AMES-PRO

Assessing the **M**etabolic **E**ffect of **S**ustainable **Pro**teins

V4.0, 21st November 2025

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FUNDERS: Bezos Centre for Sustainable Protein
STUDY COORDINATION CENTRE: Imperial College London
COLLABORATORS: MATR Foods and University of Glasgow

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Protocol authorised by:

Name & Role	**Date**	**Signature**

Study Management Group

Chief Investigator: Professor Gary Frost

Co-investigators: Aygul Dagbasi, Jennifer Pugh

Clinical Queries

Clinical queries should be directed to Gary Frost who will direct the query to the appropriate person

Sponsor

Imperial College London is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:

Research Governance and Integrity Team
Imperial College London and Imperial College Healthcare NHS Trust
5th Floor, Sherfield Building
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London, SW7 2AZ
Tel: **0207 594 9832**
Imperial College - Research Governance and Integrity Team (RGIT) Website

Funder

The Bezos Centre for Sustainble Protein.

This protocol describes the AMES-PRO study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the UK Policy Frame Work for Health and Social Care Research. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

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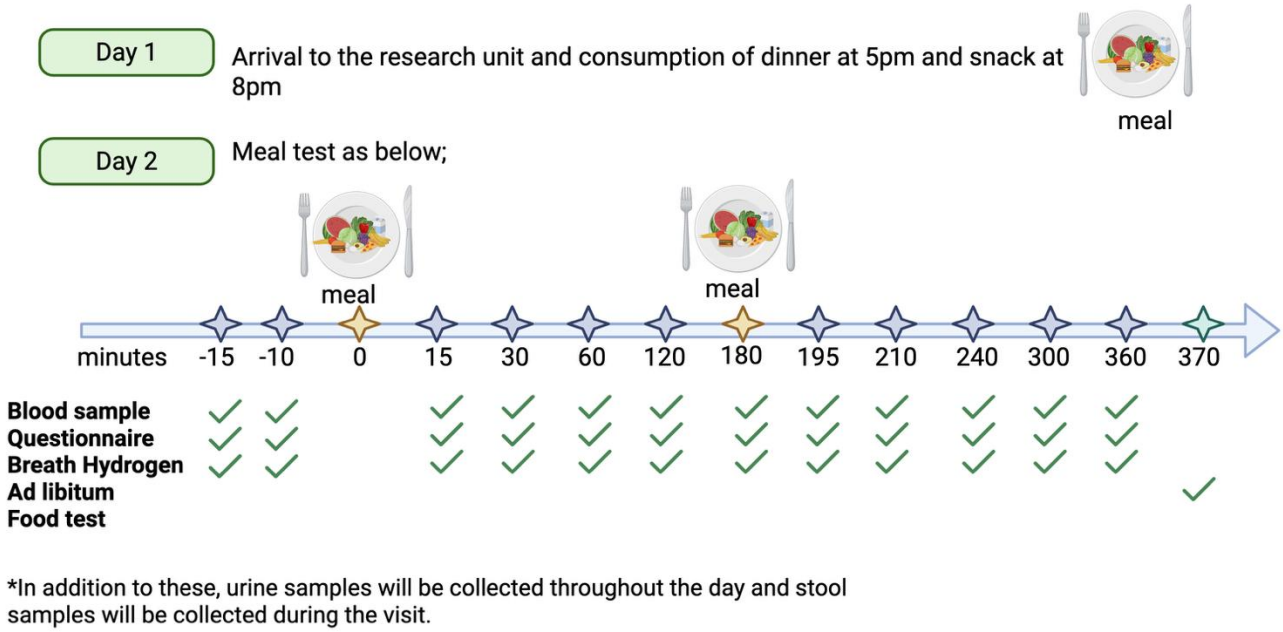
KEYWORDS

Sustainable protein, glycaemia, metabolism

STUDY SUMMARY

TITLE	**A**ssessing the **M**etabolic **E**ffect of **S**ustainable **Pro**teins
DESIGN	Pilot Study
AIMS	Assess the effects of novel, sustainable proteins on metabolism when compared to a meat control
OUTCOME MEASURES	Glucose, insulin, free-fatty acids, amino acids, faecal microbiota, urine and serum metabolomics
POPULATION	Healthy volunteers
ELIGIBILITY	• Body mass index (BMI) of 18.5-29.9 kg/m^2 • Age between 18-65 years (inclusive)
DURATION	One health screening visit and three study visits. The total duration of the study wil be one year.

REFERENCE DIAGRAM



*In addition to these, urine samples will be collected throughout the day and stool samples will be collected during the visit.

1. INTRODUCTION

1.1. BACKGROUND

The consumption of red and processed meat has increased by 88% and 153%, respectively, since 1990[1] and is projected to rise by an additional 76% by 2050.[2] However, current agricultural practices cannot sustainably meet this growing demand. Meat production is a major source of carbon dioxide emissions and highly water and land intensive.[3–5] Moreover, pollution associated with farming practices, including runoff and habitat degradation, has led to concerns about long-term sustainability.[6]

In parallel, the growing awareness of potential health risks linked to high red and processed meat consumption has prompted many individuals to seek alternative sources of protein.[7,8] The EAT Lancet Commission Report (2019) emphasised a diet consisting of plant-based proteins and minimal optional animal protein is required to feed the global population and maintain planetary health.[9] As a result, there has been a shift toward more sustainable and health-conscious protein options, such as fermented products and plant-based proteins.[10] Examples include mycoprotein (known as Quorn), derived from *Fusarium venenatum*, and soy protein.

These novel meat alternatives offer potential health benefits due to their higher fiber content and lower levels of saturated fat compared to animal products[11,12] and have been associated with reduced risks of cardiovascular disease and weight gain.[13,14]

The process of protein breakdown and absorption, alongside the acute metabolic effects of these novel meat alternatives require further investigation. Moreover, sustainable, plant-based proteins may provide other health benefits. Evidence suggests that the enhanced fibre content or specific food structures of some plant-based proteins may reduce cholesterol and improve glycaemic control, potentially improving long-term health outcomes.

This study aims to investigate how the consumption of alternative proteins (supplied by MATR foods) affects metabolic outcomes compared with meat, with the long-term goal of identifying proteins to sustain the growing requirements of the global population.

1.2. RATIONALE FOR CURRENT STUDY

The aim of the current study is to understand how novel proteins in both fermented- and non-fermented-forms, are absorbed and metabolised and any associated downstream effects, compared with a standard meat control, such as red meat.

We hypothesise that novel proteins may result in improved glycaemia and lipid concentrations over a 24-hour period compared with red meat.

2. STUDY OBJECTIVES

The primary objectives are to measure the glyceamic and triglyceride responses to novel proteins, in both fermented- and non-fermented-forms, compared with a meat standard.

The secondary objectives are:

- To assess amino acid bioavailability and absorption by measuring changes in postprandial amino acids
- To monitor changes in gut microbial fermentation using breath hydrogen
- To assess how the gut microbiome influences metabolic response by analysing stool microbiome
- To assess whether the fermented meat alternative or unfermented meat alternative have any effect on the microbiome
- To measure appetite using visual analogue scales (VAS) and energy intake via an ad libitum meal
- To assess differences in metabolites produced via urine and serum metabolomic analysis

3. STUDY DESIGN

This pilot study will be a randomised-controlled cross-over study recruiting 10 healthy participants consisting of three visits that will take place over a period of a maximum of two months.

3.1. STUDY OUTCOME MEASURES

- The primary outcomes are glucose and triglyceride incremental area under the curve (iAUC).

Secondary outcomes are:

- Weight
- Body composition (fat mass, lean mass)
- Blood pressure
- Insulin iAUC
- Free fatty acids iAUC
- Circulating amino acids
- Breath hydrogen
- Visual analogue scales of appetite
- Ad libitum energy intake
- Stool microbiome composition, pre- and post-intervention

- Urine and serum metabolite concentrations

4. PARTICIPANT ENTRY

4.1. PRE-REGISTRATION EVALUATIONS

Participants for this study will be recruited through the Imperial Clinical Research Facility database and advertised to the public using social media platforms such as Facebook and Instagram, notice boards and potentially, newspaper adverts. A study poster has been designed to advertise the study and will be displayed in medical centres, hospitals and community centres.

Once interest in the study has been expressed, interested individuals will be sent a copy of the participant information sheet (PIS) and pre-screening questionnaire via email. Potential participants will fill in a pre-screening eligibility questionnaire to assess their eligibility. By completing the pre-screening questionnaire via telephone or email, we will obtain their pre-screening consent.

Suitable participants will be invited to attend a health screening visit at the NIHR Imperial Clinical Research Facility (ICRF) at Hammersmith Hospital. They will have the opportunity to ask any questions they may have and will be provided with a hard copy of the PIS before the study is fully explained to them by the researcher and written informed consent is obtained. Consent will be obtained by the study researchers at the research facility.

The following measurements will be taken:

- Body weight (kg)
- Height (cm)
- Body composition (fat mass, water weight, lean body mass)
- Body Mass Index (BMI)
- Medical history
- Current medication
- Full blood count (FBC)
- Glycated haemoglobin (HbA1c)
- Physical activity questionnaire (IPAQ)
- Blood pressure
- Dietary habits and food allergies
- Pregnancy test – females of childbearing age

4.2. INCLUSION CRITERIA

- Body mass index (BMI) of 18.5-29.9 kg/m^2
- Individuals aged between 18-65 years (inclusive)

4.3. EXCLUSION CRITERIA

- Weight change of >3 kg in last 3 months
- HbA1c exceeding 41 mmol/mol (8.5%)
- Bowel reconstruction surgery
- Vegan or vegetarians
- Food allergies or intolerances
- Blood donation in the last three months
- Current smokers
- Substance abuse
- Excess alcohol intake (>14 units per week)
- Pregnancy or breastfeeding
- Cardiovascular disease
- Cancer
- Kidney failure
- Participation in another research study in the past 12 weeks
- Diagnosed gastrointestinal conditions
- Use of antibiotics in the past three months
- Use of anti-inflammatory drugs or steroids or thyroid hormones.
- New medication in the past three months
- Any other reason in the opinion of the investigator

4.4. WITHDRAWAL CRITERIA

- Change in health status
- Prescription of new medication which may affect study outcomes
- Unable to attend study visits/comply with protocol
- Failure to consume ≥ 85% of each intervention meal

5. ADVERSE EVENTS

5.1. DEFINITIONS

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward medical occurrence or effect that:

- **Results in death**
- **Is life-threatening** – *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 were more severe*

- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**

- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

5.2. REPORTING PROCEDURES

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

Incidental findings that require further assessment and management will be reported to the GP and clinical care teams. Incidental findings will be reported to the participant as soon as results reviewed by study team and CRF medics. If further urgent assessments are required, these will be arranged by the study team and CRF medics. All other further assessments will be conducted by the participant's GP. Letters will be sent to the GP within 3 working days of discovery via first class postage. Participants will be encouraged to contact their GP once the letter has arrived.

5.3.1 Non serious AEs

All such events, whether expected or not, should be recorded- it should be specified if only some non-serious AEs will be recorded, any reporting should be consistent with the purpose of the trial end points.

5.3.2 Serious AEs

An SAE form should be completed and emailed to the Chief Investigator within 24 hours.

All SAEs should be reported to the East of Scotland Research Ethics Service where in the opinion of the Chief Investigator, the event was:

- 'related', ie resulted from the administration of any of the research procedures; and
- 'unexpected', ie an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all related and unexpected SAEs.

Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

Contact details for reporting SAEs
RGIT@imperial.ac.uk
CI email (and contact details below)

Please send SAE forms to: g.frost@imperial.ac.uk
Tel: 020 7594 0959 (Mon to Fri 09.00 – 17.00)

6. ASSESSMENT AND FOLLOW-UP

6.1. Study Visits

Once the participant can be enrolled in the study, the participant will be invited to attend the research facility for three study visits, lasting 24 hours each (including an overnight stay). Participants will be asked to keep a food diary using the libro app (connected to Nutritics software) to record everything eaten and drank for four days leading up to each study visit.

The order of intervention will be randomly allocated using Sealed Envelope software to avoid order effects. The three interventions will be meals composed of MATR x 2 (fermented and unfermented product) or red meat, (A: fermented meat alternative, B: unfermented meat alternative or C: meat).

At the screening visit and visits 1 and 2, participants will be supplied with a stool sample collection kit to assess their microbial composition at baseline. Stool samples taken before each visit, must be taken as close to each visit as possible (i.e. no more than 3 days prior).

Each study visit will follow the same procedure, described below:

Upon arrival at the facility participant's weight, body composition and blood pressure will be taken.

Participants will be asked to void their bladder and urine samples will be collected in a large container throughout the visit for later metabolomic analysis. Female participants of childbearing age will be asked to provide a urine sample for a pregnancy test when asked to void their bladder.

Participants will then be served their evening meal containing the first serving of either fermented MATR product, unfermented MATR product or meat standard. Participants will be asked to consume the meal in its entirety within 15 minutes. Participants who do not consume ≥ 85% of intervention meals will be withdrawn from the study.

These meals will be weighed before and after to measure compliance.

Participants will be given a snack at 8 pm and they will be asked to consume it in its entirety.

In the morning, a cannula will be inserted into the antecubital vein and baseline blood samples will be taken at -15, -10 before breakfast is served, where the second meal of fermented MATR product, unfermented MATR product or meat standard will be provided. Sequence of study meal allocations will be randomly assigned using Sealed Envelope software.

After three hours, lunch will be served, providing the participant with their final meal containing the fermented MATR product, unfermented MATR product or meat standard.

Blood glucose, insulin, amino acid and triglyceride concentrations and breath hydrogen will be taken at the following time points: T -15, -10, 15, 30, 60, 120, 180, 195, 210, 240, 300, 360 mins. Blood samples will be taken 12 times per vist.

Participants will also be asked to complete subjective satiety questionnaires in the form of a visual analogue scale (VAS) at: T -15, -10, 15, 30, 60, 120, 180, 195, 210, 240, 300, 360. VAS will be completed 12 times per visit.

Breath hydrogen will be used to measure changes in microbial fermentation as the meal reaches the colon. Measurements will be taken at: T -15, -10, 15, 30, 60, 120, 180, 195, 210, 240, 300, 360, 12 times per visit.

At the end of the study visit day, T 370, an ad libitum meal consisting of pasta with a cheese and tomato sauce will be provided to the participant. They will be asked to eat until they feel comfortably full without distractions or entertainment.

Participants will be asked to provide a stool sample before leaving the facility or at home, within 24 hours of completing the study visit. Researchers will contact participants to confirm date and time of stool sample collection. Samples will posted back to the research facility as soon as they have been taken. To do this, participants will be provided with two sealed bags to store the stool sample in and a pre-paid, addressed envelope to post these back to the site. Researchers will demonstrate how to pack the sample for postage to minimise the risk of leakage or contamination.

A washout period of 7 to 21 days must occur between study visits. Participants will be requested not to make any changes to their diet or exercise routine during this period. End of study defined as last visit of last enrolled participant.

7. STATISTICS AND DATA ANALYSIS

The sample size for this pilot study will be 10 participants.

For all numerical outcomes, descriptive statistics will be summarised for each outcome for each of the three interventions.

Area under curve for glucose, triglycerides,insulin and appetite will be calculated using the trapezoidal rule.

Paired t-tests and repeated measures ANOVA will be used to determine any differences between the three interventions for all study outcome parameters using SPSS software.

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study.

8. REGULATORY ISSUES

8.1. ETHICS APPROVAL

The Study Coordination Centre has obtained approval from the East of Scotland Research Ethics Service and Health Research Authority (HRA). The study must also receive confirmation of capacity and capability from each participating NHS Trust before accepting participants into the study or any research activity is carried out. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

8.2. CONSENT

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

8.3. CONFIDENTIALITY

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act. Data will be pseudonymised. The section below details the transfer of data or samples to collaborators, the rationale for transfer and how confidentiality will be maintained:

- MATR Foods: Only fully anonymised data will be shared. The data and results of the study will be shared with this collaborator because they are a study partner and are providing all study meals.

- University of Glasgow: Fully anonymised blood and urine samples will be sent for analysis that cannot be conducted at Imperial College London. This will be using the tracer molecules in the food and tract their travel in body using urine and blood samples.

8.4. INDEMNITY

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

8.5. SPONSOR

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

8.6. FUNDING

The Bezos Centre for Sustainable Protein are funding this study. Participants will be reimbursed £75 per visit, providing a total of £225 for the three study visits.

8.7. AUDITS

The study may be subject to audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Frame Work for Health and Social Care Research.

9. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated by Aygul Dagbasi and Jennifer Pugh. Regular meetings with collaborators at MATR food and University of Glasgow will be conducted throughout the study.

10. PUBLICATION POLICY

Information concerning the study, processes or scientific data is confidential and remains the property of the Sponsor. The investigator may use this information for the

purposes of the trial only. Therefore all information obtained as a result of the trial will be regarded as confidential until appropriate analysis and review are completed.

The main results of the trial may be published in peer-reviewed journals and presented at relevant conferences. A Study Report summarising the trial results will be prepared and submitted to the REC within a year of the end of trial.

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Appendix 1. Summary of investigations, treatment and assessments

Investigation	**Visit**			
Visit	Screening	1	2	3
Visit windows		7-21 days	7-21 days	7-21 days
Informed consent	**X**			
Screening blood samples: FBC, HbA1c	**X**			
Medical History and current medications	**X**			
Dietary habits, allergies and intolerances	**X**			
Pregnancy test for women of childbearing age	**X**	**X**	**X**	**X**
Body weight and composition	**X**	**X**	**X**	**X**
Blood pressure	**X**	**X**	**X**	**X**
Visual analogue scales of appetite		**X**	**X**	**X**
Ad libitum Meal		**X**	**X**	**X**
Stool sample (2 per visit)		**X**	**X**	**X**
Urine collection		**X**	**X**	**X**
Breath hydrogen measurements		**X**	**X**	**X**
Visit blood samples: glucose, insulin, triglycerides, circulating amino acids		**X**	**X**	**X**