Participant information sheet

The effect of dietary protein intake on body composition and muscle protein turnover during a 4-week period of energy restriction and exercise in older adults with obesity

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You are being invited to participate in a research study. This information sheet forms part of the process of informed consent. After reading this document, you should have a basic idea of what the research is about and what taking part in the study will involve. Please take time to read this document carefully, and do not hesitate to ask one of the researchers named above if you have any questions.

What is the purpose of this study?
This study aims to recruit 60 participants to understand the effects of a higher protein intake on muscle mass and function during diet-induced weight loss in older adults with obesity. While diet-induced weight loss is recommended to mitigate obesity-related health consequences, weight loss comes with its own risks. In older adults with obesity, weight loss can accelerate the decline in muscle mass and function (i.e. how much muscle and how this muscle can be used), which increases the risk of falls, fractures, and hospitalisation, compromising overall health. This research aims to investigate strategies to promote 'high-quality weight loss' – preferential fat loss while maintaining muscle. Resistance and endurance exercise during weight loss has shown promise in maintaining muscle mass, improving cardio-metabolic health, and preventing weight re-gain. However, the effect of different protein intakes in combination with exercise training during short-term weight loss in older adults with obesity is not well understood. Higher protein intakes may enhance the beneficial effects of exercise on muscle protein turnover during weight loss, leading to maintenance of muscle mass during weight loss. The findings of this study could contribute valuable insights into improving weight loss strategies for older individuals, reducing potential risks associated with weight loss.

Who can take part?
Due to the nature of the study, all of the following must apply for you to be eligible:

- Male or female
- Aged 65-80 years
- BMI ≥28 kg/m^2 or waist circumference ≥102 cm (≥40.2 inches) for males or ≥88 cm (≥34.6 inches) for females
- Generally healthy (as determined through your response to a General Heath Questionnaire [GHQ] – a questionnaire provided to you by the research team)

- Weight stable (weight loss or gain <2 kg in the 6 months prior to enrolment)
- Able and willing to attend six testing visits and 12 training visits at SportExR or MoveWell

Who cannot take part?
Due to the nature of the study, if any of the following apply you will NOT be eligible:

- Habitual smoker or vaper
- Participated in a body mass loss programme and/or resistance exercise training programme in the 5 years before being enrolled in the study
- Currently performing regular, structured exercise on >2 days per week
- Uncontrolled hypertension
- Cardiovascular or neuromuscular disease

This study includes 2 x skeletal muscle biopsies separated by 4 weeks. Each muscle sample is equivalent to approximately the size of half a pea. Participants will not be eligible to undergo skeletal muscle sampling if they regularly use non-steroidal anti-inflammatory drugs (NSAIDs, including aspirin) or anticoagulants (e.g. warfarin, rivaroxaban), but may otherwise be eligible to participate in the study.

If needed, please contact Archie Belfield for more information around your eligibility.

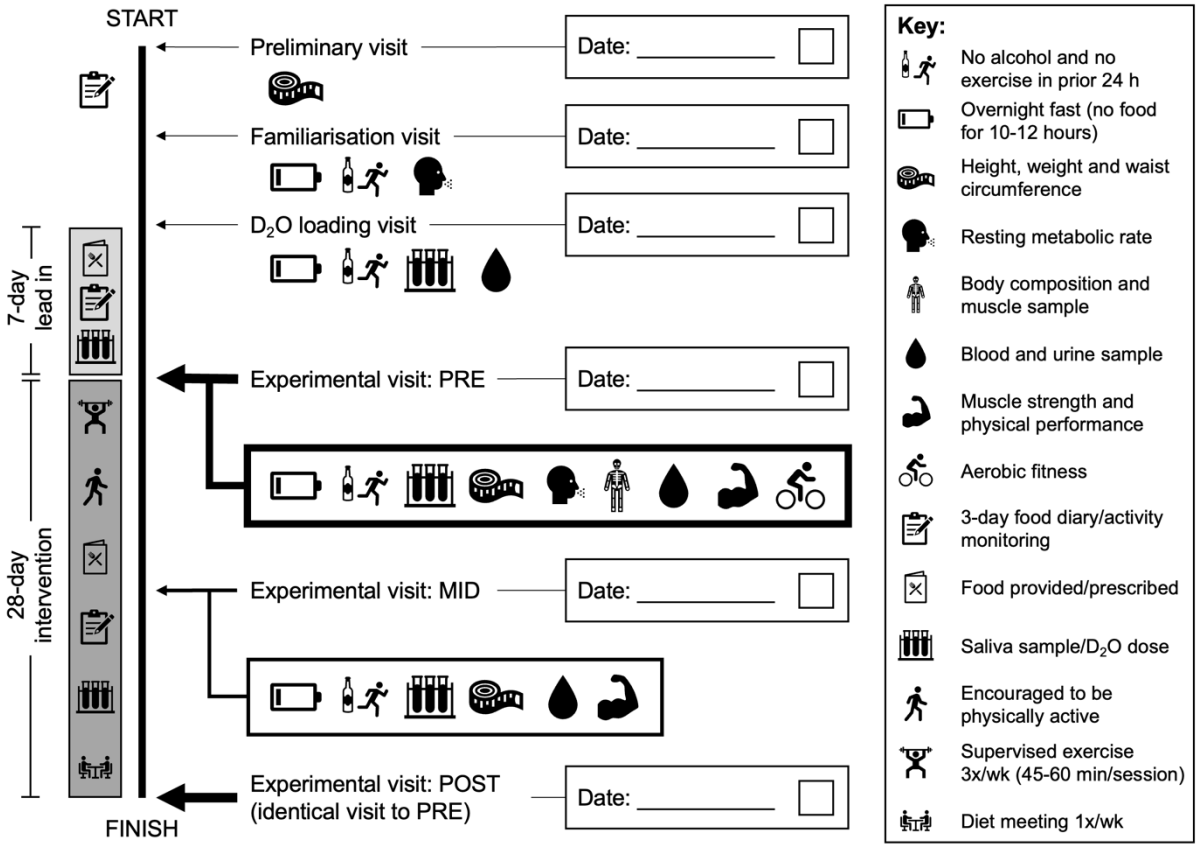
Do I have to take part?
No. It is completely up to you to participate. Before you decide, we will describe the study and go through this information sheet with you. If you agree to take part, we will ask you to sign a consent form. However, if at any time you decide you no longer wish to take part in this study you are free to withdraw, without giving a reason.

What will taking part in the study involve?
Summary: You will be asked to attend a total of six testing visits and 12 training sessions at the University of Birmingham: either at School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, UK (SportExR) or the Centre for Movement and Wellness, University of Birmingham, UK (MoveWell). You will be asked to attend a *Preliminary visit* (visit 1/6) to confirm your eligibility, to ensure you understand what the study will involve, and to obtain initial informed consent. Providing you are eligible, and consent to taking part, you will be invited to a *Familiarisation visit* (visit 2/6) to familiarise you with the study procedures. You will have the opportunity to ask questions about the study during these visits. The total duration of the intervention will be 35 days and will be separated into two phases.

The first phase will last 7 days and will act to establish a baseline. Before the first phase, you will be invited to attend a *D_2O loading visit* (visit 3/6), where you will be provided a ‘loading’ dose of deuterium oxide (D_2O) to consume orally and will be

provided with food to consume during the first phase (see *D_2O loading visit* below for more information about D_2O).

The second phase will last 28 days, with different groups receiving specific dietary interventions with the same exercise training intervention (training sessions 1-12). You will be invited to attend three *Experimental visits* before (visit 4/6), during (visit 5/6), and after (visit 6/6) the 28-day intervention. At the before and after experimental visits, we will assess your body mass, height, waist circumference, body composition, blood pressure, muscle strength, physical performance, and aerobic capacity, and obtain muscle and blood samples. At the mid-way visit, we will assess all outcomes except body composition, aerobic capacity and muscle sampling. Below is a graphical summary and a more detailed overview of each visit.



<u>*Experimental visit standardisation*</u>: Due to the high control of the research study, there are several instructions you will be asked to adhere to during the study.

24-hours before the *Familiarisation visit, D_2O loading visit,* and *Experimental visits*:

1. Do not drink alcohol
2. Do not perform any vigorous physical activity or exercise

On the morning of the *Familiarisation visit, D_2O loading visit,* and *Experimental visits:*

1. Do not perform any vigorous physical activity or exercise prior to your arrival
2. Please do not consume any caffeine-containing drinks prior to your arrival (i.e., no tea, coffee, energy drinks etc.)

3. Please arrive via the same mode of transport to ensure consistent activity prior to each visit
4. Please arrive in the morning at ~0700 after a 10-12 hour overnight fast (i.e., having not consumed any food or drinks, except water, since the evening before). The exact time will be confirmed with you prior to each visit

During the *Intervention*:

1. Please do not consume any food or drinks not provided or not prescribed to you by the research team
2. Please record and let a member of the research team know if you do consume any food or drinks not provided or not prescribed to you
3. Please record all food and drinks that you do consume using a 3-day weighed food dairy provided to you for each week of the intervention
4. Please do not engage in any other weight loss programme/intervention

Preliminary visit (Testing visit 1/6)*:* Following a telephone screening, you will attend a ~1-hour preliminary visit at SportExR or MoveWell. A member of the research team will explain the study, and you will have the opportunity to ask questions. Providing you are eligible; you will then provide initial informed consent and will complete a general health questionnaire. The research team will then obtain your date of birth, ethnicity, gender, blood pressure, body mass, height, body mass index (BMI), and waist circumference. You will be provided with a food diary and a set of weighing scales to record all food and drink you consume for a selected 3 days before the *Familiarisation visit*. The food diary helps us understand your usual diet and food preferences that will be considered when providing food during the intervention. Before leaving, you will be fitted with a wrist-worn pedometer and an accelerometer to measure daily steps and physical activity. We use this to estimate the amount of energy you expend during a typical day.

Familiarisation visit (Testing visit 2/6): You will be asked to attend a ~1-hour familiarisation visit at SportExR or MoveWell following a 10-12 hour overnight fast (i.e. having not consumed any food or drinks, except water, since the evening before). An overnight fast is required to accurately gain information about your metabolism. You will return the completed 3-day weighed food diary, the pedometer, and the accelerometer. The research team will then assess your resting metabolic rate (RMR) using indirect calorimetry, requiring you to lie down for 20-30 minutes while we collect breath samples. We measure RMR to determine your energy requirements during the intervention. You will then be familiarised with the dietary intervention foods, the process of reporting food intake, the exercise equipment and exercises that will be used during the intervention. Before leaving, you will be provided with a container to collect a 24-hour urine sample.

*D_2O loading visit (*Testing visit 3/6*):* You will be asked to attend a ~3.5-hour visit to SportExR or MoveWell, following a 10-12 hour overnight fast to ensure accurate data

on body composition and metabolism. You will return the 24-hour urine sample to one of the research team and provide a blood sample (about 20 mL or 1.5 tablespoons worth) to determine markers of inflammation and metabolic disease risk. A saliva sample will also be obtained for metabolic analysis.

You will then orally consume your first dose of D_2O (150 mL). D_2O is a naturally occurring, non-radioactive stable isotope tracer used to assess muscle protein synthesis. Stable isotopes are non-toxic and occur naturally in the body. The tracer doses used in this study will be very small and only detectable in the samples we collect using highly sophisticated equipment. In rare circumstances, nausea can be experienced following the consumption of D_2O. You will be supervised by a member of the research team for 3 hours to ensure there are no side effects. During this time, you will be free to perform seated activities, such as reading a book or watching TV.

This visit also acts as the start of the 7-day lead-in period (see *Intervention* below). You will be provided with food to consume for the next 7 days and empty saliva pots to provide a saliva sample upon waking, as well as a log to record when the saliva sample was provided.

Experimental visits (Testing visits 4-6/6)*:* You will be asked to attend an experimental visit before the intervention (visit 4/6) and after the intervention (visit 6/6). The procedures for both these visits will be identical. You will arrive to SportExR or MoveWell following a 10-12 hour overnight fast. An overnight fast is critical to the outcomes of these visits to accurately gain information about your body composition and metabolism.

During these experimental visits, we will assess RMR (resting metabolic rate) again, where you will lay down while we collect breath samples for 20-30 minutes. We will then assess body composition using techniques called dual-energy X-ray absorptiometry (DXA) and bioelectrical impedance analysis (BIA). We use body composition assessments to look at the composition of muscle, fat, bone, and water. We will assess your body composition at the same time of day under similar conditions. Deviations in time and feeding or hydration status can lead to false interpretations of data that we collect from you. A small sample of skeletal muscle (approximately the size of half a pea) will then be obtained from your thigh muscle (*vastus lateralis*) using a muscle biopsy technique frequently used by our research group. This technique is safe and will be performed by a trained member of the research team. The procedure will be performed under local anaesthetic, where administration of the anaesthetic may be associated with some discomfort. Thereafter, you will not experience any pain, but the technique may elicit feelings of pressure. Full aftercare instructions, as well as steri-strips and sterile bandages will be provided following the muscle biopsy. A blood sample (about 20 mL or 1.5 tablespoons worth) will then be obtained by a trained phlebotomist. You will then complete a short questionnaire assessing your hunger, food preferences and gastrointestinal comfort. You will then be provided with a breakfast to consume prior

to the assessments of muscle strength, physical performance, and aerobic capacity. A series of muscle strength and physical performance assessments will be performed, such as handgrip strength and the short-physical performance battery (SPPB) test. Your aerobic capacity will also be assessed using an Ekblom VO_2peak test. This test involves a series of cycling bouts and uses work rate and heart rate to estimate your aerobic fitness.

After the pre-experimental visit, you will then begin the 28-day diet and exercise intervention. You will also be asked to attend a shorter experimental visit (~30-minutes) mid-way through the intervention (visit 5/6) at SportExR or MoveWell following a 10-12 hour overnight fast. This visit will be performed on the same day as one of your exercise training sessions during the second week of the diet and exercise intervention phase (see *Intervention* below). During this visit, we will obtain a blood sample (about 20 mL or 1.5 tablespoons worth) and urine sample, and we will assess muscle strength and physical performance, as described above. After the post-experimental visit, you will have completed the full study.

Intervention (Training visits 1-12)*:* The intervention consists of two phases. The first phase is a 7-day period where you will be provided with all your food to consume. The second phase is a 28-day period of diet provision or diet prescription, and exercise training. You will be provided with food for 14 days (pre-prepared and pre-packaged meals), and prescribed food to consume for the other 14 days (recipes and food lists), with the composition of the diet depending on your treatment group.

7-day diet lead in: At the *D_2O loading visit*, you will be provided with all foods to consume and a 3-day food diary to record your intake. Foods will be provided with consideration for your preferences. You will have a telephone discussion with a member of the research team to discuss your food diary and adherence to the diet, discuss challenges you are facing with the diet, and the research team will provide support where appropriate. We ask you to maintain your normal activity level during this phase, which will be monitored using a pedometer and/or accelerometer.

28-day diet and exercise intervention: You will be provided with food for 14 days, and prescribed food for 14 days, based on your food preferences. You will be asked to complete a 3-day food diary every week for the intervention to monitor compliance with the diet throughout the study. You will also meet weekly with a trained member of the research team to discuss the dietary intervention. In these sessions, we will discuss your food diaries and adherence to the diet, discuss any challenges you are facing with the diet, and provide support to aid your adherence throughout the intervention. You will be asked to attend three exercise training sessions per week at MoveWell (total 12 supervised exercise sessions). Two sessions will be solely resistance exercise, and the other session will involve both resistance and endurance exercise. These sessions will be supervised by a trained member of the research team, who will record your progress and adjust the intensity throughout the

intervention. You will also be encouraged to be physically active during the intervention period, which will be monitored using activity monitoring devices.

Optional follow-up visit (Testing visit 7):
Following completion of the study, you will be invited to attend a follow-up visit at the Sport, Exercise and Rehabilitation Sciences department, University of Birmingham, approximately 3 months after the date of your POST visit. This follow up visit is optional. Before the follow up visit, you will be asked to complete a 3-day weighed food diary and an exercise diary. During the follow-up visit, you will undergo anthropometric assessments (body mass, height, and waist circumference), as well as the assessment of RMR and body composition via DXA and BIA. A blood sample (20 mL or 1.5 tbsp) will also be obtained. Handgrip strength and physical performance (SPPB) will also be assessed. The follow-up visit will last ~1.5 hours.

Are there any possible benefits to taking part?
Individualised exercise training programme: You will receive an individualised exercise training programme. Exercise sessions will also be supervised by a trained member of the research team who will monitor your progress.

Individualised diet programme: You will receive a dietary intervention that will be designed to promote weight loss, or that will help to improve health while maintaining weight. This diet will be individualised to you, and you will receive diet-related support from a trained member of the research team.

Food provision: You will be provided with food to consume for 21 days of the 35-day intervention (3 main meals/day, as well as snacks in the form of pre-prepared or pre-packaged food). For the remaining 14 days, you will be provided with recipe cards and foods lists from the research team.

Health information: You will receive access to important health outcomes that we will obtain during the study, including your progress during the study, your body composition analysis (body fat, muscle mass, and bone mineral density), biomarkers of metabolic health (glucose, insulin, cholesterol), and muscle strength.

Study results: Upon completion of the study, the results will be shared with you in a 'results evening'. Here the research team will present the main findings of the study. You will also have access to a summary booklet containing an overview of the main findings and all scientific posters relating to the study.

Will there be financial reimbursements for participating in this study?
Upon completion of the full intervention, you will receive £100 for your time commitment to the current research study. If you choose to withdraw from the study or are withdrawn from the study before completing the full intervention, you will receive an amount reflected by the number of visits completed and progress through

the intervention at the time of withdrawal (approximately £5-10 per visit). Transport and parking expenses will be reimbursed to participants in addition to the £100. Participants who complete the follow-up visit will also be reimbursed an additional £25 Amazon gift voucher.

Are there any possible disadvantages and risks of taking part?
We have taken every precaution to minimise the disadvantages and risks of taking part in this research study. Nevertheless, there are some possible disadvantages and risks of taking part in this study that you should be aware of.

Dual-energy x-ray absorptiometry: Dual-energy X-ray absorptiometry (DXA) will be used to assess your body composition before and after the intervention, as well as an additional scan at the optional follow-up visit (up to 3 scans in total). A DXA scan involves exposure to a very low dose of ionising X-ray radiation. The amount of radiation during a DXA scan is equivalent to 1.5 days of normal background radiation in the UK or 75 miles of air travel. So, whilst exposure to large doses of radiation can cause damage to living tissue, which may result in radiation sickness and/or cancer, taking part in this study is associated with only a very small risk of this. For example, where 50% of the general population are likely to develop a form of cancer over their lifetime, taking part in this study only adds a small chance (0.0001%) of this happening to you. Furthermore, risks will be minimised by safe practice, and testing will be conducted by trained members of the research team.

Skeletal muscle sample: This study involves the collection of a small sample of skeletal muscle. You may experience some discomfort during administration of local anaesthetic (similar to blood withdrawal). Thereafter, feelings of pressure and/or discomfort may be felt at the biopsy site, but only for a short time. The procedure is quick and will be performed by a member of the research team who has received appropriate training to conduct the sampling. Risks can include bruising, infection or insensitivity of the skin, although these risks are very small. Aseptic conditions and the provision of detailed written instructions for aftercare of the biopsy site greatly reduce risk associated with the procedure.

Ingestion of D_2O: During the intervention, we will ask you to drink a weekly dose of D_2O. D_2O is a naturally occurring, non-radioactive stable isotope tracer that is safe for human consumption. However, consuming large doses of D_2O (>200 mL/dose) can result in dizziness and nausea, and occasionally vomiting in susceptible individuals. These side effects are rare, and usually subside within 1-2 hours. This study will provide a lower dose (<200 mL) to reduce the risk of side effects, and you will be supervised for 3 hours at the D_2O loading visit. If you do feel dizzy or nauseas after consuming D_2O, please inform a member of the research team.

Blood sampling: We will obtain blood samples by inserting a small needle into a vein in your forearm. Blood sampling may be associated with minor pain. However, this

sensation, described as a ‘short, sharp scratch’, is relatively short-lived and blood samples will be performed by trained phlebotomists to minimise discomfort.

Exercise testing and exercise training sessions: Assessments of muscle strength, physical performance, and aerobic capacity and resistance and endurance exercise training may be a little uncomfortable, especially if you are not accustomed to this type of activity. However, if you experience any pain or want to stop, you may do so immediately. A clinician and a trained exercise physiologist will be present throughout testing and will monitor your heart function continuously via an electrocardiograph (ECG) during the aerobic capacity test for which we will place small adhesive pads on your chest.

How will we use information about you?
We will need to use information from you for this research project. This information will include your:

- Initials.
- Name.
- Contact details.
- General health information.

People will use this information to do the research or to check your records to make sure that the research is being done properly.

People who do not need to know who you are will not be able to see your name or contact details. Your data will have a code number instead.

The University of Birmingham is the sponsor of this research, and is responsible for looking after your information. We will keep all information about you safe and secure by:

- Making the data we collection from you anonymised using a code number.
- Storing your personal information in a locked cabinet or on a password-protected document, only accessible to the research team.

International transfers:
Your data will not be shared outside the UK.

Who will have access to the information that I provide?
Only the research team will have access to the information you provide. Some of your information will be sent to the study’s external funders in anonymised form. The funders will adhere to our rules about keeping your information safe and will only receive anonymised data. All records will be treated as confidential.

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. We need to manage your records in specific ways for the research to be reliable. This means that we won’t be able to let you see or change the data we hold about you.

Where can I find out more about how my information is used?
You can find out more about how we use your information by:

- Asking one of the research team
- Sending an email to a one of the research team (contact details on front page)
- Contacting the University of Birmingham's Data Protection Officer (dataprotection@contacts.bham.ac.uk)

What will happen to the data collected and results of the study?
All data collected during the study will be treated as confidential and will be stored electronically on a password-protected computer and on the University of Birmingham OneDrive server. Paper-based results sheets and informed consent forms will be stored in a locked place within the School of Sport, Exercise and Rehabilitation Sciences. This storage of data will be done in accordance with GDPR 2018. Recorded data will not be kept longer than 10 years, as per University of Birmingham regulations. The name or any other identifying information of participants will not be disclosed in any presentation or publication of the study results. As aforementioned, you will be invited to a 'results evening' where the research team will present the study findings, and share a summary booklet containing an overview of the main findings and all scientific posters relating to the study. Attendance to this event is optional and you will be asked to indicate if you wish to be invited back for the event on your informed consent form.

What will happen to my samples collected during the study?
Any samples collected during this study will be stored in an anonymised form within the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham. These samples will be stored for a maximum period of 10 years. You will be asked to consent to your samples being analysed in future studies, which may take place within the UK, EU, or USA. All data will be shared in an anonymised format, so that you will not be identifiable in any way. If any samples remain after the 10-year storage period, these will be destroyed, in accordance with the Human Tissue Act.

Who has reviewed this study?
This study has been reviewed by an independent Research Ethics Committee.

Who is sponsoring and funding the study?
This work is being sponsored by the University of Birmingham and will be supported by the Biotechnology and Biological Sciences Research Council (BBSRC) and University of Birmingham funded Midlands Integrative Biosciences Training Partnership (MIBTP) BB/T00746X/1.

How can I withdraw from the study?
If you wish to stop participating in this study before you have completed all experimental trials, you can inform the doctoral researcher, Archie Belfield. You can

withdraw your participation in this study at any time, without giving a reason for doing so and without repercussions. If for any reason you wish to withdraw your data, please contact Archie Belfield within 4 weeks of your participation. After this timescale it may not be possible to withdraw your data. Your individual results will not be identifiable in any way in any presentation or publication of this study.

What if something goes wrong?
Although the research team will make every effort to ensure your safety during the study, below is some information in the unlikely event of an incident. Compensation for harm arising from an accidental injury and occurring as a consequence of your participation in the study will be covered by the University of Birmingham. If you are harmed and this is due to someone's negligence, then you may have grounds for legal action for compensation against the University of Birmingham. Please note that insurance covers negligence only, and not 'no fault' compensation.

If you have any concerns about the study you should speak to a member of the research team in the first instance, contact details are available within this PIS.
If you remain unhappy with their response and wish to make a complaint you can do so by contacting the Sponsor's Research Governance representatives on researchgovernance@contacts.bham.ac.uk. If you have any concerns about how your information has been handled, you can contact the University's Data Protection Officer on dataprotection@contacts.bham.ac.uk

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Thank you for taking the time to read this information sheet. If you wish to find out more about the study, please contact the doctoral researcher, Archie Belfield (AEB845@student.bham.ac.uk).