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# The Fish and Meat study

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| **Submission date**<br>20/06/2025 | **Recruitment status**<br>Recruiting | [_] Prospectively registered<br>[_] Protocol |
| **Registration date**<br>07/07/2025 | **Overall study status**<br>Ongoing | [_] Statistical analysis plan<br>[_] Results |
| **Last Edited**<br>07/07/2025 | **Condition category**<br>Circulatory System | [_] Individual participant data<br>[X] Record updated in last year |

## Plain English summary of protocol

Background and study aim:
Eating fish can protect against heart disease, but also increase blood levels of a chemical associated with unhealthy diets high in red meat, which has been linked to heart disease.
This study aims to compare the effect of meat-, fish- and vegetarian-based diets on heart health outcomes.
The study will determine whether this chemical is a cause for concern for those who eat a diet high in fish, or whether fish should be considered a healthy alternative to eating meat-based diets in terms of heart health.

Who can participate?
Healthy men and women, aged between 30 and 65 years old, who are classed as overweight (BMI 25-29.9 kg/m2). Volunteers should have elevated blood lipids, sugar or blood pressure, but not be on any related medication.

What does the study involve?
Before the intervention, participants will take part in a health screening at the Human Intervention Study Unit at the Rowett Institute to make sure they are suitable for the study. This will include a review of their medical history, height, weight, waist and blood pressure measurements, and checking their blood fat and sugar levels with a small fingerprick blood test. In addition, the amount of energy the participant's body uses while at rest (resting metabolic rate) will be measured through a quick and painless procedure looking at the air they breathe in and out. Participants will also be asked to complete a four-day food diary before their next visit.
The intervention itself will last for 14 weeks and involves a further 7 visits to the Human Intervention Study Unit.
Following successful screening, participants will be randomly allocated to one of three different diet groups. Study diet A is a meat-based diet; study diet B is a fish-based diet; Study diet C is a plant-based diet.
For the first week, participants will be asked to eat a control diet, similar to what people eat on average in the UK. Then, they will follow their assigned diet (A, B or C) for 6 weeks. After that, they will return to the control diet for 1 week before switching to a different diet for another 6 weeks (A if you started with B or C, or B or C if you started with A).
During the intervention, participants will undergo various measurements, including urine and stool sampling, fasted blood tests, 24-hour blood pressure monitoring, and weight checks. Blood vessel stiffness will be assessed using a painless ultrasound, and body fat will be measured using

a Bod Pod machine. Additionally, a small sensor worn on the skin will track how their bodies process sugar throughout the day and after drinking a sugar solution, using continuous glucose monitoring.

What are the possible benefits and risks of participating?
The dietary changes required for this study are relatively small, and no major side effects are expected. However, if any occur, participants should contact the study team. There are no known risks associated with the diets, although there is a small chance of changes in bowel movements, stool consistency, bloating, or constipation after modifying the diet. If this occurs, guidance will be provided to help eliminate these side effects. When providing a blood sample, participants may experience slight discomfort during collection and may feel light-headed. Some bruising may also occur.
The study may not provide direct benefits to participants, but the information collected may contribute to finding ways to reduce the risk of diseases such as heart disease in others. A confidential personal feedback report will be provided at the end of the study, including recorded measurements of blood pressure, weight, blood glucose, and cholesterol. Additionally, participants' general practitioners will be informed of their participation in the study, along with their cholesterol and blood pressure results.

Where is the study run from?
The Rowett Institute, University of Aberdeen (UK)

When is the study starting and how long is it expected to run for?
October 2024 to December 2027

Who is funding the study?
Biotechnology and Biological Sciences Research Council (UK)

Who is the main contact?
1. Prof. Jules Griffin, jules.griffin@abdn.ac.uk
2. Prof. Frank Thies, f.thies@abdn.ac.uk

# Contact information

### Type(s)
Public, Scientific, Principal investigator

### Contact name
Prof Frank Thies

### Contact details
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# Additional identifiers

### Clinical Trials Information System (CTIS)
Nil known

### Protocol serial number
Sponsor number 1196765

# Study information

### Scientific Title
Defining the dietary context of TMAO as a cardiovascular risk factor

### Acronym
FAMOUS

### Study objectives
It has been known for some time that diets rich in fish and seafood are also associated with high blood plasma TMAO concentrations, which is at odds with TMAO being a universal marker of CVD risk considering that a number of diets that are associated with lower CVD risk are promoting the consumption of fish, particularly oily fish. Fish consumption is also associated with improved foetal development during pregnancy and reduced cognitive decline in the elderly. Thus, concerns about diets high in TMAO and TMA may impact nutritional advice around fish consumption, with some already urging caution for the consumption of fish high in TMAO in those at risk of CVD.

It is hypothesised that the increase in cardiovascular risk factors previously associated with high blood plasma TMAO is diet-dependent, and is not detectable for diets high in fish.

### Ethics approval required
Ethics approval required

### Ethics approval(s)
approved 19/02/2025, Rowett Ethics Panel (The Rowett Institute, University of Aberdeen, Ashgrove Road West, Aberdeen, AB25 2ZD, United Kingdom; +44 (0)1224 438708; morven.cruickshank@abdn.ac.uk), ref: 5417862

### Study design
Single-centre two-way randomized cross over trial

### Primary study design
Interventional

### Study type(s)
Other

### Health condition(s) or problem(s) studied
Cardiovascular disease

### Interventions

We will conduct a randomised control trial design to investigate how a fish diet modulates blood and urinary levels of TMAO, alongside blood plasma concentrations of cholesterol and triglycerides, and blood pressure in an at-risk population. Two 2-way randomised cross-over design interventions (6 weeks) will be used to examine meat-, fish- and vegetarian-based diets, which are otherwise identical in terms of nutritional quality. Metabolomics and lipidomics will be used alongside clinical chemistry measures to deep phenotype volunteers. We will take particular interest in the pathways that are thought to contribute to heart disease, including how the microbiome may be altered. By comparing these outcomes, we will be able to determine whether TMAO is a cause for concern with those who eat a high-fish diet, or whether fish should be considered a healthy alternative to eating meat-based diets in terms of heart disease.

The volunteers will be allocated to the two possible sequences in small randomised sets using a programme created by Dr Graham Horgan from BioSS (Biomathematics and Statistics Scotland). The HISU nurse will carry out the randomisation. As this is a crossover study with treatment assessed within volunteers, stratification of allocation to treatment sequences will be done within gender only, and by sequence of joining the study (to account for any seasonal or other order effects).

Two complementary studies using the same design as described above will be conducted in parallel.
In study 1, which will compare the effects of a fish-rich diet with a meat-rich diet, volunteers will be randomised to one of the following diets for 6 weeks:
1. A standard UK diet containing typical consumption levels of meat
2. A fish-based diet where the protein content of the meat is replaced by fish (excluding seafood, such as shellfish, to specifically investigate the relationship between fish and CVD). At least one portion of oily fish (e.g. salmon, mackerel, anchovies, sardines, herring) per week will be included, as recommended by the Science Advisory Committee on Nutrition (SACN). Fish will be selected according to the most common fish eaten within the UK as determined by the National Diet and Nutrition Survey (NDNS) and recent reviews. Cod, haddock, halibut and salmon are all reasonably high in TMAO and TMA, while tuna and trout are relatively low, so we will have a variety of daily exposures to fish-based TMA and TMAO to as closely reflect a ‘normal’ fish-based diet that might be consumed in the UK.

In study 2, which will compare the effects of a meat-rich diet to a plant-based diet, known to provide cardiovascular health benefits, volunteers will be randomised to receive:
1. The standard meat-based diet
2. A plant-based diet where plant protein replaces the meat component in 1.

For the first week, participants will be asked to eat a control diet, similar to what people eat on average in the UK. Then, they will follow their assigned diet for 6 weeks. After that, they will return to the control diet for 1 week before switching to a different diet for another 6 weeks.

The numbers of volunteers are based on power analyses for a significant increase in TMAO following fish consumption and previous dietary intervention studies conducted by the applicants. We have powered the fish-meat intervention with more volunteers than the meat-plant intervention as the latter has been previously investigated by others. This will allow us to place both studies in the context of previous results but also use these previous results in a meta-analysis of all RCTs to increase the power of study 2. While a three-way crossover would have been preferred in terms of statistics, this would require 22 weeks (with a washout period), which from past experience would significantly impact study engagement and retention.

### Intervention Type
Behavioural

### Primary outcome(s)
Measured before the run-in period, at baseline, mid-point and at the end of the three diets (day 0, 7, 28, 49, 56, 77, 98):
1. Blood and urinary TMAO concentrations determined in samples using liquid chromatography-mass spectrometry (LC-MS)
2. Plasma total and HDL cholesterol and TAG concentrations measured by standardised automated procedures. LDL cholesterol will be calculated using the Friedewald equation.

### Key secondary outcome(s))
Measured before the run-in period, at baseline and at the end of the three diets (day 0, 7, 28, 49, 56, 77, 98) unless specified otherwise:
1. Blood pressure determined using an automated oscillometric sphygmomanometer and over 24 hours using 24-hour ambulatory blood pressure monitors
2. Markers of inflammation and endothelial function (hsCRP, slCAM1, IL-1β) measured by immunoassays
3. Urine sodium, calcium and potassium concentrations assayed by elemental analysis
4. Plasma lipidomics analyses carried out using an Agilent Ultra High Performance Liquid Chromatography QToF 6560 Mass Spectrometer
5. Plasma metabolomic analyses performed using LC-MS/MS on a Thermo Quantiva to profile ~200 core metabolites representing amino acid metabolism, β-oxidation, the citric acid cycle, glycolysis, gluconeogenesis, products of reactive oxygen species (oxidised amino acids and nucleotides) and anti-oxidants (glutathione and vitamin C).
6. Short-chain fatty acids and bile acids from blood samples, as well as the gut microbiota-derived metabolites hydroxyindole sulfate, phenylacetylglutamine, indoxyl-sulfate, p-cresol sulfate, 4-methylcatechol sulfate and methionine sulfone, and the meat biomarker 1-methylhistidine, determined using LC-MS.
7. Similar analysis for the gut microbiome metabolites mentioned above will be conducted in urine samples
8. Augmentation index and central blood pressures measured by applanation tonometry using Sphygmocor at day 7, 49, 56 and 98
9. Faecal DNA will be extracted and 16S rRNA amplicon sequencing carried out to assess microbiota compositional changes in response to diet. Quantitative PCR (qPCR) will also be employed to establish changes in total bacteria in absolute terms, as well as for selected microbes that show diet-specific changes and/or that have previously been identified as TMA producers, as qPCR allows for higher phylogenetic resolution than short amplicon sequencing.
10. Short-chain fatty acids extracted from stool samples will be measured by gas chromatography
11. Blood glucose concentrations measured using standardised automated procedures
12. Body composition assessed using BodPod at days 7, 49, 56 and 98
13. Oral glucose tolerance test (OGTT) self-performed using automated continuous glucose monitoring (CGM) as a measure of insulin resistance at days 7, 49, 56 and 98.
14. Dietary intake measured using a 4-day food diary before starting the intervention
15. Resting metabolic rate assessed at screening by indirect calorimetry

### Completion date
31/12/2027

## Eligibility

## Key inclusion criteria
1. Overweight (body mass index [BMI] 25-29.9 kg/m2)
2. Aged 30-65 years
3. Mild, non-treated dyslipidaemia (fasted triglyceride [TG] between 1.7 and 8.5 mmol/l and/or cholesterol between 5.6 and 6.6 mmol/L
4. Fasting glucose between 5.6 and 6.9 mmol/L and/or systolic blood pressure (SBP) between 91 and 159mm Hg and/or diastolic blood pressure (DBP) between 61 and 89 mm Hg and glucose
5. Volunteers with low HDL cholesterol (<1.04 mmol/L for men, <1.29 mmol/L for women) and /or hypertension (>130/85 mmHg) and/or central obesity (waist circumference >102 cm for men, >88 cm for women) and/or if they have moderate hypercholesterolemia (total cholesterol between 5.6 and 6.6 mmol/L) will also be included

## Participant type(s)
Healthy volunteer

## Healthy volunteers allowed
No

## Age group
Other

## Lower age limit
30 years

## Upper age limit
65 years

## Sex
All

## Key exclusion criteria
1. Stage 2+ hypertensive (SBP >159 mmHg and/or DBP >99 mmHg)
2. Existing or active/prior health conditions including diabetes (or fasting glucose >7.0 mmol/L) or CVD
3. Taking medication to control BP or lipid levels
4. Unwillingness to comply with study protocol including stopping vitamins/minerals that may affect the trial
5. BMI>29.9 or BMI <25 kg/m2
6. consuming more than 14 alcohol units per week
7. People with thyroid gland disorders or eating disorders
8. People taking regular medication or supplements known to affect any dependant variable measured
9. SBP <90 mmHg and /or DBP <60 mmHg
10. Pregnant women
11. Individuals with bowel disorders (Crohn’s, IBS, coeliac)

## Date of first enrolment
01/04/2025

## Date of final enrolment

31/03/2027

# Locations

**Countries of recruitment**
United Kingdom

Scotland

**Study participating centre**
**The Rowett Institute**
Foresterhill
University of Aberdeen
Aberdeen
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AB25 2ZD

# Sponsor information

**Organisation**
University of Aberdeen

**ROR**
https://ror.org/016476m91

# Funder(s)

**Funder type**
Research council

**Funder Name**
Biotechnology and Biological Sciences Research Council

**Alternative Name(s)**
UKRI - Biotechnology And Biological Sciences Research Council, Agricultural and Food Research Council, Biotechnology & Biological Sciences Research Council, BBSRC, BBSRC UK, AFRC

**Funding Body Type**
Government organisation

**Funding Body Subtype**
National government

**Location**
United Kingdom

# Results and Publications

### Individual participant data (IPD) sharing plan
We will store all data in MetaboLights and make both the raw and processed data freely available to the research community. Datasets will be discoverable on the EBI MetaboLights website; this pathway for dissemination of datasets will be publicised in the project literature and also through social media (e.g. X [Twitter], LinkedIn). We will provide an overview in a metabolomics newsletter like Metabonews – a monthly newsletter for the community.

### IPD sharing plan summary
Stored in publicly available repository

### Study outputs

| Output type | Details | Date created | Date added | Peer reviewed? | Patient-facing? |
|---|---|---|---|---|---|
| Study website | Study website | 11/11/2025 | 11/11/2025 | No | Yes |