FULL/LONG TITLE OF THE STUDY

The effects of time-restricted eating (TRE) with and without additional protein on body composition, muscle function and markers of metabolic health in older adults

SHORT STUDY TITLE / ACRONYM

Time-restricted eating (TRE) and protein in older adults

PROTOCOL VERSION NUMBER AND DATE

Version 1.1

Date: 30/09/2025

RESEARCH REFERENCE NUMBERS

IRAS Number: if applicable	361019
SPONSORS Number:	SPON 2025-15
FUNDERS Number:	This research was funded by UKRI BBSRC FoodBioSystems Doctoral Training Partnership (DTP), grant number BB/T008776/1.

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KEY STUDY CONTACTS

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Funder(s)	UKRI BBSRC FoodBioSystems Doctoral Training Partnership (DTP) funded by the Biotechnology and Biological Sciences Research Council (BBSRC) - foodbiosystems@reading.ac.uk
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Committees	N/A

STUDY SUMMARY

Study Title	The effects of time-restricted eating (TRE) with and without additional protein on body composition, muscle function and markers of metabolic health in older adults
Internal ref. no. (or short title)	Time-restricted eating (TRE) and protein in older adults
Study Design	Randomised controlled parallel trial
Study Participants	Adults aged 60+
Planned Size of Sample (if applicable)	Minimum of 17 participants per study group, 4 study groups in total
Intervention duration	8 weeks

Planned Study Period	October 2025 - July 2027
Research Question/Aim(s)	The proposed study aims to investigate the effects of 8-week TRE with and without additional protein on body composition, muscle function and markers of metabolic health in older adults.

INVESTIGATORS

NAME	**Position**
Yana Petkova	**PhD student**
Dr Adam Collins	**Associate Professor of Nutrition**

STUDY FLOW CHART

Procedures		*Visits (insert visit numbers as appropriate)*					
	Screening	*Baseline*	*Week 1*	*Week 2*	*Week 4*	*Week 6*	*Week 8*
Informed consent		*x*					
Demographics	*x*						
Medical history	*x*						
Diet history	*x*						
Blood sample		*x*					*x*
DEXA scan		*x*					*x*
Grip strength		*x*					*x*
30s sit-to-stand		*x*					*x*
Food diary		*x*			*x*		*x*
Intervention			*x*	*x*	*x*	*x*	*x*
Interview							*x*

STUDY PROTOCOL

The effects of time-restricted eating (TRE) with and without additional protein on body composition, muscle function and markers of metabolic health in older adults

1 BACKGROUND

List of abbreviations

TRE – time-restricted eating

lTRE – late time-restricted eating, where the eating window is positioned in the afternoon

16:8 TRE – time-restricted eating where the duration of the eating window is 8 hours and the fasting window 16 hours

Background

The number of people aged 60 years or over is expected to double in the next two decades (1). In the UK, more than half of adults aged over 65 are overweight or obese (2). The high rates of overweight and obesity in this population are associated with a raised risk of non-communicable diseases such as cardiovascular disease and some cancers, and a lower quality of life (3). At the same time, age-related adverse metabolic changes such as a decline in muscle mass and function (sarcopenia), lower efficiency in nutrient utilization and insulin sensitivity, are associated with frailty, loss of mobility and independence, and mortality (4, 5).

Thus, strategies to promote healthy ageing are of significant interest amongst academics, policymakers and the public alike, driven by the growing recognition of the importance of extending healthspan alongside lifespan. In recent decades time restricted eating (TRE) – an eating pattern characterised by an extended fasting window of >14 hours – has emerged as a promising approach for weight loss and metabolic health (6). In human trials, TRE has been associated with small to moderate weight loss and fat mass reduction (7), improvements in fasting and postprandial glucose (8-13), insulin sensitivity (11, 13, 14) and blood pressure (13-17).

TRE in older adults

The majority of human TRE research to date has been conducted in young to middle-aged populations. However, a small number of studies with older adults have shown that TRE is safe and feasible in the short-term (10, 18-21). In a pilot study in generally healthy older adults with a normal body mass index (BMI), Martens at al. (10) found that 6 weeks of 16:8 TRE resulted in lower hunger levels, improved glucose tolerance and functional (endurance) capacity. There were no associated changes in body weight and composition, in line with the study design which included advice on maintaining habitual energy intake. Low-density lipoprotein (LDL) and total cholesterol levels increased moderately following TRE, however, this was not accompanied by changes in oxidised-LDL and other markers of cardiovascular function.

In a series of randomised controlled trials in overweight older men and women by Domaszewski et al. (18, 19), 16:8 lTRE resulted in weight and fat mass loss, without significant changes in fat-free and skeletal mass. In a recent study using the same TRE protocol, Domaszewski et al. (20) reported gender-specific effects of the intervention – despite all participants losing weight, there was a significant reduction in visceral fat and waist circumference in the male participants only. In an earlier

pilot study, Anton et al. (21) reported good tolerance and an increase in walking speed after 4 weeks of 16:8 TRE, as well as significant weight loss in a small sample of sedentary older adults with overweight.

Beyond the replicated favourable effect on weight and body composition in the studies conducted by Domaszewski et al. (18-20), there is a lack of follow up to replicate the metabolic and functional benefits observed in the pilot studies by Anton et al. (21) and Martens et al. (10) Moreover, diet composition and quality, which are closely related to body composition and metabolic health, have not been examined in older adults undertaking TRE.

Among young to middle aged adults, results regarding changes to diet quality are mixed. Although some studies assessing macronutrient composition and diet quality have not found a change with TRE (11, 22, 23), others have reported protocol-specific effects (13). Zhang et al. (13) compared early- and late TRE and found that early TRE was associated with a significant reduction in carbohydrate and protein intake compared to control, while late TRE resulted in a lower fat intake compared to early TRE and control. Interestingly, mean protein reduction in early TRE was ~20g/day, compared to ~15g/day in late TRE. Although the latter was not significantly different from control, in practice a reduction in protein intake of 15g per day could have significant implications on the nutritional status in populations with a low baseline protein intake or high protein requirements, such as older adults.

Brief description of the proposed study and population being studied

This randomised controlled trial will compare the effects of 8 weeks of 16:8 ITRE with and without additional protein on body composition, muscle function and markers of metabolic health of a sample of generally healthy adults aged over 60. It will also examine whether being allowed a protein 'snack' before the 8-hour eating window starts would affect the feasibility and compliance with TRE. Participants will be randomly allocated to one of four groups: control/no intervention, TRE, TRE with additional protein in the afternoon, during the eating window, and TRE with protein ingested in the morning, before the first meal of the day. Body composition and biochemical markers of metabolic health will be measured at baseline and at the end of the 8-week intervention. Feasibility and participants' subjective experience of their assigned protocol will be assessed using semi-structured interviews at the end of the study.

2 RATIONALE

The proposed study aims to address the following research questions:

- Does the addition of 30g protein per day during 8 weeks of TRE preserve lean body mass and muscle function whilst maintaining the metabolic benefits associated with TRE in a sample of older adults?
    - *Rationale:* Age-associated physiological changes resulting in anabolic resistance to protein ingestion, combined with a higher prevalence of reduced physical activity and appetite are thought to underlie a greater need for protein in later life (5). Expert groups have recommended a higher protein intake at 1-1.2g/kg/d than the current RDA of 0.8g/kg/d for older adults (5, 24). Similarly, several studies in older adults using the indicator amino acid oxidation (IAAO) method have suggested a protein requirement of 1.1-1.3 g/kg/d (25). These recommendations have not met with universal agreement and discourse amongst experts is ongoing (26).

In a recent systematic review and meta-analysis Hudson et al. (27) demonstrated that, although protein intake at the current RDA (~0.8g/kg/d) may be sufficient to maintain lean mass in a steady state, intake above the RDA (~1.3g/kg/d) attenuated lean mass loss under conditions of energy restriction. The latter is particularly relevant for older adults undertaking TRE, as TRE is associated with a spontaneous reduction in energy intake (and therefore potentially protein intake) in free-living conditions.
It's important to interpret the research described in this section in the context of current protein intake. Morris et al.(28) analysed data from dietary recalls in a large sample of older adults in Yorkshire and found that over a third consumed less protein than the current RDA of 0.8g/kg/d and 85% consumed less than the expert group recommendations of 1.2g/kg/d. In a recent cross-sectional study, Mao et al. (29) assessed the effect of chononutrition behaviours on muscle in older adults and reported protein intake at 0.94g/kg/d on average, but significantly lower at 0.88g/kg/d in those whose eating window was shorter than 10.3 hours, i.e. who are engaging in a form of TRE spontaneously.

- Further to the above, this study aims to answer the question of whether being allowed a protein 'snack' in the morning, 2 hours before the 8-hour eating window starts, would exert a differential effect on compliance, body composition, muscle function and markers of metabolic health compared to consuming additional protein as a 'snack' between meals in the afternoon.
    - Rationale: A more even protein distribution across the day has been linked with greater muscle strength and mass over time in a large cohort of older adults (30).Moreover, a longer feeding window has been associated with greater muscle mass in a sample of community dwelling older adults (29). When ingested in the absence of other macronutrients, protein is unlikely to 'flip the metabolic switch' from fasted to a fed metabolic state at a whole-body level (publication pending peer review). Thus, a protein 'snack' 2 hours before the start of the eating window may preserve the benefits of a 16-hour fast while conferring an additional advantage in the context of body composition and muscle function.

The study duration was determined by existing TRE research showing consistently that 6-8 weeks are sufficient to detect a change in fat mass (13, 18-20, 23). Results regarding lean mass are mixed, with some studies of this duration reporting significant change (13, 23) while others a lack thereof (18-20).

3 RESEARCH QUESTION/AIM(S)

Broad aim:

The proposed study aims to investigate whether the addition of protein to TRE has a beneficial effect on body composition, muscle function and markers of metabolic health in older adults, compared to TRE without additional protein.

Specific aims:

The primary aim of this study is to assess the effects of consuming additional 30g protein within and outside of the 8-hour eating window during 8 weeks of ITRE on body composition and muscle function and markers of metabolic health in a sample of older adults.

Secondary study aims include assessing the effect of 8 weeks of TRE with and without additional protein on markers of metabolic health including lipids, glucose control and insulin sensitivity.

Tertiary study aims include:

- assessing whether the addition of protein would affect compliance with and the long-term feasibility of TRE in this population group
- assessing and comparing changes in diet composition and quality between the study groups

3.1 Objectives

To compare the effects of 8 weeks of ITRE with and without additional protein on:

- Body composition, specifically fat-free mass/skeletal muscle mass
- Muscle function and strength:
  - Grip strength
  - 30-second sit-to-stand
- Markers of metabolic health including:
  - Lipids: TAG, TC, LDL, HDL
  - HbA1c
  - HOMA-IR
- Compliance with TRE
- Diet composition and quality

To compare the effects of additional protein consumed 2 hours before the start of the 12pm-8pm eating window and 2 hours before the last meal within the 12-8pm eating window on:

- Body composition, specifically fat-free mass/skeletal muscle mass
- Muscle function and strength:
  - Grip strength
  - 30-second sit-to-stand
- Markers of metabolic health including:
  - Lipids: TAG, TC, LDL, HDL
  - HbA1c
  - HOMA-IR
- Compliance with TRE
- Diet composition and quality

3.2 Outcome

- Primary study outcome: change in body composition and muscle function
- Secondary study outcome: change is triglycerides, cholesterol, glucose control and insulin sensitivity, and diet quality
- Tertiary study outcome:
  - difference in self-reported compliance and long-term feasibility of the different TRE protocols
  - changes in diet composition and quality between baseline and end of the intervention

4 STUDY DESIGN and METHODS of DATA COLLECTION AND DATA ANALYIS

Study design and groups

The proposed study will follow a randomised controlled parallel group design. Eligible participants will be allocated to one of four groups using a stratified approach:

- Control group
  - Participants will be instructed to maintain their habitual eating pattern for the duration of the study
- TRE
  - Participants will be asked to follow a 16:8 TRE protocol, consuming all their daily energy intake within 8 hours, between 12 and 8pm, and fast for 16 hours overnight.
  - Outside of the eating window, participants will be allowed to consume energy-free drinks only, i.e. water, black tea/coffee, 0-calorie soft drinks.
- ProtPM TRE (TRE + afternoon protein 'snack')
  - Participants will be asked to follow a 16:8 TRE protocol, consuming all their daily within 8 hours, between 12 and 8pm, and fast for 16 hours overnight.
  - Outside of the eating window, participants will be allowed to consume energy-free drinks only, i.e. water, black tea/coffee, 0-calorie soft drinks.
  - In addition, they will be asked to consume a protein drink containing 30g protein in the afternoon, either 2 hour after their first meal or 2 hours before their last meal of the day.
- ProtAM TRE (TRE + morning protein 'snack')
  - Participants will be asked to follow a 16:8 TRE protocol, consuming all their daily within 8 hours, between 12 and 8pm, and fast for 16 hours overnight.
  - Outside of the eating window, participants will be allowed to consume energy-free drinks only, i.e. water, black tea/coffee, 0-calorie soft drinks.
  - In addition, they will be asked to consume a protein drink containing 30g protein in the morning, 2 hours before the 8-hour eating window opens at 12pm (i.e. at 10am).

Figure 1 illustrates the study timeline.

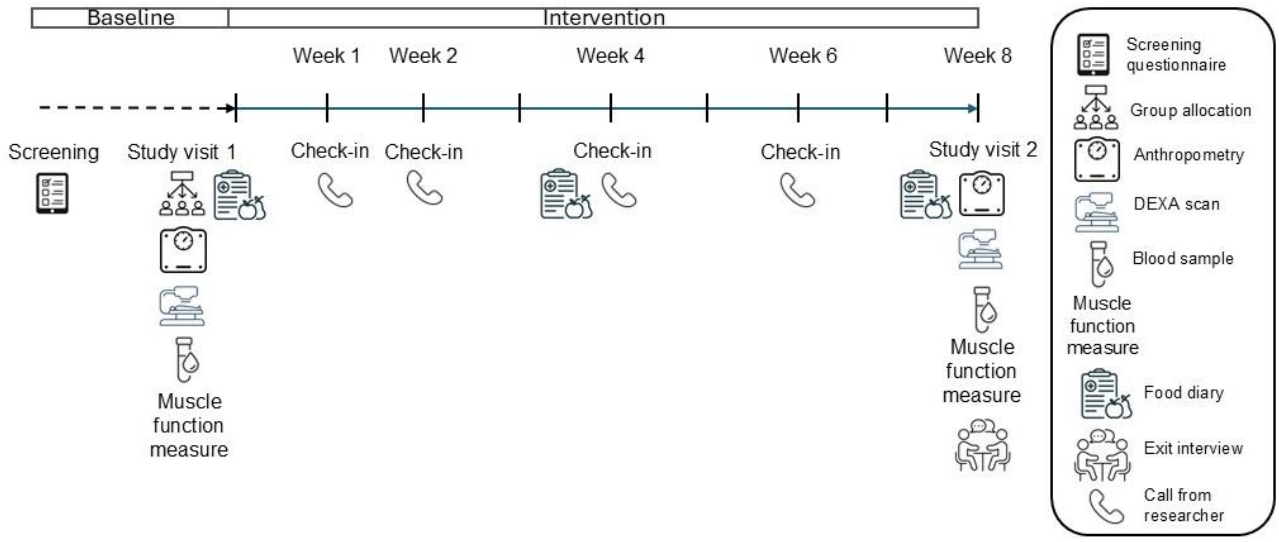

Figure 1 Study overview

Before the first study visit

Upon meeting the eligibility criteria and consenting to participate, participants will be approached by the researcher via phone/email to agree on the date of their study visits. They will be instructed to fast for 12 hours prior to their visit. All study visits will be scheduled in the morning between 8am-11am to standardise fasting duration. Participants will also be asked to note their evening meal on the day prior to their visit, so that this can be replicated in the evening before their end-of-study visit.

Study visit 1: initial study visit

Participants will arrive in the Human Research Unit (HRU) at the University of Surrey in the morning of the day of their study visit. They will be given the opportunity to ask questions, and following this, asked to sign a consent form, which they will be provided with a copy of. Participants' height and weight will be measured, and their BMI calculated. Participants will be asked to perform two validated measures assessing muscle strength and functional ability (grip strength and 30-second sit-to-stand). They will be instructed on how to perform these measures by a trained member of staff. A researcher trained in phlebotomy will then take a 10ml venous blood sample. Finally, participants will undergo a DEXA scan, carried out by a trained member of staff who possesses an IR(ME)R certification for body composition scanning. A whole-body DEXA scan will be used to assess lean, fat and bone mass.

Once all baseline measurements are concluded, participants will be told their study group allocation and receive verbal and written instructions accordingly. Those randomised to the ProtAM or ProtPM groups will receive verbal and written (including pictorial) instruction on how to prepare the protein drink and asked to drink it in front of the researcher to ensure palatability and compliance with the study. Participants will also be instructed on how to complete their food diaries. All participants will be asked to follow their assigned group protocol for eight weeks.

All participants, regardless of group allocation, will receive general healthy eating advice from a dietitian or dietetic student in line with the Eatwell guide (31).

At the end of the study visit, a date for the end of study visit will be agreed with the participants, and dates/times for the mid-study check-ins set. These will be written in an individual timetable, which participants will receive a copy of. In addition, calendar invites and email reminders will be set by the researcher. Finally, participants allocated to the ProtAM or ProtPM study groups will be provided with a pack containing the protein supplements and a shaker of the same model they practiced preparing the supplement with.

Study visit 2: End-of-study visit

Participants will be asked to arrive at the HRU at the same time as their initial study visit. Participants' height and weight will be measured, and their BMI calculated. A researcher trained in phlebotomy will then take a 10ml venous blood sample. Participants will undergo a DEXA scan and perform the same muscle strength and functional measures as in their first study visit. Finally, participants will be asked about their experience of their assigned study group in a semi-structured interview.

Mid-study check-ins

Participants will receive a phone call at the end of weeks 1, 3 and 6 from the study researcher. During the call, participants will be asked about their compliance with and tolerance of their assigned protocol and offered the opportunity to ask questions and discuss any issues. The frequency of check-ins was

set based on findings from Lee et al. who concluded that one mid-study follow up may be inadequate to ensure understanding and compliance with a TRE protocol.

Throughout the study

Participants will be asked to complete a short daily survey with the start and end time of their eating window as a measure of compliance with their assigned protocol. Those allocated to the ProtAM and ProtPM groups will also answer a question on the timing of their protein drink intake. A separate survey will be set up for each participant by the researchers using Microsoft Forms and a link emailed to them after their first study visit. They will then be asked to set a calendar reminder to complete the survey each evening for the duration of the 8-week intervention. Participants will be instructed on how to use this during their initial screening visit.

Study measures and materials

Protein supplement

The protein supplement used in this study is a commercially available vegan protein powder (Form® Pureblend), with added calorie-free flavour drops for palatability. This supplement was chosen based on unpublished data showing that when consumed after a 12 hour fast, the combination of brown rice, pea and pumpkin seed protein causes a smaller change in fasting metabolites compared to whey protein powder. Moreover, we found that adding sweetener and flavouring via calorie-free flavour drops to the vegan protein blend resulted in comparable metabolic effects.

The daily protein drink will be prepared by mixing 40g of protein powder (to provide 31g protein) with 300 ml cold water, using a shaker provided by the researchers for participants' convenience. Details of the ingredients and nutritional composition of the protein drink (per 40g daily serving) are summarised in the table below.

Ingredients	Energy (kcal)	Protein (g)	Carbohydrate (g)	Fat (g)
Organic Pea Protein Isolate, Brown Rice Protein, Pumpkin Seed Protein	156	31	0	2.2

Body composition

DEXA is considered the gold standard method for accurate body composition measurement in clinical research (32). Whole body composition including lean mass, body fat and bone density (not diagnostic) will be measured at baseline and at the end of the interventions using dual-energy x-ray absorptiometry (DEXA) using the facilities in AX00. Scans will be performed by a member of staff trained and certified in DEXA operation.

Body composition will also be measured using a Bioelectrical Impedance Analysis (BIA) digital scale (TANITA, NL) - an indirect, non-invasive measure based on body water estimation through resistance to a small alternating current (32). This will allow for a comparison between the two methods, providing an additional assessment of accuracy of the body composition measurements.

Blood samples

Trained staff members will take a venous blood sample at baseline and at the end of the interventions as outlined in the study protocol. A sample of 10ml of blood will be taken for analysis of fasting triglycerides, total, LDL- and HDL cholesterol, glucose, HbA1c, insulin and creatinine. The creatinine results will be used to calculate eGFR as a measure of kidney function, while glucose and insulin will be used to calculate HOMA-IR to assess insulin sensitivity.

The total volume taken from each volunteer over the entire study will be 20ml (10ml per study visit). Collected whole blood samples will be centrifuged in order to separate plasma for storage, and any remaining blood cells will be disposed of in the designated clinical waste bin, which will be disposed of within 7 days. Blood tubes used during the study will be labelled by participant number only to maintain participant anonymity. All samples will be handled in accordance with the Human Tissue Act 2004 in a designated HTA licensed lab at the University of Surrey.

Muscle function

Muscle function and strength will be assessed using a combination of two validated measures – grip strength and 30-second sit-to-stand. All muscle strength and function measured will be administered by a trained member of staff.

Grip strength will be conducted using a standardised protocol recommended by the American College of Sports Medicine (ACSM). The participant will be instructed on the correct positioning (upright, holding the handgrip dynamometer down by their side, ensuring there is a slight bend in the elbow and the dynamometer is not touching the body). They will be asked to squeeze the handgrip dynamometer with as much force as possible and repeat this procedure twice on the left hand and twice on the right hand, with a rest in between each attempt.

30-seconds sit to stand will be performed using a straight-backed chair with the set 17inches high. Participants start from a sitting position, holding their hands against their chest. On ‘Go’ they will rise to a full standing position and then sit back down again. This will be repeated for 30 seconds and the number of times the participant comes to a standing position counted by a researcher with a stopwatch.

Anthropometry

Participants height and weight will be measured at the start of each study visit. Height will be assessed using a standing scale and weight using a digital scale (TANITA NL). The same scale will be used at each visit to ensure consistency.

Diet composition

Participants will be asked to complete a 3-day food diary at baseline, week 4 and week 8 of the study, using a food diary template from previous research at the department of Nutritional Sciences at the University of Surrey. Food diaries will be processed by trained dietitians and nutrition and dietetic students and analysed using nutrition analysis software (Nutritics). Participant number, age and anthropometry will be entered in the platform, alongside dietary intake information from their food diaries. No personal data will be entered into the platform. A participant’s data will be deleted, should they withdraw from the study. Information from the food diaries will be used to assess macro- and micronutrient intakes at each collection time point.

Adherence

A short daily survey asking participants to note the start and end time of their eating window will be set up for each participant using Microsoft Forms to assess adherence to their assigned protocol, similarly

to that used by Martens et al. (10). Days in which a participant reported an eating window of =/<8hrs will be marked as compliant with TRE. For those assigned to the ProtAM or ProtPM groups, an additional survey question will ask participants to note the time they had the protein supplement.

Exit interviews

During their final study visit, participants will be asked about their experience of engaging in their assigned protocol in a semi-structured interview. Semi structured interviews have been used in a similar manner in existing TRE feasibility studies (33, 34). Participants will be asked pre-prepared questions, and their answers will be recorded via voice recorder device to be transcribed later. These sessions will be confidential, and data will be stored anonymously. The recordings will be kept maximum of 2 years after transcription in case the data needs to be re-assessed.

Statistical analysis

Data will be checked for normality, and appropriate parametric and non-parametric tests will be caried out using SPSS Statistics (IBM). Within participant analysis (pre- and post) will be conducted to assess whether the assigned study protocol affected weight, body composition, muscle function, diet composition and serum biomarkers. Between group analysis will be used to assess any difference between the study groups. Interviews will be transcribed and analysed using thematic analysis.

Definition of End of Study

The study will end once all plasma samples have been analysed. The target timeframe for completing the plasma samples analysis will be within 3 months of the final participant's end-of-study visit

5 STUDY SETTING

The proposed study will be conducted at the University of Surrey campus. Study visits will take place at the Human Research Unit in building AX on Stag Hill campus, which contains the relevant equipment such as a 'ward' with hospital beds and bedside tables for blood sample collection, BIA scales, DEXA scanner and lab for processing samples.

6 SAMPLE AND RECRUITMENT

6.1 Eligibility Criteria

Interested participants will be asked to complete an online survey to access eligibility based on the criteria outlines in the following sections.

6.1.1 Inclusion criteria

- Gender: male and female
- Age range: 60 years or older
- Fasting for 12 or less hours on a daily basis
- BMI range: 23-30 kg/m^2
- Weight stability: no more than 3kg weight gain or loss in the past 3 months

- Regular eating pattern, i.e. 3 meals a day
- Able to prepare the protein supplement

6.1.2 Exclusion criteria

- Any of the following present (in line with exclusion criteria used in previous TRE research in older adults (Anton et al.(21)):
    - known renal impairment
    - heart attack or stroke in the past three months
    - continuous use of supplemental oxygen to manage a chronic pulmonary condition or heart failure
    - rheumatoid arthritis
    - Parkinson's disease
    - active treatment for cancer in the past year
    - insulin dependent diabetes mellitus
- taking medications that preclude fasting for 16 h
- actively trying or planning to lose weight
- history of an eating disorder
- outside of stated age or BMI range
- fasting for longer than 12 hours on a daily basis
- unable to prepare the protein supplement
- extreme morning/evening chronotype
- sleep disorders
- regular meal skipping
- history of difficulty having a blood sample taken
- Recent exposure to high-dose radiation
- Vigorous exercise on more than 3 occasions a week

6.2 Recruitment

6.2.1 Participant identification

Participants will be recruited through posters advertising the study. Posters will be put up around the University of Surrey campus, cafes and shops in Surrey, local social clubs and charities such as Age UK Surrey. In addition, an email advertising the study will be circulated within the School of Biosciences, following relevant gatekeeper approval.

Interested participants will be able to get in touch with the study investigators using the contact details provided in the study advertising materials. They will then be emailed a copy of the Participant Information Sheet and invited to schedule a telephone call with the study investigator to discuss the nature and objectives of the study and possible risks associated with their participation. This will also be

an opportunity for interested individuals to ask questions. Those interested in participating after the phone call will be emailed a link to a series of digital screening questions, including:

- Participant demographics – gender, age, ethnicity
- Participant anthropometry – height, weight
- Self-reported health status questionnaire
    - Compiled by the study investigators with the aim of screening for the presence of any conditions and medications which may preclude participation, as outlined in the Inclusion/Exclusion criteria
- Diet history questionnaire
- Munich Chronotype Questionnaire
- Pittsburgh Sleep Quality Index questionnaire
- Physical activity: The Godin and Shephard leisure-time physical activity questionnaire

Completed screening questionnaires will be reviewed by the study investigators and individuals notified of the outcome via email. Eligible participants will be invited to schedule their initial study visit.

Study power

In order to detect a difference of 1kg change in skeletal muscle mass using a two-sided test with size = 5% and power = 80%, on the basis of the change having a standard deviation (SD) of 1kg, at least 17 participants will be required in each treatment group. A similar ratio of change to SD has been used in research on protein intake and body composition in older adults conducted by Mitchell et al. (35).

Participant benefits

All participants will receive healthy eating advice in line with government recommendations delivered by a qualified or student dietitian.

Participants allocated to the TRE study groups will have the chance to trial a strategy for metabolic health under supervision.

All participants will have the opportunity to find out their body composition (i.e. muscle mass, fat mass, bone density (non-diagnostic), as well as visceral fat in a report which they can receive at the end of their participation should they wish to. They will also have the option to opt in to receive a dietary analysis based on their food diaries once the study data has been processed, including macro- and micronutrient intakes.

Participants will have the opportunity to enter a prize draw for a chance to win 1x £200, 1x £100 or 2x £50 voucher. The value of the prizes is based on funding available for this project. The prizes will be drawn as soon as the study has concluded on July 30th 2027. Entry to the prize draw is optional and independent of completing the study.

6.2.2 Consent

Interested individuals will be emailed a copy of the participant information sheet outlining the details of the study and offered the opportunity to ask any questions before being invited to complete the screening questionnaires.

At the start of the first study visit, the investigator will go over the study protocol with the participants again, checking that they fully understand what their participation will involve and answering any questions they may have. Participants will then be asked to sign two copies of an REC-approved written consent form – they will receive a copy, whilst the second one will be kept with their study records and data.

If there is any uncertainty of a participant's ability to understand and retain the relevant study information, they will be asked to withdrawal from the study instead.

In the (unlikely) event of a loss of capacity, the research team would retain tissue and personal data collected and continue to use it confidentially in connection with the purposes for which consent is being sought.

6.2.2 Withdrawal of Consent

Prospective participants will be informed in writing in the Participant Information Sheet and in the Consent Form that they have the right to withdraw from the study by informing the investigator verbally or in writing. They will be asked to inform the researcher of their intention to withdraw within one month after completing their last study visit, as their samples are likely to have been processed and analysed after ~1 month. Participants will be further be asked their permission to use data collected prior to their withdrawal.
Participants will be withdrawn by the investigator if:

1) A participant experiences an adverse reaction to the intervention and/or intervention products (i.e. severe allergic reaction or gastro-intestinal intolerance)
2) The participant develops a medical condition either prior to entering or during the study, which may compromise their safety when undertaking the intervention and/or adversely affect the outcome of the study.
3) A participant is non-compliant with their assigned protocol as evaluated during the mid-study check-ins in weeks 3 and 6

Participants who withdraw will be replaced in order to reach a minimum target number of 17 participants per study group who have completed the study.

7 ETHICAL AND REGULATORY CONSIDERATIONS

7.1 Assessment and management of risk

DEXA

Study participants will undergo two DEXA scans within 8 weeks. They will be exposed to a minor amount of ionising radiation – 0.01 mSv per scan, which is equivalent to 1 day's natural background radiation. Participants will have two DEXA scans – the cumulative effective radiation dose will be 0.02 mSv. Therefore, the radiation exposure participants will receive is negligible, given that the average annual radiation exposure from normal background sources is ~2.7 mSv.

The results from the DEXA scan will inform participants about their body fat, lean mass and bone density (although not diagnostic). Furthermore, the Hologic Horizon DEXA scanner at the University of Surrey provides an estimate of visceral fat, which is well correlated with CT scanning. Increased visceral fat is associated with multiple chronic diseases including heart disease, type 2 diabetes and stroke. Consequently, the research team feel that the data generated from the DEXA scans in this study will be valuable for our participants to assess their body composition and overall health. We feel that the benefit of this information far outweighs the radiation risk. DEXA scans will be carried out in accordance with the Ionising Radiation (Medical Exposure) Regulations 2017.

Summary of ethical considerations

Identified risks	Likelihood	Potential impact	Risk Management/Mitigating Factors
Discomfort upon blood sample collection	Possible	Participant: Physical and visual discomfort, pain, tenderness, bruising, allergic reaction to dressings, light-headedness/ dizziness, fainting	Participants will be asked if they had any previous difficulties, or fainting episodes when having blood taken. All blood sample collection will be performed by a trained researcher whilst participants are positioned semi-supine on a bed. Some minor bruising can occur on the site of the blood draw which may feel sensitive for a few days. All participants will be clearly informed about this. The site of sampling will also be such that it will reduce the likelihood of bruising and cause minimal discomfort to participants Researchers performing cannulation will be trained and follow the relevant SOPs, and signed off as competent by senior clinical staff in the Clinical Research Facility Good hygiene standards will be maintained at all times.
Adverse reaction to intervention products (protein supplement)	Unlikely	Participant: allergic reaction, food intolerance	All participants will be screened prior to the study to ensure they do not have any food allergies or intolerances All protein supplements used in this study are commercially available and thus regulated, and prepared in the University of Surrey teaching kitchen by staff that

			have a Level 2 food hygiene safety qualification.
Adverse reaction to the intervention (TRE)	Possible	Participant: Light-headedness, dizziness, sickness, headache during the fasting window	All participants will be encouraged to consume plenty of water during the fasting window to stay hydrated, which has previously been shown to resolve some adverse reactions to TRE (21). Participants will be advised to contact the investigators in the event of an adverse reaction, so that this can be reviewed and an appropriate mitigating strategy can be agreed on. Where mitigation may not be possible, individuals may be asked to discontinue their participation.
Biological hazard	Unlikely	Staff: Contact with infected blood	All participants will be screened prior to taking part and are unlikely to be carrying an infection as they will have been advised not to take part if they are aware of any infections Investigators collecting and handling blood samples will all receive appropriate lab training for the task and be Hep B vaccinated. Appropriate PPE will be available for all staff to wear.
Ionising radiation exposure (DEXA)	Very likely	Researcher, participant	The DEXA operator will be positioned >1 meter away from the DEXA machine during scanning; therefore, minimising radiation exposure. The manufacturer's data indicates that in a typical whole-body scan, the participant will receive an effective dose of 0.01 mSv per scan, equivalent to 1 day's natural background radiation. The fan beam scanner ensures a fast scan time with as low as possible radiation dose. Participants will complete a DEXA specific consent form prior to each scan. Participants will declare they have not had an MRI or CT scan involving contrast by mouth or intravenous (radio-opaque dye) in the two days prior, or a barium meal in the three days prior and they have not had a DEXA scan in the 8 weeks prior.
Sample transportation	Unlikely	Environment contamination	Plasma samples will be transported to the laboratory in the same building (AX-AY) to be stored in sealed unbreakable boxes. Aliquoted plasma samples will be double contained; screw cap microtubes and cryoboxes.

			All samples will be clearly labelled at every step from collection to analysis. Transport/storage boxes will have the necessary information about the samples such as, contents of the box, number of samples, collection date, contamination risks, names of researchers and supervisors, study code etc.
Data loss/breach	Unlikely	Loss/corruption of research data; participant privacy breach	Any digital participant personal information will be stored on a password-protected file on a password-protected computer Any hard copy research and participant data will be stored in a locked cupboard in a locked room with limited access

7.2 Research Ethics Committee (REC) and other Regulatory review and reports

7.2.1 Approvals. Before the study commences a favourable opinion on the protocol and associated documents will be sought from the UK Health Departments Research Ethics Service, the HRA, and confirmation of capability and capacity from NHS sites where necessary. All correspondence with the REC will be retained.

7.2.2 Amendments. Should any amendments to the protocol be required the researcher will contact the University RIGO office (Sponsor) to determine if its substantial or non-substantial, upon clarification of the amendment categorisation, the researcher will submit the amendment as per current HRA practice, the amendment will not be implemented until all approvals have been completed.

7.2.3 Annual Progress reports. An annual progress report (APR) will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the study is declared ended following HRA current practice

7.2.4 The researcher will notify the REC of the end of the study with 90 days of the last data collection point using HRA current practice.

7.2.5 End of study report. Within one year after the end of the study, the researcher will submit a final report with the results, including any publications/abstracts, to the REC.

7.3 Supervisor/ Peer review

This study protocol has been reviewed by the investigator's supervisors who hold academic positions in the fields of nutrition and chronobiology at the University of Surrey. It has also been reviewed by the Research, Innovation and Impact Assurance team at the University of Surrey.

7.4 Patient & Public Involvement (optional but recommended)

The conception of this study did not involve any patient or public involvement. We aim to disseminate this research in a variety of ways to allow members of the public to learn about our findings. We hope to publish our results in peer-reviewed journals and present our results at relevant meetings and conferences.

7.5 Protocol compliance, adverse events reporting and breaches

Protocol deviations, non-compliances, or breaches are departures from the approved protocol.

7.5.1 Minor protocol deviations will be documented on the relevant forms and reported to the Chief Investigator or supervisor and Sponsor.

7.5.2 Adverse events will be discussed with the researcher's supervisor and the sponsor, adverse events will be documented in the trial files. Serious adverse events will be reported to the Sponsor as per the sponsor SOP.

7.5.3 Serious Breaches of the protocol, where a participant has been put at risk by a deviation from the approved protocol will be reported to the sponsor as per SOP

7.6 Data protection and patient confidentiality

The following guidelines for data handling will be adhered to:

- All personal data related to subjects will be held in accordance with the General Data Protection Regulation (2018).
- All samples and data will be anonymized by assigning each subject with a unique subject ID number.
- All electronic data will be stored on a password protected computer and database.

The key linking participant personal details with their identification number will be kept in a folder separate from other study data on a password-protected laptop for the duration of the study. Once the study has concluded, the key will be deleted.

Data collected during screening of participants who do not meet the inclusion criteria will be deleted once the individual has been informed of the outcome of their expression of interest.

Any paper data forms will be kept in a locked cupboard in a locked room 28AY03 for the duration of the study. The room can be accessed by a limited number of postgraduate students and the researcher is the sole person with access to the cupboard where the study data will be held. All paper data will be scanned to store digitally on a password protected laptop and then destroyed using confidential paper waste bins.

Any personal details collected to compensate participants (i.e. email address for online vouchers) will be kept separate from research data in an Excel spreadsheet stored on a password-protected computed and deleted one distribution has taken place.

7.6 Indemnity and Insurance

The sponsor has in place relevant insurance for the design, conduct and the management of the study. The sponsor has arrangements in place for payment of compensation in the event of harm to the research participants where no legal liability arises.

7.8 Access to the final study dataset

Access to the full dataset will be limited to the named study investigators, namely Y. Petkova, A. Colins, and potential final year BSc and MSc students who may be analysing a subset of the data as part of their final year projects.

8 DISSEMINIATION POLICY

8.1 Dissemination policy

Any data generated from this study is owned by the University of Surrey. The PhD thesis will constitute the final study report. This is open access and due to be completed by end of 2027.

This research will be submitted for publication in scientific journals and presented at scientific meetings and/or conferences. Any published findings will maintain participants' confidentiality and anonymity.

Any publication will need to include the following acknowledgement, as required under the conditions of the studentship funding Y. Petkova's PhD research: 'This research was funded by UKRI BBSRC FoodBioSystems Doctoral Training Partnership (DTP), grant number BB/T008776/1.'

8.2 Authorship eligibility guidelines and any intended use of professional writers

The principal investigator and supervisors will be granted authorship of the final report. Other contributors to the research such as BSc or MSc students or researcher assistants may be granted authorship or would be included in the acknowledgements section of the published manuscript.

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10 Protocol History

Amendment No.	*Protocol version no.*	*Date issued*	*Author(s) of changes*	*Details of changes made*
1	1.1	30/09/2025	Yana Petkova	Study location: CIU changed to HRU as unit name updated