PROTOCOL FOR AN OBSERVATIONAL STUDY

Gastrointestinal transit time at the population level: A cross-sectional intervention study

GITTPop

Version number: V3 – Date*21/09/2026*

Internal ref. nbr: S70908

Sponsor

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LIST OF PARTICIPATING SITES

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Confidentiality Statement

The information in this document is strictly confidential and is available for review to Investigators, potential Investigators and appropriate Ethics Committees, Institutional Review Boards or Competent Authorities. No disclosure should take place without written authorization from the Sponsor.

SIGNATURES

*For multicenter studies this page is to be **created/repeated for every Participating Site** and signed by each Principal Investigator.*
There can only be one Coordinating Investigator, whereas multiple Principal Investigators (i.e. one Principal Investigator per Participating Site) are possible.

Title: Gastrointestinal transit time at the population level: A cross-sectional observation study

Protocol: Version 1

The undersigned confirm that the above referenced protocol has been acknowledged and accepted, they agree to conduct the Study in compliance with the approved protocol, and will adhere to: the ICH guidelines, the most recent version of the Declaration of Helsinki, the EU General Data Protection Regulation 2016/679 (GDPR), relevant Belgian laws implementing the GDPR, the Belgian Law of August 22nd 2002 on patient rights, and any other regulatory requirements and Standard Operating Procedures (SOPs), as applicable.

The undersigned agree not to disclose the confidential information contained in this document for any purpose other than the evaluation or conduct of the Study, without prior written consent of the Sponsor.

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.....	.....	.....
Name & Title	Signature	Date

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FUNDING AND SUPPORT

Funder	Type of Financial or Non-Financial Support
KU Leuven	Own funding (no specific grant) and CSC Scholarship (202406350030)

ROLES AND RESPONSIBILITIES

The Principal Investigator (PI) is responsible for the conduct of the Study at his/her Participating Site, and for protecting the rights, safety and well-being of Study participants. As such the PI must ensure adequate supervision of the Study conduct at the Participating Site. If any tasks are delegated, the PI will maintain a log of appropriately qualified persons to whom he/she has delegated specified Study-related duties. The PI will ensure that adequate training is provided and documented for all Study staff, prior to conducting assigned Study-related activities. Even when certain activities are delegated, the PI will ultimately remain responsible for the conduct of the Study at his/her Participating Site.

It is the Coordinating Investigator's (CI's) responsibility to supervise the general conduct (e.g. Study progress, communication, protocol training and support of the participating sites, annual reporting to the Ethics Committee (EC), end of Study notification(s) and results reporting…) of the Study. The CI fulfils both Investigator and Sponsor responsibilities, as outlined in International Conference on Harmonisation – Good Clinical Practice (ICH-GCP) E6(R2) and applicable regulations.

PI and CI shall each be referred to as «Investigator(s)».

STUDY SYNOPSIS

Title of clinical Study («Study»)	Gastrointestinal transit time at the population level: A cross-sectional intervention study
Protocol Short Title Acronym	GITTPop
Sponsor name	UZ Leuven
Coordinating Investigator	Prof. Dr. Christophe Matthys
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Coordinating Investigator	Prof. Dr. Christophe Matthys
Medical condition or disease under investigation	Obesity
Study rationale	Gastrointestinal transit time (GITT) can be highly variable, by conducting this study we want to map the variability and distribution of GITT in the population
Primary objective	To measure gastrointestinal transit time in a large group of participants at population level
Secondary objective(s)	To 1. explore the association of gastrointestinal transit time with potential exploratory variables as **weight, body composition, age, dietary pattern, dietary supplement, rest energy expenditure, bowel movement frequency, hydration level, physical activity levels, psychological stress levels, sleep quality, genetic variants and stool moisture.** 2. classify gastrointestinal transit time into different phenotypes (fast, medium, and slow) based on its distribution.
Endpoints	Gastro-intestinal transit time
Sample size	932 participants
Maximum duration a research subject remains in the study	5 days
Participating research sites	UZ Leuven
Third parties	None

1. Background and Rationale

Obesity remains a growing global health challenge, with its prevalence expected to reach 1.2 billion by 2030.(1, 2) It significantly increases the risk of cardiometabolic diseases, such as hypertension and type 2 diabetes.(3) Treatment for obesity remains a complex problem that requires the concerted efforts of the whole community.(4) Bariatric surgery effectively reduces obesity-related health risks but often leads to nutritional deficiencies, highlighting the need for continuous dietary support.(5) GLP-1 agonists like Liraglutide and Semaglutide have gained popularity for weight loss. However, they come with high costs, potential side effects, and rapid weight regain upon discontinuation. Studies suggest that combining these medications with dietary interventions yields better long-term results. (6-8) For the time being, dietary intervention remains a required strategy to achieve weight management.(9)

Traditional "one-size-fits-all" diets have proven ineffective due to individual variability in metabolism, genetics, and gut microbiota.(10, 11) Personalised nutrition (PN) offers a tailored approach by optimising nutrient intake based on individual data.(12) Large-scale PN projects, such as PREVENTOMIC and Food4Me, aimed to refine dietary recommendations.(13, 14) Despite their potential, current PN strategies are often expensive, complex, and time-consuming, limiting their real-world application.(15) To make PN accessible, practical, and scalable, future strategies must balance scientific precision with feasibility, ensuring personalised approaches can be widely implemented for effective weight management.

A potential weight control strategy is to alleviate hunger by enhancing or prolonging satiety, thereby regulating appetite, and reducing energy intake. When food enters the gastrointestinal tract, it triggers neural signals that induce the sensation of satiety. Simultaneously, the gut secretes incretin hormones (such as GLP-1), which further extend satiety. This regulatory process is known as the Satiety Cascade. Rapid gastric emptying and transit time can lead to reduced satiety, increased appetite, and ultimately higher food and energy intake.(16-18) Our research team has also found that gastrointestinal transit time (GITT) exhibit distinct population distribution characteristics. (19)Therefore, one possible approach is to tailor diet based on individual gastrointestinal physiological to regulate satiety cascade responses effectively.

To achieve this, we first need to comprehensively understand the distribution of GITT at the population level. Therefore, measuring GITT in a large sample is the primary objective of this study. The blue dye method is a practical and feasible approach for measuring GITT due to its simplicity and potential applicability at the population level. We have applied this technique during our recent 600 years KU Leuven Citizen Project – the Health Pass (The "Health Pass" aims to obtain a general and integrated cross-section of visitors' health based on 14 simple tests (e.g., cardiac, respiratory, physical, mental, gastrointestinal)). The Health Pass resulted in a mapping of the "general health" of visitors to the 600 years KU Leuven event. From 987 participants, 931 GITT datapoints were obtained, demonstrating the feasibility of the Blue Muffin method and providing aggregate GITT data. However, due to inconsistent adherence to eligibility criteria (e.g., fasting status), the overall results and interpretations lacked robustness. Therefore, we optimized the methodology in this study through stricter condition controls.

Through this study, we aim to collect GITT data from approximately 1,000 residents (932 participants – see section ***Study Population***), construct a GITT distribution curve for the region, and analyse its correlations with BMI, age, and other factors (see section ***Statistics and Data Analysis***). This will enable us to establish reference standards and address the current knowledge gap in GITT at the population level. Furthermore, this research will lay a foundation for future personalized nutrition studies. In the next phase, we will develop personalized dietary strategies based on gastrointestinal phenotypes, aiming to effectively regulate energy intake and metabolism, thereby achieving cardiometabolic health management goals.

Study Objectives and Design

2.1 Study objectives

The aim of this study is to measure and define gastrointestinal physiology by using real-life techniques, establish gastrointestinal transit time baseline in citizens and classify gastrointestinal phenotypes.

Several validated and well-accepted techniques to measure gastrointestinal function exist, however the applicability in large population studies or in real-life remains debatable.(20) Therefore, our project aims to develop a standardized method (based on blue dye method) that can be applied in real life, and use this method to understand the gastrointestinal profile of the population.

To meet the aim the following objectives are stated:

Primary objective:

To measure gastrointestinal transit time.

Secondary objectives are to:

i. Explore the association of gastrointestinal transit time among participants with potential exploratory variables as **weight, body composition, age, dietary pattern, dietary supplements usage, rest energy expenditure, bowel movement frequency, hydration level, physical activity levels, psychological stress levels, sleep quality, and stool moisture**. Identify genetic variants associated with GITT. Therefore we emphasize an open, hypothesis-generating perspective.
ii. Classify gastrointestinal transit time into fast, medium, and slow phenotypes based on its distribution.

2.2 Primary Endpoints

The primary endpoint of the study is the gastrointestinal transit time measured by blue-dye method.

2.3 Secondary Endpoints

Secondary endpoints include:

i. Anthropometric parameters (weight, height, waist circumference, fat mass and body composition).
ii. Biochemistry outcome (blood sugar, total cholesterol, HDL, LDL, and triglyceride).
iii. Results of relevant questionnaires (the Bristol Stool Form Scale, Urine color chart, the International Physical Activity Questionnaire, the 10-item Perceived Stress, the Pittsburgh Sleep Quality Index, Dietary Supplement Questionnaire).
iv. Genetic variants associated with GITT, assessed through DNA analysis of saliva samples.

2.4 Study Design

This explorative study is designed as a cross-sectional observational investigation aimed at establishing population-level reference values for gastrointestinal transit time (GITT). Adult participants from the general population will undergo GITT measurement using the blue-dye method. Following a two-day

lifestyle standardization period (including dietary and activity guidance) to minimize potential confounding effects on gastrointestinal motility, participants will attend a clinical visit where they will ingest a blue muffin. GITT will be determined by measuring both the time interval between ingestion of the blue muffin and the first appearance of blue-colored stool, and the duration from its first appearance to its complete disappearance.

The blue muffin method has been well-established in previous research for GITT assessment. Building upon the methodology developed by Berry *et al.*, we will introduce stricter pre-test condition controls (includes dietary advice and lifestyle recommendations) and optimized the blue muffin formulation.(21) These improvements allow for reliable measurement with a single muffin administration, which has been evaluated through KU Leuven Health Pass in a large sample size(S-70168).

To investigate potential determinants and correlates of GITT, we will collect saliva sample for DNA analysis, biochemical parameters, fecal moisture, and resting metabolic rate. Additionally, participants will complete 24-h diet record and health questionnaires assessing bowel movement, physical activity habits, stress levels, sleep quality and hydration condition for subsequent correlation analyses.

2.4.1 Study Diagrams

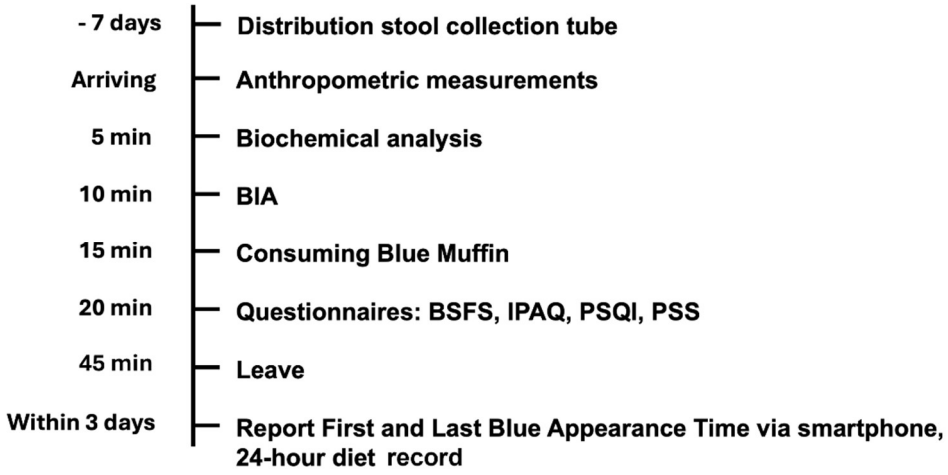


Figure 1: Study design.

BIA: Bioelectrical Impedance Analysis. BSFS: Bristol Stool Form Scale; UCC: Urine colour chart; IPAQ: International Physical Activity Questionnaire. PSQI: Pittsburgh Sleep Quality Index. PSS: Perceived Stress Scale.

2.4.2 Trial Procedures

Recruitment

Study invitations will be circulated through the YONAS platform (Your Online Nutrition Assistant; www.yonas.be), which is a publicly accessible website developed by our team, with the aim of conducting citizen science projects and allowing the recruitment of participants for nutrition related research programmes, targeting both active users in obesity management and general wellness communities. To diversify recruitment across populations flyers will be spread in designated public locations in the centre of Leuven, on the campuses of KU Leuven, and at the University Hospitals Leuven. Additionally, study information will be posted online through the UZ Leuven intranet and social media platforms, such as Facebook (https://www.facebook.com/uzleuvenweet/), X

(https://x.com/NutritionObesi1), BlueSky (https://bsky.app/profile/nmadkuleuven.bsky.social), Instagram (https://www.instagram.com/uzleuvenw_eet/) and LinkedIn (https://www.linkedin.com/in/cmatthys/). Patients who previously consented to be included in the UZ Leuven Obesity Clinic repository (ClinicalTrials.gov Identifier NCT04614961) will be pre-screened according to the study criteria.

The inclusion criteria and exclusion criteria are listed in **3.1**and **3.2**.

Participant information and informed consent

Potential participants will first register via the YONAS website using their email address. We will then contact them via email to register for either an individual or group video call (via Microsoft Teams). During the scheduled session, we will explain the study process, objectives, and potential risks in detail, and provide participants with the opportunity to ask any question. For those attending a group session, an additional opportunity will be provided to speak individually with the investigator afterward. After completion, participants will be informed of the options for providing informed consent—either electronically via REDCap e-Consent ICF (eICF) or by requesting a paper version to be sent by mail. In case of eICF, participants will get an email confirmation with a link to the eICF within RedCap, then participants can read and sign the eICF, a qualified electronic signature as defined in the eIDAS regulation will be requested to sign. The study investigator will sign the eICF following the signing of the participant after which the document/PDF will be locked. Next, the participant will receive this locked eICF which is signed by the participants and the investigator through email. After signing, participants will receive a printed or electronic read-only copy of the signed and dated ICF for their records. All signed ICFs will be safely stored in the REDCap environment as PDF format, only authorized users with fixed roles will have access to the ICFs (Prof. Christophe Matthys and Mr. Wei Li).

Study day

Participants will be advised to maintain a light, easily digestible diet in the two days before the experiment, avoiding high-fat, high-fibre, and spicy foods, and ensuring adequate fluid intake. Participants will also be suggested to avoid engaging in intense physical activities.

On the study day, participants will visit the ACRONIM study unit at UZ Leuven between 7:00 to 10:00 am. Patients will be instructed to arrive for the study visit in a fasted state (fasting for at least 8 hours). Upon arrival, standardized anthropometric measurements (height, weight, waist circumference) and bioelectrical impedance analysis (BIA) will be performed under controlled conditions (ambient temperature 22–24°C; calibrated SECA scales and BIA device). Then, blood sugar, total cholesterol, HDL, LDL, and triglyceride levels will be measured using a finger prick. We will collect a saliva sample using collection tube following the manufacturer's instructions. Handgrip strength will be measured using a handgrip dynamometer. Following baseline assessments, participants will ingest a standardized blue muffin under direct supervision, the specific time point when the participant finishing the muffin will be recorded in REDcap application. During the postprandial observation period participants will complete relevant questionnaires electronically on-site (listed below). After completing all procedures and confirming no acute adverse events, participants will be discharged with instructions to maintain fasting for an additional 2 hours (water allowed) and resume normal activities. A 24-hour diet record application 'Het Digitaal Dagboek' will be used to measure intake of nutrients and water of participants for the day and next day after they consume the muffin, and they will be requested to log their first

and last appearance of 'blue poo' —— First Blue Appearance Time (FBAT) and Last Blue Appearance Time (LBAT) using a REDCap application. There will be a special button, when the participants click it, the mobile application will automatically record the date and time (hours and minutes), If this method is not available, participants can notify us by sending a text message or calling us.

Faecal sample

Participants will self-collect stool samples using pre-labeled containers(22). The self-collect tube will be distributed via postal mail 5 days before the clinic visit participants, participants will bring the sample to the study unit at study day. After participants receive the collection tube, they scan a QR code to confirm receipt status. An electronic instruction manual is provided, highlighting key steps (appendix1). Reminders are sent one day before the experiment and on the morning of the experiment to ensure proper storage and transportation of the sample. If issues arise during distribution or collection that prevent participants from bringing their sample, on-site sample collection or replacement tubes will be provided during the experiment day.

Saliva sample

Saliva samples will be collected using the Oragene OGR-500 DNA collection kit (DNA Genotek, Ottawa, Canada). Approximately 2 mL of saliva will be collected in each collection tube. The stabilizing solution contained within the collection device will subsequently be released into the tube and mixed with the saliva according to the manufacturer's instructions. The resulting mixture will be processed for genomic DNA extraction.

Anthropometry and body composition

Participants height, weight, waist circumference and body composition will be measured. Measurements will be taken using WHO standardized methodology(23). Participants will be weighed without shoes and wearing only light clothing. For the height measurement participants will be requested to remove any hair ornaments or pieces and stand with heels, buttocks, and scapulae against a stadiometer with their heads in the Frankfurt plane. BMI will be calculated as weight (kilograms) divided by height (metres)^2. Body composition will be assessed using multifrequency Bodystat Quadscan 4000 in a fasted state. Participants will be asked to remove shoes and socks and lie supine for at least 5 minutes prior to the measurement. After cleaning the electrode sites with alcohol wipes, four adhesive electrodes will be placed on the right hand and right foot according to the manufacturer's instructions. The measurement will be initiated once the participant is relaxed, and electrodes are properly attached. All data will be recorded and exported for further analysis.

Blood pressure

Blood pressure will be measured with a calibrated automatic sphygmomanometer. Three measurements will be recorded in a supine position, 1–2 min apart, and additional measurements only if the first two readings differ by >10 mmHg. Patient with blood pressure below 140/90 mm Hg will be eligible for participation.

Biochemical Analysis

Blood sugar will be measured by a Accu-Chek blood glucose monitor, and total cholesterol, HDL, LDL, and triglyceride levels will be measured using a validated portable lipid analyser Mission® Cholesterol system via finger prick capillary blood sampling. The fingertip will be cleaned with an alcohol swab, and a sterile lancet will be used to collect a small blood sample. The blood will be applied to a test strip and inserted into the analyser for immediate results.

Energy expenditure

Resting energy expenditure（REE）affects nutrient requirements, further influence the digestive and absorptive efficiency and gastrointestinal motility, therefore may affect gastrointestinal transit time. (24)REE will be calculated using the Katch-McArdle equation (REE = 370 + [21.6 × LBM (kg)]), with triplicate measurements averaged for precision. The Katch-McArdle formula prioritizes Lean body mass (LBM) over total body weight, minimizing errors in populations with altered body composition. As validated by Frankenfield *et al.*, it demonstrated superior accuracy compared to traditional weight-based equations (e.g., Harris-Benedict.)(25)

Blue muffin consuming

After the baseline measurements, participants will consume a blue muffin within 5 mins. The blue muffin used in this study is a standardized test meal produced by the UZ Leuven Hospital Kitchen Facility . Each muffin contains the following ingredients per serving: plain wheat flour (18.87 g), baking powder (1.16 g), granulated sugar (15.41 g), vegetable oil (7.70 g), water (17.72 g), vanilla extract (0.14 g), and royal blue food dye (1.50 g). The nutritional profile is precisely calibrated to 196 kcal per muffin (61% carbohydrates, 35% fat, 4% protein), with a total weight of 62 g. This formulation and was applied in the 'Health Pass' during KU Leuven's 600th-anniversary celebrations(S-70168).

Medical history and health questionnaires

Participants' medical history including current medication will be collected from the participants' medical records (KWS). Participants will additionally complete these validated instruments: 1) the Bristol Stool Form Scale (BSFS) include bowel movement frequency to categorize stool consistency (Types 1-7) over the preceding week; 2) the Urine colour chart (UCC) 3) the International Physical Activity Questionnaire (IPAQ) - Short Form quantifying physical activity duration/intensity across domains (work, transport, leisure); 4) the 10-item Perceived Stress Scale (PSS) measuring subjective stress appraisal, and 5) the Pittsburgh Sleep Quality Index (PSQI).(26-30)

Laboratory analysis

Stool samples will first be stored at −20°C overnight and then transferred to cryovials and stored at −80°C until analysis.(31) Faecal moisture will be analysed using lyophilisation.(32) DNA will be extracted from saliva samples and used for whole-genome sequencing (WGS) to investigate genetic variants associated with GITT. All samples will be stored at −80°C until batch processing to minimize inter-run variability, with sample integrity monitored via RFID temperature loggers during transport.

2.5 Expected duration of the study

This observational study includes one experiment day for each participant, and approximately two days of home observation for each participant. On the experiment day, participants are required to

visit the ACRONIM unit (Academic Centre for Research on Nutrition in huMans) at UZ Leuven to consume the test meal (= blue muffin). Afterwards, they will leave the hospital and independently monitor their stool, reporting their observations online.

Regarding the total duration of the study, based on the number of participants, we expect to have 5 participants per day, so the required ~200 clinical trial days are expected to be completed within one year (<12 months).

2.6 Translational research

Stool samples and saliva samples will be stored and analysed on site (=campus Gasthuisberg). Stool samples and extracted DNA samples for analysis will be stored at the -80°C in the Department of Chronic Diseases and Metabolism (CHROMETA) laboratory. The samples will be registered with the UZ/KU Leuven Biobank and remaining samples will be stored on their premises Health Sciences campus Gasthuisberg.

3. Study Population / Eligibility Criteria

This study will be performed at the ACRONIM unit (Academic Center for Research on Nutrition in huMans, University Hospitals Leuven). Based on the pilot study 'Health Pass' data and the sample size estimation for observational and experimental study(33, 34),

at a 95% CI, the SD value is approximately 1.01 days, with a margin of error of 0.068 days, and an anticipated 10% attrition rate, a sample size of 932 participants is expected to be recruited for this study.

The cross-sectional assessment will ensure that all participants are in a weight-stable state and free from acute gastrointestinal disturbances at the time of measurement.

3.1 Inclusion criteria

Participants eligible for inclusion in this study must meet **all** the following criteria:

1) 18-65 years old
2) 18.5 kg/m² ≤ BMI ≤ 25 kg/m² or BMI ≥ 30 kg/m² (people with normal weight or obesity)
3) Provision of informed consent to participate.

3.2 Exclusion criteria

Participants will be excluded based in the following criteria:

1) Gastrointestinal conditions which may affect nutrient absorption or gastric emptying, including coeliac disease, Crohn's disease, previous resection of the small intestine other than bariatric surgery, gastroparesis;
2) Using medications which effect gastric motility (e.g., domperidone, erythromycin, metoclopramide, prucalopride, opiates, loperamide, …)
3) The ingredients of blue muffins include gluten, nuts, baking powder, sugar, vanilla extract and sunflower oil. Participants with allergies to these ingredients;
4) Current diagnosis of cancer;

5) Advanced organ failure, including chronic kidney disease Stage 5, liver cirrhosis Child-Pugh B or C, intestinal failure, heart failure stage D, or chronic obstructive pulmonary disease stage 4;

6) Immobility;

7) Neuromuscular degenerative conditions associated with muscular atrophy, such as myasthenia gravis, muscular dystrophies, fibromyalgia, or multiple Sclerosis;

8) Unable to follow the procedures of the studies due to language problems.

4. Assessment of Efficacy

Not applicable

5. Assessment of Safety

5.1 Specification, timing and recording of safety parameters

5.1.1 Definitions

Adverse Event (AE)
An AE is any untoward medical occurrence in a patient or subject during an experiment, and which does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a product, whether or not considered related to the product. Any worsening (i.e., any clinically significant adverse change in the frequency or intensity of a pre-existing condition) should be considered an AE.

Adverse Reaction (AR)
An AR is any untoward and unintended responses to an investigational medicinal product or to an experiment and, when an investigational product is concerned, related to any dose administered.

Serious Adverse Event (SAE)
An SAE is any untoward medical occurrence that results in any of the following:

- Death
- A life-threatening [a] experience
- In-patient hospitalisation or prolongation of existing hospitalisation
- A persistent or significant disability or incapacity
- A congenital anomaly or birth defect
- Important medical events that may be considered an SAE when - based on appropriate medical judgement - they may jeopardise the subject and may require medical or surgical intervention to prevent one of the above outcomes.

[a] The term “life threatening” in the definition of SAE refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it was more severe.

5.1.2 Recording of adverse events

All study participants will be under the supervision of a physician during the study visit. Through anamnesis and physical examination, the physician will stay informed on the health status of the participants. The participant will be asked to report any adverse event related to the study-specific intervention to the study team. These reported events will be documented by the participants in the electronic source documents and collected through the (e)CRF.

The following minimum information will be recorded for each adverse reaction (AR):

- AE description
- Start and stop date of the AR
- Severity
- Seriousness
- Causality assessment to the study interventions
- Outcome

Possible adverse effects of blood sampling that have been observed are pain, bruising, nerve damage and hematoma. A bacterial infection can be the consequence of poor infection hygiene. To minimize these risks, blood sampling is done by trained nurses and/or research staff. The possibility of infection is minimized through the implementation of good hygiene practices and by following the WHO guidelines for blood sampling.

Possible adverse effects of faecal self-collection that have been observed include sample contamination, improper handling leading to bacterial exposure, and potential discomfort during collection. Inaccurate test results may arise from delayed processing or failure to follow storage guidelines. Transmission of pathogens (e.g., bacteria, parasites) could occur if personal hygiene practices are neglected. All participants will receive sterile faecal collection kits with biohazard-sealed containers and illustrated instructions adhering to WHO sanitation guidelines. Trained staff will supervise specimen handling to minimize contamination risks, with emergency decontamination supplies (e.g., 10% bleach) and 24-hour medical support available. Protocols follow CDC Biosafety Level 2 standards and CLSI GP44-A3 microbiological guidelines.

If investigations conducted for this study identity any new clinically relevant information, the patient's medical team will be informed with the patient's consent.

5.1.3. Reporting to the Ethics Committee

The Principal Investigator will report any relevant safety information that becomes available ad hoc during the study to the EC.

The Principal Investigator has the obligation to, once a year throughout the clinical trial (or on request), submit a progress report to the EC containing an overview of all SARs (Serious Adverse Reactions) occurred during the reporting period and considering all new available safety information received during the reporting period.

5.2 Treatment stopping rules

Not applicable

6. Statistics and Data Analysis

This study will establish the baseline based on the overall level of participants and perform normal regression to obtain reference values such as the mean, standard deviation, and residuals. Different GITT phenotypes will be defined based on the curve. Participants will be categorized into different subgroups based on age, gender, and weight status (BMI) to explore potential variations in GITT across demographic and physiological characteristics. The primary outcome variable, GITT, will be assessed in a single measurement session for each participant.

GITT will be determined by measuring the time difference between the ingestion of the blue muffin and the FBAT, as well as the time difference between the FBAT and LBAT of blue stool. All data from the REDcap application will be encrypted and synchronised in real-time to the REDCap database, with automated quality checks for completeness. Only the principal investigator and conductor of this study can access this data (Christophe Matthys and Wei Li).

The collected GITT data will be fitted and analysed to explore the distribution pattern of GITT and the correlation between GITT and body characteristics and other explanatory variables. Currently, there is no established gold standard for defining fast and slow GITT, based on the classification methods of Wang *et al.* and Nandhra *et al.*, we will define the values less than one standard deviation below the mean in the normal distribution as fast GITT, and values bigger than one standard deviation above the mean as slow GITT, values in between as medium.(35, 36) Correlation analysis of GITT with age, BMI, REE, and stool moisture content will also be conducted. Genome-wide association analysis (GWAS) will be performed using regression models to assess the association between individual genetic variants and GITT, adjusting for relevant covariates such as age, sex, and genetic principal components.

Data will be analysed using R version 4.2.2. (R Foundation for Statistical Computing, Vienna, Austria, 2024). Results will be reported as means ± SD. The normality of the data will be assessed using the Shapiro-Wilk test. When the W value is greater than 0.95 and the P value is greater than 0.05, GITT is considered to follow a normal distribution. Correlation analysis of GITT with age, gender, BMI, basal metabolic rate, and stool moisture content will be tested by descriptive statistics, Pearson correlation test and t-test. Multivariate linear regression will then be used to evaluate the independent effects of these factors on GITT, controlling for potential confounders, the closer the correlation coefficient R is to 1, the stronger the correlation. The statistical significance between different GITT classes, Bristol stool types will be tested by ANOVA with post hoc analysis, a p-value less than 0.05 will be considered statistically significant. Observations within subjects will be regarded as statistically depended. Differences between the current study population and a comparable population from literature will be assessed using a student's t test. Significance will be set as $P \leq 0.05$.

7. Data handling

7.1 General data handling information

Data collection, handling, processing for the purpose of this study will be performed in compliance with applicable regulations, guidelines for clinical studies and internal procedures. It remains the responsibility of the Investigator to check that all data relating to the Study, as specified in the Study protocol, are entered into the electronic Case Report Form ((e)CRF) in accordance with the instructions provided and that the forms are filled out accurately, completely and in a timely manner.

(e)CRFs are provided by the Sponsor for each participant. The Study data will be transcribed from the source records into an (e)CRF by Study Staff.

The (e)CRFs shall under no circumstances capture personal data such as but not limited to the participant or their relative(s) name, home address, contact details, full date of birth medical record number (e.g., UZ Leuven EAD number), social security number etc.

7.2 Study specific data handling information

Redcap electronic data capture tool will be used to capture study related data. Confidentiality is ensured throughout the entire study from inclusion to publication. Upon agreement to participate, the patient data will be collected anonymously by using a unique identification code. A list of the identification codes with the associated patients will be stored on a secured server of the KU Leuven and will be accessible for the principal investigators and the sub-investigators. During the study visits, the data of the patients will be entered into the (e)CRF.

The following data will be collected in the (e)CRF:

1. Demographics: age，gender;
2. Relevant medical history and study-related blood biochemistry results;
3. Dietary intake data, anthropometry, energy expenditure, and body composition;
4. Questionnaire result: 1) BSFS, 2)UCC, 3) IPAQ, 4) PSS, 5)PSQI.

7.3 Direct Data Access

The investigators will permit trial-related monitoring, audits, EC review, and regulatory inspections by providing direct access to source data and other documents.

8. Ethical and Regulatory Considerations

8.1 Ethics Committee (EC) review & reports

Before the start of the Study, this protocol and other related documents will be submitted for review to the EC for Study authorization. The Study shall not commence until such approvals have been obtained and until other relevant essential Study documents, such as duly signed contract agreements, evidence of adequate Study financing etc. are in place.

8.2 Protocol / GCP compliance

The Study must be performed in accordance with the protocol, current ICH and ICH-GCP guidelines, and applicable regulatory and country-specific requirements. ICH guidelines are an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human participants. Compliance with this standard provides public assurance that the rights, safety, and well-being of Study participants are protected, consistent with the principles that originated in the most recent version of the Declaration of Helsinki, and that the Study data are credible, reliable and reproducible.

8.3 Data protection and participant confidentiality

The Study will be conducted in compliance with the requirements of the EU General Data Protection Regulation 2016/679 (GDPR), and the relevant Belgian laws implementing the GDPR including the Belgian Privacy Act of 30 July 2018 on the protection of privacy in relation to the processing of personal data. Any collection, processing, and disclosure of personal data, such as participant health and medical information is subject to compliance with the personal data protection laws (cfr. Data Processing Annex (DPA) in Appendix). In case personal data is transferred outside the European Economic Area, safeguards will be taken by the Sponsor to ensure that appropriate protection travels with the data in accordance with the GDPR. (https://ec.europa.eu/info/law/law-topic/data-protection/international-dimension-data-protection/rules-international-datatransfers_en#documents)

Any personal data shall be always treated as confidential including during collection, handling and use or processing, and the personal data (including in any electronic format) shall be always stored securely and with all technical and organizational security measures that would be necessary for compliance with EU and national data protection legislation (whichever is more stringent). The Sponsor shall take appropriate measures to ensure the security of all personal data and guard against unauthorized access thereto or disclosure thereof or loss or destruction while in its custody.

9. Research Registration, Dissemination of Results and Publication Policy

The Declaration of Helsinki (latest version) and European and Belgian regulations require that every research Study involving human participants be registered in a publicly accessible database before recruitment of the first participant. The CI is responsible for registering the Study.

In addition, the CI will fulfil their ethical obligation to disseminate and make the research results publicly available. As such the CI is accountable for the timeliness, completeness, and accuracy of the reports. Researchers, authors, Sponsors, editors, and publishers must adhere to accepted guidelines for ethical reporting. Negative and inconclusive, as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in publication.

10. Insurance/Indemnity

In accordance with the Belgian Law relating to experiments on human persons dated May 7, 2004, Sponsor shall assume, even without fault, the responsibility of any damages incurred by a Study Patient and linked directly or indirectly to the participation to the Study and shall provide compensation therefore through its insurance.

The sponsor, UZ Leuven/KU Leuven accepts responsibility for damage caused to the participant (or his/her dependants) and linked directly or indirectly to participation in this study. In this context, the sponsor has an insurance which covers this liability. In case the participant suffers damage following his participation in the study this damage will be reimbursed conform the Belgian law concerning experiments on the human person of May 7th, 2004.

The participant will therefore be requested to report any new health problem to the investigator, after which he/she will be able to provide the participant with additional information concerning possible treatments.

If the investigator believes that the damage is possibly linked to the study (the insurance does not cover the natural progression of disease or the known side effects of normal treatment), he/she will inform the study sponsor, which will initiate the declaration procedure to the insurance company. The latter will appoint an expert - if it considers it necessary - to assess whether there is a link between the participant's new health problems and the study.

In the event of disagreement either with the investigator or with the expert appointed by the insurance company and whenever the participant feels it is appropriate, he or - in case of death – his right holders may start legal proceedings against the insurer directly in Belgium (Kristel De Jonghe, Liability Manager, Allianz Global Corporate & Specialty SE, Belgium Branch, Uitbreidingstraat 86, 2600 Berchem, België (+32 (0) 3 304 16 00)).

The law provides that the insurer may be summoned to appear either before the judge of the location where the event giving rise to the damage occurred, or before the judge of your domicile, or before the judge of the insurer's registered offices.

11. References

1. Adolph TE, Tilg H. Western diets and chronic diseases. Nature Medicine. 2024;30(8):2133-47.
2. Powell-Wiley TM, Poirier P, Burke LE, Després J-P, Gordon-Larsen P, Lavie CJ, et al. Obesity and Cardiovascular Disease: A Scientific Statement From the American Heart Association. Circulation. 2021;143(21):e984-e1010.
3. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature. 2006;444(7121):840-6.
4. Lingvay I, Cohen RV, Roux CWL, Sumithran P. Obesity in adults. Lancet. 2024;404(10456):972-87.
5. Steenackers N, Vanuytsel T, Augustijns P, Tack J, Mertens A, Lannoo M, et al. Adaptations in gastrointestinal physiology after sleeve gastrectomy and Roux-en-Y gastric bypass. The Lancet Gastroenterology & Hepatology. 2021;6(3):225-37.
6. The L. Treating obesity and diabetes: drugs alone are not enough. The Lancet. 2024;403(10421):1.
7. Wadden TA, Chao AM, Moore M, Tronieri JS, Gilden A, Amaro A, et al. The Role of Lifestyle Modification with Second-Generation Anti-obesity Medications: Comparisons, Questions, and Clinical Opportunities. Current Obesity Reports. 2023;12(4):453-73.
8. Manne-Goehler J, Teufel F, Venter WDF. GLP-1 Receptor Agonists and the Path to Sustainable Obesity Care. JAMA Internal Medicine. 2025;185(1):8-10.
9. The L. Treating obesity and diabetes: drugs alone are not enough. Lancet. 2024;403(10421):1.
10. Gardner CD, Trepanowski JF, Del Gobbo LC, Hauser ME, Rigdon J, Ioannidis JPA, et al. Effect of Low-Fat vs Low-Carbohydrate Diet on 12-Month Weight Loss in Overweight Adults and the Association With Genotype Pattern or Insulin Secretion: The DIETFITS Randomized Clinical Trial. JAMA. 2018;319(7):667-79.
11. Berry SE, Valdes AM, Drew DA, Asnicar F, Mazidi M, Wolf J, et al. Human postprandial responses to food and potential for precision nutrition. Nature Medicine. 2020;26(6):964-73.

12. Matusheski NV, Caffrey A, Christensen L, Mezgec S, Surendran S, Hjorth MF, et al. Diets, nutrients, genes and the microbiome: recent advances in personalised nutrition. British Journal of Nutrition. 2021;126(10):1489-97.
13. Jinnette R, Narita A, Manning B, McNaughton SA, Mathers JC, Livingstone KM. Does Personalized Nutrition Advice Improve Dietary Intake in Healthy Adults? A Systematic Review of Randomized Controlled Trials. Advances in Nutrition. 2021;12(3):657-69.
14. PREVENTOMIC Project https://preventomics.eu/the-gut-microbiota-a-key-factor-in-metabolic- disorders-and-dietary-interventions/.
15. Food4me programme https://www.food4me.org/about/.
16. Wolever TMS. Personalized nutrition by prediction of glycaemic responses: fact or fantasy? European Journal of Clinical Nutrition. 2016;70(4):411-3.
17. Hengist A, Ong JA, McNeel K, Guo J, Hall KD. Imprecision nutrition? Intraindividual variability of glucose responses to duplicate presented meals in adults without diabetes. The American Journal of Clinical Nutrition. 2025;121(1):74-82.
18. Stribiţcaia E, Evans CEL, Gibbons C, Blundell J, Sarkar A. Food texture influences on satiety: systematic review and meta-analysis. Scientific Reports. 2020;10(1):12929.
19. Steenackers N, Wauters L, Van der Schueren B, Augustijns P, Falony G, Koziolek M, et al. Effect of obesity on gastrointestinal transit, pressure and pH using a wireless motility capsule. Eur J Pharm Biopharm. 2021;167:1-8.
20. Rao SSC, Camilleri M, Hasler WL, Maurer AH, Parkman HP, Saad R, et al. Evaluation of gastrointestinal transit in clinical practice: position paper of the American and European Neurogastroenterology and Motility Societies. Neurogastroenterology & Motility. 2011;23(1):8-23.
21. Asnicar F, Leeming ER, Dimidi E, Mazidi M, Franks PW, Al Khatib H, et al. Blue poo: impact of gut transit time on the gut microbiome using a novel marker. Gut. 2021;70(9):1665-74.
22. Chen C-C, Wu W-K, Chang C-M, Panyod S, Lu T-P, Liou J-M, et al. Comparison of DNA stabilizers and storage conditions on preserving fecal microbiota profiles. Journal of the Formosan Medical Association. 2020;119(12):1791-8.
23. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. World Health Organ Tech Rep Ser. 1995;854:1-452.
24. Xiong Q, Sun L, Luo Y, Yun H, Shen X, Yin H, et al. Different Isocaloric Meals and Adiposity Modify Energy Expenditure and Clinical and Metabolomic Biomarkers During Resting and Exercise States in a Randomized Crossover Acute Trial of Normal-Weight and Overweight/Obese Men. J Nutr. 2022;152(4):1118-29.
25. Wang Z, Ying Z, Bosy-Westphal A, Zhang J, Heller M, Later W, et al. Evaluation of specific metabolic rates of major organs and tissues: comparison between men and women. Am J Hum Biol. 2011;23(3):333-8.
26. Mentes JC, Wakefield B, Culp K. Use of a urine color chart to monitor hydration status in nursing home residents. Biol Res Nurs. 2006;7(3):197-203.
27. Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. Scand J Gastroenterol. 1997;32(9):920-4.
28. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35(8):1381-95.
29. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28(2):193-213.
30. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. J Health Soc Behav. 1983;24(4):385-96.
31. Santiago A, Panda S, Mengels G, Martinez X, Azpiroz F, Dore J, et al. Processing faecal samples: a step forward for standards in microbial community analysis. BMC Microbiol. 2014;14:112.

32. Procházková N, Laursen MF, La Barbera G, Tsekitsidi E, Jørgensen MS, Rasmussen MA, et al. Gut physiology and environment explain variations in human gut microbiome composition and metabolism. Nature Microbiology. 2024;9(12):3210-25.
33. Sharma S, Mudgal S, Thakur K, Gaur R. How to Calculate Sample Size for Observational and Experimental Nursing Research Studies? 2019:1.
34. Bujang MA. A Step-by-Step Process on Sample Size Determination for Medical Research. Malays J Med Sci. 2021;28(2):15-27.
35. Nandhra GK, Mark EB, Di Tanna GL, Haase A-M, Poulsen J, Christodoulides S, et al. Normative values for region-specific colonic and gastrointestinal transit times in 111 healthy volunteers using the 3D-Transit electromagnet tracking system: Influence of age, gender, and body mass index. Neurogastroenterology & Motility. 2020;32(2):e13734.
36. Wang YT, Mohammed SD, Farmer AD, Wang D, Zarate N, Hobson AR, et al. Regional gastrointestinal transit and pH studied in 215 healthy volunteers using the wireless motility capsule: influence of age, gender, study country and testing protocol. Alimentary Pharmacology & Therapeutics. 2015;42(6):761-72.