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Adipose tissue inflammation and the regulation of muscle mass

Submission date	Recruitment status	[X] Prospectively registered
26/03/2025	Recruiting	[_] Protocol
Registration date	**Overall study status**	[_] Statistical analysis plan
24/04/2025	Ongoing	[_] Results
Last Edited	**Condition category**	[_] Individual participant data
06/05/2025	Nutritional, Metabolic, Endocrine	[X] Record updated in last year

Plain English summary of protocol

Background and study aims
Many people experience muscle loss as they get older. This can lead to muscle weakness and, in some people, it can eventually lead to frailty and loss of independence. A key factor that leads to age-related muscle loss and frailty is a raised level of inflammatory molecules in the body, but the main cause of this inflammation has always been uncertain. Adipose tissue (body fat) produces hundreds of molecules that can affect a person's health. The research team have demonstrated that adipose tissue becomes inflamed and produces large amounts of inflammatory molecules in some older people. The production of some of these molecules predicts whether people have less muscle. Based on these findings, they believe that adipose tissue is a major source of inflammation in ageing in some people, and that when adipose tissue is inflamed, it negatively affects the ability to maintain normal amounts of muscle. This project will directly assess how well older men and women with different levels of adipose tissue inflammation produce muscle. It will also try to work out which cells are producing the molecules causing inflammation. It is hoped that this project will pave the way for new treatments to avoid loss of muscle and frailty with ageing, thus helping people to reduce frailty and live independently for longer.

Who can participate?
Healthy volunteers aged 65-75 years and 20-30 years

What does the study involve?
The project involves a team of researchers from three Universities (Bath, Exeter and Birmingham), with study visits taking place at Bath and Exeter. All participants will attend the University of Bath for 2 visits. On the first visit participants will complete questionnaires, undergo body composition scans, assessment of cognitive and physical function, measurement of resting metabolism and a short treadmill walk. They will be sent away with food and sleep diaries as well as a physical activity monitor to wear between visits. They will be asked to maintain their normal lifestyle during this time. The second visit involves 2 adipose (fat) tissue biopsies taken from the fat tissue underneath the skin on either side of the belly button. We will also place a cannula (a small plastic tube) in a forearm vein that will allow us to take regular blood samples over the visit. As well as the assessment of inflammatory markers in these samples, blood and fat tissue samples collected as part of visit 2 will also be used to look at the

effect of inflammation levels on the development of muscle cells grown in the laboratory. During the visit, participants will be asked to perform an oral glucose tolerance test (OGTT), which measures blood sugar control and involves consuming a flavoured sugary drink followed by regular blood samples. Visit 2 is the end of the study for the younger participants (20-30 years).

Older participants (65-75 years) will have an additional visit to the University of Exeter for an assessment of muscle protein metabolism in response to exercise and nutrition. During this visit participants will firstly rest in bed, before completing a short protocol of resistance exercise followed by consumption of a protein drink. This visit involves insertion of 2 cannulas, one in a forearm vein to infuse a protein "tracer" that allows us to measure muscle protein metabolism, and one cannula in the hand that allows us to take regular blood samples across the visit. In addition to these measures, the study will also collect 4 muscle biopsies from the quadriceps muscle (2 on the left leg and 2 on the right leg).

What are the possible benefits and risks of participating?
By participating in this study, participants will contribute to science and our understanding of why some people are prone to the loss of muscle mass with ageing. This, in turn, will help scientists develop strategies to improve health for the millions of people who lose a lot of muscle and become frail as they age.

Participants will be reimbursed for any travel expenses and will be provided with parking permits for the University car park for visits.
At the end of the study, participants will be given a copy of their results and personalised feedback regarding:
• Diet
• Physical activity levels
• Blood pressure
• Body composition analysis from the DEXA scan (the amount of muscle and fat that you have and your bone density)
• Cognitive performance
Even though the tests are not designed to be diagnostic, if any results are found that are outside the normal reference ranges, participants will be informed and recommended to make an appointment with their GP for specific medical advice. There is also an inconvenience payment in the form of Love2shop gift vouchers. £100 voucher for attending visits 1&2, and an additional £100 voucher for attending visit 3. Participants will also be able to opt in to receive a lay summary of the overall findings of the whole study once it has been completed.

Beyond being asked to give their time, as described above, there are inherent minor risks associated with some of the procedures involved in this study.
DEXA and pQCT: Participants will receive body composition scans (one DEXA scan and one pQCT scan), both of which they would not receive otherwise. These procedures use ionising radiation to assess body composition and provide clinical insights. Participation in this study will result in a minimal radiation exposure, equivalent to approximately two days of natural background radiation.

Blood sampling and infusion: Blood sampling via venepuncture may occasionally cause minor bruising, with very rare risks of infection or nerve damage. Cannula insertion and infusion also carry a small risk of infection or embolism (a tiny air bubble or plastic fragment that could affect blood flow), though such occurrences are extremely rare. These risks are minimized through strict adherence to best practice.

Across all study visits, the total blood volume collected will be less than 250 mL (under half a pint), which is about half the amount taken during a standard NHS blood donation. This volume is unlikely to cause any adverse effects.

Fat tissue sample: Only highly trained and experienced researchers at the University of Bath will perform the fat tissue biopsy. This process involves using a needle and so there is a small risk of infection, but this risk is minimised by our strict adherence to best practice. Some people experience a very small amount of bleeding during the hours immediately after the sample has been taken, however, participants will be closely monitored during the trial day and will be advised regarding best practice for changing dressings where necessary. We advise against any contact sports for four or five days and against swimming for three or four days. In the days following the sampling, some bruising and/or a small lump under the skin are likely, however, these will typically return to normal within a few days/weeks, respectively.

Muscle sampling: Only highly trained and experienced researchers at the University of Exeter will perform the muscle biopsy. The technique used to acquire muscle biopsies has been in common use since the early 1960s, and only minor complications are typically observed (e.g. bleeding from the skin wound, bruising and minor soreness over the days afterwards). After the biopsy, the site will be cleaned, and the incision will be closed using wound closure strips. A bandage will be placed over the site. Participants will be given spare bandages to use later if needed and will be given instructions on how to look after the area at home.

Physical Activity Monitoring: The physical activity monitor is mounted on a heart rate monitor strap or pads to be worn around the chest. It is designed to be as discrete and comfortable as possible. Should it become uncomfortable, it can be removed intermittently, and it must be removed for water-based activities (swimming or bathing).

Where is the study run from?
The main study site is the Centre for Nutrition, Exercise, and Metabolism at the University of Bath. However, visit 3 will be carried out at the Nutritional Physiology Research Unit at the University of Exeter.

When is the study starting and how long is it expected to run for?
July 2024 to September 2028

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

Who is the main contact?
Dr Harry Smith, hs565@bath.ac.uk

Contact information

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Additional identifiers

Clinical Trials Information System (CTIS)
Nil known

Integrated Research Application System (IRAS)
345960

ClinicalTrials.gov (NCT)
Nil known

Protocol serial number
CPMS 64096, Biotechnology & Biological Sciences Research Council Grant Code: BB/Y006542/1

Study information

Scientific Title
Establishing the role of adipose tissue inflammation in the regulation of muscle mass in older people

Study objectives
Adipose tissue is a major source of inflammation in human ageing, and specific proinflammatory molecules secreted by adipose tissue directly impair skeletal muscle protein metabolism.

Ethics approval required
Ethics approval required

Ethics approval(s)
approved 11/09/2024, Wales Research Ethics Committee 7 (-, Carmarthen, -, United Kingdom; -; Wales.REC7@wales.nhs.uk), ref: 24/WA/0242

Study design
Observational cross-sectional study

Primary study design
Observational

Study type(s)
Other, Quality of life

Health condition(s) or problem(s) studied
Adipose tissue inflammation and muscle protein metabolism in older adults

Interventions
This study will recruit older (65-75 years) and younger (20-30 years) participants to investigate adipose tissue inflammation and its potential relationship with muscle protein metabolism using in vivo and ex vivo techniques. Both groups will undergo initial screening (Visit 1) for eligibility at the University of Bath, including body composition scans (dual energy x-ray absorptiometry and peripheral quantitative computed tomography) to determine age-specific fat mass index (FMI) and leg muscle cross-sectional area. Provided FMI criteria are met, participants will then complete both cognitive (Montreal Cognitive Assessment, Digit Symbol Substitution Task, Trail Making Task, Stroop Test and Digit Span Test) and physical (short physical performance battery; SPPB) function assessments. Participants will then be asked to record their diet and sleep, and wear a device to measure physical activity levels for 3 days.

Eligible participants will then undergo a second visit (Visit 2) at the University of Bath in which 2 adipose tissue biopsies will be collected from abdominal subcutaneous tissue for comprehensive immuno-metabolic phenotyping using bulk RNA sequencing, proteomics, spectral flow cytometry, single-cell RNA sequencing, western blotting, and adipocyte characterisation. Moreover, adipose tissue explants from both age groups will be cultured to generate conditioned media for subsequent in vitro cell studies. Inclusion of the younger cohort also allows for comparison of comprehensive adipose phenotypes between age groups. During this visit, participants will also provide fasted blood samples, which will be analysed for a panel of inflammatory and metabolic markers, before undergoing an oral glucose tolerance test and completing questionnaires related to sleep and quality of life.

All older participants will then be invited back for a third visit (Visit 3) that will be conducted at the University of Exeter. During this visit, muscle protein metabolism will be examined under fasted, fed, and exercised conditions. This design will allow us to investigate whether older individuals with higher adipose tissue inflammation (based on unsupervised hierarchical clustering of bulk RNA sequencing data) exhibit blunted muscle protein metabolism relative to those with lower adipose tissue inflammation. Using adipose, muscle, and blood samples collected from participants across these visits we will be able to perform a series of in vitro cellular studies that will allow us to directly examine how differences in inflammatory markers secreted from the adipose tissue between younger and older adults influences markers of muscle metabolism, including muscle cell growth (diameter), as well as molecular pathways related to protein synthesis and breakdown.

Intervention Type
Other

Phase
Not Specified

Primary outcome(s)
1. Skeletal muscle (synthesis and breakdown) and whole-body (oxidation and net balance) protein metabolism measured using Stable Isotope Tracers over 3 hours (Older people only - Visit 3).
2. Adipose tissue expression and secretion of cytokines and adipokines in older people and younger adults in the post-absorptive state measured using RNAseq, multiplex antibody-based assays and/or proteomics from biopsies collected at visit 2

Key secondary outcome(s))
1. Adipose tissue immune cell phenotype, function and activation in the post-adbsorptive state measured using spectral flow cytometry from biopsies collected at Visit 2 (All)
2. Single-cell RNA sequencing of adipose stromavascular fraction (non-adipocytes) in the post-absorptive state from biopsies collected at Visit 2 (subset of participants).
3. Measures of muscle protein metabolism and development (diameter) in skeletal muscle cell models when cultured in vitro with adipose explant media and serum from young and old participants collected at Visit 2.
4. Concentrations of cytokines and adipokines in human serum collected in the post-absorptive state, measured using antibody-based electro-chemiluminescence from blood samples collected at Visit 2 (All).
5. Skeletal muscle gene expression before and after the resistance exercise protocol in Visit 3 measured using PCR (Older only).
6. Skeletal muscle gene expression before and after ingestion of protein beverage in Visit 3 measured using PCR (Older only).
7. Post-prandial change in plasma glucose over 2 hours measured using a clinical metabolite analyser (Visit 2 - All).
8. Post-prandial change in plasma insulin over 2 hours measured using enzyme-linked immunosorbent assay (Visit 2 - All).
9. Postprandial change in plasma non-esterified fatty acids (NEFA) over 2 hours measured using a clinical metabolite analyser (Visit 2 - All).
10. Postprandial change in plasma glycerol over 2 hours measured using a clinical metabolite analyser (Visit 2 - All)
11. Postprandial change in energy expenditure over 2 hours measured using indirect calorimetry (Visit 2 - All).
12. Postprandial change in substrate oxidation measured using indirect calorimetry (Visit 2 - All).
13. Physical activity energy expenditure measured using combined accelerometry and heart rate.
14. Energy intake measured using weighed food record over 3 days between Visit 1 and Visit 2.
15. Subjective sleep quality measured using the Pittsburgh Sleep Quality Index (PSQI) at Visit 1.
16. Sleep duration measured using combined accelerometry and heart rate monitoring over 3-days (verified with physical sleep diary) between Visit 2 and Visit 3.
17. Physical function measured using the Short Physical Performance Battery (SPPB) involving gait speed, chair stand and balance test at Visit 1.
18. Cognitive function measured attention, processing speed and short-term memory recall. The tests include the Montreal Cognitive Assessment (MoCA), Digit Symbol Substitution Task, Trail Making Task, Stroop Test and Digit Span Test at Visit 1.
19. Body composition and bone mineral density measured using dual-energy x-ray

absorptiometry at Visit 1.
20. Calf and quadriceps cross-sectional area, muscle density, adipose tissue infiltration, bone mineral density and bone strength measured using peripheral quantitative computed tomography at Visit 1.
21. Systemic (blood) immune cell phenotype, function and activation in the post-absorptive state, measured using spectral flow cytometry on Visit 2 (All).
23. Skeletal muscle immunohistochemistry (IHC) with specific targets to be determined.

Completion date
30/09/2028

Eligibility

Key inclusion criteria
Older People
1. Age 65 to 75 years
2. Postmenopausal women must be > 1 year since last menses
3. Able to provide informed consent
4. Willing and able to comply with all study procedures, including maintenance of habitual dietary intake, exercise, and medication use during the study period.

Younger People
1. Age 20-30 years
2. Able to provide informed consent
3. Willing and able to comply with all study procedures, including maintenance of habitual dietary intake, exercise, and medication use during the study period.

Participant type(s)
Healthy volunteer

Healthy volunteers allowed
No

Age group
Mixed

Lower age limit
20 years

Upper age limit
75 years

Sex
All

Key exclusion criteria
Older People
1. Living in a residential care home
2. Unstable or clinically active pulmonary, cardiac, hepatic, renal, endocrine, hematologic, immunologic, neurologic, psychiatric or biliary disorders. ‘Unstable’ refers to complications of a

condition that are not controlled by medication or lifestyle and which require frequent monitoring and testing by a health professional. Stable chronic disease is not an exclusion criterion unless specified.
3. Diagnosed Type 1 or Type 2 Diabetes Mellitus or other metabolic disease(s) that would affect study outcomes
4. Diagnosed autoimmune condition
5. Diagnosed osteoarthritis affecting more than one joint
6. Past or current cancer diagnosis and treatment that required systemic treatment
7. Severe hypertension (systolic blood pressure >/= 180 mmHg and/or diastolic blood pressure > /= 120 mmHg), as defined by blood pressure measured at Visit 1
8. Current tobacco or recreational drug use
9. Reported changes to use of thyroid, antihypertensive, antidepressant or statin medications within 30 days of Visit 1
10. Taking medications that will interfere with the study outcomes
11. Regular participation in resistance training (at least once a week).
12. High levels of non-recreational exercise (> 6h per week of high-intensity exercise or sport)
13. Fat Mass Index (FMI) < 3 and > 12 kg/m2 (Men) and < 5 and > 16 kg/m2 (Women)
14. Known negative reaction to lidocaine anaesthetic and/or taking warfarin
15. Not weight stable in the prior 3 months (> 5% weight change)
16. Presence of injuries or conditions that would prevent completion of resistance exercise
17. Unable to converse in English

Younger People
1. Unstable or clinically active pulmonary, cardiac, hepatic, renal, endocrine, hematologic, immunologic, neurologic, psychiatric or biliary disorders. 'Unstable' refers to complications of a condition that are not controlled by medication or lifestyle and which require frequent monitoring and testing by a health professional. Stable chronic disease is not an exclusion criterion unless specified.
2. Diagnosed Type 1 or Type 2 Diabetes Mellitus or other metabolic disease(s) that would affect study outcomes.
3. Diagnosed autoimmune condition.
4. Diagnosed osteoarthritis affecting more than one joint.
5. Past or current cancer diagnosis and treatment that required systemic treatment.
6. Severe hypertension (systolic blood pressure ≥ 180 mmHg and/or diastolic blood pressure ≥ 120 mmHg), as defined by blood pressure measured at Visit 1.
7. Current tobacco or recreational drug use.
8. Reported changes to use of thyroid, antihypertensive, antidepressant or statin medications within 30 days of Visit 1.
9. Taking medications that will interfere with the study outcomes.
10. Regular participation in resistance training (at least once a week).
11. High levels of non-recreational exercise (> 6h per week of high-intensity exercise or sport).
12. Fat Mass Index (FMI) < 3 and > 10 kg/m² (Men) and < 5 and > 12 kg/m² (Women).
13. Known negative reaction to lidocaine anaesthetic and/or taking warfarin.
14. Not weight stable in the prior 3 months (> 5% weight change).
15. Unable to converse in English.

Date of first enrolment
29/04/2025

Date of final enrolment
30/09/2028

Locations

Countries of recruitment
United Kingdom

England

Study participating centre
University of Bath
Claverton Down
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BA2 7AY

Sponsor information

Organisation
University of Bath

ROR
https://ror.org/002h8g185

Funder(s)

Funder type
Government

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

Stored in a publicly available repository
Available on request
Data sharing statement to be made available at a later date

IPD sharing plan summary

Stored in publicly available repository, Available on request, Data sharing statement to be made available at a later date

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