TRIAL PROTOCOL

PRODREX

The effects of dietary protein distribution on the responsiveness to resistance exercise training in older adults

Reference numbers for the trial can be included in the table below. Examples of the reference numbers that could be included are given. Add/delete as appropriate. Note that for CTIMPs the Sponsor reference number must be listed in the protocol.

EudraCT number (CTIMP only)	
Sponsor reference number	
ISRCTN number	
REC reference number	

Protocol development and sign off

Protocol Contributors

The following people have contributed to the writing of this protocol:

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CI Signature Page

This protocol has been approved by:

Trial Name:	The effects of dietary protein distribution on the responsiveness to resistance exercise training in older adults
Protocol Version Number:	Version: _6.0_ __
Protocol Version Date:	10/04/17
CI Name:	Danielle Thomas
Trial Role:	Chief Investigator
Signature and date:	 _____ __ 10/04/17

Sponsor statement:

Where the University of Birmingham takes on the sponsor role for protocol development oversight, the signing of the IRAS form by the sponsor will serve as confirmation of approval of this protocol.

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TRIAL SUMMARY

Title

The effects of protein distribution and resistance exercise training on the muscle of older adults

Trial Design

Exploratory randomised trial and feasibility trial

Objectives

Principal objective: Investigate the effect of dietary protein distribution on muscle protein synthesis (MPS) over two weeks, with and without RET, in the thigh muscle of older adults

Secondary objectives:

(i) Investigate whether protein distribution influences serum inflammatory markers

(ii) Consider the feasibility of manipulating daily protein distribution using diet plans, and investigating participant compliance to the diets

(iii) Determine whether the effect of RET on muscle strength is influenced by protein distribution

(iv) Investigate whether changes in responsiveness of MPS predict changes in muscle strength

Participant Population and Sample Size

We will recruit women aged 65 years or over and ambulatory (with or without walking aid) from the Birmingham 1000 Elders database, and through local community groups and leaflets in public areas

As an exploratory and feasibility trial, a power calculation to determine sample size has not been completed due to lack of previous data. A sample size of n=10-12 has been decided upon for each group, giving a total sample size of n=20-24.

Outcome Measures

All outcome measures will be made by members of the study team (i.e., research nurses, the PhD student)

The primary outcome measure will be muscle protein synthesis

Secondary outcomes are:

- Serum inflammatory markers (IL-1, IL-6, IL-8, TNF-alpha, and the anti-inflammatory cytokine IL-10)
- Leg extension strength in each leg
- Food diaries
- Outcomes relating to trial feasibility, including participants' willingness to be randomised, number of eligible participants, number of participants recruited, compliance with the study diets, adherence to exercise and the time taken to collect data

Muscle biopsies will be taken from the thigh at baseline and 2 weeks to measure muscle protein synthesis; at baseline a sample from one thigh will be taken, and at 2 weeks a sample from each leg will be taken, for comparison between exercised and non-exercised legs (see intervention). Saliva samples will also be taken at these times, and an additional saliva sample at 1 week, to allow calculation of MPS. 1-RM leg extension strength and serum inflammatory markers will be measured at baseline and after 2 weeks. 3-day food diaries will be completed at baseline and at the end of the intervention to determine compliance to the diet plan, and participants will be asked to complete a daily questionnaire to confirm whether they have eaten one of the study meals for each meal of the day (yes/no answers). All assessments will take place in the Wellcome Trust Clinical Research Facility.

Key Eligibility Criteria

Inclusion criteria are: Aged 65 years of over; female; ambulatory (with or without walking aids).
Exclusion criteria are based upon previously published criteria for exercise studies and include:

- Already engaging in regular exercise (at least twice a week)
- History of myocardial infarction within previous 2 years
- Cardiac illness: moderate/ severe aortic stenosis, acute pericarditis, acute myocarditis, aneurysm, severe angina,
- Clinically significant valvular disease, uncontrolled dysrhythmia, claudication within the previous 10 years
- Thrombophlebitis or pulmonary embolus within the previous 2 years
- History of cerebrovascular disease (CVA or TIA) within the previous 2 years
- Treatment with anticoagulants (Warfarin, rivaroxaban, apixaban, dabigatran) and antiplatelets (dipyridamole, clopidogrel, prasugrel, ticagrelor, glycoprotein IIb/IIIa antagonists). Nb. those regularly taking aspirin will be asked to stop for 3 days prior to biopsies and restart the day after
- Acute febrile illness within the previous 3 months
- Severe airflow obstruction
- Uncontrolled metabolic disease (e.g., thyroid disease or cancer)
- Significant emotional distress, psychotic illness or depression within the previous 2 years
- Lower limb fracture sustained within the previous 2 years; upper limb fracture within the previous 6 months; non arthroscopic lower limb joint surgery within the previous 2 years
- Any reason for loss of mobility for greater than 1 week in the previous 2 months or greater than 2 weeks in the previous 6 months
- Resting systolic pressure >200 mmHg or resting diastolic pressure >100mmHg
- Poorly controlled atrial fibrillation
- Poor (chronic) pain control
- Moderate/ severe cognitive impairment (MMSE <23)
- Renal impairment (Stage 4 or 5)

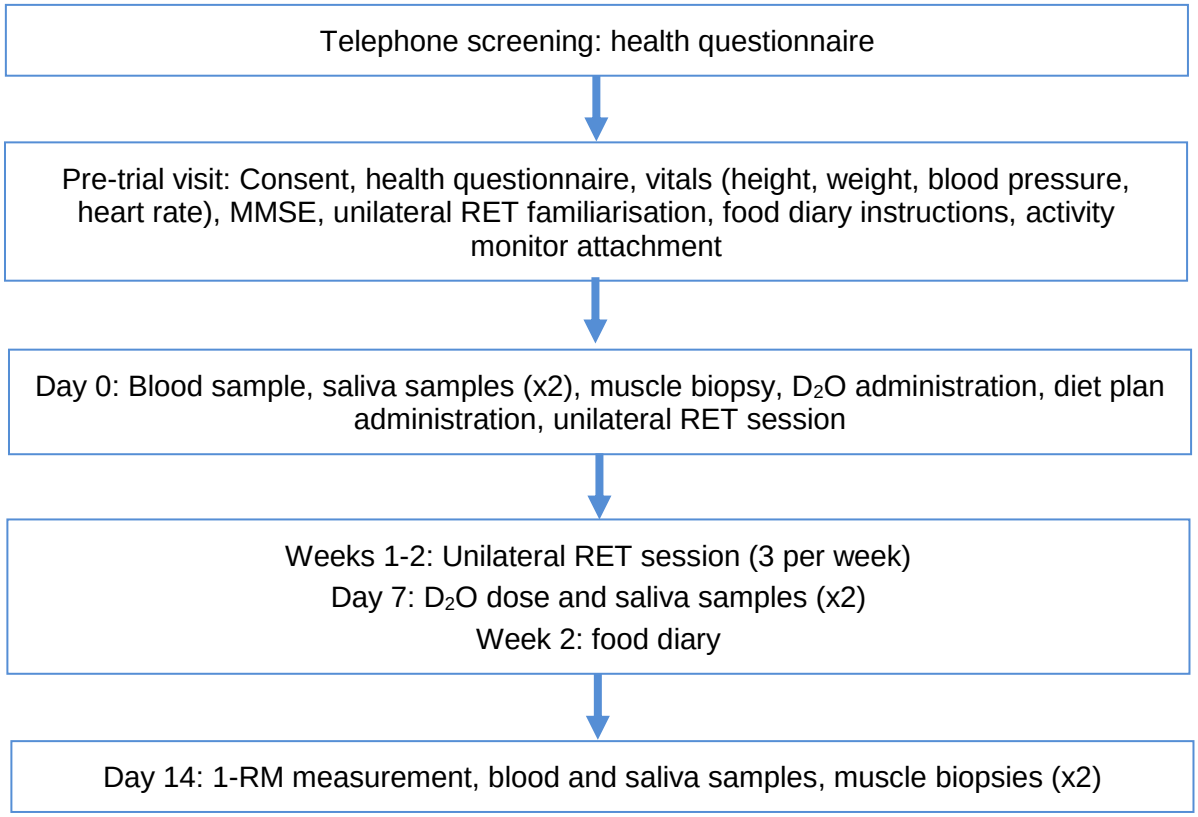
Intervention

Participants will be randomised to RET (x3 per week) + Pulse protein distribution or RET + Spread distribution.

Protein distribution: Daily protein intake will either be consumed in a Pulse distribution (meals of 10%, 80% and 10% of daily intake) or a Spread distribution (3 x 33%). This will be achieved using a diet plan which, if followed, will give these distributions, and will provide 1.2 grams of protein per kilogram of body weight per day. There will be a level of flexibility in the diets to allow for dietary requirements and personal preferences, and preliminary data indicate that protein supplementation may be required for some individuals in order to reach a protein intake of 1.2 $g.kg^{-1}.day^{-1}$. A commercial protein supplement will be used for this.

Resistance exercise training: Throughout the trial participants will complete 3 RET sessions per week of a unilateral leg extension model (6 x 8 repetitions at 75% 1-repetition maximum). The use of a unilateral model will allow comparison between exercised non-exercised legs, as well as between treatment groups.

Trial Schema



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1. Background and Rationale

1.1. Background

Life expectancy in the UK is increasing, meaning the number of adults aged over 70 years is also growing, however this rate of increase is not matched by that of healthy life expectancy. Therefore, issues associated with healthy ageing are of great interest. One such issue which influences health in older age is sarcopenia, defined as the age-related loss of skeletal muscle mass and function. Sarcopenia is an important public health problem with a range of consequences. Loss of functionality makes it more difficult to carry out day-to-day activities and can result in loss of independence, which affects both quality of life and social care. Lower muscle mass is also associated with increased risk of falls[1], which can have serious health consequences for older adults, as well as disability, infection and mortality[2-4]. Hence, the design of effective interventions to combat sarcopenia has been a research goal for 30 years.

Although the causes of sarcopenia are multifactorial and not fully defined, one of the factors which is thought to contribute is the rate of muscle protein synthesis (MPS), and the responsiveness of MPS to anabolic stimuli. The basal rate of MPS in older adults is not different to that of younger adults[5], but the rate in response to anabolic factors, such as protein or essential amino acid (EAA) ingestion or resistance exercise, is blunted compared to younger adults[6,7]. This effect, known as anabolic resistance, translates into chronic maladaptation to anabolic factors[8], and is thought to be one of the mechanisms behind sarcopenia. As a probable cause of sarcopenia, anabolic factors may also be a source of interventions to prevent sarcopenia; if the delivery of anabolic factors can be optimised to maximise MPS, this may translate into long term improvements in muscle mass and function. One component of delivery to consider is the distribution of protein intake. It has been suggested that ingesting protein in many small doses may repeatedly stimulate the MPS response, giving overall higher levels of synthesis. However, there is also the size of these doses to consider; division of the same amount of protein into a smaller number of larger doses may stimulate higher levels of MPS with each dose, giving overall higher output. This effect has been studied acutely in younger adults; participants fed three protein meals over 24 hours showed greater nitrogen retention than those fed smaller hourly meals[9,10], and in another study participants fed four protein doses of intermediate size had higher MPS than those fed either eight smaller or two larger meals[11]. Studies testing this effect in older adults are relatively sparse. Arnal et al.[12] compared the effects of a Pulse diet, which provided 80% of daily protein at noon, with a Spread diet in which protein was spread more evenly over four meals, in 15 older women for two weeks, and found greater nitrogen balance, protein turnover and fat free mass (FFM) in the Pulse group. Bouillanne et al.[13] (2001) compared similar distributions in 66 older adults for six weeks, and reported greater improvements in indices of lean body mass (LBM), appendicular skeletal muscle mass (ASMM) and body cell mass (BCM) in the Pulse group. These two studies alone are not sufficient to develop recommendations regarding protein distribution, however they do indicate a possible role of protein distribution in preventing sarcopenia.

Resistance exercise training, another anabolic stimulus, has been more widely studied, and has consistently demonstrated improvements in muscle-related outcomes in older adults[14]. However, this response is still blunted compared to that of younger adults, and changes to the delivery of dietary protein, such as the distribution, may help to optimise the response and further improve the effects on muscle health. To date, no study in older adults has

compared the protein distributions described above with a resistance exercise training programme. This study aims to fill this knowledge gap, which will contribute to the development of lifestyle interventions to act against sarcopenia.

Furthermore, as research in this area is so sparse, little is known as to the feasibility of this trial, particularly as previous studies of daily protein distribution in older adults have been of shorter durations (2-6 weeks). Hence, another aim of this study is to consider the feasibility of the trial in terms of diet compliance, as well as exercise adherence, participants' willingness to be randomised, number of eligible participants and number recruited, and the time taken to collect the data. This information could inform the design of future studies.

1.2. Trial Rationale

1.2.1. Justification for participant population

We have selected adults aged over 65 years as this aged group are most susceptible to the effects of sarcopenia, and so stand to benefit from interventions designed to improve muscle health. We have chosen to make this a single sex study, as older women display greater blunting of adaptation to RET than older men[15], hence the inclusion of both men and women would affect the ability to interpret the results. Specifically, we have selected to only include women, as they experience greater loss of functional ability as a result of sarcopenia.

1.2.2. Justification for design

This standalone study is designed to measured acute effects of protein distribution in combination with resistance exercise. The study includes a unilateral RET component to allow measurement of effects with and without exercise. Protein distributions will be implemented through dietary manipulation to investigate whether this is feasible as a lifestyle intervention.

1.2.3.Choice of treatment

There is evidence to suggest that the distribution of protein intake may have an effect on muscle health in older adults, however this evidence is limited to two studies. Chronic resistance exercise training has been shown to be effective in improving muscle health, however no study has combined this with different protein distributions in older adults.

1.2.4. Sub-studies

None proposed

2. Aims, Objectives and Outcome Measures

2.1. Aims and Objectives

We hypothesise that there will be a significant difference in muscle health with different protein distributions. Based on previous studies, we hypothesise that improvements will be seen in both groups, however there will be greater improvement in the Pulse group.

Principal objective: Investigate the effect of dietary protein distribution on muscle protein synthesis (MPS) over two weeks, with and without RET, in the thigh muscle of older adults

Secondary objectives:

(i) Investigate whether protein distribution influences serum inflammatory markers

(ii) Consider the feasibility of such an intervention in this population

(iii) Determine whether the effects of RET on muscle strength is influenced by protein distribution

(iv) Investigate whether changes in responsiveness of MPS predict changes in muscle strength

2.2. Outcome Measures

All outcome measures will be made by members of the study team (i.e., research nurses, the PhD student).

The primary outcome measure will be muscle protein synthesis

Secondary outcomes are:

- Serum inflammatory markers (IL-1, IL-6, IL-8, TNF-alpha, and the anti-inflammatory cytokine IL-10)
- Leg extension strength in each leg
- Food diaries
- Feasibility of the trial, including participants' willingness to be randomised, number of eligible participants, number of participants recruited, compliance with the study diets, adherence to exercise and the time taken to collect data

Muscle biopsies will be taken from the thigh at baseline and 2 weeks to measure muscle protein synthesis; at baseline a sample from one thigh will be taken, and at 2 weeks a sample from each leg will be taken, for comparison between exercised and non-exercised legs (see intervention). Saliva samples will also be taken at these times, and an additional saliva sample after 1 week, to allow calculation of MPS. Serum inflammatory markers and 1-RM leg extension strength will be measured at baseline and 2 weeks, and the analysis metric will be change from baseline. Physical activity level will be measured before the start of the study to characterise the population. 3-day food diaries will be completed at baseline and in the final days of the intervention to determine compliance to the diet plan, and participants will be asked to complete a daily questionnaire to confirm whether they have eaten one of the study meals for each meal of the day (yes/no answers). All assessments will take place in the Wellcome Trust Clinical Research Facility.

3. Trial Design and Setting

3.1. Trial Design

This is an exploratory randomised trial and feasibility trial of RET plus Pulse protein distribution versus RET plus Spread protein distribution.

3.2. Trial Setting

The trial will take place in the Wellcome Trust Clinical Research Facility. Participants will be recruited through the Birmingham 1000 Elders database, a list of adults aged 65 years and over, who have given their permission to be contacted regarding research studies. The custodian of the database will provide contact details for a list of potentially eligible participants based on inclusion criteria. Potential participants will be sent a copy of the participant information sheet along with an invitation letter, which will include a telephone number and email address to contact the study team directly. Potential participants may also be reached through local community groups and leaflets left in public places, and again this will include details to contact the study team directly. Researchers will ask participants to complete a screening health questionnaire over the phone to determine eligibility, and those eligible will be invited to attend a meeting at the Clinical Research Facility. At this meeting, participants will be given the opportunity to ask any further questions, and consent will be obtained. Blood pressure will be measured and the MMSE completed to determine eligibility. After consent, the participant will be asked to complete a 3-day food diary and to wear and activity monitor for 7 days, and a date will be scheduled to being the intervention. Following this initial meeting the participant be randomised to either RET plus Pulse distribution or RET plus Spread distribution.

4. Eligibility

4.1. Inclusion Criteria

Inclusion criteria are: Aged 65 years or over; female; ambulatory (with or without walking aids).

Dr Greig has over 25 y experience of recruiting older participants to studies of exercise and muscle function. Given that confirmation of eligibility in this low-risk study does not require the interpretation of medical notes/ history or a physical examination, Dr Greig is suitably qualified to confirm eligibility. However, any queries about eligibility will be forwarded to the study team's medical expert (Dr Thomas Jackson) before a decision is taken.

4.2. Exclusion Criteria

Exclusion criteria are based upon previously published criteria for exercise studies and include:

- Already engaging in regular exercise (at least twice a week)
- History of myocardial infarction within previous 2 years
- Cardiac illness: moderate/ severe aortic stenosis, acute pericarditis, acute myocarditis, aneurysm, severe angina,
- Clinically significant valvular disease, uncontrolled dysrhythmia, claudication within the previous 10 years

- Thrombophlebitis or pulmonary embolus within the previous 2 years
- History of cerebrovascular disease (CVA or TIA) within the previous 2 years
- Treatment with anticoagulants (Warfarin, rivaroxaban, apixaban, dabigatran) and antiplatelets (dipyridamole, clopidogrel, prasugrel, ticagrelor, glycoprotein IIb/IIIa antagonists). Nb. those regularly taking aspirin will be asked to stop for 3 days prior to biopsies and restart the day after
- Acute febrile illness within the previous 3 months
- Severe airflow obstruction
- Uncontrolled metabolic disease (e.g., thyroid disease or cancer)
- Significant emotional distress, psychotic illness or depression within the previous 2 years
- Lower limb fracture sustained within the previous 2 years; upper limb fracture within the previous 6 months; non arthroscopic lower limb joint surgery within the previous 2 years
- Any reason for loss of mobility for greater than 1 week in the previous 2 months or greater than 2 weeks in the previous 6 months
- Resting systolic pressure >200 mmHg or resting diastolic pressure >100mmHg
- Poorly controlled atrial fibrillation
- Poor (chronic) pain control
- Moderate/ severe cognitive impairment (MMSE <23)
- Renal impairment (Stage 4 or 5)

5. Consent

It will be the responsibility of the Investigator to obtain written informed consent for each participant prior to performing any trial related procedure. Written, informed consent will be obtained from potential participants by one of the investigators (i.e., the CI, the research nurse or the PhD student after appropriate training). A Participant Information Sheet (PIS) will be provided to facilitate this process. Investigators will ensure that they adequately explain the aim, trial treatment, anticipated benefits and potential hazards of taking part in the trial to the participant. They will also stress that participation is voluntary and that the participant is free to refuse to take part and may withdraw from the trial at any time. The participant will be given at least one week to read the PIS and to discuss their participation with others outside of the site research team. The participant will be given the opportunity to ask questions. If the participant expresses an interest in participating in the trial they will be asked to sign and date the latest version of the Informed Consent Form (ICF).

The Investigator will sign and date the form. A copy of the ICF will be given to the participant, a copy will be filed in the medical notes, and the original placed in the Investigator Site File (ISF). Once the participant is entered into the trial, the participant's unique trial identification number will be entered on the Informed Consent Form maintained in the ISF.

At each visit the participant's willingness to continue in the trial will be ascertained. Throughout the trial the participant will have the opportunity to ask questions about the trial. Any new information that may be relevant to the participant's continued participation will be provided. Where new information becomes available which may affect the participants' decision to continue, participants will be given time to consider and if happy to continue will be re-consented. Re-consent will be documented in the medical notes. The participant's right to withdraw from the trial will remain. With the participant's prior consent, their General Practitioner (GP) will also be informed that they are taking part in the trial.

Details of all participants approached about the trial will be recorded on the Participant Screening/Enrolment Log and with the participant's prior consent, their General Practitioner (GP) will also be informed that they are taking part in the trial.

6. Enrolment and Randomisation

6.1. Enrolment/Registration

Prior to consent, participants will complete a health questionnaire over the telephone to determine eligibility. This will be repeated during the consent meeting to ensure no information has been missed, and blood pressure will be measured and the MMSE completed. After consent and prior to randomisation, participants will complete a 3-day food diary and wear an activity monitor for 7 days. In terms of retention, the study is relatively short.

6.2. Randomisation

Participants will be randomised in advance by Dr Peter Nightingale (UHB Statistician) using a computer generated programme, and concealed in sequentially numbered opaque envelopes. Blocked randomisation will be used for the first 20 participants to ensure at least n=10 in each group.

6.3. Blinding

Due to the nature of the intervention, blinding will not be used. The intervention is protein distribution, and as this is to be achieved by dietary manipulation, participants will be aware of which protein distribution they are receiving.

7. Trial treatment / intervention

7.1. Treatment

This is not a cTIMP/NIMP study. The protein distribution intervention will be implemented through the manipulation of the participants' existing diet. Protein supplementation may be used for individuals requiring more protein to reach the desired intake, however these will be commercial dietary supplements.

7.2. Treatment Supply and Storage

7.2.1. Treatment Supplies

N/A

7.2.2. Packaging and Labelling

N/A

7.2.3. Drug Storage

N/A

7.3. Dosing Schedule

The protein distribution interventions will be administered through the participants' diets. Participants will be given a diet plan to follow, which will provide 1.2 $g.kg^{-1}.day^{-1}$ protein in either a Pulse or a Spread distribution.

RET will be consist of a unilateral leg extension programme, delivered in one-on-one sessions with a member of the study team three times per week.

7.4. Drug Interaction or Contraindications

N/A

7.5. Accountability Procedures

Compliance with RET will be assessed by recording attendance to each session. Adherence to diet plans will be monitored using a 3-day food diary completed in the final days of the intervention, and a daily questionnaire in which participants will be asked to state whether or not they have consumed a meal from the diet plan for each daily meal.

7.6. Treatment Modification

N/A

8. Trial procedures and assessments

8.1. Summary of assessments

Figure 1 PRODREX summary of assessments

	TRIAL PERIOD						
	Enrolment	Allocation	Post-allocation				Close-out
TIMEPOINT**	***-28 to -14 days***	***-14 days to baseline***	***day 0***	***week 1***	***day 7***	***week 2***	***day 14***
ENROLMENT:							
Eligibility screen (health questionnaire, blood pressure, MMSE)	X						
Informed consent	X						
Vitals (height, weight, heart rate)	X						
Unilateral RET familiarisation	X						
Allocation		X					
INTERVENTIONS:							
Protein distribution diet			♦—	—	—	—	—♦
Unilateral RET			♦—	—	—	—	—♦
ASSESSMENTS:							
1-RM strength	X						X
3-day food diary		X				X	
7-day activity monitoring		X					
Blood sample			X				X
Saliva sample			X		X		X
Muscle biopsy			X				X
D_2O administration			X		X		
Daily compliance questionnaire			♦—	—	—	—	—♦

8.2. Schedule of Assessments

-Screening (telephone call): Screening health questionnaire

-Pre-trial visit (visit 0): Consent, health questionnaire, blood pressure measurement, MMSE, vitals measurement (height, weight, heart rate), unilateral knee extension exercise familiarisation (including 1-RM tests), food diary introduced, physical activity monitor attached for 7 days

-Visit 1 (day 0): Muscle biopsy, blood sample, saliva samples, D_2O administration, unilateral RET session

-Visit 2-6 (weeks 1-2): Unilateral RET session

-Visit 7 (day 14): Blood and saliva samples, muscle biopsy (x2), leg extension 1-RM

At-home activities:

-Day 0: Saliva sample

-Day 7: D_2O administration and saliva samples (x2) (or in CRF if scheduled for training visit)

-Weeks 1-2: Daily compliance questionnaire

-Week 2: 3-day food diary

8.3. Trial Procedures

Muscle biopsies; performed by trained member of the study team. If participants regularly take aspirin they will be asked to stop this 3 days prior to each biopsy and restart the day after the biopsy.

Venepuncture; performed by trained research nurse.

1-RM leg extension strength test; established procedure; incremental increases in load until only one repetition can be completed

Physical activity (ActivPAL accelerometry); assessors will be trained.

Serum inflammatory markers will be measured using the Luminex system, and assessors will be trained.

8.3.1. Sub studies

None proposed

9. Adverse Event Reporting

9.1. Reporting Requirements

Non-CTIMPs

The collection and reporting of Adverse Events (AEs) will be in accordance with the Research Governance Framework for Health and Social Care and the requirements of the National Research Ethics Service (NRES). Definitions of different types of AEs are listed in the table of abbreviations and definitions. The Investigator should assess the seriousness and causality (relatedness) of all AEs experienced by the trial participant (this will be documented in the source data) with reference to the protocol.

9.2. Adverse Events

AEs are commonly encountered in participants aged 65 y and over.

D_2O has been known to cause vertigo and nausea although this is very unusual.

Any AEs as a consequence of the exercise programme over and above the expected and common muscle stiffness which occurs after the initial exercise sessions in those unaccustomed to regular exercise will be recorded.

The study team will use the UHB NHS Foundation Trust reporting system embedded within their governance structure.

9.3. Serious Adverse Events

Investigators will report AEs that meet the definition of an SAE

In this trial, an SAE must fulfil at least one of the following criteria:

- Is fatal
- Is life-threatening
- Requires in-patient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/ incapacity

9.3.1. Events that do not require expedited (immediate) reporting

N/A

9.3.2. Events that do not require reporting on a Serious Adverse Event Form

N/A

9.3.3. Monitoring pregnancies for potential Serious Adverse Events

N/A

9.4. Reporting period

Details of all AEs will be documented and reported from the date of informed consent, as participants will undergo an exercise familiarisation session prior the commencement of the protocol defined treatment (protein distribution diets) until the final day of administration of the treatment.

9.5. Reporting Procedure – At Site

9.5.1. Adverse Events

Information on AEs will be collected on an AE Form (and where applicable on an SAE Form). An AE Form will be completed and returned to the study team medical expert (and copied to the CI). The study team medical expert will be included in all AE/ SAE reporting processes.

9.5.2. Serious Adverse Events

AEs defined as serious and which require reporting as an SAE should be reported on an SAE Form. When completing the form, the Investigator will be asked to define the causality and the severity of the AE.

On becoming aware that a participant has experienced an SAE, the Investigator (or delegate) will complete, date and sign an SAE (DATIX) form. The form will automatically be sent (scanned and emailed – not faxed which is no longer considered best practice) to the Clinical Research Facility's Clinical Manager for review as soon as possible and no later than 24h after first becoming aware of the event. All SAEs will be reviewed formally every 2

weeks during Clinical Research Facility operations meetings which include representation from the Trust R&D office.

The SAE reference number will be quoted on all correspondence and follow-up reports regarding the SAE and filed with the actual SAE in the site file.

For SAE forms completed by someone other than the Investigator, the Investigator (or delegate) will countersign the original SAE to confirm agreement with the causality and severity assessments. The form will be returned to the Clinical Research Facility Manager and the copy kept in the site file.

The Investigators will report SAEs to their own Trust via the Clinical Research Facility in accordance with local practice using an electronic reporting system.

9.5.3. Provision of follow-up information

Participants should be followed up until resolution or stabilisation of the event. Follow-up information should ideally be provided on a new SAE Form.

9.6. Reporting Procedure – Trials Office

This is a low-risk study of exercise plus a dietary supplement versus exercise plus placebo. The Trials Office will not be involved. However we will report AEs and SAEs according to the UHB NHS Foundation Trust's electronic reporting system

Http://doha/datix/live/index.php?form_id=20&module=INC

The PI will monitor any forms completed and reported through the CRF Clinical Manager / CRF operations meetings for trends. Additional input will be provided by University of Birmingham Clinical Research Compliance Team.

9.7. Reporting to the Competent Authority and Research Ethics Committee

9.7.1. Suspected Unexpected Serious Adverse Reactions

N/A

9.7.2. Serious Adverse Reactions

N/A

9.7.3. Unexpected and Related Serious Adverse Events

All events categorised as Unexpected and Related SAEs will be reported to the REC and the University of Birmingham (Sponsor) research governance team (researchgovernance@contacts.bham.ac.uk) within 15 days.

9.7.4. Adverse Events

N/A

9.7.5. Other safety issues identified during the course of the trial

The REC will be notified immediately if a significant safety issue is identified during the course of the trial.

9.8. Investigators

Details of all Unexpected and Related SAEs and any other safety issue which arises during the course of the trial will be reported to Principal Investigators. A copy of any such correspondence should be filed in the Site File.

9.9. Data Monitoring Committee

The study team considers this to be a low risk study and as such have taken the decision to not commission a Data Monitoring Committee for this study.

9.10. Reporting to third parties

N/A

10. Data Handling and Record Keeping

10.1. Source Data

In order to allow for the accurate reconstruction of the trial and clinical management of the subject, source data will be accessible and maintained. Some data may be entered directly onto the CRF these are clearly identified and detailed below.

Type of Data	**Source Document**
Informed consent	Signed Informed Consent Form
Relevant Medical History and Current Medical Conditions	Health Questionnaire
Demographics	CRF
MMSE	CRF
Vital signs including blood pressure, heart rate, height and weight	CRF
1-RM leg extension strength	CRF
Physical activity measurement (ActivPAL) times and results	Printed report
Food diary	Printed report
Compliance questionnaire	Printed report
Adverse events	AE form
Trial Termination details	CRF

Diet plan/ exercise compliance	CRF

10.2. CRF Completion

Data reported on each Case Report Form will be consistent with the source data and any discrepancies will be explained. All missing and ambiguous data will be queried. Staff delegated to complete CRFs will be trained to adhere to UHB NHS Foundation Trust governance guidelines

In all cases it remains the responsibility of the site's Principal Investigator to ensure that the CRF has been completed correctly and that the data are accurate. Where applicable for the trial this will be evidenced by the signature of the site's Principal Investigator on the CRF.

The completed originals will be kept by the CI and a copy filed in the Investigator Site File.

10.3. Data Management

Individual data sets will be checked by the CI at regular intervals and any discrepancies highlighted and listed. These will be viewed and discussed by the supervisory team.

10.4. Archiving

Data will be archived according to UHB NHS Foundation Trust governance guidelines.

11. Quality control and quality assurance

11.1. Site Set-up and Initiation

This is a low risk single-centre study which the study team do not consider is reliant upon Trials Office support. An Investigator Site File will be maintained and which will include essential documentation, instructions, and other documentation required for the conduct of the trial.

11.2. Monitoring

11.2.1. On-site Monitoring

The study team will allow Trials Office staff to monitor the study, including access to source documents as requested.

11.2.2. Central Monitoring

This is a small single-site study and the study team considers that central monitoring is not applicable.

11.3. Audit and Inspection

The investigator will permit trial-related monitoring, quality checks, audits, ethical reviews, and regulatory inspection(s) at their site, providing direct access to source data/documents. The investigator will comply with these visits and any required follow up.

11.4. Notification of Serious Breaches

In accordance with the Research Governance Framework for Health and Social Care 2005, the sponsor of the trial is responsible for notifying the REC and R&D departments of any

serious breach of the conditions and principles of GCP in connection with that trial or the protocol relating to that trial.

Any serious breaches will also be reported to the University of Birmingham's (the Sponsor's) research governance team (researchgovernance@contacts.bham.ac.uk)

Further recruitment to the study may be suspended in the event of serious and persistent non-compliance with the protocol and/or GCP, and/or poor recruitment. Any major problems identified during monitoring may be reported to the Trial Management Group and the REC.

12. End of Trial Definition

The end of trial will be 12 months after the last data capture. The Trials Office will notify the REC the trial has ended and a summary of the clinical trial report will be provided within 12 months of the end of trial.

13. Statistical Considerations

13.1. Definition of Outcome Measures

13.1.1. Primary outcome measures

See section 2.2.

13.1.2. Secondary outcome measures/exploratory endpoints

See section 2.2.

13.2. Analysis of Outcome Measures

All data will be entered into a database. Using SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp., data analysis will be conducted by the PhD student on both an intention to treat and per protocol basis (i.e. values for all measurements, completed at least 75% of exercise sessions, and complied with the study diet). Comparisons are planned between Pulse and Spread distribution groups for change in all outcome measures. In the case of MPS, a comparison between trained and untrained legs is also planned. All data will be checked for normality and appropriate log transformations applied prior to *t*-tests for primary and secondary outcome measures.

13.2.1. Planned Randomisation Methodology

Participants will be randomised (by UHB statistician Dr Nightingale) to RET (x3 per week) + Pulse protein distribution diet or RET + Spread distribution diet for 12 weeks. Participants will be randomised in advance using a computer function, and blocked randomisation will be used for the first 20 participants to ensure equal numbers in each group.

13.2.2. Planned Sub Group Analyses

None proposed

13.2.3. Planned Interim Analysis

None proposed

13.2.4. Planned Final Analyses

Final analysis will be when all 12 week outcome measures have been collected

13.2.5. Power Calculations

As an exploratory trial, the study has not been powered due to lack of previous data. Data from this trial will inform sample size calculations for future studies; the MRC Framework for Development and Evaluation of RCTs for Complex Interventions to Improve Health states that “the exploratory trial may be a vital source of estimates for the sample required for a main trial, an essential step since, as is frequently lamented, all too many trials prove to be under-powered”. Specifically, a sample size of n=20-24 has been chosen due to practical considerations of time and cost.

14. Trial Organisational Structure

14.1. Sponsor

The University of Birmingham is the Sponsor of this study

14.2. Trial Management Group

As this is a small, low risk study, the trial will be overseen by the CI and the PhD supervisor. There will not be a Trial Steering Committee

14.3. Data Monitoring Committee

This is a low-risk single centre trial and thus the study team do not consider the support of a Data Monitoring Committee to be necessary

14.4. Finance

This is an investigator-initiated and investigator-led trial funded by the Medical Research Council and Arthritis UK Centre for Musculoskeletal Ageing Research (CMAR), as a PhD studentship. Funding will be managed by CMAR.

15. Ethical Considerations

The trial will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects, adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, June 1964, amended at the 48th World Medical Association General Assembly, Somerset West, Republic of South Africa, October 1996 (website: http://www.wma.net/en/30publications/10policies/b3/index.html).

The trial will be conducted in accordance with the Research Governance Framework for Health and Social Care, the applicable UK Statutory Instruments, (which include the Medicines for Human Use Clinical Trials 2004 and subsequent amendments and the Data Protection Act 1998.

The protocol will be submitted to and approved by the REC prior to circulation.

Before any participants are enrolled into the trial, the Principal Investigator at each site is required to obtain local R&D approval. Sites will not be permitted to enrol participants until written confirmation of R&D approval is received by the Principal Investigator.

It is the responsibility of the Principal Investigator to ensure that all subsequent amendments gain the necessary local approval. This does not affect the individual clinicians’ responsibility to take immediate action if thought necessary to protect the health and interest of individual participants.

16. Confidentiality and Data Protection

Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the Data Protection Act 1998.

Participants will always be identified using only their unique trial identification number on the Case Report Form and correspondence between the participating site.

The Investigator will maintain documents in strict confidence. In the case of specific issues and/or queries from the regulatory authorities, it will be necessary to have access to the complete trial records, provided that participant confidentiality is protected.

17. Insurance and Indemnity

The University of Birmingham has in place Clinical Trials indemnity coverage for this trial which provides cover to the University for harm which comes about through the University's, or its staff's, negligence in relation to the design or management of the trial and may alternatively, and at the University's discretion provide cover for non-negligent harm to participants.

With respect to the conduct of the trial at Site and other clinical care of the patient, responsibility for the care of the patients remains with the NHS organisation responsible for the Clinical Site and is therefore indemnified through the NHS Litigation Authority.

The University of Birmingham is independent of any pharmaceutical company, and as such it is not covered by the Association of the British Pharmaceutical Industry (ABPI) guidelines for participant compensation.

18. Publication Policy

Results of this trial will be submitted for publication in peer reviewed journals. The manuscript will be prepared by the study team led by Dr Carolyn Greig and authorship will be determined by mutual agreement.

Any secondary publications and presentations prepared by Investigators will be reviewed by Dr Carolyn Greig. Manuscripts will be submitted to her in a timely fashion and in advance of being submitted for publication, to allow time for review and resolution of any outstanding issues. Authors will acknowledge that the trial was performed with the support of the MRC-ARUK Centre for Musculoskeletal Ageing Research (CMAR).

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Abbreviations and Definitions:

Term	Description
RET	Resistance exercise training
MPS	Muscle protein synthesis
1-RM	1 repetition maximum
EAA	Essential amino acids
ASMM	Appendicular skeletal muscle mass
BCM	Body cell mass
For non-CTIMPs	
Adverse Event (AE)	Any untoward medical occurrence in a participant or clinical trial subject participating in the trial which does not necessarily have a causal relationship with the treatment received. Comment: An AE can therefore be any unfavourable and unintended sign (including abnormal laboratory findings), symptom or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.
Related Event	An event which resulted from the administration of any of the research procedures.
Serious Adverse Event (SAE)	An untoward occurrence that: • Results in death

	• Is life-threatening* • Requires hospitalisation or prolongation of existing hospitalisation • Results in persistent or significant disability or incapacity • Consists of a congenital anomaly/ birth defect • Or is otherwise considered medically significant by the Investigator** Comments: The term severe is often used to describe the intensity (severity) of a specific event. This is not the same as serious, which is based on participants/event outcome or action criteria. * Life threatening in the definition of an SAE refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe. ** Medical judgment 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 be considered serious
Unexpected and Related Event	An event which meets the definition of both an Unexpected Event and a Related Event
Unexpected Event	The type of event that is not listed in the protocol as an expected occurrence.
Source data	All information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial
Trials Office	The team of people, including the Chief Investigator, responsible for the overall management and coordination of the trial.

Appendices