# Participant information

GutEnergy

**English title:**
Why calories are not the same – a gut explanation? (GutEnergy)

**Danish title:**
Hvorfor kalorier ikke er ens - findes forklaringen i tarmen? (GutEnergy)

Version 1.3 / April 2025

**Please read this information material carefully before deciding whether to participate.**

If you have further questions, you can contact the
trial coordinator:
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# Information about participation in a scientific study

We would like to ask if you would like to participate in a health science study, named: “Why calories are not the same – a gut explanation? (GutEnergy)”

Your participation is completely voluntary. You can withdraw from the study/trial at any time, and without giving a reason, even if you have signed a consent form. This will not have any consequences.

Before you decide whether to participate in the trial, you must fully understand what the trial is about and why we are conducting the trial. We therefore ask you to read this participant information carefully. We also recommend that you read the appendix “The rights of the subject in a health science research project”, which was prepared by the Danish Research Ethics Committee System in January 2023. The appendix is attached to this participant information.

You will be invited to an information meeting where this participant information will be explained in more detail. Here you will have the opportunity to ask any questions you have about the trial. You are welcome to bring a family member, friend or acquaintance with you to the information meeting. If for practical reasons you cannot attend the information meeting at the study site and wish to have the oral information and answers to any questions you may have over the telephone or video call, this can be arranged.

If you decide to participate in the trial, we will ask you to sign a consent form. Remember that you have the right to 24 hours to think about it before deciding whether to sign the consent form.

The trial runs from April 2025 to December 2031 and is being conducted at the Department of Nutrition, Exercise and Sports, Rolighedsvej 26, 1958 Frederiksberg C. The principal investigator is Associate Professor Henrik Munch Roager. The clinically responsible doctor is Christian Mølgaard, and the daily project manager is PhD student Paula Rodríguez-García. The trial has been approved by the Research Ethics Committee of the Capital Region (H-25015424).

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# Background

Why, if consuming the same foods and the same number of calories, do some people gain weight while others don't?

Weight gain occurs when you eat more calories than you burn. But not all the calories we eat are absorbed by the body – some are lost in our stools. There is a big difference in how many calories people excrete through their stools: some excrete up to 15% of the calories they eat, while others excrete only 1-2%. If we can understand why these differences exist, we will be able to better understand why people have different propensities to gain or lose weight.

One possible explanation for this difference in calorie excretion through stool may lie in our gut, where a complex community of microbes - known as the gut microbiome - influences our digestion and metabolism. The gut microbiome varies from person to person, and research has shown that differences in the gut microbiome can affect weight gain in mice, which is partly due to how much energy we get from food. However, this has only been proven in mice. In humans, we have recently shown that the gut microbiome, as well as the environment in the gut, likely plays a role in how many calories there are in the stool. However, no one has investigated whether the gut microbiome affects energy excretion by precisely measuring how many calories are eaten and excreted over a specific period of time (e.g. several days). There are also very few studies that have examined how different diets affect energy excretion through stool mediated by the gut microbiome. We know that the amount of the gas methane, which we can measure in exhaled breath, reflects differences in the gut microbiome. We hypothesize that people with low and high levels of methane in exhaled breath excrete different amounts of energy through their stool.

Therefore, in this study, we will investigate how differences in the gut microbiome influence energy extraction from the diet by studying how many calories are excreted in the stool relative to the calories consumed, under two different types of diets. Acquiring new knowledge about the role of the gut microbiome in energy extraction from the diet could potentially lead towards personalized dietary recommendations that can support weight loss, prevent weight gain, and improve overall health.

# Purpose

The purpose of GutEnergy is to investigate how differences in the human gut microbiome influence the excretion of energy in stools in the context of two calorie-controlled diets.

# The trial

We will need 60 volunteers for the trial (Figure 1). The trial will begin with a 4-day screening period, where exhaled breath measurements and defecation patterns will be recorded. This will be followed by a period when you will eat a provided diet for three days and collect all stools for 3-5 days (depending on the transit time of your food in the gut). The two diets contain the same amount of calories and macronutrients (i.e. carbohydrates, protein, fat), but one diet will have high content in fiber, resistant starch and large food particles, while the other diet will be more similar to a Western diet (low in fiber, resistant starch and with small food particles). After the diet and stool collection period there will be a washout period of at least 10 days before you begin the second period, where you will again eat a provided diet for 3 days and collect all stools for a few more days. There are 5 visits in total: one screening visit before the screening period, two visits before the two intervention periods (visits 1 and 3), and two visits after the two intervention periods (visits 2 and 4). We are recruiting continuously and expect the trial to last approximately 4-5 weeks per participant.


***Figure 1. Overview of the trial, visits and timeline.***

**Before inclusion in trial – screening visit**

Once you have given your informed consent, you will be invited to a screening visit where the inclusion and exclusion criteria will be evaluated (see "Criteria for participation"). We will measure your height and weight, and we will record your medical and medical history – all to assess whether you meet the inclusion and exclusion criteria that determine whether you can participate in the trial. However, we cannot make the final assessment until we have collected data over a longer period. Unless you do not meet the criteria that we can assess at the visit, you will be given the following equipment:

- A breath measurement device (AIRE 2, FoodMarble Digestive Health Limited, Dublin, Ireland) with access to the associated mobile app (FoodMarble© 2021 FoodMarble Digestive Health Ltd.)
- Access to a mobile app (MyFood24, University of Leeds, UK) for dietary recording
- A defecation diary
- A medication and supplement diary
- Materials for collecting, storing and transporting urine and stool samples

- Instructions regarding breath measurements, dietary recording, and urine and stool collection
- An overview calendar of activities and visits throughout the study. You will also receive reminders (via email or SMS) about visit days and other activities during the trial.

Before the trial begins, we will ask you not to change your diet (except during the intervention periods) and physical activity level throughout the trial.

**Before inclusion in the trial – screening period**

After the screening visit, you will undergo a 4-day screening period. The purpose of the screening period is to find out whether you excrete low or high amounts of methane in your breath. During the 4 days, you will measure your exhaled breath by breathing into portable breath analyzer three times a day, in the morning (before breakfast, 06:00-09:00), in the afternoon (after lunch, between 12:00-15:00) and in the evening (before bedtime, between 21:00-23:00). You will also complete a medication and supplement diary and a defecation diary. You will measure your exhaled breath and fill in the defecation diary for 4 days, while you will fill in the medication and supplement diary throughout the study.

After the 4-day screening period, we will use your methane measurements to place you in either the low or high methane producer group. Based on our experience, there is a higher proportion of low methane producers than high methane producers (2:1). Therefore, we expect to recruit all low methane producers more quickly, and at some point in the trial, it is possible that we will only need participants who are high methane producers. At that point, it will not be possible to include you in intervention periods if it turns out that you are a low methane producer. In such a case, you will be informed, asked to return the portable breath analyzer and questionnaires, and compensated accordingly, as described in the “Compensation” section. If we need your participation, after the screening period you will be called in for the first visit, which will typically take place between 3 days and 2 weeks.

**After inclusion in the trial, before each intervention period**

Before Visit 1 and before Visit 3, you will record your food intake via the MyFood24 app for three days: two working days and one weekend day.

**Visits 1 and 3**

*Unless otherwise stated, all events described in this section will occur in both Visit 1 and Visit 3.*

Within 48 hours before Visit 1 and Visit 3, you will collect a stool sample and store it in your own freezer until you bring it to the study site. In addition, you will be asked to fast from 9:00 PM the night before, drink 400-500 ml of water the night before and the morning of the visit day, avoid intense physical activity and alcohol for 24 hours before the visit day, and avoid tobacco for 8 hours before the visit day. On the morning of the visit day, you will collect a morning urine sample and store it in the cooler bag with freezing elements until you bring it to the study site.

You should use the least physically strenuous form of transport to get to the study site. Upon arrival, you will hand over to the staff your stool and urine samples, which you have brought in the cooler with freezing elements and which should remain frozen. You will be asked whether you have followed the procedure so far. Afterwards, your weight will be measured, and experienced bio-analysts will take a blood sample; a maximum of 40 ml of blood will be taken. In addition, your hydrogen and methane concentration in fasting exhaled air will be measured using both an instrument on-site and the portable device you already know.

Only in Visit 1, you will have your body composition measured by a DXA scan. The DXA scan allows us to measure your body composition in relation to fat, muscle and bone mass in a non-invasive way. The scan takes approximately 15 minutes and uses X-ray technology. A small amount of low-dose radiation will be sent through the body (read more in “Risks”). If you are a woman in fertile age, you will be tested for pregnancy before the DXA scan for safety reasons, as even a small dose of radiation could theoretically be harmful to the fetus. If the test is positive, you will be excluded from the study. At Visit 1, you will also return the defecation diary provided at the screening visit and you will complete a questionnaire about physical activity.

Once all measurements have been recorded, you will receive the meals for the three intervention days (three breakfast, lunch, dinner and snacks), as well as two blue-colored muffins. Before leaving the site, you must eat the first blue muffin and record the time of consumption in the defecation diary (date + hour + minutes).

In addition to the meals and muffins, you will receive:

- Materials for collecting, storing and transporting urine and stool samples
- A scale to weigh potential food scraps

- A food return form
- A questionnaire about gastrointestinal symptoms
- A new stool diary

**Intervention and stool collection periods**

During the two intervention periods, you must eat all the meals provided at Visit 1 and Visit 3 respectively. It is very important that you eat all the food as much as possible and, most importantly, avoid consuming any other food. This is because we need to know exactly how many calories you have eaten. If you have any leftover food, you must weigh the leftovers using the scale and report it on the food return form. Please do not eat anything other than the food provided. If you are going to eat food other than the meals provided, you must report it on the food return form. On the third day of the intervention period, after you have eaten your last meal (i.e. dinner), you will consume the second blue muffin and record the time of consumption in the stool diary. After consuming the second blue muffin, you should not eat anything for the rest of the day until the next morning.

After consuming each of the two muffins, you will note the time of defecation when you can see a change in stool color to blue/green, and the subsequent time of defecation when the blue/green color is no longer visible.

After consuming the first blue muffin, you will collect all stool samples in separate airtight containers. If the first stool samples have not changed color to blue/green, discard the sample. From the stool sample after the first blue muffin, where you first see a blue/green color, you will collect and store the sample and all subsequent samples. Stool collection continues until the stool – after the second blue muffin – where the blue/green color is no longer visible, i.e. that stool is the last sample you should collect. You can see examples of the stool collection process in Figure 2. You should store the stool samples in your own freezer (or in a cooler bag equipped with freezing elements until they are stored in the freezer) until the visit after the stool collection period (i.e. visit 2 or visit 4). You also have the option of continuously delivering the stool samples to the study site (in a cooler bag with freezing elements), if this is easier for you.

After the 3-day dietary intervention period, you will eat your habitual diet.

You will collect a morning urine sample for the first four days of the two periods. In addition, you will complete the gastrointestinal symptoms questionnaire the day after the dietary intervention has ended (i.e. day four of this period). You will also take breath measurements three times daily (as described in the screening period) until you have collected the last stool sample in each period. Finally, you will complete the defecation diary and the medication and supplement diary until you have collected the last stool sample in each period.

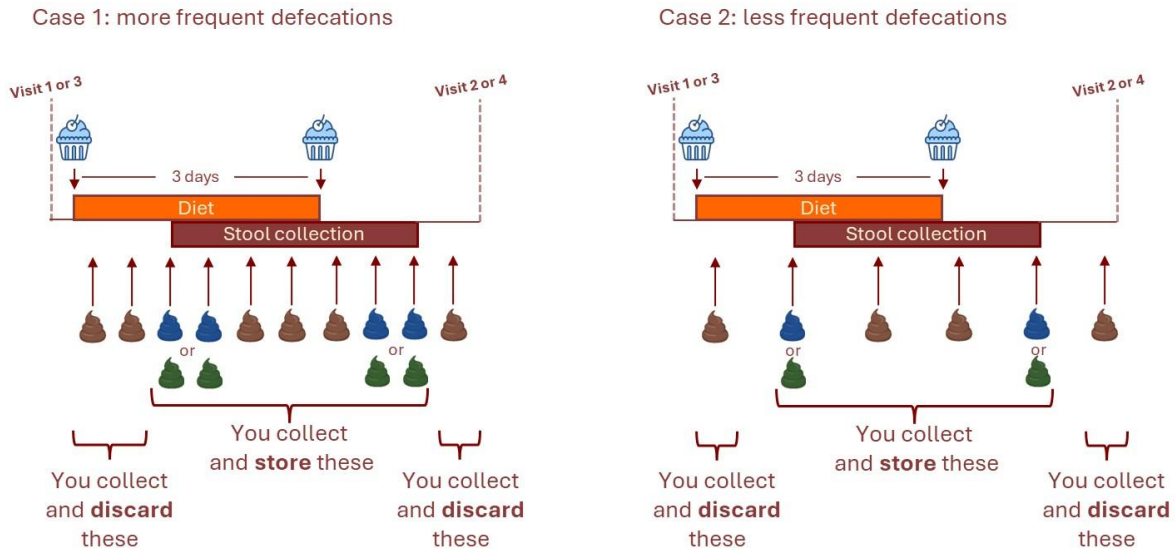


***Figure 2. Examples of the stool collection process, depending on the participants' defecation frequency.***

**Visits 2 and 4 (i.e. after stool collection period)**

*Unless otherwise stated, all events described in this section will occur in connection with both Visit 2 and Visit 4.*

Visit 2 and Visit 4 take place approximately 7 days after Visit 1 and Visit 3, ensuring that you have had sufficient time to collect all the necessary stool samples, regardless of the transit time of your food in the gut.

Upon arrival, you will hand over to the research staff the collected stool and urine samples that you have brought in the cooler bag with freezing elements and which should remain frozen. You will be asked whether you have followed the procedures so far. You will return the complete stool diary, the questionnaire about gastrointestinal symptoms and the food return form.

At Visit 4, you also return the portable breath analyzer, the scale, and the medication and supplement diary.

**Washout period**

Between the two 3-day dietary interventions, you will undergo a "washout period" where you eat your habitual diet. This will last for at least 10 days, allowing sufficient time for the effects of the first intervention period to wear off. Therefore, Visit 3 will be scheduled no earlier than 14 days after Visit 1.

# Samples and analyses

All biological samples and data are collected for specific purposes. The blood samples are analyzed for relevant biomarkers related to metabolism, appetite, immune function, as well as molecules related to your diet and gut microbiome. Body composition measurements allow us to estimate your basal metabolic rate and thus your energy expenditure in a non-invasive way. The exhaled breath is analyzed for concentrations of hydrogen and methane, which will provide information about fermentation in the gut. The urine samples are analyzed for molecules that can be related to your diet, environmental factors in the gut, the gut microbiome, and your metabolism. The stool samples will be used to analyze energy content (from food residues, gut microbiome, and secreted gut cells), to profile the composition and activity of your gut microbiome, and to measure environmental factors in the gut (e.g. pH and water content). If you are interested in more details about how we will analyze your biological samples, please refer to Table 1 in the appendix.

# Criteria for participation

You may participate in the trial if you meet the inclusion criteria and none of the exclusion criteria below. In addition, you must be willing to fast from 9:00 PM on the day before visits 1 and 3 until the relevant samples have been collected at the study site the following morning.

**Inclusion criteria**

- 18-65 years
- BMI 18.5-29.9 kg/$m^2$
- Have one bowel movement at least every other day
- Willing to collect urine and stool samples at home and able to temporarily store the samples in their own freezer in provided storage containers
- Willing to exclusively eat the food provided
- Own a smartphone (iOS 11.0 and later or Android 5.0 and later) with access to the internet
- Speak and understand Danish or English

You cannot participate in the trial if you meet one or more of the following:

**Exclusion criteria**

- Current pregnancy or lactation
- Following a specific dietary program or diet (e.g. vegetarian, vegan, gluten-free) or unable to consume the food provided
- Diagnosis of small intestinal bacterial overgrowth (SIBO), inflammatory bowel disease (IBD), intestinal obstruction, or ischemic colitis
- Diagnosed chronic constipation
- Regular use of diarrhea inhibitors or laxatives
- Any chronic disease that may affect the results of the study
- Taking medications that potentially alter the pH of the stomach (proton pump inhibitors, histamine receptor antagonists, antacids)
- Intake of medications that potentially alter gastro-intestinal motility(e.g., prokinetics, antie-metic agents, anticholinergic agents, narcotic analgesics, nonsteroidal anti -inflammatory drugs, peroral glucocorticoids, and GLP-1-related medications such as semaglutide and liraglutide)
- Taking antibiotics or other medications that may affect the results of the study within the past three months
- Participation in other intervention trials at the same time as this one
- Any condition that makes the investigator doubt whether the participant can complete the trial

**Discontinuation and exclusion of the project**

You may be excluded from the trial if the principal investigator finds it medically necessary or if the procedures, as described in the trial protocol, are not sufficiently followed.

# Biobank

**Research biobank**

Two blood samples (each max. 40 mL, total max. 80 mL), 6 to 12 stool samples (depending on participants' gut transit time, each approx. 20 mL, total max. 240 mL) and 8 spot urine samples (3 mL each, total max. 24 mL) will be stored from each participant for analysis related to the study. The samples will be processed according to standard procedures, after which they will be stored at -20°C or -80°C, with a number identifying the participant and sampling date, in a research biobank at the study site. The research biobank is registered at the University of Copenhagen on statutory records available to the Danish Data Protection Authority.

Stool samples will be sent to the study collaborators who will assist with the analyses. This includes Wageningen University & Research in the Netherlands. In addition, stool samples and data may be sent for analysis by other researchers, e.g. the Technical University of Denmark. All data processors will handle biological material and data according to a written agreement/contract with the University of Copenhagen and/or Wageningen University & Research. Urine and blood samples will be analyzed within the University of Copenhagen.

All samples will be stored and processed in accordance with applicable legislation on data controllers/processors and their processing of participants' personal data and biological material (GDPR – EU Data Protection Regulation 2016/679 of 27 April and Act No. 502 of 23 May 2018 on supplementary provisions to the Regulation on the protection of natural persons with regard to the processing of personal data and on the free movement of such data (Data Protection Act)). The samples will be stored in the research biobank until all analyses have been completed, after which they will be destroyed. Destruction will take place no later than 31 December 2031, unless you have voluntarily chosen to donate your biological material (feces, urine and blood) to the CUBE biobank (see below).

**CUBE biobank**

After all planned analyses have been completed, any remaining material from all biological samples will be stored. It is voluntary whether you want to donate biological material to the CUBE biobank, and we will ask you to give separate written consent for this. These samples will be stored at NEXS for up to 15 years after the last participant has completed the last visit to our experimental biobank (CUBE) for use in any relevant future research. If the material in the biobank still linked to your participant ID number, it can only be used for new research projects that have been previously approved by the Research Ethics Committee System. After the destruction of the link between your biological material and your participant ID number, it will no longer be possible for the Biobank to be registered with the University of Copenhagen on statutory records available to the Danish Data Protection Authority. If you do not want to give permission to donate biological material to the CUBE biobank, this will not affect your participation in the study or your current or future right to treatment, and remaining material from the research biobank will be destroyed.

## Confidentiality and protection of your data

You must give separate, written consent to your data and sensitive information being processed in the trial to participate. When you consent to participate in the study, you also consent to the authorities that control clinical research projects, such as the Ethics Committee and the Danish Data Protection Agency, having access to your personal information.

All personal data is treated confidentially and stored in accordance with applicable legislation, the EU-GDPR/General Data Protection Regulation and Danish Data Protection Act No. 289 of 08-Mar-2024. Data and biological samples are only handled and processed encoded with a project ID. The “key” that links the project ID to the personal data is either stored separately from your data in locked rooms or in a secured system that complies with the legislation.

The research project is registered at the University of Copenhagen, and statutory records are kept of data controllers/processors and their processing of the participants' personal data and biological material. Pseudonymized data will be shared, in accordance with the EU-GDPR, with Prof. Michiel Klerebeezem (Wageningen University & Research, The Netherlands), and Martin Laursen (Technical University of Denmark).

When publishing the results of the trial, your identity will not be revealed. You will receive information about your rights and the data controller's obligations in relation to the processing of your personal data, including samples.

## Risks and safety

All blood samples are taken in the laboratory by an experienced bio-analyst. You may experience some discomfort when the needle is inserted into your skin, and there may be some bruising at the site of the injection, but this will usually go away within a few days. It is estimated that there is minimal discomfort involved in collecting stool, urine, blood, and hydrogen and methane breath samples. Storing stool and urine samples in your own freezer is risk-free when you use the materials provided and follow the instructions for safe and hygienic storage.

Furthermore, it is assessed that there are no risks associated with consuming the provided diet, as the diet consists of ordinary foods. However, you may experience increased gas in the stomach because of consuming one of the diets that contains high amounts of fiber.

DXA scans expose you to a small amount of radiation: 0.001 mSv. DXA scans are routinely used in clinical trials and clinical practice, and this amount of radiation is very limited: for comparison, this is equivalent to 1/3 of the dose during a 1-hour flight. If the DXA scan measurements fail, one additional scan might be conducted.

There may be risks that we are not aware of. Therefore, we would like to ask you to tell us if you experience any problems with your health while participating in the trial. It is possible that during the trial, new information about the diet being studied may become available. If this happens, the trial staff will inform you of this and ask you if you want to continue in the trial. If it has a significant impact on your health and safety, you will be asked to give a new informed consent based on both written and oral information about this new information.

## Insurance

You are covered by the Danish Workers' Compensation Act in accordance with the current insurance conditions at the Faculty of Science, University of Copenhagen. Throughout the trial, you will be covered by the Danish Act on Access to Complaints and Compensation within the Health Service (cf. LBK no. 962 16/08/2024).

## Financial aspects

The project is initiated by Associate Professor Henrik Munch Roager and is funded by the Novo Nordisk Foundation. The project has a total budget of 4 million DKK at the Department of Nutrition, Exercise and Sports. The grant covers salary for technical personnel and PhD, supplies, meals, analysis, equipment, remuneration, data management and communication and dissertation of results. The project account will be subject to audit by the Danish authorities. There are no commercial interests in the project. The researchers, project managers and the medical responsible have no personal financial connection to the foundation that funds the project. The sponsor has no influence on the design, methods, data generation and analysis or publication of results.

## Compensation

By participating in the trial, you will receive free food for two periods of 3 days (3 meals per day) and a gift card of DKK 500 upon completion of each of the two intervention periods. If, after completing the screening period, you cannot be included in the trial, you will receive a gift card of DKK 250. If you discontinue the trial, you will be compensated proportionally with a gift card in relation to the time spent, inconveniences and risks you have had. In addition,

you will be offered your body composition results (DXA scan), and your energy excretion via stool (relative to the participants' average) as well as your baseline fecal microbiome family profile (relative to the participants' average) at the end of the trial.

## Publication of trial results

The project will be registered on the publicly available database www.clinicaltrials.gov. Positive, negative and inconclusive results will be published in international scientific peer-reviewed journals, in non-scientific journals, in student projects, at conferences and in other relevant media, including the department's website. The researchers at the University of Copenhagen have all rights to publish the results from the project.

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We hope that this information has given you sufficient insight into what it means to participate in this trial. We also ask you to read the attached material "Forsøgspersonens rettigheder i et sundhedsvidenskabeligt forskningsprojekt". If you would like to know more about the study, you are very welcome to contact the trial coordinator. The contact information can be found on the front page.

Kind regards,
Henrik Munch Roager, PhD
Associate Professor and Principal Investigator

# Appendix

***Table 1 – List of collected biological samples and planned analyses***

| Sample | Analysis | Purpose |
|---|---|---|
| **Blood** – Serum/Plasma | Biomarkers of glucose metabolism (insulin, glucose, HbA1c) and lipid metabolism (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides). | Before-intervention characteristics |
| | Appetite biomarkers (glucagon, GLP-1, GLP-2, PYY, leptin, ghrelin, GIP, CCK). | Appetite and satiety |
| | Inflammatory biomarkers (CRP, IL-6, TNFα, other cytokines), LBP | Inflammation and intestinal permeability |
| | Metabolomics | Systemic circulation |
| **Breath** | Lactotest 202 Xtend ($H_2$, $CO_2$, $CH_4$)<br>AIRE 2, FoodMarble personal device ($H_2$, $CH_4$) | Colonic fermentation |
| **Urine** | Metabolomics<br>Creatinine | Systemic circulation<br>Normalization |
| **Feces** | Energy density<br>Microbial load<br>Vand<br>pH<br>Particle size<br>Short-chain fatty acids (SCFA)<br>Carbon-nitrogen (C:N) ratio<br>Redox potential<br>Calprotectin<br>Zonulin<br>Total human DNA concentration<br>Metabolomics<br>16S rRNA sequencing<br>Shotgun metagenomics sequencing | Energy excretion<br>Number of bacterial cells<br>Stool consistency<br>Colonic fermentation<br>Colonic fermentation<br>Colonic fermentation<br>Colonic fermentation<br>Colonic fermentation<br>Intestinal inflammation<br>Intestinal permeability<br>Intestinal barrier function<br>Gut microbial metabolism<br>Gut microbiome composition<br>Gut microbiome composition |