PARTICIPANT INFORMATION LETTER

TITLE OF PROJECT: Effects of exercise training on the deleterious effects of a high fat diet on circadian metabolomics

PRINCIPAL INVESTIGATOR: Professor John Hawley
STUDY CO-ORDINATOR: Dr Trine Moholdt
ASSOCIATE INVESTIGATORS: Dr Brooke Devlin, Dr Evelyn Parr
PHD STUDENT: Mr Samuel Pinto
RESEARCH ASSISTANT: Miss Bridget Radford

Dear Participant,

You are invited to participate in a research project conducted by Australian Catholic University (ACU) described within this Participant Information Letter.

This Participant Information Sheet/Consent Form informs you about the research project. It explains the tests and research involved. Knowing what is involved will help you decide if you want to take part in the research.

Please read this information carefully and ask questions about anything that you don't understand or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or local doctor.

Participation in this research is voluntary. If you don't wish to take part, you don't have to. You will receive the best possible care whether or not you take part.

If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:

- Understand what you have read
- Consent to take part in the research project.
- Consent to the tests and research that are described
- Consent to the use of your personal and health information as described.

You will be given a copy of this Participant Information Letter and Consent Form to keep.

What is the project about?

You have been invited to participate in this research study because you are aged between 30 and 45 years and belong to an important target group for evaluating how dietary intakes and exercise change metabolic measurements of circadian rhythm. This age group is an important time to intervene to prevent diabetes and cardiovascular disease.

The body has a circadian clock that regulates the 24-hour rhythm of sleep, body temperature, and metabolism. Metabolic changes influence the circadian rhythm and exercise has a major impact on the whole-body metabolic health profile. The effect of exercise on circadian metabolism has not yet been thoroughly examined in humans. Therefore, this study aims to quantify, measure and investigate the effects of exercise on the deleterious circadian effects of a high-fat diet. Furthermore, the study will provide insight into the effects of exercise in the morning compared to in the evening.

Who is undertaking the project?

This project is being conducted under the supervision of Professor John Hawley in the Centre of Exercise and Nutrition within the Mary MacKillop Institute for Health Research at ACU. The co-ordinators for this study will be Dr Trine Moholdt and Ph.D student Samuel Pinto, with the assistance of Dr Brooke Devlin, Dr Evelyn Parr and Research Assistant Miss Bridget Radford. Dr Trine Moholdt is a visiting post-doctoral research fellow from Norwegian University of Science and Technology with extensive research conducting exercise intervention studies. Dr Brooke Devlin is a Researcher Officer at ACU, an Accredited Practising Dietitian (APD), a qualified DXA technician and the research dietitian within the Centre of Exercise and Nutrition. Dr Evelyn Parr is a post-doctoral research fellow at ACU whose research has involved all of the measures being taken in the current study. Dr Parr is experienced in venepuncture (with certification), regular blood drawing for oral glucose tolerance tests and is a qualified DXA technician. All researchers that are involved in this research project have current first aid certification.

Dr Andrew Garnham is the GP employed on this project. Dr Garnham's primary role will be to take the muscle biopsy samples on trial days and follow up with participants as and if required. Dr Garnham has previously taken muscle biopsies from >16 studies from our laboratory group alone and he is the leading muscle biopsy specialist in Melbourne (works for VU, Deakin, RMIT and Melbourne Universities where needed).

The project has been approved by the ACU Human Research Ethics Committee (2016-254H) and is supported by a grant from the Novo Nordisk Foundation to Professor Hawley for preventative type 2 diabetes work in collaboration with Professor Juleen Zierath (Karolinska Institutet) and Professor Paulo Sassone-Corsi (University of California).

What does participation in this research involve?

Twenty-four sedentary (<150 min/week moderate-intensity exercise for >3 months and >5 hours sitting each day), but otherwise healthy, volunteers will be required for this study. The study is seeking males who are 30-45 years old, overweight or obese (BMI 27 – 35 kg/m^2) and therefore at a higher risk of developing chronic diseases such as diabetes.

Screening: Initial screening will take place via telephone. This screening will comprise medical history, age, self-reported height/weight (in order to calculate BMI) and self-reported activity levels.

Consent form: If you decide to participate in this study and meet the selection criteria, a consent form will need to be signed prior to any assessments being performed. Prior to signing, you will be given the opportunity to ask any questions and have any concerns clarified.

Participation in this project will involve **a minimum of SIX and a maximum of ELEVEN (for participants allocated to training)** separate visits to the Centre for Exercise and Nutrition at the Australian Catholic University (Fitzroy, Melbourne). **Figure 1** outlines the experimental protocol.

In summary:

Visit 1: Forms, Resting Metabolic Rate, Dual-Energy X-Ray Absorptiometry (DXA), peak oxygen uptake test, blood pressure measurements, anthropometry measurements, instructions to complete a three-day food record, exercise pre-screening questionnaire.

Visit 2: Continuous glucose monitor inserted and physical activity monitors put on.

Visit 3 and 4 (morning and evening visits for both visits): muscle biopsies and blood collection, blood pressure measurements, and collection of study diet foods (all provided).

Visit 5-9 (for participants allocated to training): Exercise training sessions, either in the morning or in the eventing, depending on group allocation. All exercise will be completed on an exercise bike at the laboratory.

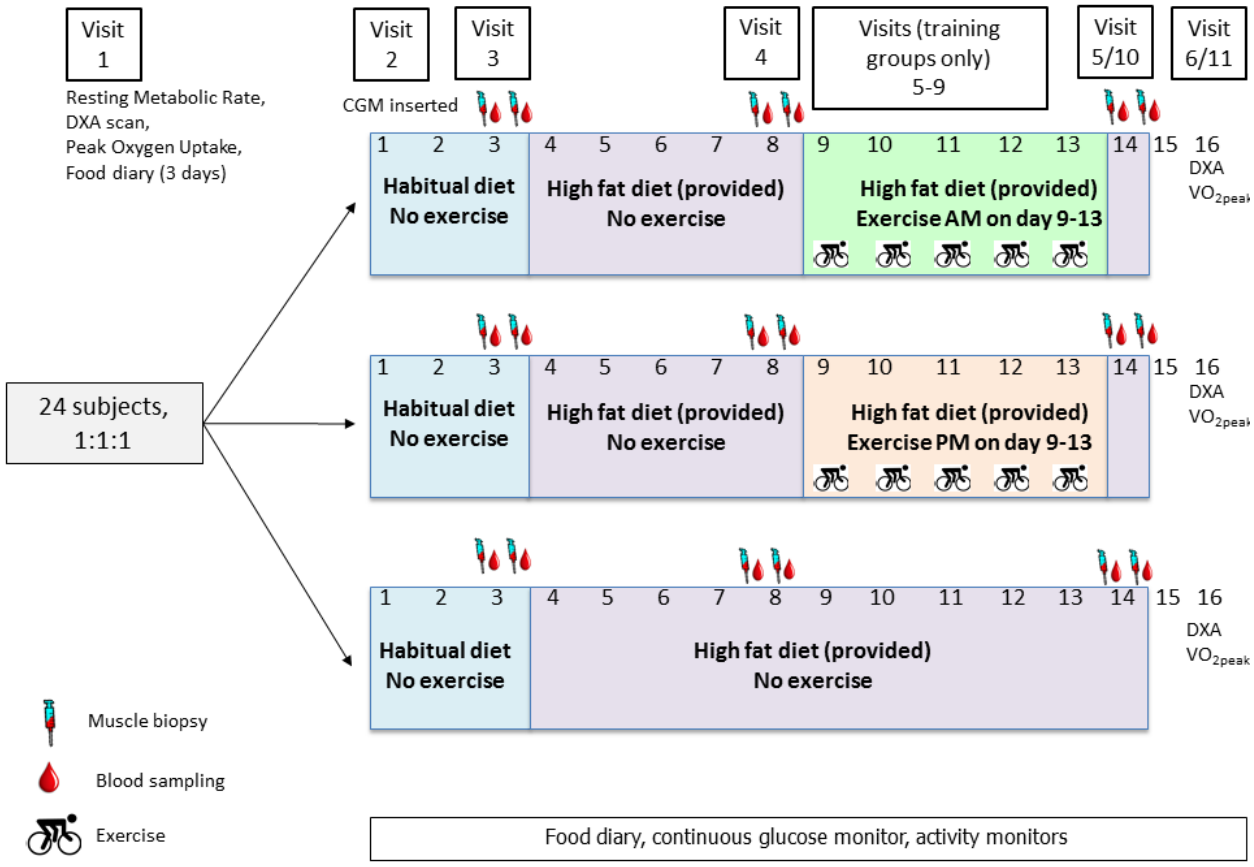
Visit 5 (for participants in the control group)/Visit 10 (for participants in the training groups) Morning and evening visit: muscle biopsies and blood collection, blood pressure measurements

Visit 6 (for participants in the control group)/Visit 11 (for participants in the training groups) DXA scan, peak oxygen uptake test and removal of continuous glucose and activity monitors.

Specific details of each visit, the time commitment and procedures are outlined below.

We are located in the Daniel Mannix Building (17 Young Street or 8 Brunswick Street, Fitzroy) and the initial testing will take place on Level One in the Centre for Exercise and Nutrition's research lab – Room 1.03. To find the room, take the lift to level 1 and exit to your left, follow the signs and you will find room 1.03 where measurements will occur.

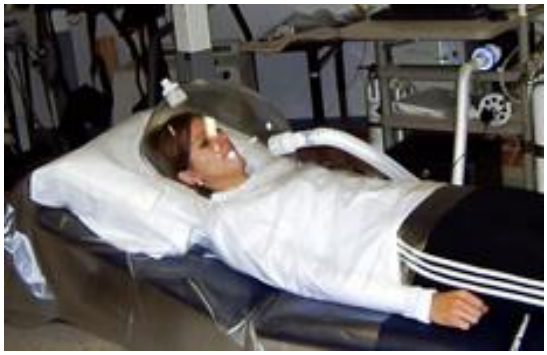
Figure 1. Experimental protocol.



VISIT 1 (total time ~2 h):

At the first visit, you will meet the trial coordinator, go through the participant information letter and sign the consent form. At this stage, you will also participate in the following measures:

1. **Body composition assessment (~30 minutes):** A dual-energy x-ray absorptiometry scan (DXA) is a specialised x-ray technique to provide a measure of body composition. This is similar to a normal x-ray, with no pain involved and will obtain a measure of total body mass, fat mass and lean mass. Participants are required to lay supine on the scanning bed for the duration of the scan, which is approximately 15 minutes and will be one to two scans depending on your body shape. The machine uses **small doses (<1% of the yearly radiation dose) of radiation** to estimate tissue density. This test **requires you to be fasted** with no food, fluid or exercise/activity prior to the test and requires you to wear light clothing with no metal items (i.e. zips, domes, clips, underwire etc.) and remove all jewellery. Please check with the research staff if you are unsure. All measures will be taken by trained researchers, Dr Evelyn Parr and Dr Brooke Devlin.
2. **Resting Metabolic Rate (~25 minutes):** This assessment also involves you being fasted and therefore will be completed immediately after the DXA san. For 25 minutes you will be lying in a quiet, dim-lit room where we can measure your resting metabolic rate using an automated gas analyser. A hood, as in the picture, will go over your head and is connected to the gas analyser. There is a 10 minute rest period followed by a 15 minute period where the data will be collected. The information from this test will also be used to give an assessment of estimated energy requirements for comparison with your dietary intake information.



3. **Anthropometric measurements (~5 minutes):** Height will be measured using a wall mounted stadiometer. Weight will be measured using digital scales. Hip, waist and neck circumferences will be measured with a metal tape measure. All measures will be taken in duplicate.
4. **Blood pressure measurement (~5 minutes):** Blood pressure will be measured via an automated blood pressure machine. Three measures will be taken. Each measure is approximately 3 minutes in duration. A cuff will be applied to the upper portion of the arm. This will inflate and tighten around the arm and then slowly release.
5. **Three-day food record:** You will be asked to record ALL food and fluid consumed over a three-day period prior to the study to establish baseline and habitual dietary intake. To assist with compliance, and reduce the burden on you, household measures will be recorded. A comprehensive document will be provided and this will be explained to you in detail at this visit by the study dietitian.
6. **Exercise pre-screening:** You will be asked to complete the Exercise and Sports Science Australia pre-screening tool. This tool includes a set of questions designed to identify individuals with signs or symptoms of underlying disease, or who may be at higher risk of an adverse event during exercise.
7. **Maximum effort cycling exercise test (VO_2 max).** After a light 10-minute warm-up on a cycling ergometer (exercise bike), you will pedal at a fixed intensity (watts) that will increase every 1.0-2.5 min until volitional fatigue. During this test you will wear a

mouthpiece and headpiece that is connected to a tube for the collection of expired air. This test is done to measure how much oxygen you can take up and transport to your working muscles during the test. The test lasts for ~10-15 minutes after the warm-up, where the last few minutes should be perceived as exhaustive.

VISIT 2: Total time ~ 45 min

You will attend the laboratory at a time that suits to have the following take place:

1. **Insertion of a sensor for a continuous blood glucose monitor (CGM) (~10 minutes):** The minimally invasive Medtronic iPro2 blood glucose monitoring system will enable us to get blood glucose measures every 5 minutes whilst you are able to conduct normal daily activities whilst wearing the monitor. The CGM will be worn for the whole baseline period (7 days) to gain an insight into your blood glucose regulation prior to the dietary intervention. With each period of wearing the CGM, you will be provided with a hand-held blood glucose monitor to measure blood glucose with finger prick samples four times a day (pre-breakfast, -lunch, -dinner and pre sleep). The CGM is the size of a 50 cent piece and the associated sensor inserted rather painlessly into the subcutaneous tissue of the lower back. Following the insertion, you will remain in the laboratory for an hour to be able to calibrate the machine with the first finger prick and also to learn how to use the hand-held glucose monitor (the following other measurements will take place during this hour).
2. **Activity monitors:** To assess habitual physical activity, you will be fitted with ActivPal (worn on the thigh) and an ActiGraph (worn around the waist). Additionally, you will be asked to complete an activity and sleep record. These monitors and record will need to be completed for seven days.

   The **ActivPal inclinometer**, records information regarding the frequency and duration of times spent sitting, standing and walking. This will be fixed to the front of your upper thigh with a micropore dressing and will be worn for the entire study period. The research staff will provide you with replacement micropore tape if you need to switch legs because of irritation with the dressing. If this occurs, please consult one of the research team.

   The **ActivGraph accelerometer,** records information regarding the speed of movement patterns. This will be worn around the waist and will be worn for the entire study period.

   The **SenseWear armband** records sleep patterns. This will be worn around the triceps muscle (upper arm) for the entire study period.

VISIT 3, 4, and 5 (for participants in the control group) or 10 (for participants allocated to training). Two visits per day (~07:00 – 08:15 and ~ 18:30 – 20:00)

For visit 3, 4, and 10, you will be required to arrive at the morning visit fasted at 07:00, and at the evening visit at 18:30. At both of these time points, the following will take place:

1. **Blood pressure measurement (~5 minutes):** Blood pressure will be measured via an automated blood pressure machine. Three measures will be taken. Each measure is approximately 3 minutes in duration. A cuff will be applied to the upper portion of the arm. This will inflate and tighten around the arm and then slowly release.
2. **Blood sampling (~10 minutes):** A single venepuncture will be taken from an appropriate forearm vein using a butterfly needle set. Three 6 mL tubes will be sampled.
3. **Muscle biopsy sample (~20 minutes):** In preparation for a biopsy, a small amount of local anaesthetic is injected under your skin, which may result in a mild burning sensation while the fluid is injected. A small, 4-5 mm incision is then made into the skin to create an opening for the biopsy needle. There is often a small amount of bleeding from the incision; however, this bleeding is generally minimal. The biopsy needle is then inserted through the incision site. You may feel the sensation of deep pressure in the biopsy site, and on some occasions this is moderately painful. However, the discomfort very quickly passes and participants are capable of performing exercise and daily activities within minutes. There may also be some minimal bleeding when the needle is removed which may require the application of pressure for a few minutes.

   After each biopsy, the incision will be closed with sterile tape and wrapped with a bandage. Once the anaesthetic freezing wears off, your leg may feel tight and often there is the sensation of a deep bruise. Painkillers such as paracetamol (e.g. Panadol) or Ibuprofen (e.g. Advil or Neurofen) are acceptable to use if you do experience pain associated with the biopsy. Periodically applying an ice pack to the biopsy site the following day reduces any local swelling and/or residual soreness. Your leg may feel uncomfortable the following day when walking down stairs and to some lesser extent activities that involve forceful movements of the abdomen. To allow the incisions to heal properly and minimize any risk of infection, participants should avoid prolonged submersion in water for 4 days. Daily showers are acceptable, but baths, swimming, saunas etc. should be avoided for at least 4 days following the biopsy. We do encourage to visit Dr. Andrew Garnham 7-10 days following the experimental trial day to check the biopsy safely and re-apply dressings/ bandages, should it be required.

   A total of 6 muscle biopsies will be taken over the study period (one in the morning and one in the evening of each of visits 3, 4, and 10).

   After the morning muscle biopsy sampling, you will be provided with a standardized high fat breakfast at Visits 4 and 10, whereas you will consume your habitual breakfast at Visit 3, and then be able to leave the laboratory and continue your normal daily activities. You will then be asked to return to the laboratory at 18:30. At this visit, you will get to consume your evening meal (provided for Visit 4 and 5/10 and you will be asked to bring along dinner to the lab for Visit 3) at standardized time prior to a second biopsy to be obtained at 19:30 that evening. The biopsy collection will follow the same procedures as in the morning, with a forearm venepuncture taken at this time point as well (18 mL total). At the conclusion of these measures, you will be able to return home.

4. **Provided with food package:** On visit 3 and visit 4, food packages will be provided that include the standardised high fat diet to be consumed.

VISIT 5, 7, and 9: Total time ~40 min each – for participants allocated to training only:

If you are allocated to exercise in the morning (AM group), you will meet at 07:00, and if you are allocated to exercise in the evening (PM group), you will meet at 18:00.

Exercise: After a 10-minute warm up at moderate intensity, you will undertake ten one-minute high intensity intervals, separated by one-minute recovery at light intensity.

VISIT 6: Total time ~50 min– for participants allocated to training only:

If you are allocated to exercise in the morning (AM group), you will meet at 07:00, and if you are allocated to exercise in the evening (PM group), you will meet at 18:00.

Exercise: You will undertake a 40 min bout of moderate intensity exercise.

VISIT 8: Total time ~70 min – for participants allocated to training only:

If you are allocated to exercise in the morning (AM group), you will meet at 07:00, and if you are allocated to exercise in the evening (PM group), you will meet at 18:00.

Exercise: You will undertake a 60 min bout of moderate intensity exercise.

At all training sessions you will be asked to fill out a questionnaire about how you perceive the exercise (your subjective rating of enjoyment).

VISIT 6 (for participants in the control group) or 11 (for participants allocated to training). Total time ~60 min

DXA scan, blood pressure measurement and peak oxygen uptake tests will be performed, as described under visit 1. At this visit we will also remove your glucose monitor and the activity monitors.

Are there any risks associated with participating in this project?

As a participant you will be exposed to some risks arising from the study. Appropriate measures will be taken to provide you with the safest possible testing environment. The invasive procedure (blood sampling) will be conducted by a certified phlebotomist under sterile conditions and using single use sterile equipment.

The risks associated with participation in this study are:

Blood sampling – 18 mL blood samples will be obtained twice on three separate days, making a total volume of 108 mL for the whole study. This total volume collected across 2 weeks is minimal compared to the standard 500 mL for a Red Cross donation. This may cause some discomfort or possible bruising. Sometimes, the blood vessel may swell, or a clot may develop in the blood vessel or there can be a minor bleeding. Although the possibility of significant bruising or bleeding is small, if by chance it does eventuate, consult your doctor immediately and inform the study coordinators (Dr Brooke Devlin or Dr Trine Moholdt).

Dual-energy x-ray absorptiometry – this procedure provides an estimate of your body composition (fat and lean mass). The scan takes ~15 minutes and will be one to two scans depending on your body shape. The machine uses **small does (<1% of the yearly radiation dose) of radiation**.

Muscle biopsies – sampling of the muscle can result in a small amount of bruising, swelling, and bleeding and there is a low risk of nerve injury. All biopsies will be completed following the use of a small amount of local anaesthetic, sterile needles, sterile consumables and an extremely experienced doctor (Dr. Andrew Garnham) to reduce any risk of feeling uncomfortable/pain. Dr Garnham has previously completed >10 000 muscle biopsies.

Continuous Blood Glucose Monitoring – this will involve having a tiny sensor inserted into the skin of your abdomen or lower back. Insertion is quick and virtually painless. The sensor sits just beneath the skin, is comfortable to wear and generally becomes unnoticeable over time. The sensor is connected to a data monitor, which continuously records the blood glucose readings. Irritation or inflammation may occur at the sensor site, but this is unlikely as the device is minimally invasive. If you do have any issues, then please contact the study coordinator immediately.

Activity monitors - Some participants may experience a minor skin irritation with having the micropore tape over the ActivPal. If this occurs, participants can remove the ActivPal and adhere it to the other leg for the remaining period. Participants will be supplied with spare micropore, as well as two clear, plastic dressings (Opsite) for this purpose.

VO_2 Peak Testing – This test will require maximal exertion and therefore can result in some discomfort. The Exercise and Sport Science Australia Pre-Screening Tool will be completed beforehand to ensure that you are suitable to undertake the test. If you are not cleared to participate by the screening tool you will need to obtain approval form a general practitioner to participate. In the unlikely event that you experience discomfort beyond what is expected with exercise, please notify research staff. You are able to end the test at any time.

Exercise Training – Similar to VO_2 peak testing, exercise training may result in some discomfort. In the unlikely event that you experience discomfort beyond what is expected with exercise, please notify research staff. You are able to end the test at any time.

Participation in this study can be suspended or terminated if a medical issue or distress occurs. There may be additional unforeseen or unknown risks.

What are the benefits of the research project?

We cannot guarantee or promise that you will receive any benefits from this research. However, potential personal benefits may include an understanding of your current blood profiles and measurements of body composition, resting metabolic rate and cardiovascular fitness (VO_2peak). We anticipate that this research will establish a circadian profile in a healthy middle aged population, which will act to inform future diet and exercise training studies. Participating in supervised exercise training may also be of benefit.

Can I withdraw from the study?

Participation in this study is completely voluntary. You are not under any obligation to participate. If you agree to participate, you can withdraw from the study at any time without adverse consequences. However, if you decide to leave the project, the researchers would like to keep the personal and health information about you and any blood samples that have been collected. This is to help them make sure that the results of the research can be reported properly. If you do not want them to do this, please advise them before you join the research project.

Will anyone else know the results of the project?

All information that you provide as part of your participation in the study will remain confidential and private and no publications arising from the study will contain names or other identifying information. As this project is collaborative, some of your muscle tissue samples will be sent to our collaborators at the University of California under the supervision of Professor Paulo Sassone-Corsi. Prof Sassone-Corsi will receive a single spreadsheet from our research group containing the randomly assigned code for your samples as well as the non-identifiable physiological data (i.e. age, sex, body composition, blood analytes etc.). The analysis that his team will complete will never identify you and it is anticipated that the analyses will use all of the muscle tissue provided. Where this is not the case, samples will be destroyed in line with the ACU privacy policy following publication or 5 years post investigation.

Only at your request, will study personnel disclose medical results to your primary care physician or specialist. Data records will be kept securely in a non-identifiable format and will only be accessible by research staff. These records will be retained by the University for five years following publication, or five years following the completion of the project. After this period, data will be disposed of in accordance with the ACU's privacy policy.

Will I be able to find out the results of the project?

Once the study has been completed you will be provided with a summary of both your individual results and group data by mail. If the study results are published in a scientific journal you will also be sent a copy of the publication. You are free to ask questions of our research staff at any time during the study.

Who do I contact if I have questions about the project?

The primary research co-ordinator for this study, Trine Moholdt, can be contacted from 8:30 am to 5:30 pm on 0429-111-151 or via email Trine.Moholdt@acu.edu.au. Alternatively, you can contact the principal researcher, Professor John Hawley, on (03) 9953-3552.

Reimbursement for costs

There are no costs associated with participating in this research project, nor will you be paid. However, you will be reimbursed $800 for participating in the research project to cover any costs incurred with the appointment schedules and travel/parking. If you are allocated to the control group in the study, you will be offered a training program similar to the one in the study protocol after conclusion of the last visit.

What if I have a complaint or any concerns?

The study has been reviewed by the Human Research Ethics Committee at Australian Catholic University (2016 254H). If you have any complaints or concerns about the conduct of the project, you may write to the Manager of the Human Research Ethics Committee care of the Office of the Deputy Vice Chancellor (Research).

Manager, Ethics
c/o Office of the Deputy Vice Chancellor (Research)
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Any complaint or concern will be treated in confidence and fully investigated. You will be informed of the outcome.

I want to participate! How do I sign up?

If you would like to participate in this research study, please contact Trine Moholdt on 0429-111-151 or trine.moholdt@acu.edu.au or Bridget Radford on 0431-474-121 or bridget.radford@acu.edu.au to determine if you are a suitable candidate and have a pre-screening phone call.

Yours sincerely,

Dr Trine Moholdt
Study Co-ordinator

Mr Samuel Pinto
PhD Student

Dr Evelyn Parr
Dr Brooke Devlin
Associate Investigators

Miss Bridget Radford
Research Assistant

Professor John Hawley
Study Principal Investigator