hours
- > 8 hours

<u>SECTION D</u> : Reproductive Health – For female Participants Only

In this section, we ask questions to evaluate your **reproductive health .** All the information we receive from you will remain confidential and will be treated as such.

ROH 1 How many times have you been pregnant in the past? Number of pregnancy _ _ _

ROH 2 How many of these pregnancies ended with delivery of a baby? Number of deliveries _ _ _

ROH 3 Which type of contraceptives do you use currently? (pick all relevant answers)

Type of contraceptive			**Since (year)**
None	o No	o Yes	
Condoms	o No	o Yes	
Hormonal interuterine device	o No	o Yes	
Non-hormonal interuterine device	o No	o Yes	
Arm birth control implant (Nexplanon)	o No	o Yes	
Oral contraceptive	o No	o Yes	
Hormonal ring	o No	o Yes	
Contraceptive injection	o No	o Yes	
Spermicides	o No	o Yes	
Contraceptive patch	o No	o Yes	
Surgical sterilization	o No	o Yes	

ROH 4 Do you still have a menstrual cycle?

- No
- Yes

ROH 5 Is your menstrual cycle regular?

- No
- Yes, length of total cycle:_ _ _ , days of bleeding: _ _ _

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE!

Appendix 5: Dietary Questionnaire

KILIMANJARO CHRISTIAN MEDICAL UNIVERSITY COLLEGE

(A Constituent College of Tumaini University Makumira)

Participants Study ID ☐☐☐☐☐☐

Participants initials ☐☐☐

Date of recruitment (dd/mm/yyyy) ☐☐/☐☐/☐☐☐☐

Place of recruitment :----------

Questionnaire filled/distributed by:

Name: _____ Initials: _____

SECTION 1	**Involves a recall of foods that you have consumed in the last 24 hours** A 24 hours food recall will be documented on 3 non-consecutive days. (Example; On Tuesdays, Thursdays and Sundays) in a period of one-week preceding intervention phase. These will include at least one day on weekend or during festivity. (We will use a 4 stage multipass technique to collect the information of food intake).

	Food	**Portion**
		Big - Medium - Small
Morning	- - -	
Afternoon	-	

	- -	
Evening	- - -	

SECTION 2	Information about your dietary behaviour. For this, we ask you about **your eating behaviour eg your diet, how many maeals , where do you normal take your meals etc.**

Eating behaviour
1- How do you define your diet? • My diet include **meat, fish, poultry and vegetable**s? • I eat **fish, dairy products, eggs and vegetables but do NOT** eat **meat and poultry?** • I eat **dairy products, eggs and vegetables but do NOT** eat **meat, fish and poultry?** • I eat mostly **plant-based diet** but also eat **meat, but once a month at most?** • I eat **ONLY plant-based diet** and **NEVER** eat **meat, poultry**, **fish**, **eggs and dairy products**?
2- **How many meals do you generally have a day** • 3 meals a day: which times of the day (……) • 2 meals a day: which time of the day (……) • 1 meal a day: which time of the day (……)

3- How often (in a typical week) do you eat food prepared away from home eg; from the street vendors, cafeteria, restaurants etc

- Never
- Once per week
- 2 – 3 times a week
- everyday

4- How often do you eat fried foods that has been prepared away from home?

- Never
- Once per week
- 2 – 3 times a week
- everyday

5- **How often do you eat fried food that has been prepared at home?**

- Never
- Once per week
- 2 – 3 times a week
- everyday

6- **Do you often add extra salt / table salt to foods on your plate?**

- Yes
- No

7- Do you normally have lunch at home?

- No
- Yes

8- Do you have dinner at home?

- no
- yes

SECTION 3	Food Frequency Questionnaire (FFQ)

Food item	Swahili	Serving size	Never	Once per day	1-2 times per week	3-6 times per week	Everyday
Cereals and cereal products							
Maize, stiff porridge (wholegrain)							
Maize, stiff porridge (milled)							
Millet dishes / porridge							
Mixed porridge flour (without sugar)							
Mixed porridge flour (with sugar)							

Maize, green cooked							
Sorghum							
White Rice							
Brown rice							
Spiced rice, pilau with meat							
Rice biriani							
Rice bun							
Pasta eg:spaghetti, macaroni							
Pancakes							
Bread roll							
Bread, brown							
Chapati							
African doughnut (mandazi)							

Roots, tubers and bananas) including local dishes							
Banana fried							
Banana, roast							
Fried bananas							
Fried potatoes (chips)							
Fried sweet potatoes							
Cassava crisps (salted)							
Cassava, fried							
Cassava stiff porridge							
Cassava, boiled							
Potato crisps (salted)							
Sweet potatoes (boiled)							
Yams (boiled)							

Banana porridge with meat							
Banana porridge, plain							
Banana with kidney beans							
Cooked banana with meat							
Boiled banana							
Pulses, nuts and seeds							
Groundnuts							
Almonds							
Cashew nuts							
Green beans							
Cooked beans, with coconut milk							
Cooked beans, without coconut milk							
Peas							
Sweets and sugars							
Homemade juice with added sugar							

Homemade juice without sugar							
Boxed / commercial juices							
Soda							
Sweets, candies							
Cookies							
Cake							
Chocolates							
Meat, poultry and fish							
Beef, fillet							
Beef with fat							
Beef, with bone							
Beef tripe							
Beef liver, roasted							
Goat meat							

Lamb meat, mutton							
Pork							
Chicken, fried							
Chicken, boiled or roast							
Eggs, boiled							
Eggs, fried							
fish (fried)							
fish (sardines)							
Fruits and fruit juices							
Avocados							
Banana, ripe							
Watermelon							
Mangoes							
Oranges							
Vegetables							

African egg plant							
Amaranthus							
Egg plant							
cabbage							
Okra							
Spinach							
Lettuce							
Carrots							
Pumpkin							
Brocolli							
Cauliflower							
Cucumber							
Fast food snacks (from restaurants / home)							
Meat samosas							

Meat barbeque							
Kababs							
Sausages							
Omelet, Spanish with potato							
Chicken chips							
***Canned foods**							
Milk and milk products							
Yoghurt, plain whole milk							
Milk, cow, whole							
Low fat milk (commercial)							
Low fat milk (home reduced)							
Oils and Fats / spreads							
Olive oil							

Coconut oil							
Vegetable oil							
Peanut oil							
Margarine							
Butter							
Breakfast							
Tea with sugar (black)							
Tea without sugar (black)							
Milk tea with sugar							
Milk tea without sugar							
Coffee with sugar							
Coffee without sugar							
Alcohol							

Beer, commercial							
Local brew (mbege)							
Liquor							
Wine							

Appendix 6: Intervention Recipes

Low Fat and sugar, High Fibre, unprocessed food 'plant-based' (Traditional Tanzania Foods).

	Breakfast	**Lunch**	**Dinner**
Day 1; Monday	Millet and sorghum porridge mixed with sour milk w boiled taro + berries + spinach	Maize and kidney bean with + cabbage + cucumbers + fruit: orange	Cooked plantain with meat + amaranthus, fruit: avocado
Day 2; Tuesday	Fat reduced milk tea with brown bread + low fat spread (little butter) + spinach	Banana porridge with beans + amaranthus + cucumber sticks (eg: a piece of watermelon	Whole grain rice (brown) with lentils (green), or yellow lentils), collard + orange
Day 3; Wednesday	Black tea with boiled cassava + vegetable sticks (carrots, cucumber)	Maize stiff porridge with fish stew mixed with okra and eggplants, potato leaves + oranges	Whole grain rice (brown) rice with kidney beans+ fruits + spinach + ripe banana
Day 4; Thursday	Millet and sorghum Porridge with sour milk with boiled sweet potato + vegetable mix (cabbage, carrots, zucchini)	Brown spaghetti, free range chicken stew / with boiled peas + carrots + watermelon	Banana + white yams + mashed with little lean meat + broth + papaya
Day 5; Friday	Black tea with boiled cassava, vegetable sticks (cucumber, carrots)	Banana porridge w beans + vegetables: cabbage + carrots, green beans + fruits: oranges	Sorghum stiff porridge (with fish and vegetables + fruit: papaya + avocado

Day 6; Saturday	Millet and sorghum Porridge with sour milk with grilled plantain + (cabbage, carrots, zucchini)	Mashed potatoes with beef stew + vegetable mix: carrots, zucchini, peas, green beans + orange	Brown macaroni with vegetable sauce: peas + carrots + fruit: papaya