METABOLIC (GLYCEMIC, INSULINEMIC & SATIETY) CHARACTERISTICS OF A NOVEL COMMON BEAN (*Phaseolus vulgaris L.*) PRODUCT

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List of acronyms and abbreviations

AMREF-ESRC	African Medical Research Foundation-Ethics & Scientific Review Committee
BIA	Bioelectrical impedance analysis
EAR	Estimated average requirement
EP	
FII	Food insulin index
GI	Glycemic index
GL	Glycemic load
iAUC	Incremental area under the curve
IUC	Inter-university cooperation
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KEMRI	Kenya Medical and Research Institute
KU Leuven	University of Leuven
LCEFoNS	Legumes Centre of Excellence for Food and Nutritional Security
MoH	Ministry of Health
NACOSTI	National Commission for Science, Technology and Innovation
CoV	Co-efficient of variance
SEM	Standard error of means
SOP	Standard operating procedure
T2DM	Type-2 diabetes mellitus
VAS	Visual analog sales
VUB	University of Brussels

1. INTRODUCTION/BACKGROUND

Kenya is classified as having three main forms of malnutrition: overweight, anaemia and stunting [1], exhibiting a double burden of malnutrition nutritional landscape [2]. Indicators of overweight and obesity, and diabetes in both adult men and women either show no progress or are worsening when looking at trends from the 80's to present [3]. Iron and zinc, whose deficiencies (acute or chronic) manifest as iron deficiency anaemia and stunting are micronutrients of public health concern in Kenya [4]. Type-2 diabetes is one of the priority non-communicable diseases (NCDs) [5] in Kenya with a prevalence of 5.8% and 6.2% in men and women [6].

Type 2 diabetes (T2DM) is the most common form of diabetes. It is a chronic metabolic disease occurring when the pancreas is unable to produce sufficient amounts of insulin or when the body cannot effectively utilize produced insulin, or both. Insulin regulates the amount of glucose in blood and T2DM is characterized by hyperglycemia [7]. Diabetes due to its nature has an inflammatory disease character, with higher inflammatory processes as a result of diseases duration and glycemic control [8]. Iron homeostatsis is affected by hepcidin levels. Hepcidin levels are abnormally high during inflammation, resulting in a decrease in iron uptake in the gut [9]. Investigators have observed positive associations between serum ferritin and blood glucose levels, body mass index (BMI) and lipid profiles [11, 12]. This suggests that the incidence of anemia is likely to increase in poorly controlled diabetes. Therefore, reducing blood glucose levels could help reduce the risk of anemia in diabetic populations. Zinc is found in highest concentrations in the pancreatic β-cell in the human body [12]. Dysregulation of zinc is known to occur in both type 1 and type 2 diabetes. However there is no conclusive evidence as to whether zinc deficiency causes the diabetes or is merely a consequence of the disease [13]. Suggested potential beneficial mechanisms of action of zinc among diabetes patients are its antioxidant role. Studies have reported the association of high zinc levels with reduced oxidative stress (lowering lipid peroxidation [14], chronic hyperglycemia, insulin resistance and β-cell loss [15]).

We conducted a baseline study during quarter 4, 2018 in Makueni County to study the double burden of malnutrition at the individual level in T2DM (N=168). Preliminary reports from the study show anaemia as present in 34.4% and 24.8% in men and women respectively. 142 participants had a random blood sugar of >8.5mmol/L. 72.2% of the participants were also

hypertensive (reported current use of hypertension medication). Diets consumed were characterized by low sources of readily bioavailable iron and zinc. Diet quality is at the core of both diabetes management and the double burden of malnutrition. From the daily consumption data, we found the intake of both iron and zinc to be lower than their respective estimated average requirement (EAR). Further considering the characteristic low bioavailability diet, improvements in the quality of diets among this population is thus crucial.

This study aims to study the associated glucose, insulin and appetite responses associated with the consumption of a novel common bean product. This product profiling is key to inform dietary management of T2DM patients using this specific product.

2. PROBLEM STATEMENT AND JUSTIFICATION

Problem Statement

Though prevalence of T2DM in Makueni County is unknown, a high number of diabetes-related outpatient morbidity cases has been reported [16]. The double burden of malnutrition at the individual level has hardly been little studied and is less well understood than the prevalence of the double burden of malnutrition observed at country or household level.

Zinc is found in very high concentrations in pancreatic beta-cells [17]. Low zinc levels have been reported elsewhere among diabetes patients as compared to non-diabetic patients [18]. Associations between dysregulation in zinc homeostasis and pancreatic beta-cell function has been suggested [19]. Evidence is however still inconclusive as to whether zinc deficiency causes the disease or is a consequence of the disease [15]. Studies suggest that inflammatory state associated with obesity lead to alterations in iron homeostasis. However, work on iron metabolism during inflammation has been focused on studies on obesity combined with anemia in women, or in persons undergoing bariatric surgery [20, 21]. The metabolic basis and complications associated with T2DM result in dysregulation in zinc, iron and copper homeostasis. Dietary management of T2DM has concentrated on macronutrient consumption[22, 23].

Problem justification

Micronutrients are cofactors in β-cell function, glucose metabolic pathways, and in insulin signaling. Baseline data shows us inadequate mean daily zinc and iron intakes in a characteristic low bioavailable diet. Additionally, presence of anaemia was >20% in T2DM patients in Makueni,

Kenya (N=168). The successes and challenges of diet and food-based approaches have been addressed before [24]. Despite the knowledge that several nutrients are implicated in glycaemic control: 1) limited studies and evidence on micronutrient status among T2DM patients in Kenya is available, and, 2) limited evidence on targeted improved micronutrient consumption has been conducted in Kenya. To tackle both, micronutrient deficiency and metabolic health a new common bean product has been developed. We hypothesize that the newly developed product will have improved micronutrient bioaccessibility and that it will evoke desirable satiation and metabolic responses. Additionally, common beans contain soluble and insoluble fibre, phytonutrients and slowly digestible starch which are beneficial to glycaemic and lipaemic control.

3. REVIEW OF LITERATURE

3.1 Double burden of malnutrition at the individual level

T2DM in Kenya is on a rise with about 458,900 people diagnosed in 2017 and a 2.5 fold projected increase [25]. It is one of the NCDs accounting a big proportion of NCD-related mortality[26]. Risk factors of T2DM in Kenya are: age, physical inactivity, adoption of unhealthy lifestyles [5, 20]. Chronic hyperglycemia associated with T2DM results either in production of reactive oxygen radicals and/or compromises the antioxidant defense system activity [28]. In T2DM patients, zinc improves oxidative stress by a plethora of mechanisms: reducing chronic hyperglycemia, acting as a cofactor of the superoxide dismutase enzyme [29], regulating glutathione metabolism and metallothionein expression [30], and promoting phosphorylation of insulin receptors[31]. Copper is a necessary pro-oxidant for the catalytic activity of superoxide dismutase [32]. Zinc and copper are important for balanced redox mechanisms. Imbalances in redox mechanisms may contribute to diabetic complications. The importance of antioxidant nutrients in the management of this disease should be further explored.

Dysregulation of iron homeostasis is closely linked to the body's inflammatory response and T2DM. As yet, the link between dietary iron and metabolic dysfunction is incompletely understood [25, 26]. Hepcidin controls dietary iron absorption and distribution in metabolic tissues. Hepcidin binds ferroportin in iron storage tissues inhibiting export of iron. Several studies have found high ferritin levels in T2DM patients. In enterocytes, inhibition of ferroportin reduces the absorption of dietary iron into the blood by trapping it within the enterocyte [35]. With both iron deficiency and

overload associated with metabolic disorder, it is important to maintain iron levels within a narrow physiological range.

3.2 Nutritional profile of common beans

Several studies report that common bean is a food with a high content of proteins, carbohydrates, diet fiber, minerals and vitamins [36]. Depending on the common bean variant, protein ranges between 14% and 33%. They contain amino acids such as lysine, phenylalanine and tyrosine. They lack in methionine and cysteine (sulphur amino acids). Beans contain slowly digestible carbohydrates and a high proportion of non-digestible carbohydrates. Fiber (soluble and non-soluble) in beans would be beneficial to T2DM patients by reducing macronutrient absorption, satiety increase, increased sensibility to insulin, effects on inflammatory markers and on intestinal microbiota [37]. Common beans are important sources of iron and zinc. Moreover, they contain several bioactive compounds (anthocyanins, condensed tannins [38] and flavonoids [39]) possessing anti-inflammatory, antioxidant and other biological activity [40]. Due to their composition and effects, common beans have attractive properties for T2DM patients.

3.3 Estimated Average Requirements (EARs) for Zinc, Iron and Copper

The EARs for zinc, iron, and copper differ depend on age, body weight, sex, for pregnant and/or lactating women and on the bioavailability of the nutrient in a diet. Based on these factors approximate EAR values for adults' ≥18 years and ≥70 years are shown in **Table 1** below:

Table 1: Zinc, Iron and Copper EAR Summary, Ages 18 years and older

Micronutrient	Male	Female
Zinc(mg/day)	8.5 – 9.4	6.8 – 7.3
Iron (mg/day)	6 – 7.7	5 – 8.1
Copper (μg/day)	685 - 700	685 - 700

Source: adopted from Institute of Medicine (US) Panel on Micronutrients (2001)

It is important to remember that dietary reference intake values are set for healthy individuals, and nutrient requirements are likely to be differ in disease.

3.4 Satiety and satiation

Satiety is defined as the extent to which feelings of hunger and desire to eating are reduced between meals (prompts termination of eating). Satiation refers to the amount of food consumed within a meal (fullness that persists after eating) [41]. Both are key factors in appetite control and energy intake. Satiety determines the length of time until next eating occasion and satiation the amount consumed at one sitting. Few studies have been conducted on satiety in the context of legumes and weight management. Studies suggest short term improvements in satiety [37,38] and favorable influence on appetite sensations and energy intake in legume based meals when compared to animal based meals[44]. Satiety can be subjectively measured using visual analog scales (VAS) while satiation can be assessed by *ad libitum* consumption of test foods.

3.5 Insulin and glucose responses to food consumption

Following food consumption, absorbed carbohydrates give a rise in blood glucose concentrations. In response insulin is released causing blood glucose to fall. Different food components evoke different responses e.g. simple sugars give both rapid increases and falls. Glucose response refers to the degree of rise and fall in blood glucose levels and the time interval within which it occurs. Slowly digestible carbohydrates show a slower and more prolonged increase in blood glucose levels (with a lower peak) and vice versa. The glycemic index (GI) shows the average glucose concentration in the blood after consumption of a certain food (usually for 2 hours) relative to a standard food. The test foods usually contain 50 grams (or 25 grams if portion size is very large) of available carbohydrate and the reference food (anhydrous glucose powder, 50g or dextrose, 55g) has a GI of 100 [45] . Available carbohydrate of a food is estimated by difference: *100 - water (g/100 g EP) - total fat (g/100g EP) - total protein (g/100 g EP) - total dietary fibre (g/100 g EP) - ash (g/100 g EP)*. The GI is obtained from the area under the 2hour curve (iAUC)[1] [46] as: $\frac{\text{iAUC of the test food}}{\text{individual subjects' average iAUC of the reference food}} * 100$. Overall GI is calculated as mean (±SEM) GI value for ≥10 participants. From the GI, the glycaemic load (GL) can be derived. It is calculated

[1] The incremental area under the curve has been calculated as the incremental area under the blood glucose response curve (iAUC), ignoring the area beneath the fasting concentration. This can be calculated geometrically by applying the trapezoid rule. When a blood glucose value falls below the baseline, only the area above the fasting level is included.

as: $\frac{\text{GI}}{\text{Total available carbohydrate in the food portion}}$/100. GL gives information on glycaemic quantity as a factor of food portion size, and may be used as an indicator of insulin demand.

Insulin release can be triggered by other food components such as proteins and fats. The food insulin index (FII) allows for testing of foods with no or low carbohydrate content. It shows the observed (plasma) insulinemic response to consumption of a particular food compared to an iso-caloric portion of the reference food over a 2 hour period. It is obtained as: $\frac{\text{iAUC for 1000kJ test food}}{\text{iAUC for 1000kJ reference food}}$ * 100. FII has previously been reported to have potential of reducing postprandial hyperinsulinaemia in T2DM management [47].

4. RESEARCH OBJECTIVES

4.1 General objective

1. To assess the appetite, insulin and blood glucose responses associated with consumption of an acceptable novel common bean product

5. CONCEPTUAL FRAMEWORK AND OPERATIONALIZATION

5.1 Conceptual framework

Common beans in type 2 diabetes patients could have many health benefits such as modulation of glucose metabolism, reduction of low density lipoproteins, and satiety [48,49] . The conceptual framework (Fig. 1) we have adopted proposes that short term metabolic responses are a factor of baseline characteristics of an individual, common bean product portion and feeding frequency [50].

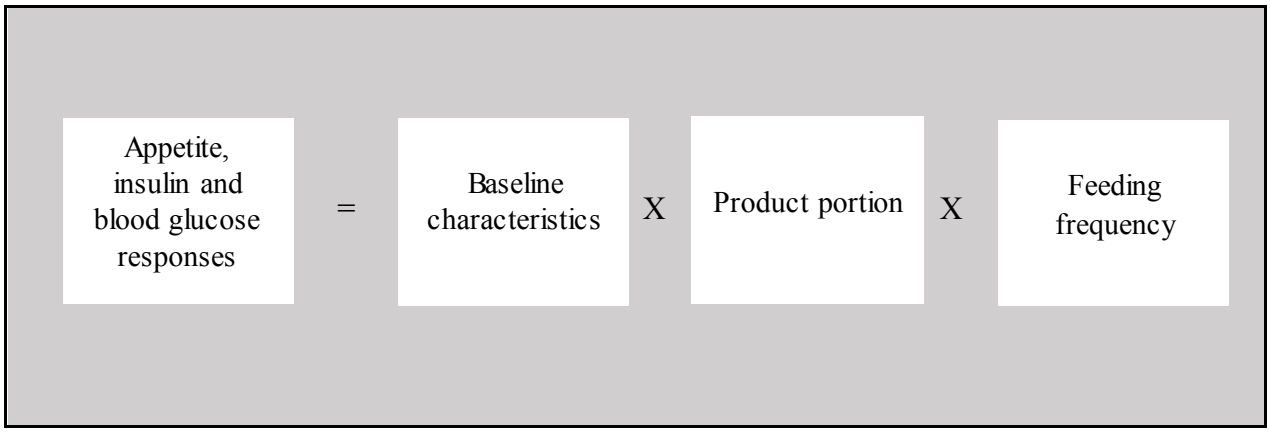


Figure 1: Conceptual framework on how common beans can increase nutrient intake, and potentially improve metabolic responses in T2DM patients

For T2DM patients, portion control is one of the pillars in dietary management. Based on the main ingredient (common bean), form (solid), nutrient composition (available carbohydrate, fibre and protein) and oral processing needed (high sensory exposure/chewing time), we aim to develop a product that is highly satiating. The portion will also be influenced by the product packaging size of approximately 150-200 grams.

The frequency and timing of the common bean product is also key. From the baseline study, majority of the T2DM were between 40-69 years. Age is associated with loss of appetite. In such a population group, small portions and frequent meals are recommended. We aim to provide a product that allows for optimization of the intake of vitamins and minerals in adults [48, 49].

5.2 Operational definition of variables

For the assessment of metabolic responses associated with consumption of the common bean product, Figure 2 below illustrates the relationship between the dependent (blood sugar, insulin) and independent variable (common bean product consumption):

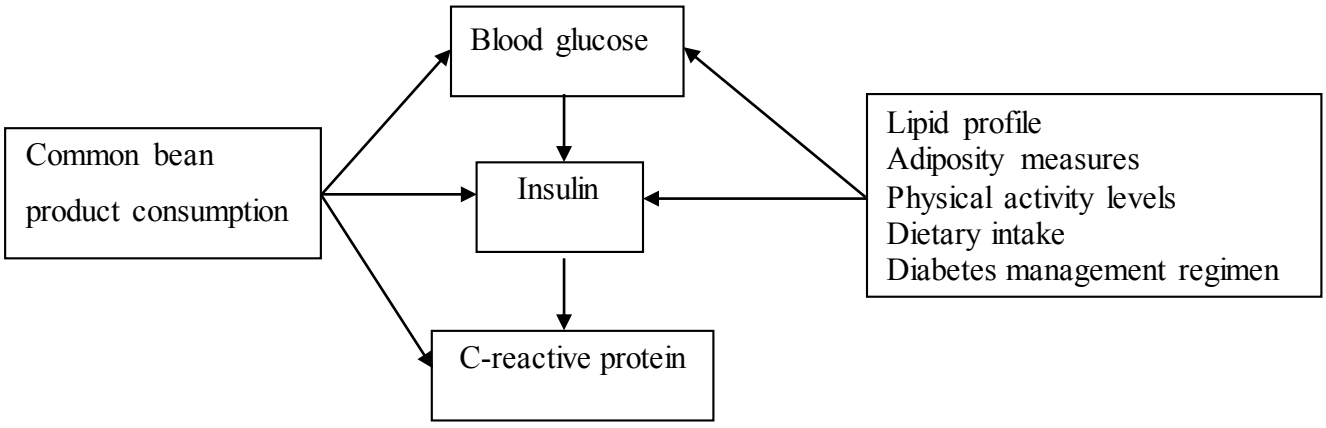


Figure 2: Factors influencing glycemic and insulinemic responses

Recent evidence suggests that CRP levels are higher in people with diabetes compared with those without diabetes. However, less is known about whether CRP in people with diabetes is related to level of glycemic control. Poor glycemic control is significantly associated with the development of macrovascular complications of diabetes. We will be using the CRP data to evaluate association with glycemic control in this particular population. The lipid profile, dietary intake and bio-electrical impedance data are some of the variables that will be used to explain observed blood glucose and insulin measurements.

6. HYPOTHESES AND RESEARCH QUESTIONS

6.1 Hypotheses

H1: the new common bean product is not associated with desirable satiety, glycemic and insulinemic responses in the T2DM patients.

6.2 Research questions

1. What are the post prandial insulin, blood glucose and appetite responses associated with consumption of the common bean product in:
    a. healthy volunteers?
    b. T2DM patients?

7. STUDY DESIGN AND SAMPLING STRATEGY

7.1 Glycemic index (GI) and glycemic response study

The GI evaluates the blood-raising ability of equi-carbohydrate portions of different single foods or mixed meals. To assess the GI of the common bean product *in vivo*, 10 healthy volunteers will be recruited. The selection of the number of participants, and the inclusion and exlusion criteria is based on the ISO_26642_2010 standard [53].

The 10 healthy volunteers will be recruited from Jomo Kenyatta University of Agriculture and Technology (JKUAT) and handled at the JKUAT University Hospital.

Inclusion criteria:

- Male or female 18-69 years
- No known food allergies or intolerances
- No prescription medication[2] (excluding oral contraceptives) known to affect glucose intolerance (e.g. steroids, protease inhibitors, antipsychotics e.t.c.)

Exclusion criteria

- Known history of diabetes mellitus or use of anti-hyperglycaemic medication or insulin
- Alcohol or tobacco use in the evening preceding the test
- Vigorous exercise on the morning of the test
- Undergone major medical/surgical event needing hospitalization within the preceding 3 months
- Presence of disease or drug influencing digestion and absorption of medicine

Glycemic response associated with consumption of the common bean product will be assessed in 10 T2DM patients in Makueni County. The participants will be recruited from the Makueni Level-5 and Mbooni sub-county hospitals which run a diabetes outpatient clinic.

Inclusion criteria:

- Male or female aged 18-69 years
- Previously diagnosed with type 2 diabetes
- Consenting to participate

Exclusion criteria:

- Use of prescription medication
- History of eating disorders and irregular eating habits
- Use of tobacco or alcohol
- Females experiencing menstrual period or adverse premenstrual symptoms

For the reference food, we will use either anhydrous glucose powder, 50g or dextrose (glucose monohydrate), 55g. The reference food will be dissolved in 250ml of water and refrigerated for use within 72 hours after preparation. Participants will be fed with an equi-carbohydrate portion of the common bean product to the reference food.

[2] Stable doses of oral contraceptives, acetylsalicylic acid, thyroxin, vitamins and mineral supplements or drugs to treat hypertension or osteoporosis are acceptable

The GI of the food will be measured over a 120 min period starting at the ingestion of the common bean product. The test food and reference food will be assessed in each subject on separate days with a 1 week period between each study day.

The GI value of the common bean product will be calculated as follows: = [iAUC of the common bean product/individual subject's average iAUC of reference food]*100. The overall GI will then be obtained as: The mean GI value for the 10 participants.
The test conditions, sample size and methodology are as adopted from the international standard ISO 26642:20110(E)[3] [53].

7.2 Food insulin index (FII) and insulin response

To assess the FII of the common bean product *in vivo*, 10 healthy volunteers will be recruited from Jomo Kenyatta University of Agriculture and Technology. Insulin response associated with consumption of the common bean product will be assessed in 10 T2DM patients in Makueni County. Participants will be fed with iso-caloric (240 kilocalorie) portions of the test food and reference food.

Inclusion and exclusion criteria for the FII and insulin response used will be as cited in GI study (see section 7.1).

The FII of the food will be measured over a 120 min period starting at the ingestion of the common bean product. The test food and reference food will be assessed in each subject on separate days with a 1 week period between each study day.

The FII value of the common bean product will be calculated as follows: = [iAUC of the common bean product/individual subject's average iAUC of reference food]*100. The overall FII will then be obtained as: The mean FII value for the 10 participants.

[3] The standard was issued under licence to Christophe Matthys (co-principal investigator)

Insulin response associated with consumption of the common bean product will be assessed in 10 T2DM patients in Makueni County.

7.4 GI and FII study procedures

Researchers will obtain signed consent from the participants before testing. For the GI and FII study, the common bean product will be served with a standard 250 ml portion of water. Participants will first receive the reference food and then the test food on separate mornings after a 10-12h overnight fast. The conditions and testing times during the test days will be as similar as possible. Participants will be asked beforehand to maintain their usual diet; avoid alcohol; and not engage in unusual or excessive physical activity on the day preceding the test. For the night preceding the test day, participants will be asked to eat a standard evening meal that allows for the 10h fasting period prior to the study and refrain from eating and/or drinking (except for water).

On the test day, arrival time to the hospital will be 0700h. The researchers will assist participants in filling out a 24 hour dietary recall. Anthropometric measurements (weight, height, waist and hip circumferences); blood pressure; and body composition using bioelectrical impedance analysis (BIA) will be assessed. The subsequent tests will be conducted while the participants are in a rested position. A catheter will be introduced in the cubital vein to obtain venous blood by a phlebotomist. Two blood samples will be obtained within a five minutes interval in the fasting state, and their average taken as the baseline blood glucose concentration (time -5 and time 0). Participants will then be given the test food and 250ml water all presented in standard bowls and/or glasses. Participants will be asked to eat the whole portion in 12 to 15 minutes, at an even pace. Time taken to eat the entire portion will then be recorded. Participants will remain seated and will be allowed to read or use their phones. They will however not be allowed to sleep or eat or drink until the end of the 2h of study period.

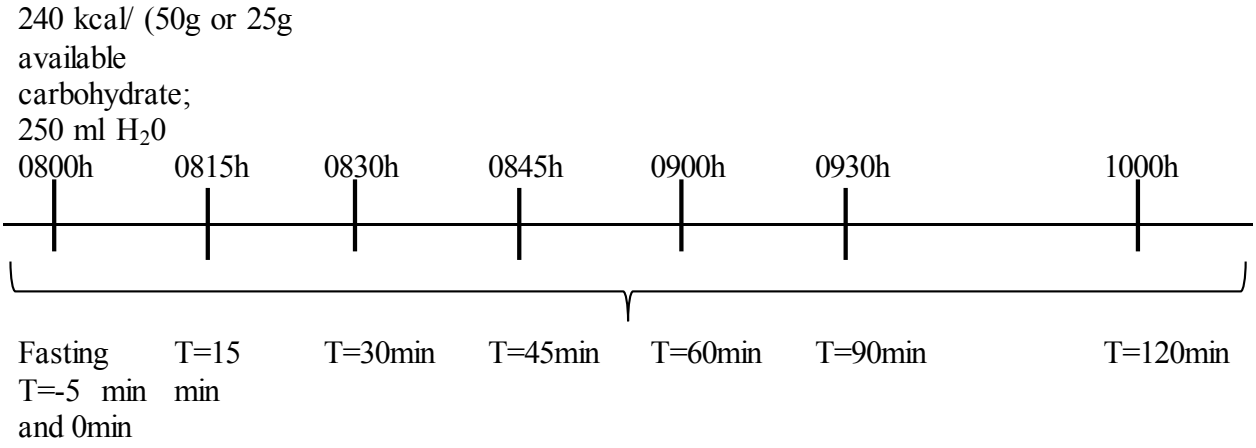
Postprandial blood samples will be collected after the feeding at six time-points, 15, 30, 45, 60, 90 and 120 min. For each time point, ~2 ml of blood will be obtained. Whole-blood samples will be labelled using codes allocated to each participant for proper identification. Blood glucose content will be measured in either whole blood or plasma samples. Centrifugation will be done within 30 minutes of blood collection using portable centrifuges carried to the field. Blood plasma or serum

will then be pipetted into aliquots in cryovials, labelled, wrapped with aluminium foil and stored in liquid nitrogen awaiting transportation to LANCET laboratories for analysis. The blood samples will be analysed for blood glucose, insulin, lipid profile and C-reactive protein.

7.5 Appetite scores using Visual Analog Scales (VAS)

Appetite scores will be used to assess the satiety power of the test (common bean) product among T2DM participants. The appetite score will be taken on one study day, that is, after consumption of the bean product. Subjective measures of appetite will be taken prior to, and at predetermined time intervals after consumption of the test meal. Appetite score refers to the average between hunger, desire to eat, prospective consumption and a 100-fullness score. The appetite score will be assessed at before and after consumption of test product at time points 0, 15, 30, 45, 60, 90 and 120 minutes. Participants will be given a short questionnaire to assess satiety.

A single vertical mark best expressing the participants' response will be made on an anchored 100 mm VAS scale. The anchor on the left and right will express the most positive and the most negative rating respectively (see **Appendix 7**). The response will be quantified by measuring the distance between the left anchor and the marking made. The questionnaires will be made as a small brochure booklet showing one question at a time [54][55]. Participants will be asked to remain silent while filling out their VAS. Between evaluations, they will be allowed to read or use their phones. The participants will not be allowed to discuss with each other or refer to previous ratings when filling out the VAS.



Blood draw + Appetite scores

Figure 3: Summary of protocol for the assessment of postprandial glycemic, insulinemic and satiety responses

8. DATA COLLECTION

8.1 Data collection processes

Glycemic index and glycemic response (*Appendix 2*)

a. Equi-carbohydrate (50g or 25g) portions of the common bean product
b. 24-hour of dietary recall
c. Anthropometry: weight, height, waist & hip circumference
d. Body composition: BIA
e. Biochemical samples: Blood

Food insulin index (FII) and insulin response (*Appendix 2*)

a. Iso-caloric (240kJ) portions of the common bean product
b. 24-hour of dietary recall
c. Anthropometry: weight, height, waist & hip circumference
d. Body composition: BIA
e. Biochemical samples: Blood

Appetite scores (*Appendix 3*)

a. Visual analog scales
b. Timers

BIA will be assessed with the Bodystat 500 (Bodystat, UK). Raw data on impedance, resistance, reactance and phase angle will be added to already validated equations to calculate fat free mass, body fat and hydration [56].

For blood draw, handling and processing; phlebotomists from the Kenya Medical and Research Institute (KEMRI) will be engaged. A catheter will be place in the cubital vein. Blood samples will be collected at seven time-points; 0, 15, 30, 45, 60, 90 and 120 minutes. For each bleeding moment ~2 ml of blood will be drawn from the participant. Plain tubes will be used for blood collection. After collection, whole-blood samples will be labelled with codes with participant

codes and separated based on time-collection point. A drop of blood will then be obtained for determination of haemoglobin concentration using the Hemocue 301 portable device. The tubes will then be placed immediately into insulated coolers containing ice/cold packs at around 4^{o}C awaiting centrifuging. Centrifuging will be within 30 minutes of sample collection. After centrifuging, serum will be separated and divided in aliquots. Cryovials containing the aliquots will be labelled (with participant code and test to be run), wrapped with aluminum foil (to reduce chances of light degradation) and secured together with rubber bands. They will then be put in a portable freezer. At the end of sample collection and separation, the aliquots will be dropped into a liquid nitrogen tank awaiting transportation to analytical laboratories (LANCET), for further analysis. Necessary precautions will be taken to ensure a dust and light free environment during the blood sample collection and handling.

Single use for alcohol swabs, clean disposable latex-free gloves, disposable needles, catheters and plain tubes will be observed. Biological waste and sharps will be disposed appropriately in biological hazard bags and biohazard box respectively and later on disposed of together with the hospital waste at the participating hospital facilities in Makueni County and JKUAT hospitals.

8.2 Recruitment and Training of Research Assistants

Enumerators will be recruited to assist in screening, recruitment and data collection. The research team will engage persons with previous field study experience and knowledge of the local language. The nurse/nutrition officer in charge of nutrition support in the clinic will be requested to assist the researchers in recording blood glucose levels of the T2DM patients. A two day training will be designed by the research team to introduce the study, study objectives and applicable methods to the enumerators. The training will encompass taking of anthropometric measurements, interviewing techniques and data collection procedures, code of ethics, data management and proper interpretation of questions to avoid the error of individual subjectivity during the interview.

Practical demonstrations, discussions and role play will be used to ensure standardization of interviewing skills, taking of body measurements, and will also help in familiarization with the research questions. The enumerators' competence will be assessed by the research team a day after completion of the training session.

8.3 Recruitment and Training of Phlebotomists

Phlebotomists from KEMRI will be engaged. Through a meeting with the research team, the study, study objectives, blood collection procedures, biomarkers for analysis, standard operating procedures (SOPs), biomarker quantification methods, equipment needs and any other practicalities will be reviewed. This will amount to the development of a laboratory analytical plan for this study.

8.4 Pre-testing of the tools

Questionnaires to be used for the studies will be pre-tested before the commencement of the studies. Participant with similar characteristics will be used for the pre-testing.

8.5 Reliability

Other than pre-testing of the questionnaires, training of research assistants on the questionnaire administration and anthropometric measurements will be done. Calibration of anthropometric equipment will be done routinely to enhance reliability. An average of two readings will be used to record anthropometric measurements. Appetite scores will be assessed using Cronbach's alpha. Approved and standardized SOPs will be followed for different procedures in the different studies.

8.6 Ethical Considerations

Ethical clearance will be sought from the African Medical Research Foundation-Ethics & Scientific Review Committee (AMREF-ESRC). A Research permit will be obtained from National Council for Science, Technology and Innovation. Local authority/opinion leaders and Ministry of Health & hospital management will be briefed by the researcher on the intended activities prior to the start of the study. Approval for all data collection procedures and tools will be sought from the aforementioned committee.

Information on the study will be explained to the local administration, hospital administration and study participants in open sessions during outpatient clinic days. The study will be introduced to the hospital management with an introductory letter and approvals obtained. A pre-visit will be made to the hospitals. The T2DM patients will be issued information sheets containing all information pertaining to the study. Consenting individuals will be recruited only after signing of the informed consent.

During recruitment, unique codes will be used for identification and all data obtained from study subjects will be handled with utmost confidentiality. Study documents containing personal information will be stored in a separate repository. In this case, this information will be removed from study documents where possible. Data collected will strictly be used for study purposes only. The process will consider interests of the study participants and will aim to protect their privacy, dignity and integrity. Access to files will be limited to the investigators.

The data collection procedures will have no serious risks to participants. Placement of the catheter will cause only a little discomfort at the site of the needle prick. Necessary care will be taken to stop the discomfort in a short while within the same day. Their participation in this study will enable us assess responses to the new product from the intended (real) or potential consumers. This will be vital to enable product optimization if need be, and in deciding on the use of this product for a food intervention.

For any unforeseen events due to participation in the study, T2DM participants will be immediately referred from the diabetes outpatient clinic to the casualty department of the same health facility for further examinations, treatment and follow up. The participants from JKUAT will be referred to the University hospital.

A lunch meal will be provided to the study participants after the end of the activity. This is considering the participants come for the research early in the morning in a fasted state.

8.7 Quality Control

The research team will be trained and assessed based on standardized methods and tools as in this protocol. Biochemical analysis will be done at the KEMRI laboratories which participate in a series of inter-laboratory calibration exercises to validate analytical results. All equipment will be well maintained and internally calibrated during the entire study period.

9. DATA PROCESSING AND ANALYSIS

9.1 Laboratory Analyses

Biochemical samples will be assessed for blood glucose, lipid profiles, insulin and C-peptide in serum, all in the fasted state.

9.2 Glycemic and insulinemic tests

Allowable mean within-subject co-efficient of variance (CV) for the reference food is ≤30%. The laboratory's inter-assay analytical variation on standard solutions should be <3.6%. The laboratory's minute-to-minute analytical variation in human subjects for 20 or more duplicate measurements of fasting glucose should generally be <5 %. Glycemic and insulinemic responses will be obtained from the iAUC (incremental area under the curve). iAUC is calculated by applying the trapezoid rule (sums of the areas of the triangles and trapezoids) [53]. iAUC is reported in millimole minutes per litre. Below is an example to how the iAUC and GI will be arrived at:

Table 2: Sample blood glucose responses to the ingestion of a 50g carbohydrate portion

Food sample	**Time of sampling, min**							**iAUC**
	0	**15**	**40**	**45**	**60**	**90**	**120**	
Reference food replicate 1	4.3	6.3	7.9	5.3	4.1	4.6	4.9	114
Reference food replicate 2	4.0	6.0	6.7	5.5	5.3	5.0	4.2	155
Test food	4.0	5.0	5.8	5.4	4.8	4.2	4.4	93

The iAUC for the Reference food replicate 1 = sums of the areas of the triangles and trapezoids A to F obtained after plotting the blood glucose responses at the different time intervals on a graph (Fig. 4).

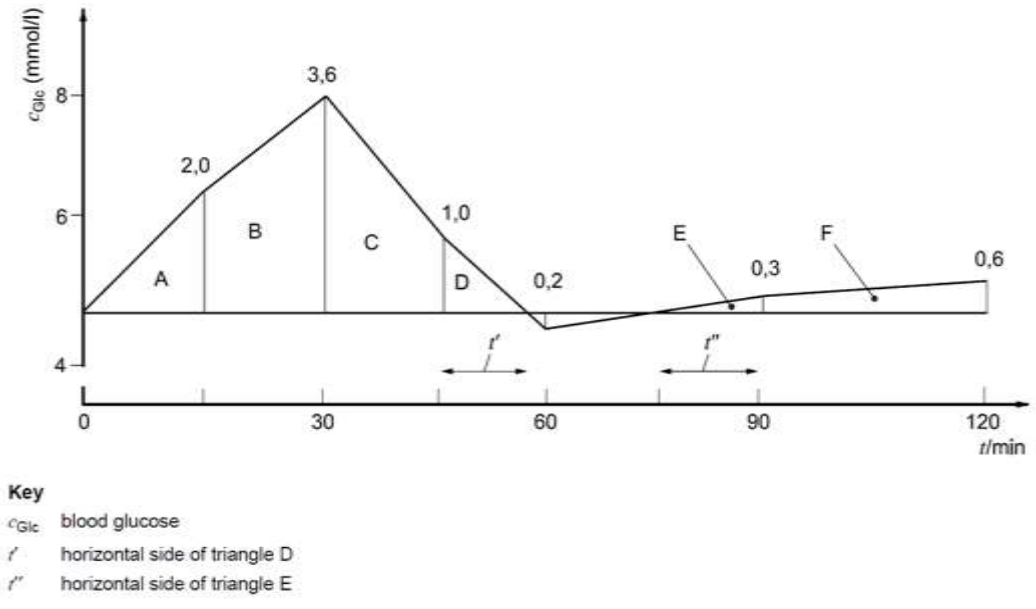


Figure 4: Graph of blood glucose responses to the ingestion of a 50g carbohydrate portion over a 2h period

Calculation for GI

- Mean iAUC for the reference food= (iAUC reference food replicate 1+ iAUC reference food replicate 2)/ 2= 135±29 mmol min/l
- The CoV is thus 29/135*100 = 22% (Test condition: CV for the reference food for the group should be ≤30%).
- iAUC for test food is 93 mmol min/l. For this subject, GI of the test food is thus 93/135*100 = 69%.

9.3 Appetite scores

Averages of markings on the VAS for all the participants will be calculated. Descriptive data will be expressed as means and/or standard errors or deviations. Cronbach's alpha will be used to assess validity of the appetite scores. ANOVA, Tukey's significant difference test and Pearsons correlation will be used to analyze the effects of product consumption and composition on appetite scores and to assess the repeatability.

Data will be coded, entered and analysed using either/or SPSS, R, Excel or STATA software.

10. STUDY FINDINGS COMMUNICATION PLAN

Varied approaches (based on intended audience) will be used to disseminate salient study findings. Findings will be published in peer-reviewed journals and also presented in conferences, research seminars and workshops through oral or poster presentations. Reports will also be shared with Ministries of Health, participating hospitals, LCEFoNS programme platform (hosted by JKUAT website). Letters of thanks to participating hospitals with study updates will be done upon completion of data analysis [57].

Key stakeholders to be included in dissemination of the study include Ministry of Health (MOH), Kenya; Kenya Medical Research Institute (KEMRI) and relevant Non-Governmental Organisations, (participating) T2DM patients, T2DM patient support groups where they exist (at community level this are psychosocial support groups to raise the level of self-care and adherence to treatment), nutritionists/personnel in charge clinics where participants will be recruited from and community leaders.

11. STUDY LIMITATIONS AND RISKS

Limitations include assessment of one test product. Additionally, when determining the GI and FII, a limitation is in the inter-individual, day-to-day, and even within-day variance in glycemic and insulinemic response to a particular food. For this, we will adhere to recommendations from the ISO 26642:2010 (e.g. testing the reference food twice to reduce day-to-day variability within individuals, follow recommendations as in protocol section 9.2 among other guidelines).

12. MANAGEMENT AND ORGANIZATION OF THE STUDY

12.1 Team, members and roles

This study is being implemented through The Legumes in Human Nutrition and Health Project, which is one of the four projects of the Legumes Centre of Excellence for Food and Nutritional Security (LCEFoNS). LCEFoNS is an Inter-University Cooperation (IUC) programme between Jomo Kenyatta University of Agriculture and Technology (JKUAT), Kenya; University of Leuven (KU Leuven), Belgium and University of Brussels (VUB), Belgium. The IUC is funded by VLIR-UOS, Belgium. Dr. Florence Kyallo is the project leader in Kenya (South) while Prof. Christophe Matthys is the project leader in Belgium (North). Prof. Tara Grauwet, Dr. Beatrice Kiage and Prof. Bart Van der Schueren are co-investigators in the study. Implementation of the study will be managed by Dr. Florence Kyallo, while Prof. Christophe Matthys will oversee the technical aspects of the study. All investigators are involved in the design and development, and implementation of study protocols. Ms.Linet Mutwiri, the PhD student in the study, will be responsible for data collection, analysis and reporting under guidance of the other 5 investigators.

12.2 Work plan

Year 2020 - 2021	July-Sept 20	Oct-Nov 20	Dec 20-Jan 21	Feb-Mar 21	Apr-July 21
Work Package 1: Protocol preparation					
Proposal writing					
Submission of proposal for ethical clearance					
Expected response from ethical body					
Submission to NACOSTI and expected response					
Work Package 2: Metabolic studies					
Review of data collection tools					
Introductory visit to community/local (opinion) leaders/MoH Makueni/JKUAT/JKUAT hospital to inform on research intent					
Recruitment and training of research assistants					
Gathering all data collecting tools					
Pre-test, review of data collection tools, and pre-test report					
Issuance of consent forms and data collection in the various study sites					
Data collection					
Analysis at LANCET and receipt of results					
Work Package 3: Data analysis					
Data compilation and analysis					
Report submission and dissemination (stakeholders)					

12.3 Budget

Item	Unit(s) required	Unit Price(KSh)	Amount (KSh)
Ethics and Scientific Review			
Ethical clearance fee (AMREF)	-	-	5,000
Research approval by NACOSTI	-	-	2,000
Sub-total	**-**	**-**	**7,000**
Protocol costs			
ISO 26642 standard	1	9,000	9,000
Sub-total	-	-	**9,000**
Training Costs			
Training of 2 enumerators (2 days)	2	2,200	4,400
Meeting with phlebotomy team	2	6,300	12,600
Transport	6	600	3,600
Sub-total	-	-	**20,600**
Data collection costs			
Phlebotomy (4 days & 2 phleobotomists)	8	7,640	61,120
Team leader (4 days)	2	7,640	15,280
PhD student (4 days)	4	7,640	30,560
Enumerators (4 days)	8	2,540	20,320
Driver (4 days)	4	5,135	20,540
Fuel		20,000	20,000
Participant lunch (3 days)	30	500	15,000
Community mobiliser (4 days)	4	2,240	8,960
Stationery (paper for VAS scores)	1	500	500
2 dozen pens	2	300	600
Water (crates)	4	800	3,200
Airtime (4 study days)	4	500	2,000
Subtotal			**198,080**
Equipment and Consumables Costs			
Blood pressure measurement			
OMRON blood pressure monitor (digital, M2)	1	8,500	8,500
Subtotal			**8,500**
Collection, handling, storage and transfer of blood samples			
Butterfly needles (preferably size 21G;22G;23G)	80	1500/100pc pkt	1,500
Flexible IV tube	80	200	16,000
Vacutainer tubes	2	1,200/100pcs	2,400
Vacutainer holder	10	250	2,500
Alcohol swabs	80	180/100 pieces	180
Disposable rubber gloves	1	250/100 pieces	250

Bio-hazard box	2	50/pc	100
Biological waste disposal bag (20l)	3	15	45
Hand sanitizer	1	500	500
Cryogenic vials (freezable)	2	1,200/100pcs	2,400
Disposable pipettes or pipettes with changeable apex	2	4,500	9,000
Blue tips	1	1,200/1000pcs	1,200
Centrifuge, 12 tubes with timer	1	20,000	20,000
Permanent marker pens	4	250	1,000
Cool boxes (Thermos, 28lit)	2	3,000	6,000
Ice packs	10	400/pc	4,000
Liquid nitrogen	50	480/kg	24,000
Subtotal			**91,075**
Anthropometric and body composition measurements			
Tefal Weight scale	4	4,000/pc	16,000
Seca portable/wall mounted stadiometer with movable head piece, or measuring rod	4	7,000/pc	28,000
Waist circumference tape	10	1000/pc	10,000
Bodystat 500	1	80,00032	80,000
Subtotal			**134,000**
Laboratory Blood Specimen Analysis Costs			
Blood glucose (30 fasting, 180 random)	210	649	136,290
Plasma insulin (fasting)	30	3,849	115,470
Plasma insulin (non-fasting)	180	4,149	746,820
C-peptide (fasting/non-fasting)	20	2,949	58,980
HbA1C	20	2,949	58,980
Lipid profile (Cholestrol, HDL,LDL,Trig+Ratio calculations)	20	2,949	58,980
Subtotal			**1,175,520**
TOTAL			**1,643,775**
Miscellaneous (flat rate)			**10,000**
GRAND TOTAL			**1,653,775**

Funding: VLIR-UoS

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13.2 Appendices

Appendix 1a: Information sheet and informed consent form for metabolic (glycemic, insulinemic and satiety) characteristics of a novel common bean product study

Study №:

Study title: Metabolic characteristics of a novel common bean product (*shortened version*)

Information sheet

Introduction:

We are researchers under the Legume Centre of Excellence for Food and Nutrition Security (LCEFoNS), a programme by Jomo Kenyatta University of Agriculture and Technology (JKUAT), University of Leuven (KU LEUVEN), and, University of Brussels (VUB). You are being invited to take part in a study investigating the glycemic and food insulin indices, and metabolic responses of a novel common bean product at Jomo Kenyatta University of Agriculture and Technology University Hospital.

Your participation is voluntary. You have the right to refuse to participate in this study. Before you decide whether to participate in the trial, you need to fully understand what the study is about and why we conduct the trial. This consent form explain why the research is being done, what we will ask you to do during the study, the possible risks and benefits of participating, and the amount of time the study will require. Please take time to understand the following information carefully before you decide. Once you understand the study, you will be asked to sign this form if you wish to participate. If you decide to participate, you may still choose to withdraw from the study at any time without giving any reason. Withdrawal from the study will not result in any negative consequences to the services to which you are entitled or are presently receiving.

Purpose of the study: This study aims to study the associated glucose, insulin and appetite responses associated with the consumption of a novel common bean product.

Procedure: If you agree to take part in this study, you are expected to attend all the 3 study visits, each lasting approximately 140 minutes. The reference food will be tested two (2) times and the test food (1) time in each participant on separate days within a 3 month period. During some of the study visits you will be asked to consume a reference food, which will be either a anhydrous glucose powder or dextrose (glucose monohydrate) beverage. On other study visits you will be asked to consume the test food, a food grade novel common bean product(s) from food grade ingredients though the application of conventional food processing technologies.

Each study visit will include the following procedures: • Avoid all food and beverages including alcohol at least 10 hours before the study visit, and avoid vigorous exercise on the morning of the visit • Provide two fasting blood samples • Consume a specified portion of the reference or test food during various timepoints of the study (water will also be available) • Provide a blood sample at 15, 30, 45, 60, 90, and 120 minutes after beginning to eat the food

Possible harms and discomforts: The blood collection procedure may cause some discomfort and slight bruising at the site of the lancet poke. After the blood draw you will immediately be given a ball of cotton dipped in surgical spirit to cover the spot where the blood was taken. There are no other known risks involved with participating in this research.

Potential benefits of participating: There are no direct benefits to participating in this study. We hope that the information on the common bean product will be used in the future to help consumers make informed food choices.

Withdrawal to participate: You can withdraw participation at any time without giving reasons. If you consent to participate but decide to withdraw at a later moment, you can request the withdrawal of your information collected during the study. However, in cases where the data and/or samples are no longer identifiable/ cannot be linked back to your identity/data has been merged the request to withdraw data and/or samples may not be possible.

Confidentiality: Your confidentiality will be highly respected. As a study participant, you will be issued a unique study code. Only this code will be used on any research related information collected about you. Information on your identity will remain only with the Principal Investigator and/or designate. The list that matches your name to the unique study number that is used on your research-related information will not be removed or released without your consent unless required by law.

Who to Contact: If you have any questions, you can ask them now or later. If you wish to ask questions later, you may contact **Linet Mutwiri**, Mobile: +**254714223507**.

Participant consent

By signing this form:

- I have read and understood the information in this information and consent form.
- I have had enough time to think about the information provided.
- I have been able to ask questions and have had satisfactory responses to my questions.
- I understand that the purpose of this study is to assess the associated glucose, insulin and appetite responses associated with the consumption of a novel common bean product
- I understand that all of the information collected will be kept confidential and that the results will only be used for scientific purposes.
- I understand that my participation in this study is voluntary. I understand that I am completely free at any time to refuse to participate or to withdraw from this study at any time, and that this will not change the quality of care that I receive.
- I will receive a signed copy of this consent form for my own records.
- I consent to participate in this study.

Participant signature	Participant name	Date
_____	_____	_____
Signature of person obtaining consent	Name and role in study	Date
_____	_____	_____

Appendix 1b: Information sheet and informed consent form for metabolic (glycemic, insulinemic and satiety) characteristics of a novel common bean product study
Study №:

Study title: Metabolic characteristics of a novel common bean product (*shortened version*)

Information sheet

Introduction:

We are researchers under the Legume Centre of Excellence for Food and Nutrition Security (LCEFoNS), a programme by Jomo Kenyatta University of Agriculture and Technology (JKUAT), University of Leuven (KU LEUVEN), and, University of Brussels (VUB). You are being invited to take part in a study investigating the glycemic and food insulin indices, and metabolic responses of a novel common bean product at _____ Hospital.

Your participation is voluntary. You have the right to refuse to participate in this study. Before you decide whether to participate in the trial, you need to fully understand what the study is about and why we conduct the trial. This consent form explain why the research is being done, what we will ask you to do during the study, the possible risks and benefits of participating, and the amount of time the study will require. Please take time to understand the following information carefully before you decide. Once you understand the study, you will be asked to sign this form if you wish to participate. If you decide to participate, you may still choose to withdraw from the study at any time without giving any reason. Withdrawal from the study will not result in any negative consequences to the services to which you are entitled or are presently receiving.

Purpose of the study: This study aims to study the associated glucose, insulin and appetite responses associated with the consumption of a novel common bean product.

Procedure: If you agree to take part in this study, you are expected to attend 1 study visit, lasting approximately 140 minutes. During study visit you will be asked to consume a test food, a food gradenovel common bean product(s) from food grade ingredients though the application of conventional food processing technologies.

Each study visit will include the following procedures: • Avoid all food and beverages including alcohol at least 10 hours before the study visit, and avoid vigorous exercise on the morning of the visit • Provide two fasting blood samples • Consume a specified portion of the reference or test food during various time points of the study (water will also be available) • Provide a blood sample and give an appetitie score for the product at 15, 30, 45, 60, 90, and 120 minutes after beginning to eat the food

Possible harms and discomforts: The blood collection procedure may cause some discomfort and slight bruising at the site of the lancet poke. After the blood draw you will immediately be given a ball of cotton dipped in surgical spirit to cover the spot where the blood was taken. There are no other known risks involved with participating in this research.

Potential benefits of participating: There are no direct benefits to participating in this study. We hope that the information on the common bean product will be used in the future to help consumers make informed food choices.

Withdrawal to participate: You can withdraw participation at any time without giving reasons. If you consent to participate but decide to withdraw at a later moment, you can request the withdrawal of your

information collected during the study. However, in cases where the data and/or samples are no longer identifiable/ cannot be linked back to your identity/data has been merged the request to withdraw data and/or samples may not be possible.

Confidentiality: Your confidentiality will be highly respected. As a study participant, you will be issued a unique study code. Only this code will be used on any research related information collected about you. Information on your identity will remain only with the Principal Investigator and/or designate. The list that matches your name to the unique study number that is used on your research-related information will not be removed or released without your consent unless required by law.

Who to Contact: If you have any questions, you can ask them now or later. If you wish to ask questions later, you may contact **Linet Mutwiri**, Mobile: **+254714223507**.

Participant consent

By signing this form:

- I have read and understood the information in this information and consent form.
- I have had enough time to think about the information provided.
- I have been able to ask questions and have had satisfactory responses to my questions.
- I understand that the purpose of this study is to assess the associated glucose, insulin and appetite responses associated with the consumption of a novel common bean product
- I understand that all of the information collected will be kept confidential and that the results will only be used for scientific purposes.
- I understand that my participation in this study is voluntary. I understand that I am completely free at any time to refuse to participate or to withdraw from this study at any time, and that this will not change the quality of care that I receive.
- I will receive a signed copy of this consent form for my own records.
- I consent to participate in this study.

Participant signature	Participant name	Date
_____	_____	_____
Signature of person obtaining consent	Name and role in study	Date
_____	_____	_____

Appendix 1c: Research Assistant Confidentiality Agreement for metabolic (glycemic, insulinemic and satiety) characteristics of a novel common bean product study

A. CONFIDENTIALITY OF A RESEARCH STUDY:

Confidentiality is the treatment and maintenance of information that an individual has disclosed in a relationship of trust and with the expectation that it will not be divulged to others in ways that are inconsistent with the understanding of the original disclosure (the consent form) without permission. Confidential information relating to human subjects in a research study may include, but is not limited to:

- Name, date of birth, age, sex, address, and contact information;
- Current contact details of family, guardian etc.;
- Medical or educational history and/or records;
- Sexual lifestyle;
- Personal care issues;
- Service records and progress notes;
- Assessments or reports;
- Ethnic or racial origin;
- Political opinions, religious or philosophical beliefs.

As a research assistant you will have access to confidential information pertaining to the research study. Many participants have only revealed information to investigators because principal investigators have assured participants that every effort will be made to maintain confidentiality. That is why it is of the upmost importance to maintain full confidentiality when conducting a research study. Below is a list of expectations you will be required to adhere to as a research assistant. Please carefully review these expectations before signing this form.

B. EXPECTATIONS FOR A RESEARCH ASSISTANT

In order to maintain confidentiality, I agree to:

1. Keep all research information that is shared with me (e.g. flash drives, notes, transcripts, data, etc.) confidential by not discussing or sharing this information verbally or in any format with anyone other than the principal investigator of this study;

2. Ensure the security of research information while it is in my possession. This may include:

- Keeping all documents and/or data related to the research study on a password protected computer with password protected files;

- Closing any programs, documents, or data files related to the research study when away from the computer;
- Keeping any printed documents and/or data related to the research study in a secure location such as a locked filing cabinet;
- Permanently deleting any digital communication containing documents and/or data related to the research study.

3. Not make copies of documents and/or data related to the research study unless specifically instructed to do so by the principal investigator;

4. Give all research information/data and research participant information/data back to the principal investigator upon completion of my duties as a research assistant;

5. After discussing it with the principal investigator, erase or destroy all research information that cannot be returned to the principal investigator upon completion of my duties as a research assistant.

By signing this form I acknowledge that I have reviewed, understand, and agree to adhere to the expectations for a research assistant described above. I agree to maintain confidentiality while performing my duties as a research assistant and recognize that failure to comply with these expectations may result in disciplinary action.

Name of Research Assistant: Signature:

Mobile number:

Name of Principal Investigator: Signature:

Appendix 2: Questionnaire for glycemic and insulinemic response study.
Study №:

Participant code #: Date:

Answer the following questions. Please tick [√] one

1. Do you have a history of eating disorders and/or irregular eating habits? Yes [ ] No [ ]

2. Do you currently use tobacco or alcohol? Yes [ ] No [ ]

3. Are you on your menstrual period or having adverse premenstrual symptoms? Yes [ ] No [ ]

4. Are you currently consuming any supplements? Yes [ ] No [ ]
   (What, when, dose, manufacturer/brand)

5. Are you currently on any medication? Yes [ ] No [ ]
   (What, when, dose, manufacturer/brand)

Participant information, anthropometry and body composition

Gender (tick one)	DOB (DD/MM/YY)	Blood pressure	Waist circ.	Hip circ.
M[ ] F[ ]	___/____/____	_____mmHg	_____cm	_____ cm
Weight	Height	BMI	Impedance	Resistance
_____kg	_____ cm	_____kg/m^2		

24 hour dietary recall

1. Is this a typical day? Please tick [√] one. Yes [ ] No [ ]

If not, give an example of a typical day after yesterday's record if you wish.

E.g.

Time	*Quantity eaten*	*Details of food and drink*
7:15am	*1 cup*	*Tea*
		Milk
	1½ teaspoons	*White sugar*
	½ glass	*Water*
10am	*1 mug*	*Wheat porridge*
	2	*Medium sized yellow sweet potatoes (boiled)*

Blood draw

Time of catheter introduction: _____ Time of first blood draw: _____

Start of test food consumption: _____ Completion of test food consumption: _____

Appendix 3: VAS in assessment of appetite sensations

	How hungry do you feel?	
Not at all hungry		Extremely hungry
	How full do you feel?	
Not at all full		Extremely full
	How satiated are you?	
Not at all satiated		Extremely satiated
	How strong is your desire to eat?	
Not at all strong		Extremely strong
	How much could you eat right now?	
Nothing at all		A very large amount of food
	Would you like to eat something sweet?	
Yes, very much		No, not at all
	Would you like to eat something salty?	
Yes, very much		Yes, very much
	Would you like to eat something fatty?	
Yes, very much		Yes, very much

Hunger: A compelling need or desire for food; the painful sensation or state of weakness caused by the need of food

Satiety: Fullness that persists after eating; length of time until next eating occasion

Appendix 4: CVs of Team Members

CV of Dr. Florence Kyallo

Proposed Position in Research:	PI
Proposed role in the study:	PI
Organisation:	Jomo Kenyatta University of Agriculture and Technology, Kenya
Name of Staff:	Dr. Florence Mumbi Muthiani Kyallo
Profession:	Nutritionist and Lecturer
Date of Birth:	
Years with Organisation:	13
Nationality:	Kenyan
Membership in Professional Societies:	• Kenya Nutritionists and Dieticians' Institute (KNDI) • Member of technical working groups at the Ministry of Health in Kenya (Nutrition and HIV, Agri-Nutrition and Healthy Diets
Detailed Tasks Assigned in Project:	• Budget Preparation and Management • In charge of and accountable for activities on in the research project. • Reporting and dissemination • Proposal development and submission • Ensure compliance proposal to regulatory requirements • Responsible for the overall scientific, fiscal, and administrative conduct of the research

Education & Qualifications:

Qualification	Awarding Institution	Country	Year
Doctor of Philosophy in Applied Human Nutrition	University of Nairobi	Kenya	2013
Master of Science in Applied Human Nutrition	University of Nairobi	Kenya	2005

Employment Record:

From (year)	To (Year)	Position	Employer
2006	Present	Academic (Lecturer, Support Lecturer, Assistant lecturer)	Jomo Kenyatta University of Agriculture and Technology (JKUAT), Kenya.
2004	2005	EUREPGAP Inspector	Africert Limited, Nairobi.
2004	2005	Academic (Lecturer, Curriculum Developer)	Eastern Institute for Health Research and Training, Nairobi.
1995	1997	Agriculturalist, Project manager	Tana and Athi Rivers

			Development Authority (TARDA).

Summary of research experience:

- 2012-2016: Development of a nutritious composite flour from pearl millet (Pennisetum glaucum) and pumpkin (Cucurbita pepo-variety styriaca) (with Kindiki Maryanne and Dr. Arnold Onyango)
- 2014 to Date: Development of elearning courses for nutrition education and counseling, and Nutrition in Emergency Situations. Project funded by EU-Edulink in collaboration with German, Ethiopian, Ugandan and Kenyan Universities.
- 2013-date: Effect of school feeding on nutritional status of pre-school children in Ganze Division of Kilifi County, Kenya. (with Mungai Beatrice)
- 2013-Date: A case control study of clinical and socio-demographic risk factors associated with severe acute malnutrition (sam) among children admitted at Lubango hospital-Angola (with Ketha F)
- Jan 2012-2014: Nutritional Vulnerability of People Living With HIV and AIDS in Kenya. A collaboration between Kenya Medical Research Institute (KEMRI), National AIDS aand STI control Programme (NASCOP), World Food Programme (WFP).
- 2007 – 2012: The Impact of Nutrition Education on overweight and obesity and the associated risk factors and among school children aged 9-14 years in Nairobi, Kenya.
- 2011 – 2013: Effect of Soy bean based diets on nutrition status of children below five years in Rwanda. A collaboration between Rwanda Agricultural Board, RUFORUM and JKUAT.
- June 2009 – 2011: Committee member, HENNA project on Harmonization of Applied Human Nutrition curricula within Eastern Africa Universities. A collaboration between JKUAT, University of Nairobi and Egerton University in Kenya, Hawassa University in Ethiopia, Makerere University in Uganda, University of Ulm and Giessen University in Germany. (Project funded by EU)
- 2008: Trainer, developing Monitoring and Evaluation systems, Kenya HIV/AIDS Business Council (KHBC), Nairobi
- February – April 2007: Co-researcher. The Role of Probiotics in the Health and Nutrition of the Maasai of Kajiado District, Kenya. A collaboration between JKUAT and FriedrichSchiller-University, Germany.
- 2003 - 2005: Danida - Community Based Nutrition Programme, Nairobi. Consultant on Monitoring and Evaluation of Nutrition Interventions and Food Security Projects. Was involved in data collection, data analysis and report writing of nation-wide and regional surveys including household baseline and follow up surveys and orphan surveys carried out in 13 districts in Kenya.
- April - July 2003: Research assistant. Food Consumption, Vitamin A and Nutritional status of children 6 - 59months in Machakos district, Kenya. A study conducted under the Applied Nutrition Programme, University of Nairobi.
- March - February 2003: Research assistant. Food Security, Health and Nutritional Status of Under-Fives in Kisii District, Kenya. A survey carried out by the Applied Nutrition Programme, University of Nairobi.

Publications:

- 2016: Abdikadir S. Omar, Joseph K. Mutai, Florence M. Kyallo, Musa Otieno Ngayo. Family planning utilization and correlates; perspective of women aged 15-49 years from Mandera County of North Eastern Kenya. Journal of Health, Medicine and Nursing Vol.26, 2016 156-168.

- 2015: Maryann Mukethi Kindiki, Arnold Onyango, Florence Kyallo: Effects of Processing on Nutritional and Sensory Quality of Pearl Millet Flour. Food Science and Quality Management Vol.42, 2015 13-20. 2014: Niyibituronsa M, Kyallo F, Mugo C and Gaidashova. The Effects of Household Food Practices and Diseases Prevalence on Nutritional Status of Under-Five Children in Ruhango District, Rwanda 2014: Niyibituronsa M, Kyallo F, Mugo C and Gaidashova. Improving the nutritional status of Malnourished Chldren in Rwanda. AJFAND 14:4.
- 2013: Kyallo F.M., Mwangi A.M.M and Makokha A.O. (2013). Overweight and Obesity among Public and Private school children in Nairobi, Kenya. Health.
- 2012: Catherine Wachuka Mutie and Florence Kyallo (2012). Empowering Women Through Better Healthcare and Nutrition: The Case of Kenya: In Empowering Women Through Better Healthcare and Nutrition in Developing Countries. The Centre for Science and Technology of Non-Aligned and Other Developing Countries (NAM S&T Centre), India Page 151-160.
- 2012: Louise Ngugi, Jasper Imungi, Jaswant Sehmi and Florence Kyallo (2012). Knowledge on and Consumption of Traditional Green Leafy Vegetables among 13-14 year olds – A Case Study of two Primary Schools in Peri-Urban Nairobi. Paper presented at the 24th Nutrition Congress Africa 01-04 October 2012 Bloemfontein, South Africa.
- 2011: Andere N.A and Kyallo F.M. (2011). Nutritional Status, Nutrition Knowledge and Attitudes of students in Jomo Kenyatta University of Agriculture and Technology. Paper presented at the Jomo Kenyatta University of Agriculture and Technology Conference, October 2011.
- 2011: Nadja Knoll, Katrin Kuhnt, Florence M. Kyallo, Beatrice N. Kiage-Mokua, and Gerhard Jahreis. High content of long-chain n-3 polyunsaturated fatty acids in red blood cells of Kenyan Maasai despite low dietary intake. Lipids in Health and Disease. 10:141.
- 2010: Kyallo F.M., Mwangi A.M and Makokha A.O. Demographic and socioeconomic determinants of overweight and obesity among school children in Nairobi. Paper presented at the 4th ANEC Conference. Safari Park Hotel, Nairobi.
- 2010: Kyallo F.M. Imungi J.K. Sehmi J. and Ngugi L.W. Utilization of Traditional Leafy Vegetables in Kalama Division, Machakos, Kenya. Paper Presented at the 2nd ITROMID Scientific Conference. AICAD, Kenya. 25-28th May 2010.
- 2010: Macharia-Mutie C.M and Kyallo F.M. Empowering Women Through Better Healthcare and Nutrition; Preliminary Findings of the 2008-9 Kenya Demographic and Health Survey (KDHS). Paper presented at the International Conference on Empowering Women in Developing countries Through Better Heathcare and Nutrition. BITS Pilani, Rjasthan, India on 22-24th April 2010.
- 2009: Kyallo F.M., Mwangi A.M. and Makokha A.O. Obesity among School Children In Nairobi. Paper presented at the UNICEF Nutrition Research Symposium. Pan Afric Hotel, Nairobi. 29-30th June 2009. 2009: Kyallo F.M., Mwangi A.M. and Makokha A.O. Nutrition Status of School children In Nairobi. Paper presented at the 3rd East African Health and Scientific Conference. KICC. Nairobi, Kenya on 27-29th March 2009.

Language proficiency:

Language	Speaking	Reading	Writing
English	Proficient	Proficient	Proficient
Swahili	Proficient	Proficient	Proficient
German			
Kikamba	Proficient		

I, **FLORENCE KYALLO**, certify that the information provided here in is correct to the best of my knowledge as of **(____/_____/2019).**

CV of Prof. dr. Christophe Matthys

Proposed Position in Research:	PI
Proposed role in the study:	PI
Organisation:	UZ Leuven/ KU Leuven
Name of Staff:	Christophe Matthys
Profession:	Professor
Date of Birth:	05th March, 1975
Years with Organisation:	8
Nationality:	Belgian
Membership in Professional Societies:	• Member of the Scientific Committee of the Belgian Food Consumption Survey • Member of the Scientific Committee of the Belgian Federal Agency for the Safety of the Food Chain (2013 – present) • Member of the Working Group on Food Additives of the European Food Safety Authority (2013 – 2014) • Member of the scientific Committee of the VITADEK study (2014 – present) • Member of the scientific Committee of the Sweeteners-study conducted by the Belgian Institute of Public Health (2015) • Member of the scientific Committee of the Belgian Nutrition and Health Conference • Member of the editorial board of the Nutrition Bulletin and Food Science & Law • Member of the European Nutrition Leadership Platform • Founding member and active member of the Belgian Nutrition Society • Member of the Vlaamse Vereniging voor Klinische Voeding en Metabolisme • Member of the Belgian Endocrine Society
Detailed Tasks Assigned in Project:	• Budget Preparation and Management • In charge of and accountable for activities on in the research project. • Reporting and dissemination • Proposal development and submission • Ensure compliance proposal to regulatory requirements • Responsible for the overall scientific, fiscal, and administrative conduct of the research

Education & Qualifications:

Qualification	Awarding Institution	Country	Year
PhD in Medical Sciences, Faculty of Medicine and Health Sciences	Ghent University	Belgium	
Master of Bioscience Engineering - option Tropical Agriculture	Ghent University	Belgium	
Continuing Education: several courses in statistics, epidemiology, leadership skills and management skills in technology transfer, education courses on teaching at university level			

Employment Record:

From (year)	To (Year)	Position	Employer
October, 2011	2017	Academic (Assistant Professor, Scientific Co-ordinator)	KU-Leuven, Leuven, Belgium
April, 2009	September, 2011	Senior Scientific Project Manager	ILSI Europe, Brussels, Belgium
July 2008	March 2009	Post-doctoral Researcher	Ghent University, Belgium
July, 2007	May, 2008	Lecturer	Institute of Food, Nutrition and Human Health, Massey University, New Zealand
August 2005	July, 2007	Assistant Academic Staff	Ghent University, Belgium
September, 1998	July, 2005	Research Staff	Ghent University, Belgium

Summary of research experience:

Christophe Matthys has (inter)national research experience in the different domains of human nutrition (eg food consumption and nutrition surveys, nutrition policy and public health nutrition, nutritional epidemiology, experimental studies in nutritional epidemiology, food safety).

From an international perspective, he is currently collaborating with several universities in the context of research and education projects. There is a stong contact with the following universities: Muhumbili University (Dar Es Salaam, Tanzania), Stellenbosch University (Stellenbosch, South Africa), Massey University, (Palmerston North, New Zealand) and University of Bergen (Bergen, Norway).

Publications:

https://lirias.kuleuven.be/cv?u=U0076391

- 65 publications in international peer-reviewed journals
- >100 presentations at national and international conferences - number of citations: 875 – without self-citation: 840 (January 2016)
- h-index: 19 (January 2016)

Language proficiency:

Language	Speaking	Reading	Writing
English	Fluent	Fluent	Fluent
Dutch	Fluent	Fluent	Fluent

French	Good	Good	Moderate
German	Moderate	Moderate	Moderate
Spanish	Moderate	Moderate	Moderate

I, **CHRISTOPHE MATTHYS**, certify that the information provided here in is correct to the best of my knowledge as of **(___/____/2019).**

CV of Dr. Beatrice N. Kiage

Proposed Position in Research:	Co-I
Proposed role in the study:	Co-I
Organisation:	Jomo Kenyatta University of Agriculture and Technology, Kenya.
Name of Staff:	Beatrice N. Kiage
Profession:	Lecturer
Date of Birth:	26th February, 1976
Years with Organisation:	7
Nationality:	Kenyan
Membership in Professional Societies:	Member of the Kenya Nutritionists and Dieticians Institute (KNDI) Board member in the board of post graduates studies in the Faculty of agriculture at JKUAT Departmental quality assurance member in the Department of Food Science and Technology at JKUAT
Detailed Tasks Assigned in Project:	Supervise the doctoral student in research activities related to: Data collection Handling of raw data Statistical analysis Publications and oral, poster and seminar presentations Study results dissemination stakeholders

Education & Qualifications:

Qualification	Awarding Institution	Country	Year
PhD. Nutritional Biochemistry/ Dietetics	Kiel	Germany	2013
M.Sc. Food, Nutrition and Dietetics	Kenyatta University	Kenya	2005
M.Sc. Food, Nutrition and Dietetics	Kenyatta University	Kenya	1999

Employment Record:

From (year)	To (Year)	Position	Employer
2013	Present	Lecturer	Jomo Kenyatta University of Agriculture and Technology
2010	2013	Doctoral Researcher	Max Rubner Institute
2006	2013	Assistant Lecturer	Jomo Kenyatta University of Agriculture and Technology
2006	2013	Part time Lecturer	Kenyatta University
2000	2005	Graduate Student Researcher & Nutritionist/Dietician	Kenyatta University & Kenyatta National Hospital

Summary of research experience:

2008-2014: Graduate Student Researcher, Kenyatta National Hospital surgical wards

• Research focused primarily adult patients admitted to the ward for abdominal surgery

• Techniques used to determine nutritional status included 24/hour recall, diet history, food frequency, anthropometric assessments :height, weight, BMI, waist circumference, skin fold callipers, and laboratory measurements of serum albumin levels and serum creatinine levels

• Demonstrated strong data collection/analysis, problem solving, written and verbal skills through thesis and scientific presentations

2010-2013:Doctoral Researcher, Max Rubner Institute, Kiel, Germany

• Conducted in vitro and in vivo studies using plant extracts to treat obesity, diabetes and metabolic syndrome and other cardiometabolic disorders

• Analysed large data sets using exploratory, descriptive and multivariate analysis techniques Processed and analysed large datasets using SPSS, SAS and R

• Planned and coordinated laboratory experiments and animal trials and interpreted results

• Provided oral explanations of my data analysis at several local, national, and international scientific conferences

• Prepared manuscripts for submission to scientific journals

• Assisted in the development of a randomized clinical trial which is ongoing at CRC, Kiel, Germany Research at Kajiado, Kenya

• Assisted in writing the proposal

• Conducted household surveys of the Maasais

• Collected food samples from the households

• Assisted with manuscript preparation for publication

Publications:

In Vitro Anti-Diabetic Activities and Phytochemical Analysis of Bioactive Fractions Present in Meriandra dianthera, Aloe camperi and a Polyherb. Mussie Sium, Patrick Kareru, Beatrice Kiage-Mokua, Kaushal Sood, John Langley, Julie Herniman. 2017. American Journal of Plant Sciences 8: 533-548.

Effect of soaking and germinating techniques on the macronutrient content of Hyptis spicigera Lamiaceae seeds in Morobo County, South Sudan. Grace Ayite Banja, Kiage-Mokua Beatrice and Okoth Judith Kanensi1. 2016. Sky Journal of Food Science 5(3), 019-023.

Lapacho Tea (Tabebuia impetiginosa) Extract Inhibits Pancreatic Lipase and Delays Postprandial Triglyceride Increase in Rats. Kiage-Mokua BN, Roos N, Schrezenmeir J. 2012. Phytotherapy Research 26: 1878-1883.

High content of long-chain n-3 polyunsaturated fatty acids in red blood cells of Kenyan Maasai despite low dietary intake.Knoll N,Kuhnt K,Kyallo FM, Kiage- Mokua BN,Jahreis G. 2011. Lipids in Health and Disease 10: 141.

Published a chapter on nutrition in a book by African Medical Research Foundation (AMREF) on "Non-communicable Diseases". 2006

The effect of Nutritional Status on Clinical outcome of abdominal Surgery patients at Kenyatta National Hospital, Kenya. Published at the graduation proceedings of Kenyatta University (14th October 2005).

2005

Language proficiency:

Language	Speaking	Reading	Writing
English	Fluent	Fluent	Fluent
Kiswahili	Fluent	Fluent	Fluent
German	Fluent	Fluent	Fluent

I, **BEATRICE N. KIAGE**, certify that the information provided here in is correct to the best of my knowledge as of **(____/____/2019).**

CV of Prof. Dr. Bart Van der Schueren

Proposed Position in Research:	Co-I
Proposed role in the study:	Co-I
Organisation:	KU Leuven
Name of Staff:	Bart Van der Schueren
Profession:	Associate professor
Date of Birth:	
Years with Organisation:	8
Nationality:	Belgian
Membership in Professional Societies:	• The Committee for Medicinal Products for Human Use (CHMP) at the European Medicines Agency (EMA)from Sep 2013 – Present • Belgian Alternate Member from Sep/2013 to Sep/2016; • Belgian Member from Sep/2016 to date; • Rapporteur for 4 products/year in the field of endocrinology, vaccines and oncology; • Member of the Cardiovascular Working Party
Detailed Tasks Assigned in Project:	Co-promoter of the doctoral student for doctoral studies

Education & Qualifications:

Qualification	Awarding Institution	Country	Year
PhD degree			
Master's degree	KU Leuven	Belgium	2002
Bachelor's degree	Facultes universitaires Notre-Dame de la Paix		1998

Employment Record:

From (year)	To (Year)	Position	Employer
2011	Present	Professor Dr in endocrinology/ Associate professor	University Hospital Leuven/ KU Leuven
2010	2011	Postdoctoral \fellow	Columbia University College of Physicians and Surgeons/ Belgium American Educational Foundation

Summary of research experience:

Publications:

Scientific output: 75 publications in the field of obesity and bariatric surgery (h-index 16; 721 citations);

Language proficiency:

Language	Speaking	Reading	Writing
English	Fluent	Fluent	Fluent
Dutch	Fluent	Fluent	Fluent

I, (name), certify that the information provided herein is correct to the best of my knowledge as of **(dd/m/yr).**

CV of Linet N. Mutwiri

Proposed Position in Research:	PhD student
Proposed role in the study:	Co-I
Organisation:	Meru University of Science and Technology, Kenya.
Name of Staff:	Linet Nkirote Mutwiri
Profession:	Human Nutritionist and Tutorial Fellow
Date of Birth:	14th August, 1988
Years with Organisation:	7s
Nationality:	Kenyan
Membership in Professional Societies:	KNDI
Detailed Tasks Assigned in Project:	Preparation of a PhD thesis: Literature study; collection of data and samples and making analyses; studying methodologies to analyse and interpret study results; conclude research results. Defence of a PhD thesis about the research process to a scientific jury Networking at congresses and scientific for a of our research field; present our "work in progress"; get feedback; build a network of contacts Publishing in scientific journals; dissemination of study findings to stakeholder platforms

Education & Qualifications:

Qualification	Awarding Institution	Country	Year
PhD. Biomedical Science	Jomo Kenyatta University of Agriculture and technology & Katholieke Universiteit Leuven	Kenya & Belgium	2017-Present
Msc. Human Nutrition and Rural Development-main subj. Human Nutrition	Ghent University	Belgium	2015
Bsc. Food Science and Nutrition	Jomo Kenyatta University of Agriculture and Technology	Kenya	2012

Employment Record:

From (year)	To (Year)	Position	Employer
2013	Present	Academic (Teaching Assistant, Tutorial Fellow)	Meru University of Science and Technology

2012	2013	TOT	Aphia Plus KAMILI-Upper Eastern Region

Publications:
Dietary Practices and Disease Management Practices in Diabetes' Patients with Good and Poor Glycemic Control at Kenyatta National Hospital (Published in Ghent University Library, 2015)

Summary of research experience:
I have conducted field research work, facilitated community training programs and dissemination at community level while attached to Kenya Red Cross Society and APHIA-Plus KAMILI (now defunct).

I have also collected research for my project and dissertation at both undergraduate and graduate study level respectively.

Language proficiency:

Language	**Speaking**	**Reading**	**Writing**
English	Proficient	Proficient	Proficient
Swahili	Proficient	Proficient	Proficient
Kimeru	Proficient	Good	Elementary

I, **LINET N. MUTWIRI**, certify that the information provided here in is correct to the best of my knowledge as of **(____/____/2019).**

Appendix 6: Ethics Training Certificates

Ethics Training Certificate for Dr. Florence Kyallo

fhi360
THE SCIENCE OF IMPROVING LIVES

FHI 360

certifies that

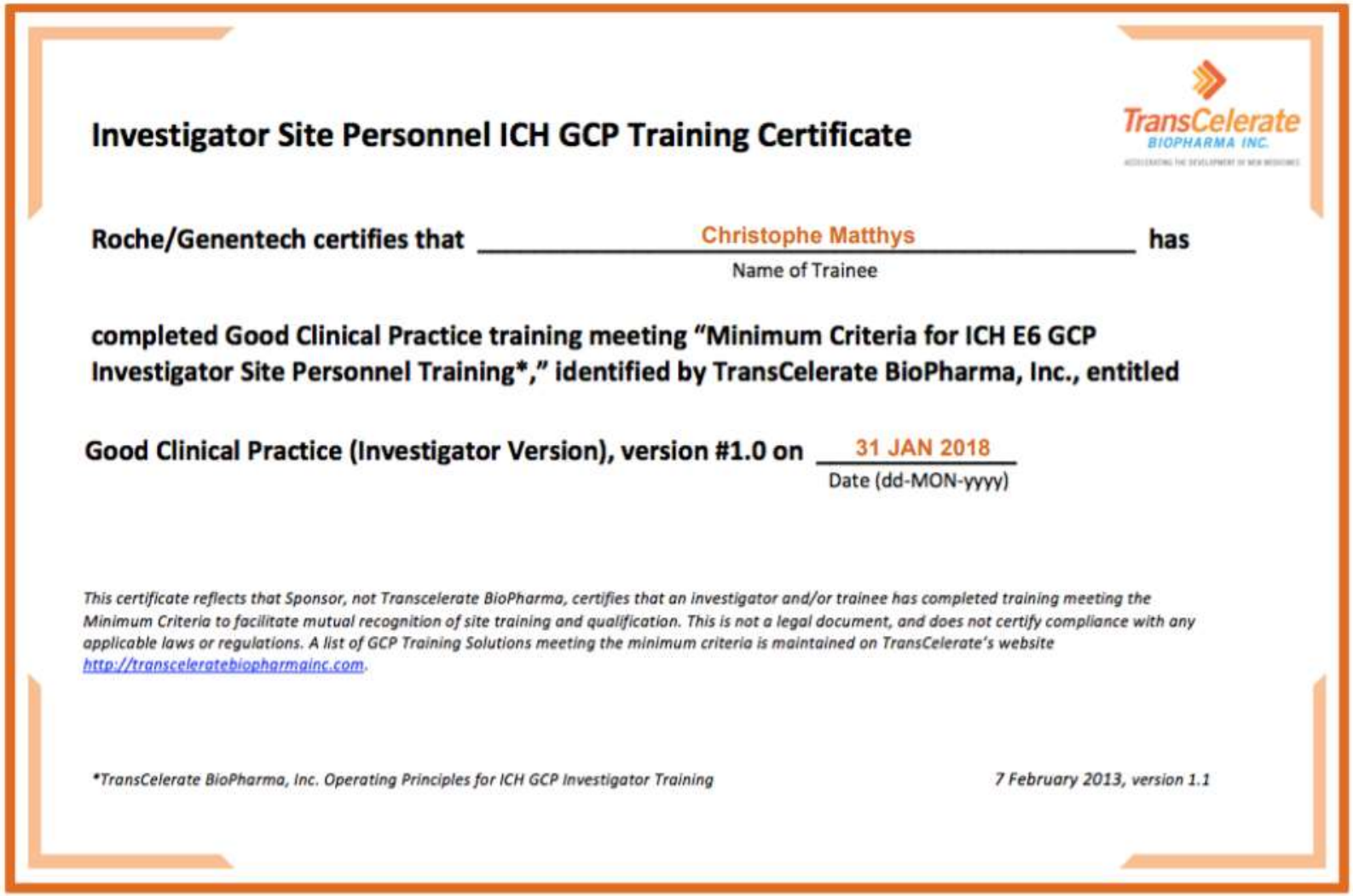
DR. FLORENCE KYALLO

has completed the

RESEARCH ETHICS TRAINING CURRICULUM

April 1, 2019

Ethics Training Certificate for Prof. dr. Christophe Matthys



Investigator Site Personnel ICH GCP Training Certificate

TransCelerate BIOPHARMA INC.

Roche/Genentech certifies that Christophe Matthys has

Name of Trainee

completed Good Clinical Practice training meeting "Minimum Criteria for ICH E6 GCP Investigator Site Personnel Training*," identified by TransCelerate BioPharma, Inc., entitled

Good Clinical Practice (Investigator Version), version #1.0 on 31 JAN 2018

Date (dd-MON-yyyy)

This certificate reflects that Sponsor, not Transcelerate BioPharma, certifies that an investigator and/or trainee has completed training meeting the Minimum Criteria to facilitate mutual recognition of site training and qualification. This is not a legal document, and does not certify compliance with any applicable laws or regulations. A list of GCP Training Solutions meeting the minimum criteria is maintained on TransCelerate's website http://transcelerate biopharmainc.com.

**TransCelerate BioPharma, Inc. Operating Principles for ICH GCP Investigator Training* *7 February 2013, version 1.1*

Ethics Training Certificate for Dr Beatrice Kiage

fhi360
THE SCIENCE OF IMPROVING LIVES

FHI 360

certifies that

Dr. Beatrice N. Kiage

has completed the

RESEARCH ETHICS TRAINING CURRICULUM

May 10, 2019

Ethics Training Certificate for Linet Mutwiri

fhi360
THE SCIENCE OF IMPROVING LIVES

FHI 360

certifies that

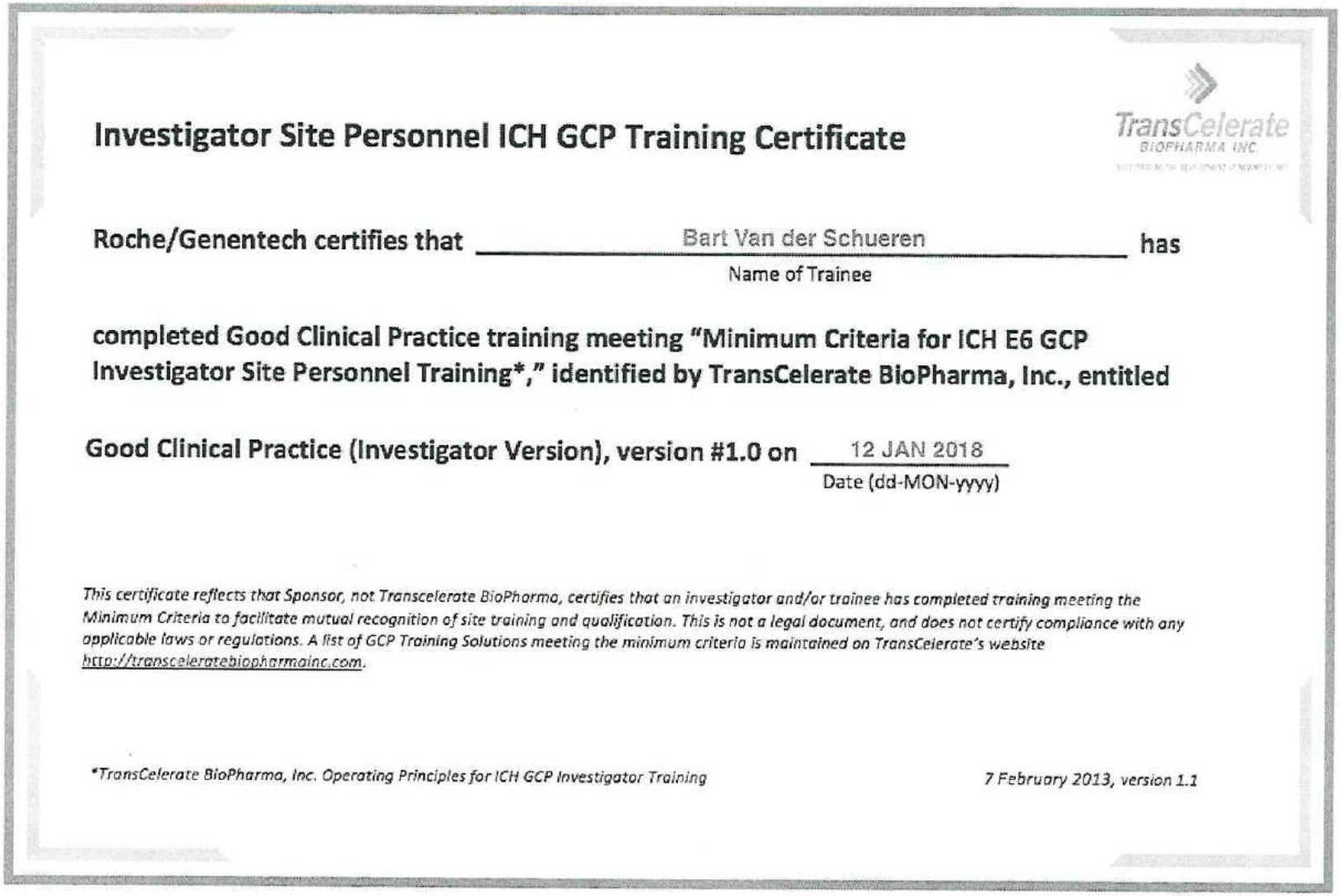
Linet Nkirote Mutwiri

has completed the

RESEARCH ETHICS TRAINING CURRICULUM

January 22, 2018

Ethics Training Certificate for Prof. Bart van der Schueren



Investigator Site Personnel ICH GCP Training Certificate

TransCelerate BIOPHARMA INC

Roche/Genentech certifies that Bart Van der Schueren **has**

Name of Trainee

completed Good Clinical Practice training meeting "Minimum Criteria for ICH E6 GCP Investigator Site Personnel Training*," identified by TransCelerate BioPharma, Inc., entitled

Good Clinical Practice (Investigator Version), version #1.0 on 12 JAN 2018

Date (dd-MON-yyyy)

This certificate reflects that Sponsor, not Transcelerate BioPharma, certifies that an investigator and/or trainee has completed training meeting the Minimum Criteria to facilitate mutual recognition of site training and qualification. This is not a legal document, and does not certify compliance with any applicable laws or regulations. A list of GCP Training Solutions meeting the minimum criteria is maintained on TransCelerate's website http://transcelerate biopharmainc.com.

**TransCelerate BioPharma, Inc. Operating Principles for ICH GCP Investigator Training* 7 February 2013, version 1.1*

Appendix 7: Adherence measures COVID 19 prevention and control regulations (adopted from Ministry of Health, Kenya)

Considering that the COVID-19 disease has now been classified as a pandemic we are taking the following precautionary measures during research activities:

1. Regular and thorough washing of hands with soap and water, or alcohol-based hand sanitizer provided by the researches.

2. Maintaining a distance of at least 1 meter (5 feet) between yourself and anyone (who is coughing or sneezing), avoiding handshakes or hugging.

3. Persons with a cough or sneezing should stay home or keep a social distance, but avoid mixing with others in a crowd.

4. Maintain good respiratory hygiene by covering your mouth and nose while coughing and sneezing with a handkerchief, tissue, or into flexed elbow.

5. Stay at home if you feel unwell with symptoms like fever, cough and difficulty in breathing.

6. All study participants should at all times wear a face mask to reduce the chances of transmission of the virus.

7. Any cases regarding the disease will be reported using the call line facility number 719. Correct messages to be relayed to the participants (if need be) will be received from *719#

8. Surfaces used during the research activities (e.g. desks, tables) and objects will be wiped with disinfectant regularly.