Participant Information Sheet

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Concurrent multi-organ responses to CHronic physical Activity and Inactivity intervention, to increase research discovery in human health and wellbeing

REC Project ID: FMHS 103-0124

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We would like to invite you to take part in our research study. Before you decide, we would like you to understand why the research is being done and what it would involve for you. One of our team can go through the information brochure with you and answer any questions you have. Talk to others about the study if you wish. Ask us if there is anything that is not clear.

This study requires a significant commitment from those volunteering to take part, so please read through this information sheet carefully to help you understand what participation entails. If you have any questions, or would like an informal visit to the laboratory to help you understand what the study involves, then please don't hesitate to contact researchers (contact details at the end of this document). If you wish to visit the laboratory you would be under no obligation to participate and would NOT be asked to make any decision about participation at this meeting.

What is the purpose of the study?

Life expectancy has been increasing at approximately 2 years per decade for the last 150 years. However, in the last 30 years, the maintenance of good health has not kept pace with increased lifespan, and on average, UK adults spend the last decade of their life in poor-health. This has major consequences for health and social care services, employment, the individual and their family. It is therefore vital for individual wellbeing and the wider society that more adults reach old age in better health and maintain a good quality of life for a greater proportion of their older age. Key to achieving this is a need to understand what drives the trajectory of ill health as we age, and this project aims to significantly contribute to this enterprise.

From research which has looked at characteristics associated with general health and lifespan within large populations, habitual physical inactivity is thought to be a key contributing factor to a risk of developing poor health and a decline in the physical and/or brain functioning required to carry out activities of daily living. It is therefore of significant concern that sedentary behaviours are now extremely common in the UK; most middle-aged adults spend over half their waking day (8+hrs) being sedentary, with the average step count being between 3000-4000 steps/day.

The biological changes and processes in the body which cause this relationship between physical activity levels and health, and the time frames over which these changes occur across different tissues and organs (in relation to each other) are not clear. Indeed, inactivity has rarely been studied in an integrated way in humans. The general assumption is that the effects of being sedentary on the way that body works, are simply the reverse of what is seen when we increase our physical activity. However, initial research suggests that this is not the case. To be able to successfully assess the efficacy of future therapies (lifestyle and drug) to address age-related decline in health and devise robust public health messages to help individuals reach older age in better health, it is essential that the complex effects that activity and inactivity have on the body are characterised.

This project aims to address some of the limitations of previous research studies by identifying (a) changes that occur in the structure and functioning of multiple tissues and organs in the body, (b) the biological processes controlling those changes, and (c) the time frame that changes occur in response to increasing and decreasing physical activity levels.

In summary, the biological processes that underpin the relationship between physical activity levels and health are not clear, and research examining changes in the body across tissues and organs in response to physical activity and inactivity are absent. Addressing these knowledge gaps has been recognised as vital to assist scientists and medical doctors working in the fields of human chronic diseases and aging to help more adults reach old age in better health and maintain a good quality of life for longer.

Why have I been invited?

You are being invited to take part because you have expressed an interest in participating in the study and have indicated that you are aged 55-65 years and that currently you are not very physically active. We are recruiting 20 participants like you to take part (Group 2). Your results will be combined with results from other participants in your group to investigate changes that occur across the body after decreasing physical activity levels.

In addition, we will be recruiting 20 people who are moderately active and will be asking them to reduce their activity levels (Group 1). Results from both groups will be compared to understand differences in how activity and inactivity bring about changes in the body.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information brochure to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. This would not affect your legal rights.

What will happen to me if I take part?

Summary: This study needs you to increase your physical activity by attending the Medical School at Queen's Medical Centre, Nottingham, three times a week for six months to undertake a supervised exercise program. Before and during this 6-month period we will ask you to make some measurements at home and attend the University of Nottingham to have multiple assessments made. Figure 1 shows the outline of the study, which will last a maximum of 28 weeks (7 months), including the screening and baseline period.

Including attendance for exercise training sessions, the study will require you to come to the University on **81** occasions over 7 months, with assessments being made on **33** of these. The schedule of these visits and measurements being made at each visit are shown in Figure 4.

Assessment visits will take place between Monday and Friday, will last a minimum of 1 hour and a maximum of 7 and a half hours, and will incorporate time for breaks in between measurements. The length of individual visits is outlined in the appendix. Please note that before the intervention begins and in weeks 6, 12, 18 and 24 of the intervention, you will need to attend the laboratory on several days during those weeks.

Exercise training sessions will take approximately an hour and can be scheduled Monday to Friday between the hours of 7am and 7pm (last appointment 6pm), or between 9am and 12pm on Saturday morning (last appointment 11am).

Each study day, measurement and procedure are described in greater detail in a participant brochure which can be obtained by contacting Abhishek Sheth or Aline Nixon, whose contact details are given at the end of this document.

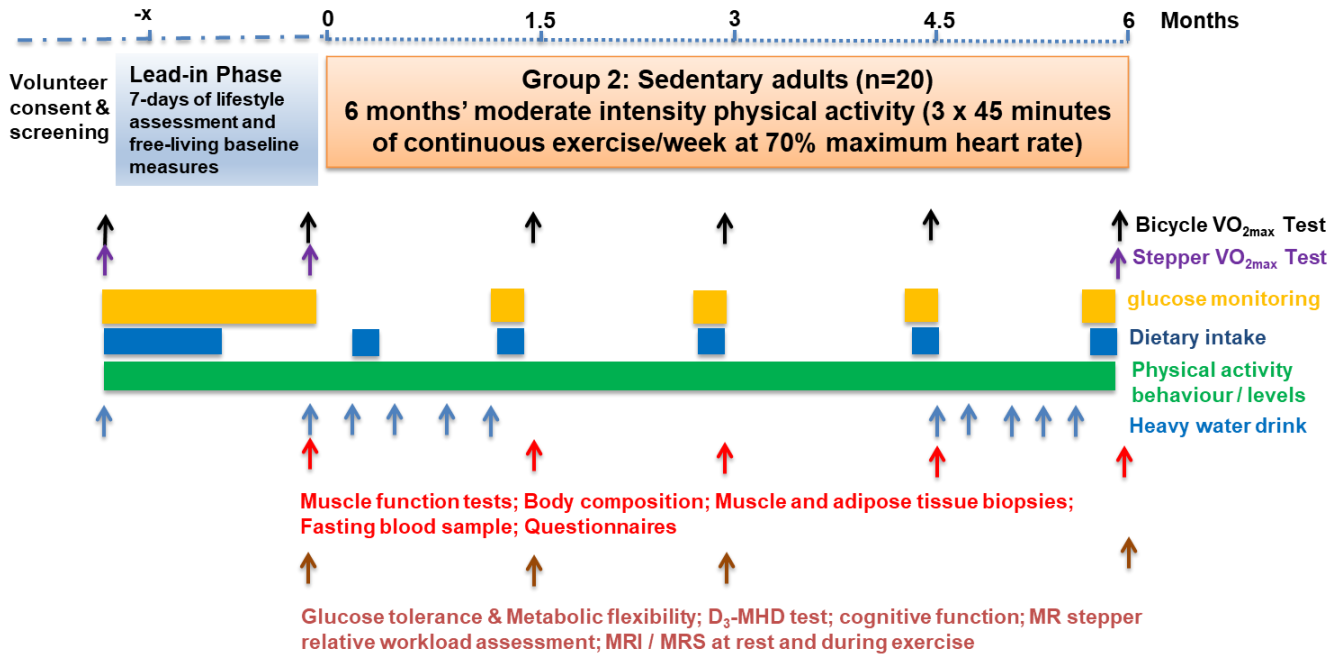

Figure 1: Study schematic. *Timeline not drawn to scale.*

'Tracers': In the description of the study which follows, you will be introduced to the term 'tracer'. This is a substance which is given to you in small amounts and can be identified in blood, saliva or tissue samples using specialist chemical analysis techniques. This is due to the presence of small differences when compared with similar molecules found in the body. The tracers that we use in this study are water (H_2O) and the amino acid (building block of protein) '3-Methylhistidine' which have been 'labelled' by replacing hydrogen atoms with a heavier form of hydrogen, called deuterium. Deuterium is found in nature (we already have it in our bodies) and is known as a 'stable isotope' of hydrogen as it is not radioactive. The deuterium-labelled water ('heavy water') and D_3-3-methylhistidine (D_3-3MH) behave chemically in the same way in the body as non-deuterium versions. However, the small difference in their weight allows us to distinguish the 'labelled' tracers we give you from water or 3-methylhistidine that is already in your body. By analysing the amount of heavy water that has been incorporated into your muscle tissue we can calculate the rate that proteins in the muscle are made during the study, and the D_3-3MH tracer allows us to quantify the rate that the protein in your muscles is being broken down.

Consent and screening (visit 1): If you are interested in participating in the study, you will be invited to attend the David Greenfield Human Physiology Unit (Medical School, Queens Medical Centre) at a convenient time (Mon-Fri, 8am – 5pm) for a consent and screening visit (V1). This visit will check that you are suitable to be included in the research study and should last no longer than 60 minutes. If you are found to be ineligible for the study, we will let you know the reason why you are not being recruited and we will not collect any further data from you.

7-day lead-in period: You will be asked to attend the David Greenfield Human Physiology Unit (Visit 2) before entering a 7-day 'lead-in phase' (12-21 days prior to the study intervention begins). At this laboratory visit, you will be familiarised with the exercise equipment that we will be using during the study and will undertake two fitness measurements; once on a stationary bicycle and once when lying down using a stepper machine. Over this visit, you will also be asked to consume some 'heavy water' tracer (D_2O; 3mls per kg body weight split into 3 drinks) and provide a saliva sample.

Free-living assessments during the lead-in phase: During the 7-day lead-in phase, we will ask you to wear 2 activity monitors; one is attached to your thigh using a waterproof dressing (ActivPal), and the other is worn on

your chest (at the level of your heart) attached to two ECG adhesive pads (ActiHeart). You will also record everything that you eat and drink over three weekdays and one weekend day. Finally, we will assess the way that your body handles the sugars found in your diet using a glucose sensor which is attached onto your upper arm. It is important that you do not change your usual activity and eating habits over this recording period. Again, if you are found to be ineligible for the study after the lead-in phase, we will let you know the reason why you are not continuing to the intervention and we will not collect any further data from you.

Pre-intervention laboratory assessments: After the lead-in phase, you will undergo a series of detailed measurements which will take place across 3 days at the David Greenfield Human Physiology Unit and over 1 day at the Sir Peter Mansfield Imaging Centre on the University of Nottingham main campus.

Day One (Visit 3):

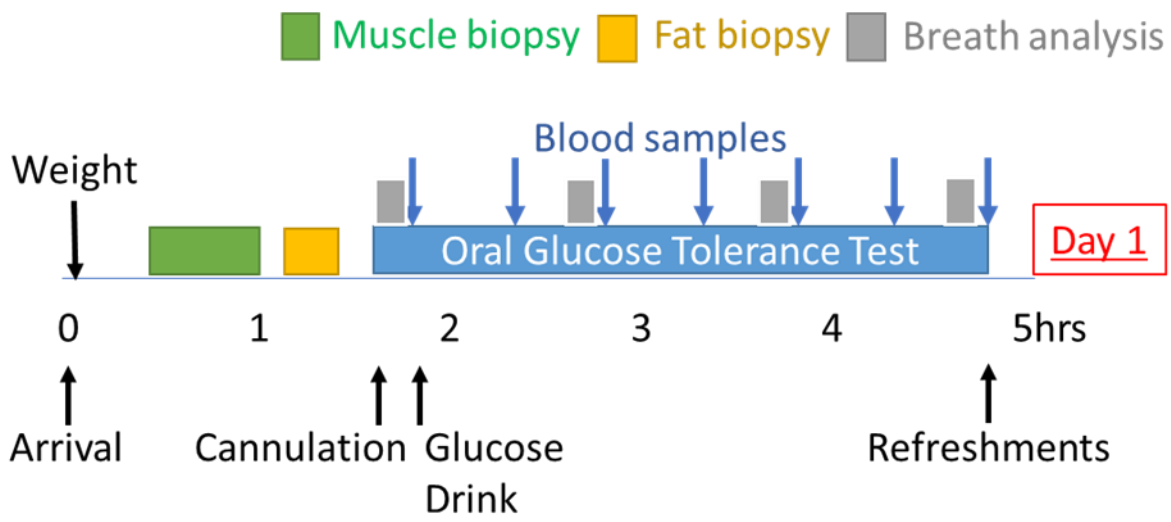

Figure 2: Overview Day 1

You will be asked to arrive at the David Greenfield Human Physiology Unit between 8am and 9am in the morning after fasting from midnight the night before, having also avoided alcohol and strenuous exercise for the previous 24hrs and caffeine for the previous 18hrs. This visit will take approximately 5 hours (see Figure 3). Initially you will be weighed and then we will then ask you to rest on a hospital bed. The study doctor will take a biopsy from a muscle in your thigh and collect some fat from the adipose tissue on your tummy (at the level of your belly button), both after applying local anaesthesia. These procedures will take about an hour in total.

You will then undergo an oral glucose tolerance test, which is similar to the test that is used in the hospital to diagnose diabetes, and will take ~3.5 hours. During this time, we will take some blood samples and also measure how many calories you burn when lying down (resting energy expenditure) and the amount of fat and carbohydrate that your body is using to provide energy (fuel oxidation) by analysing the amount of oxygen and carbohydrate in your breath. At the end of the 3-hour period, you will be offered refreshments before you leave (Figure 2).

Day Two (Visit 4):
You will be asked to arrive at the David Greenfield Human Physiology Unit in the morning between 7am and 9am in the morning to undergo the '3-methylhistidine test'. You should have nothing to eat or drink (other than water) for 10 hours prior to the test, and also have avoided alcohol for the previous 24hrs. This visit will take approximately 7.5 hours (Figure 3). A cannula will be placed into a vein in your arm and blood samples will be taken over the day.

During the study day we will also ask you to complete 3 questionnaires, which will take about 30 minutes in total, and carry out 6 puzzles on a computer which test different ways that the brain functions ('cognitive function tests'). Each test lasts for between 5 and 10 minutes and you will be given breaks between each one. Over the study day we will also take the opportunity to talk with you about the project, and discuss the exercise training sessions that you will carry out over the 6-month study. At the end of the 3-methylhistidine test you will be offered a meal for you to have before you leave the laboratory.

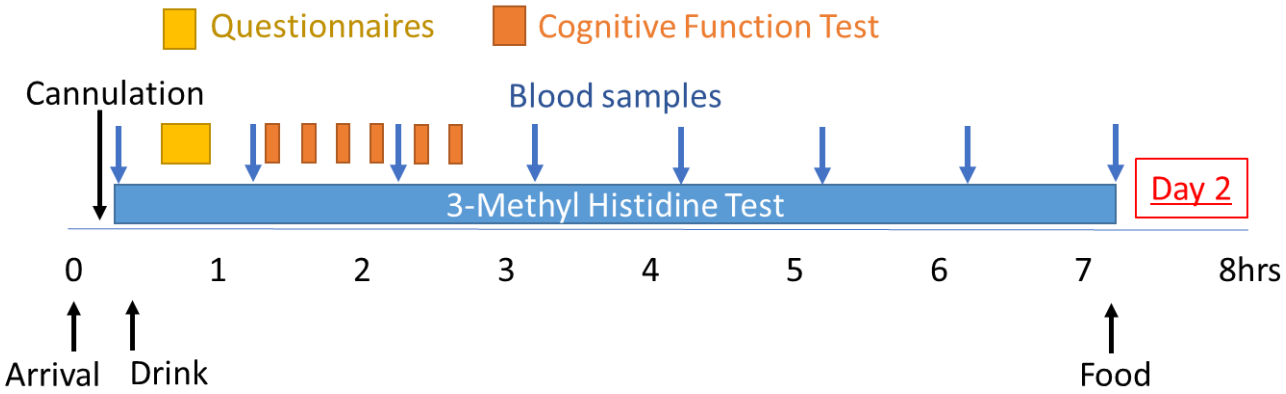

Figure 3: Overview Day 2

Day Three (Visit 5): This visit can be scheduled in the morning or afternoon to fit your availability and does not require you to be fasted. You will repeat the 2 exercise tests that you had at visit 2 (before the lead-in phase began); VO_2 max tests on the stationary bicycle and the stepper. These will be followed by assessments of your thigh muscle strength and muscle function.

Finally, on Day 3 you will be given a 'top-up' heavy water tracer drink to consume and be provided with a tube to collect a saliva sample at home. This saliva sample should be given to the research team on your scheduled Day 4 visit.

Day Four (Visit 6): This visit will take place at the Sir Peter Mansfield Imaging Centre located on the University of Nottingham main campus. You will need to be fasted for at least 2 hours before this ~4-hour session, and the visit can be scheduled to take place in the evening (starting at 4pm), or the morning (starting at 8am) to fit in with your availability. However, the time of the day (morning / evening) that you schedule Visit 6 will be kept the same for Day 4 assessments at each measurement timepoint during the intervention.

This visit involves having a series of MRI scans made when you are lying quietly in the MRI scanner and having some measurements taken when you are exercising on the stepper machine. In between each series of scans, you will be able to come out of the MRI scanner for a break and during the scanning you can listen to the radio.

Study Intervention:

After these baseline series of assessment days have been completed, you will then start the 6-month intervention period. Over this time, you will need to come into the David Greenfield Human Physiology Unit 3 times a week to undertake 45 min of cycling on a stationary bike. This will provide 150 minutes of moderate activity over the week, which is the recommended weekly amount of exercise to do. On assessment weeks (weeks 6,12, 18 and 24 of the intervention), you will not need to attend exercise sessions due to the measurements that will be made. We will ask you to also wear the ActivPAL activity monitor continuously over the 6 months (being removed when having the magnetic resonance scans) so that we can track the total activity that you do each week, but you will not be asked to do extra exercise sessions outside of the supervised sessions that we run.

At different timepoints during the intervention the measurements described across days 1 to 4 will be repeated. Which measures will be taken at which time points during the intervention are outlined in Figure 4.

What if we notice something abnormal in one of the scans?

Since you are healthy, it is unlikely that a MRI scan will show any abnormality. Even if there were an abnormality, it is unlikely that we would notice it since we are undertaking the assessments for scientific research, which are not the same as scans collected by doctors for medical purposes. Furthermore, the pictures will not be looked at by a radiologist (a doctor qualified to find abnormalities in scans).

If we did notice anything abnormal on your scan, the investigator would arrange for an appropriately qualified doctor, from a healthcare provider e.g. an NHS Trust or a private doctor, to look at them. That specialist doctor would contact your GP to explain the findings, so that your GP could then advise you.

What if we notice something in one of the other assessments?

All the assessments undertaken for this study are for research purposes only and are not intended to be used for health care However, if something is noticed that we feel you should be aware of we will let you know and pass on the information to your GP with your permission.

Expenses and payments

Participants will be paid an allowance of £350 after attending assessment visits at each of the 5 timepoints (before, and at weeks 6,12,18 and 24 of the intervention) to meet out of pocket expenses (total allowance £1750). An additional £250 will be offered at study completion (week 24) as a thank you for giving up your time to participate. These inconvenience allowance payments are not taxable and would not impact welfare benefits.

What are the possible disadvantages and risks of taking part?

Time commitment: The study requires many visits to the University for the assessment visits and the supervised exercise program at the end of the intervention, and these represent a considerable time commitment for participants. It is important that you carefully consider the requirements of the study and whether it would be possible to incorporate this commitment into your life before you decide to volunteer.

Being asked to reduce your activity may impact your wider social life and it is recommended that you take time to consider this possible impact of taking part and whether it would be acceptable, including discussing with friends and family, before deciding to volunteer for this study.

Biopsies: At each assessment time point (before, and at weeks 6,12,18 and 24 of the intervention), a biopsy will be taken from a muscle in your thigh and a 'fat' biopsy will be taken from the adipose tissue found under the skin near to the belly button. Prior to these procedures, local anaesthetic will be given to numb the area to make the procedure as painless as possible, but there is a risk that you will develop some bruising at the site of the biopsies. Pressure above the insertion sites will be applied after the biopsies have been taken to reduce any bleeding, and you will be asked to wear a compression bandage around your thigh for at least 4 hours after the muscle biopsy. In the last 12 years (932 muscle biopsies taken), we have had 2 occasions where participants have experienced a 'haematoma' (collection of blood) at the muscle biopsy site. You may experience some discomfort at the biopsy sites (adipose and muscle) for 48 hrs following the procedure, but this can be relieved using the over the counter painkiller, Paracetamol. If you cannot take this medication, please tell the experimenters. There is a very small risk (<1 in 1000) that a biopsy site could become infected and the study doctor will check them when you come into the laboratory for scheduled study visits in the days following the procedures. However, we have not had any infections occur with these methods in our laboratory previously. After a muscle biopsy, there is also a very small risk of damage to the small nerve branches which lie near to the muscle we are sampling from. This damage can result in skin sensations (numbness or tingling) or in very rare cases, partial weakness of a muscle in the thigh. Nerve injuries like this would not impact your day-to-day

activities and usually resolve in 8-12 months, but there is a theoretical risk of a residual mild weakness in this area. Again, since the introduction of the 'micro' muscle biopsy method 12 years ago, we have not had this reported. It is also important to note that the incision for the muscle biopsy will leave a small scar which will fade over time. If you have experienced pronounced scarring, called a 'keloid' scar after an injury previously, then we would not recommend that you take part in the study.

Discomfort from blood sampling: Blood samples taken during the oral glucose tolerance test and 3-methylhistidine test will be taken via a plastic tube (cannula) inserted into a vein. This removes the need for you to have multiple needle sticks, but some minor discomfort may be experienced when the cannula is inserted. However, a small amount of local anaesthetic will be given before the cannula is put in to make the process as comfortable as possible. At the screening visit and in week 18 of the intervention, a blood sample will be taken from the arm using a needle. This is similar to having a blood sample taken at your GP practice or at the hospital. The needle feels sharp and may sting when it enters the skin, but the procedure will take less than a minute to do. When the cannula or needle is removed, there is a risk that a bruise will develop. We ensure that pressure is placed above the puncture site for 5 mins after a cannula or needle are removed to reduce the risk that this occurs. There is also a very small risk that the puncture site made following blood sampling or cannula insertion could become infected. However, in the last 30 years (since records have been kept), the research team have not had this occur.

Consumption of Heavy water: At several timepoints during the study you will be asked to drink water which contains a heavier type of hydrogen in the molecule, called Deuterium. This is found naturally in the body, is not radioactive and behaves chemically like regular water in the body. However, some people (fewer than 1 out of 30) may initially feel light-headed, dizzy, or experience vertigo after they have consumed the first drink, because when the heavy water initially becomes distributed around the body it changes the density of the liquid in your inner ear. When this does occur, it is a temporary feeling which resolves within 2-4 hours and is not experienced with additional consumption. Heavy water has been extensively tested in humans over the past 70 years and there are no risks associated with consuming the amount that you will be given during the study.

MRI scans: Although the magnetic resonance imaging machine that we use has a wider diameter than traditional scanners found in the NHS, you do go inside a tube for measurements to be made, and this can make people feel claustrophobic. If you know that you can feel claustrophobic in small spaces, then we would recommend that you do not take part in this study. However, you may not know this until you go into the MRI scanner, so we take measures to reduce the risk of you feeling uncomfortable during the scanning. You will be able to communicate with experimenters throughout the scanning process and will be taken out immediately if you feel uneasy or wish to stop at any point. We also have glasses which you can wear, which enable you to see out of the scanner tunnel and help to reduce feelings of claustrophobia. MRI machines can also be very noisy, so we will provide you with earplugs and ear defenders to wear during the scanning periods.

Some people experience vertigo (dizziness) when moving quickly through a magnetic field, so we will move you in and out of the magnet slowly to reduce these feelings, and people with balance problems will not be recruited onto the study. There is also a small chance that you experience muscle twitches during a scan. This is related to the changes in the magnetic field that occurs during MRI and it will stop when the scan is stopped. You may be withdrawn from the study if you experience these twitches, or if they only occur with a specific scan sequence, we may ask whether you would be happy to continue with the study, but to drop that specific measurement.

Finally, laying in an MRI machine can be boring and may become uncomfortable after long periods of time. We will make sure that you are comfortable on the table before scanning begins (using padding, pillows and supports) and frequent breaks and position changes are built into the visit. You can also listen to the radio, or bring in your own CDs to listen to when in the scanner.

What are the possible benefits of taking part?

We cannot promise that the study will help you, but the information we get from this study will contribute significantly to scientific knowledge which should positively impact the health of older people in the future. It will help us to understand the changes that occur to the body when we become more active and how these changes affect our health in the longer term. The detailed measurements that we are making in this study will allow us to identify the key assessments that future researchers should be making to evaluate the effects of their treatments, and could mean that future research participants need fewer measurements to be made.

You may benefit from having detailed assessments of your diet, glucose regulation, physical fitness and body composition. You will also have a personal exercise training plan and may get physically fitter over the course of the study. This input and access to trainers could be more motivating than simply attending a gym and could be a benefit.

From experience in previous studies, we find that participants become part of a supportive study community of other participants and researchers, which may positively impact your sense of wellbeing.

What happens when the research study stops?

Once all participants have completed the intervention period, analysis of your biological samples will begin and all the data that we have collected on you will be collated into a single database which is stored securely and locally on a University of Nottingham server. These data will only be identified using your unique study code and no information which has the potential to identify you (e.g. date of birth, postcode) will be entered onto this database.

If you would like us to let you know the results of the study, we will ask for your consent to hold your contact details to enable us to do so. It may be several years after you have finished the study that we have all these results. If you have consented to be contacted after the study ends, you will be invited to a public meeting where initial results of the study will be shared. You will also be sent a summary of the main findings of the study (via email or post as per your preference) in case you cannot attend the meeting.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. The researchers' contact details are given at the end of this information sheet. If you remain unhappy and wish to complain formally, you can do this by contacting the University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee administrator , $^{c}/_{o}$ The University of Nottingham, Faculty Hub, E41, E Floor Medical School, QMC Campus, NG7 2UH. E-mail: FMHS-ResearchEthics@nottingham.ac.uk

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against the University of Nottingham but you may have to pay your legal costs.

How will we use information about you?

The University of Nottingham are the sponsor of this study. This means we are responsible for looking after your information and using it properly. We will need to use information from you for this research project. This information will include your initials, name, and contact details which will be held securely by the research team at the University of Nottingham. People will use this information to do the research or to check your records to make sure that the research is being done properly. If you agree, we will keep your contact details to send you the findings of the study. People who do not need to know who you are will not be able to see your name or contact details. Your research data will be identified by a code number instead.

We will keep all information about you safe and secure. We will write our reports in a way that no-one can work out that you took part in the study.

We may share our research data with researchers in other Universities and organisations (which may include commercial organisations), including those in other countries, for research in health and social care. Sharing research data is important to allow peer scrutiny, re-use (and therefore avoiding duplication of research) and to understand the bigger picture in particular areas of research. Data shared in this way will be anonymised.

What are your choices about how your information is used?

You can stop being part of the study at any time, without giving a reason, but we will keep information about you that we already have as we need to manage your records in specific ways for the research to be reliable. This means that we won't be able to delete or let you change the data we hold about you. However, if you withdraw from the study, you can choose for your data to be removed from the research analysis.

Where can you find out more about how your information is used?

You can find out more about how we use your information by:

- reading our privacy statement https://www.nottingham.ac.uk/utilities/privacy/privacy-information-for-research-participants.aspx
- asking one of the research team
- sending an email to liz.simpson@nottingham.ac.uk or
- ringing us on 0115 8230343.

What will happen if I don't want to carry on with the study?

Your participation is voluntary, and you are free to withdraw at any time without giving any reason, and without your legal rights being affected. If you would like to withdraw, contact the researchers (details at the end of this information sheet) and they can organise this for you. If you withdraw, or are withdrawn from the study for any reason, we will no longer collect any information about you, or from you, but we will keep the information about you that we have already obtained and we will seek your permission for us to use this in the research analysis. Similarly, if you withdraw, or are withdrawn from the study, we will no longer collect any biological samples from you, but we will seek your permission for us to use collected samples in the analysis of this study and for further research purposes. However, if you wish, we will destroy any biological samples which have been collected from you.

Involvement of the General Practitioner/Family doctor (GP)

Your GP (or other health care practitioner) will not routinely be notified of your participation in this study, unless you wish us to do so. However, at the consent visit, we will record your GP's details and will seek your consent for us to contact them if we discover anything in the course of the study that indicates that you may have a health condition. If this occurs, the study doctor will discuss this with you, and we will contact your GP to pass on any relevant information.

What will happen to any samples I give?

Blood, saliva, muscle and adipose tissue samples will be analysed in the UK either at Nottingham or Derby Medical Schools, or Queens Medical Centre Pathology Department.

1. 14 ml blood sample taken at screening and at visits 4, 11, 18, 25 and 31:
   Assessments that will be made on this blood, to check that you are in good health, are;

(a) *Full blood count*

(b) *Liver function test*

(c) *Urea, electrolytes* (e.g. sodium and potassium concentrations) *and 'glomerular filtration rate'*.

(d) *Glucose (blood sugar)* concentration

(e) *Blood Clotting*

2. 5ml blood sample taken when fasted at visits 4, 11, 18, 25 and 31:

(d) *Cholesterol* (type of fat) level in the blood

(e) *Biochemistry of the blood* (metabolome). This is a large-scale biochemical analysis of small molecules, commonly known as metabolites, within the blood. Collectively, these small molecules and their interactions within a biological system are known as the metabolome.

3. 4ml blood samples that are taken before and every 30-minutes for 3 hours after consuming the glucose drink during the oral glucose tolerance test (visits 3, 10, 17 and 30). These will be analysed for;

(f) the hormone *insulin*,

(g) *triglycerides* (type of fat)

(h) *glucose* concentration.

4. 3ml blood samples that are taken every hour for 7 hours during the 3-Methylhistidine test will be analysed for the amount of *labelled and non-labelled 3-methylhistidine* and allows us to quantify the rate that the protein in your muscles is being broken down.

5. Saliva samples collected after you have drunk the heavy water will be analysed for the amount of this tracer that is present. This tells us the proportion that is in your body and will be used, together with the muscle analysis, to calculate the rate that the protein in your muscles is being formed.

6. Muscle samples that are taken at visits 3, 10, 17, 24 and 30 will be analysed for;

(i) The incorporation of the *deuterium* from heavy water into molecules found in muscle cells to indicate the rate that muscles are growing during the study.

(ii) The activity of an enzyme, called *citrate synthase*. This is used to calculate the number of structures called mitochondria that are present in muscle cells. Mitochondria are vital within all living cells as these are where energy is produced from the breakdown of fat and carbohydrate.

(iii) The expression of genes which control cell functions such as regulation of fuel breakdown, inflammation, and cell growth. Extraction of DNA and RNA from muscle will be carried out to understand alterations in gene expression which underlie or are associated with physiological changes that we see.

7. Adipose tissue taken at visits 3, 10, 17, 24 and 30 will be analysed for the expression of genes which control cell functions such as regulation of fuel breakdown, inflammation, and cell growth.

We would also like to seek your consent so that any remaining samples may be stored and used in future research. This is optional (please indicate whether you agree to this on the consent form). The samples will be stored with a code unique to you and securely at the University of Nottingham under the University's Human Tissue Research Licence (no 12265). Some of these future studies may be carried out by researchers other than current team who ran the study (people identified at the start of this information sheet), including researchers working for commercial companies. Any samples or data used in future research will be anonymised, so you will not be identified in anyway. If you do not agree to this, any samples remaining at the end of the study will be disposed of in accordance with the Human Tissue Authority's codes of practice.

Will any genetic tests be done?

Analysis of genetic material may be performed on your biological samples and involves examining genes, which are the instructions for our cells. Genes are made of molecules called DNA. The DNA can be copied by cells into a molecule called RNA. RNA provides instructions to cells to make proteins. Extraction of DNA and RNA from muscle and adipose tissue will be carried out and will be analysed to understand alterations in gene expression (genes that are activated or supressed) which underlie or are associated with the physiological changes that we are measuring. These analyses do not provide information which could have clinical implications and do not tell us whether you have any genetic diseases.

What will happen to the results of the research study?
This research study will generate results which will be published in scientific journals and presented at National and International scientific conferences so that our findings are shared widely. This will ensure that the maximum benefit is derived from the research. Presentations at conferences will occur within the first year after the study (including sample analysis) has been completed, with initial publications being submitted after this time. You will not be identified in any report/publication.

Who is organising and funding the research?
This research is being organised by the University of Nottingham and is being funded by the Biotechnology and Biological Sciences Research Council.

Who has reviewed the study?
All research in healthcare is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by the Faculty of Medicine and Health Sciences Research Ethics Committee.

Further information and contact details:

If you have any questions, would like more detailed information on all the study procedures outlined in this document, or would like to arrange a meeting with a member of the study team before deciding whether to take part in this study, then please contact:

Ms Aline Nixon (Research Nurse)
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0115 9515151 ext 19105

or

Dr Abhishek Sheth (Clinical Research Fellow)
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The lead investigator for this project is Professor Paul Greenhaff who can be contacted at the David Greenfield Human Physiology address shown above, via email (paul.greenhaff@nottingham.ac.uk), or using telephone number 0115 8230133.

Thank you for taking the time to read this information. If you have ANY questions, please don't hesitate to contact Abhishek Sheth or Aline Nixon.

Figure 4: Schedule of events

	Screening	Before 'lead-in phase'	Lead-in phase: free-living measurements	Pre-intervention lab-based measures				Intervention																																		
Week Number	-4		-3 to -2	-1				1	2	3	4	5	6				7	8	9	10	11	12				13	14	15	16	17	18			19	20	21	22	23	24			
Laboratory assessment visit N°	1	2	-	3	4	5	6	-	7	-	8	9	10	11	12	13	-	14	-	15	16	17	18	19	20	-	21	-	22	23	24	25	26	-	27	-	28	29	30	31	32	33
Consent (30 min)	X																																									
Health Screening Questionnaire (10 min)	X																																									
screening blood sample (FBC,U+E,LFT; 10min)	X																							X																X		
general health questionnaire (10 min)	X																																									
12-lead ECG (10 min)	X																																									
Blood pressure / Heart rate (10 min)	X			X									X									X									X								X			
Body Weight (1 min)	X			X					X		X		X					X		X		X					X		X		X				X		X		X			
Bicycle VO$_{2max}$ test (30 min)		X				X									X									X									X								X	
Stepper VO$_{2max}$ test (30 min)		X				X																																			X	
D$_2$O tracer consumption (1 min)		X				X		X	X	X	X	X																			X			X	X	X	X	X				
Saliva sample (2 min)		X				X		X	X	X	X	X																			X			X	X	X	X	X		X		
7-day 'ActivPAL' activity monitoring			X					X (Continuous over the intervention period)																																		
7-day 'Actiheart' activity monitoring			X									X									X									X								X				
4-day Dietary record			X						X			X									X									X								X				
7-day glucose monitoring			X									X									X									X								X				
Stepper workload test (20 min)															X									X																		
Muscle Biopsy (45 min)				X									X									X									X								X			
Adipose tissue Biopsy (20 min)				X									X									X									X								X			
Oral glucose tolerance test (3 hrs)				X									X									X																	X			
fuel oxidation (15 min x 4 per timepoint)				X									X									X																	X			
D$_3$-MH tracer administration (5 min)				X	X								X	X								X	X																X	X		
D$_3$-MH tracer test (7 hours)					X									X									X																	X		
Questionnaires (25 min)					X									X									X									X								X		
Cognitive function tests (60 min)					X									X									X									X								X		
Blood sample (safety, archive, metabolomics)					X									X									X									X								X		
Muscle function tests; strength + fatigue (30 min) iEMG (30 min)						X									X									X									X								X	
Magnetic resonance scans at rest ; muscle (30min) liver, heart, brain, adipose (40min)							X									X									X																	X
Magnetic resonance scans during exercise; muscle function (30 min) heart and brain function (30 min)							X									X									X																	X
Group 2 exercise visits (~1 hr)								XXX	XXX	XXX	XXX	XXX					XXX	XXX	XXX	XXX	XXX					XXX	XXX	XXX	XXX	XXX					XXX	XXX	XXX	XXX				