**STUDY PROTOCOL**

Study title: ***Proteome Dynamics to Inform Healthy Muscle Ageing***

Document date: ***12th December 2022***

Research Ethics Committee Reference Number: **22/SPS/075**

**PARTICIPANT INFORMATION SHEET [*Healthy, non-exercise trained adults.*]**

**Research Ethics Committee Reference Number**: 22/SPS/075 Version 2 (23rd Jan 2023)

**Title of Study:** Proteome Dynamics to Inform Healthy Muscle Ageing

You are being invited to take part in a research study. You do not have to take part if you do not want to. Please read this information, which will help you decide.

1. **What is the purpose of the study?**

Losses in muscle function are a feature of ageing that negatively impact disease risk profile, resilience to injury, societal/economic contribution and quality-of-life. This project exploits a new technique (m*uscle* p*roteome dynamics*) that is unique to our group and capable of studying the quality of thousands of proteins in human muscle. This study hopes to identify any potential differences in skeletal muscle protein turnover due to aging, we hope this work could eventually lead to better care and lifestyle advice for older adults.

2. **Why have I been invited to participate?**

You have been invited because you are an otherwise healthy adult male that does not take part in very regular physical exercise training.

Exclusion criteria – you must not participate if you:

- female
- aged <18 years, 35-65 years, or >75 years
- take part in >3 bouts of endurance-/resistance-based training per week
- are pregnant
- have any previous diagnosis of ischaemic heart disease, diabetes mellitus myopathy or any neuromuscular disorder.
- take any anticoagulant medications such as warfarin
- have a current musculoskeletal injury.
- currently unwell with cold or flu
- have a chronic connective tissue disorder
- have a cardiovascular disease
- are currently enrolled in any other research study

3. **Do I have to take part?**

No. You can ask questions about the research before deciding whether to take part. If you do not want to take part that is OK. We will ask you to sign a consent form and will give you a copy for you to keep.

You can stop being part of the study at any time, without giving a reason. You may withdraw from the study by contacting the research team.

You must not take part if you have COVID-19 or symptoms of COVID-19 - https://www.nhs.uk/conditions/coronavirus-covid-19/symptoms/

4. **What will happen to me if I take part?**

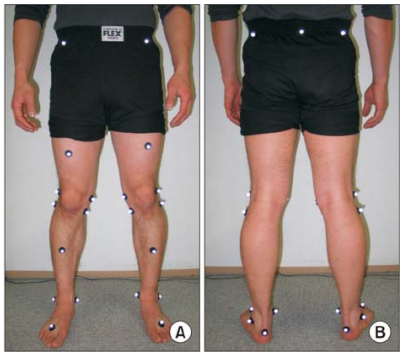
We will talk you through all study protocols and procedures prior to your participating and give you the chance to ask any questions that you may have.

**Initial assessment**
You will first undergo an initial screening questionaire to check you meet the inclusion criteria prior to commencing the study. If you are eligible for the study, the first laboratory visit will take place 7 ± 2 days prior to the start of the experimental intervention. You will complete a biopsy screening form in the presence of a trained member of LJMU staff. In addition, basic assessments will be undertaken, including height, weight and blood pressure measurements.
If you fit the inclusion criteria, we will assess your physical activity levels (**Paffenbarger physical activity questionnaire**), ability to exercise (**Readiness to Exercise questionnaire**), and typical dietary intake (**Food Frequency Questionnaire)**. You will then undertake 6 assessments (body composition assessment, walking test, 5x sit-to-stand, grip strength, isokinetic dynamometry and 10-repetition maximum leg press).

Body composition assessment - we will assess your body composition using a bioelectrical impedance analysis (BIA) machine. This procedure is non-invasive, pain free and takes approximately five minutes, from a practical point of view it is like standing on a set of bathroom scales. From the test, we attain information on your height, body mass, fat mass and skeletal muscle mass.

The **walk test** - you will be required to complete seven 6-second walking trials over a 12-metre distance, while the position of your whole body is monitored, and video recorded by multiple cameras.

You will need to wear a t-shirt and cycling shorts. Cycling shorts can be provided if you don't have any. You will then have some reflective markers attached to the skin on your legs and upper body/torso using medical grade double sided tape and elastic wrapping.




The sit-to-stand test - Next, the 5 times **sit-to-stand test** will be completed while the position of your whole body is monitored, and video recorded by multiple cameras.

The 5 x sit-to-stand test will be conducted using a standard height (43-45 cm) chair with straight back. You will start in the seated position and, after confirming your ability to perform 1 sit-to-stand action, you will be instructed to stand and sit 5 times as quickly as possible, ensuring your feet remain flat on the floor. The time taken to complete 5 sit-to-stand movements will be measured using a stopwatch.

While seated, your **grip strength** of your dominant arm will be measured using a hand dynamometer. During this test you will be instructed to grip the dynamometer as tightly as possible without moving from the seat or raising your legs from the floor. Next, isokinetic torque of the knee extensor muscles will be assessed via **isokinetic dynamometry (IKD).** You will first perform a warm-up (5 min at 50 W on a cycle ergometer) and then be seated in an upright position on the isokinetic dynamometer (similar to a gym leg-extension exercise machine). Non-extendable straps crossing the chest, abdomen and thighs will be used to minimise upper body movements during the leg exercise. The test protocol will consist of four consecutive maximal knee extension movements of each leg at two different fixed movement velocities, 1.05 rad.sec-1 (60 deg.sec$^{-1}$) and 3.14 rad·sec-1 (180 deg.sec$^{-1}$) from which peak torque (Nm) data will be recorded. Each movement will be separated by a five-minute rest.
We will then assess your single-leg leg press ten-repetition maximum (10RM) on a leg press machine, which will be the same equipment that will be used in the subsequent exercise sessions - this will help to provide familiarization to the apparatus. You will perform warm-up exercises including cycling (5 min at 50 W) and leg press (10 repetitions at <50 % of your predicted 10RM

estimated from normative data). Repetitions of leg press will be performed to a metronome-based cadence of 3 seconds for the concentric (push) phase of the lift and 3 seconds for the eccentric (return) phase of the lift. If you are able to complete 10 repetitions at a given weight, a greater weight will be selected and the test repeated after a 5 min period of rest. The greatest weight with which you are able to complete ten repetitions is recorded as the 10RM.

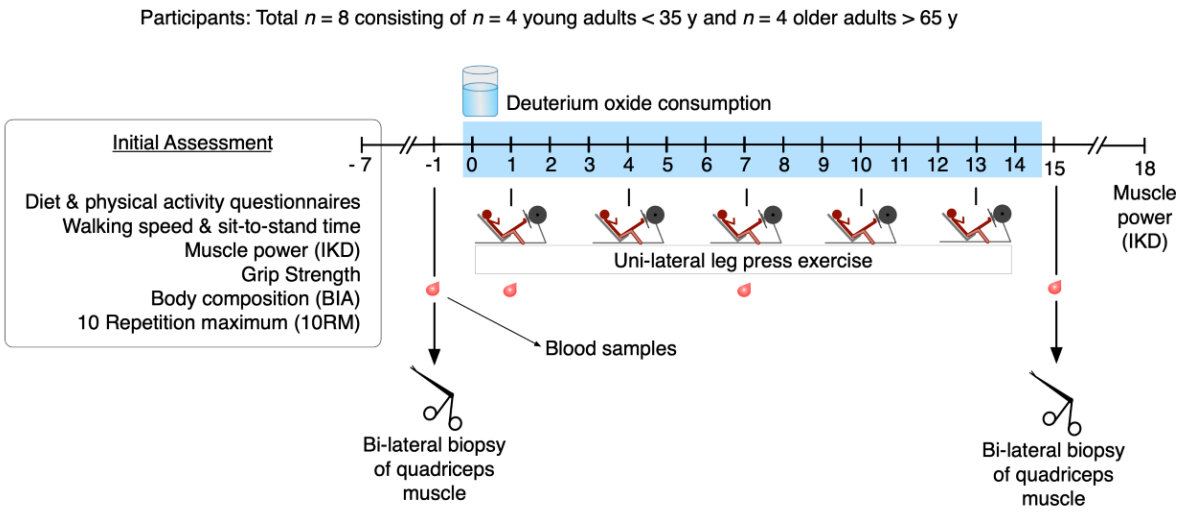



Figure 1 – schematic illustration of the experimental protocol.

**Experimental Intervention**

**Baseline muscle biopsy**

On day -1 of the experimental intervention (around 7 days after the initial assessment), you will need to arrive at the laboratory at ~8 am after an overnight fast. A blood sample (7 ml) will be collected via venipuncture and muscle samples will be collected by percutaneous biopsy of the vastus lateralis (quadriceps) of each leg (see below for more detail).

**Deuterium Oxide consumption**

On the next day (Experiment Day 0) you will consume the stable isotope, deuterium oxide (D2O – see below for more details), which is a tracer that can be used to measure the turnover rate of proteins inside you muscle. You will consume a dose of 13.3 ml D2O per kg body weight on Day 0. This equates to ~1 L of liquid for a person of 75 kg body weight. The liquid must be consumed in 4 doses of ~250 ml each at 2 h intervals, during which time you will be monitored for potential side effect (see below) by the research team, this means you will need to be available for 6 h on this day. (N.B. during this period you will have access to office and catering facilities on the LJMU Byrom Street site). On subsequent days of the experiment you will need to consume one maintenance dose of 0.67 ml D2O per Kg body weight (e.g. 50 ml for a person of 75 kg body weight) – self-administered each day, at a standardised time of day until day 14.

**Unilateral leg-press exercise**

On days 1, 4, 7, 10 and 13 of the experimental intervention, you will perform supervised single-legged resistance exercise (unilateral leg-press) at a standardised time of day. Each session will begin with a warm-up consisting of 5 repetitions at 70 % of 10RM. You will then perform three sets of 10 repetitions at a lifting intensity of 90 % of 10 RM with 2- to 3-min rest between sets. On days 1 and 7 we will collect a venous blood sample (7 ml) and a finger-prick blood sample (~0.2 ml).

**Post-intervention muscle biopsy**

On day 15 of the experimental intervention, you will arrive at the laboratory at 8 am after an overnight fast. A blood sample (7 ml) will be collected via venipuncture and muscle samples will be collected by percutaneous biopsy of the vastus lateralis (quadriceps) of each leg.

**Post-intervention muscle function test**
On day 18 of the experimental intervention, isokinetic torque of the knee extensor muscles will be assessed via **isokinetic dynamometry (IKD).** Consistent with the initial assessment (Experiment day -7), you will first perform a warm-up (5 min at 50 W on a cycle ergometer) and then complete a test protocol consisting of four consecutive maximal knee extension movements of each leg at two different fixed movement velocities, 1.05 rad.sec-1 (60 deg.sec$^{-1}$) and 3.14 rad·sec-1 (180 deg.sec$^{-1}$) from which peak torque (Nm) data will be recorded. This assessment will be scheduled at a time of day roughly equivalent to that of your initial visit.

## 5. Are there any potential risks in taking part?

The main risks to you as a participant are associated with the collection of muscle and blood samples. All these measures are, however, routinely used in research and carry a low risk (outlined below). If at any point during your participation you feel uncomfortable, unwell, or unable to continue participating for any reason, you should contact the research team immediately and testing can be ceased if necessary.

**Muscle biopsy procedure:**
In total you will have 4 muscle biopsies taken during the study (one sample from each leg at two different time points). Briefly, you will undergo a short period of discomfort whereby you receive a local anaesthetic to numb the area on the vastus lateralis (thigh) muscle. The researcher will then make a ~4-5 mm incision using a scalpel and extract an extremely small (approximately the size of a grain of rice) piece of muscle tissue for analysis.

These procedures are carried out by fully qualified personnel, who have been previously trained and signed off by a Physician. The biopsy sites will be fully dressed upon completion of the procedure, and you will be able to walk without any pain immediately after the dressing is applied. The day after the biopsies you may feel a 'dead leg' like sensation and may experience minimal amounts of bruising which is normal. If you do experience any pain, this normally disappears within 2 days of the biopsy and if you have any concerns, you can contact a member of the research team and come back in at any time to get the biopsy site checked.

There is an extremely low risk (1 in 1,000,000) of allergic reaction to the local injection. The chance of a local skin infection in less than 1 in 1000. Carefully cleaning the skin and keeping the area clean until the skin heals will minimize this. Infection can be serious and if you therefore experience a lot of bleeding from the biopsy site, swelling or infection around the biopsy site, faintness, light headedness, heart pain, chest pain or increasing pain in your leg which is not relieved by Paracetamol, you must contact the researcher who did the biopsy right away. However, if for some reason, you are not able to contact this individual then you should contact your family doctor or go to the Accident and Emergency Department. More detailed information on the muscle biopsy procedure can be found on the accompanying muscle biopsy information sheet. This information sheet will provide advice on what to do following your muscle biopsy and it is essential these guidelines are followed.

All risks can be discussed with the research team, and we advise all participants to visit their GP before consenting to take part in the study. This will give you the opportunity to discuss any medical history that may prevent you from taking part.

Because you will be given an anaesthetic, we will request your consent to notify your GP about your involvement in this study

**Blood sampling**
You will feel a sharp pain when the needle is inserted, though this will be short-lived. The researchers are accredited and experienced in this technique, so the pain you experience should be minimal. You may develop a small bruise on your arm, which can be prevented by applying pressure on the site following removal of the needle (the researcher will either do this themselves

or ask you to do so if you are comfortable to). The risk of haematoma or bruising has been shown to occur in ~12% of blood collections, with minor bruising most common at the site. There is also a small risk of fainting, which typically occurs in less than 1% of blood collections. If you develop any issues at a blood collection site, you should contact the research team, who will decide if there is an issue for medical referral.

**Deuterium (D2O) Consumption:**
You will be asked to orally consume the stable isotope, deuterium (D2O), also called 'heavy water', which is similar to regular water ($H_2O$) but contains a stable (non-radioactive) isotope of hydrogen with one more neutron ($^2H$ instead of $^1H$) and it is a bit heavier than regular water (~1.1 kg per litre instead of 1 kg per litre). Heavy water behaves like water in your body, but because of its slightly heavier isotope, we can detect how much of it is retained in your body. Also, because there is decay in the amount of circulating D2O you consume, we will ask you to consume small top-up doses on a daily basis.

D2O consumption is safe but there are risks associated with deuterium consumption when enrichment exceeds ~20%, or when large amounts (e.g. > 500 ml) are taken as a single dose. These side-effects typically include dizziness and nausea. Human research, including this study, typically aims to achieve an enrichment of 1 - 3%, which is significantly below this threshold.

The risks associated with deuterium consumption in the doses provided in this study are therefore minimal. You will be monitored during the initial enrichment (4x 250 ml drinks over 6 h on Experiment Day 0) in case you experience side-effects. During the remaining days of the experiment you will be provided with 50 ml doses to consume daily at home. In the unlikely event that you experience any side-effects whilst not in the lab, you should contact the research team immediately and if necessary, we will advise you to seek further medical advice from your GP or by dialling 999.

### 6. Are there any benefits in taking part?
The potential or hoped for benefits of the study for the wider society are to show there detectable age-related differences in the turnover of proteins in human muscle during basal conditions or when performing regular exercise. If this is the case, our new technique for assessing protein turnover in humans could help other researchers to better develop and test interventions aimed at improving healthy ageing.

### 7. Payments, reimbursements of expenses or any other benefit or incentive for taking part
You will receive up to £15 per laboratory visit for reasonable travel costs

### 8. What will happen to information/data provided?
The information you provide as part of the study is the **study data**. Any study data from which you can be identified (e.g. from identifiers such as your name, date of birth, audio recording etc.), is known as **personal data.** Your participation in this study will involve the collection/use of personal data.

We will keep personal data safe and secure. People who do not need to know who you are will not be able to see your name or contact details. The personal data collected will include:

- Contact details. With your agreement, we would like to store your contact details so that you may be contacted about future opportunities to participate in research studies.
- A record of consent (which will include your name)

Study data. We will use a code/pseudonym so that you cannot be directly identified from the data. Study data / records of consent / contact details will be kept for up to three years after the study has finished.

Once we have finished the study, we will keep some of the data so we can check the results.

**9. What will happen to the tissue samples?**

It will be necessary to retain the consent form (personal data) until the sample has been depleted or destroyed, in order to meet the traceability requirements of the Human Tissue Act.

Your DNA and tissue samples will be assigned a code and your data will also be identified only by this number. You will not be identifiable from reading the code; however, your DNA is unique to you so it can never be completely anonymous.

Your muscle biopsy sample(s) (or derivatives of) will be stored securely at LJMU indefinitely and might be shared anonymously with other investigators, in ethically approved studies that take place in hospitals, universities, non-profit institutions or commercial laboratories worldwide. If you agree to your samples being used in future studies, your consent form will be held until the samples have been used up.

**10. What if we find something unexpected?**

The investigator will not suggest the presence or absence of diagnosable conditions. The study is not a clinical or personal assessment and the investigators are not appropriately qualified to provide meaningful individual assessments. If the investigator suspects they have found something unexpected, they will consult with senior researchers and may advise you to seek appropriate advice if they think that could help you gain access to services that might be of help.

**11. Who is organising and who is funding/commissioning the study?**

This study is organised by Liverpool John Moores University and funded by the ART of Healthy Aging Network who focus on addressing novel questions on underlying biological processes during ageing.

**12. Whom do I contact if I have a concern about the study or I wish to complain?**

If you have a concern about any aspect of this study, please contact Dr Jamie Pugh, and we will do our best to answer your query. You should expect a reply within 10 working days. If you remain unhappy or wish to make a formal complaint, please contact the Chair of the Research Ethics Committee at Liverpool John Moores University who will seek to resolve the matter as soon as possible:

Chair, Liverpool John Moores University Research Ethics Committee; Email: FullReviewUREC@ljmu.ac.uk; Tel: 0151 231 2121; Research Innovation Services, Liverpool John Moores University, Exchange Station, Liverpool L2 2QP

**13. Data Protection**

Liverpool John Moores University is the data controller with respect to your personal data. Information about your rights with respect to your personal data is available from:

- https://www.ljmu.ac.uk/legal/privacy-and-cookies/external-stakeholders-privacy-policy/research-participants-privacy-notice
- by asking one of the study team or contacting us using the information below

**14. Contact details**

Principal Investigator: Dr Jamie Pugh (Member of LJMU staff)
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LJMU School/Faculty: Sport and Exercise Science
LJMU Central telephone number: 0151 231 2121