

PLAIN LANGUAGE STATEMENT AND CONSENT FORM

TO: Participants

Plain Language Statement

Title *The effects of antioxidants on vascular function and exercise capacity in peripheral artery disease.*

Protocol Number **xxxx**

**Coordinating Principal Investigator/
Principal Investigator** *Dr Hannah Thomas*

Associate Investigator(s) *A/Prof. Michelle Keske
Dr Lewan Parker
Dr Andrew Garnham*

Location: *Deakin University (Burwood Campus)*

1 Your Consent

You are invited to take part in this project which will investigate how antioxidants influence vascular function and exercise capacity in adults with peripheral artery disease. This project is being conducted by Dr Hannah Thomas, Dr Lewan Parker, A/Prof. Michelle Keske and Dr Andrew Garnham from Deakin University.

This Plain Language Statement and Consent Form contains detailed information about the research project. It explains the tests and treatments involved. Knowing what is involved will help you decide if you want to take part in the research or not.

Please read this information carefully. 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 your local doctor.

Participation in this research is voluntary. If you don't wish to take part, you don't have to. Your choice to participate, withdraw, or not participate, will have no effect on your academic grades, employment, or memberships.

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 have the tests and treatments that are described.
- Consent to the use of your personal and health information as described.

You will be given a copy of this Plain Language Statement and Consent Form to keep.

2 What is the purpose of this research?

Peripheral artery disease can result in extensive functional alterations including vascular dysfunction and reduced capacity to exercise. The progression and cause of peripheral artery disease is thought to be associated with increased oxidative stress and tissue injury related to increased production of reactive molecules and a decrease in antioxidant defences. Oxidative stress and inflammation are major features in the development of atherosclerosis and are therefore extremely relevant in the development of peripheral artery disease. Several important antioxidant defences have been shown to be deficient in the muscle of peripheral artery disease patients compared to controls. Endogenous antioxidants function as defences to remove or regulate reactive molecule production. Therefore, antioxidant deficiencies can lead to a state of excess oxidative stress which contributes to vascular dysfunction, decreased exercise capacity, and the development of peripheral artery disease, cardiovascular disease, and cerebrovascular disease. However, few studies have adequately explored antioxidant treatment in peripheral artery disease patients, especially during exercise.

The main aim of this project is:

- To test whether antioxidant treatment (via the intravenous infusion of an antioxidant) can improve vascular function and exercise capacity in patients with peripheral artery disease.

You may be eligible to participate in this study if you meet the following criteria:

1. Aged 40–75 years.
2. BMI ≤ 35 kg/m².
3. Diagnosis of peripheral artery disease.
 - Ankle brachial index ≤ 0.90 .
 - History of stable intermittent claudication >1 year.
4. Have given signed informed consent to participate in the study.

You may be excluded from participating in the study if:

1. Younger than 40 years old or older than 75 years old.
2. You have an ankle brachial index of > 0.90 .
3. You have unstable intermittent claudication.
4. You have any of/or history of the following conditions:
 - a. Cerebrovascular disease (e.g., stroke or dementia).
 - b. Any respiratory diseases that may prevent you from safely exercising.
 - c. Critical limb ischemia or foot ulcers.
 - d. Cardiovascular disease including coronary artery disease or heart valve disease.
 - e. Identification of any medical condition requiring immediate therapeutic intervention.
 - f. Uncontrolled hypertension (resting brachial blood pressure $\geq 160/100$ mmHg).
 - g. Other non-cardiovascular barriers to exercise
5. You are currently smoking or were previously smoking within the past 12 months.
6. You have undergone or plan to undergo elective major surgery during the course of the study.
7. You are pregnant or are breastfeeding.

There are no direct costs associated with participating in this research project. All tests and equipment required as part of the research project will be provided to you free of charge. You will not be paid for participation in this project, but you will be provided a gift card (\$100 shopping voucher) at the end of your participation as a thank you.

3 What does participation in this research involve?

Prior to enrolment in the study, you will be asked to complete two questionnaires to estimate your current physical activity level and to identify conditions that may exclude you from participating. If there is evidence of risk, this study may not be suitable for you.

After enrolment into the study, you will be asked to undergo/perform the following sessions and procedures:

Familiarisation and screening session (~ 1 h).

- General health, physical activity, and medical history questionnaires.
- Resting heart rate and blood pressure.
- Assessment of blood flow in the peripheral artery (ankle brachial index assessment).
- Familiarisation with ultrasound machines, treadmill and metabolic cart (facemask).

Maximal exercise testing – with/without antioxidant infusion (~ 2.5 h each).

This involves two separate sessions (one with and one without antioxidant infusion), that will be completed in a randomised order.

- Graded exercise test to exhaustion on a treadmill to measure aerobic capacity/fitness and maximal walking distance.
- Intravenous infusion of an antioxidant (glutathione) and/or infusion of a placebo (salty water solution).
- Wear a face mask to measure oxygen consumption.
- Ultrasound measurement of the chest (heart), head (brain), thigh (arterial blood flow), and muscle (microvascular blood flow).
- Blood pressure and blood sampling.

The overall time commitment for this study is around 6 hours of on-site testing, spread across 1-2 weeks. All testing sessions will take place at the Deakin University (Burwood campus) research facilities, building J, level 5.

4 What do I have to do?

Prior to attending the Deakin University research facility for each testing visit, you will be asked to avoid moderate to vigorous-intensity exercise and alcohol for 48 hours (i.e., mild walking is ok but please avoid activities that elicit a heavier than normal breathing or sweating response). You will also be asked to avoid caffeine on the day of the testing visits, and fast for two hours prior to attending the research laboratory for the two exercise sessions (i.e., no eating or drinking, except for water, in the two hours leading up to testing).

To reduce the spread and risk of COVID-19: all participants will be screened prior to participating in the research. This means that a member of the research team will contact you by phone the day before you are scheduled to take part in the research. They will ask you questions such as whether you have returned from any country outside Australia in the last 14 days, whether you have had contact with someone who has been unwell, or whether you are unwell yourself. If you answer yes to any of these questions, they may ask you additional questions to rule out the possibility of COVID-19 infection. Where the possibility cannot be ruled out, your participation in the study may need to be postponed or cancelled.

We will ask you to wash or sanitise your hands when you arrive to participate and prior to departing. While we are together, it is important that we follow social distancing guidelines. That means we will distance ourselves as much as possible during your visit, and we won't be able to shake your hand or engage in any other form of physical contact unless required by the research. It's not necessary for you to wear a protective mask but we do ask that you cover your nose and mouth with a tissue if you cough or sneeze. If you do not have a tissue, you should cough or sneeze into your upper sleeve or elbow. After each participant leaves, we will sanitise all work surfaces, door handles etc. to avoid the risk of infection.

Familiarisation and screening session (~ 1 h)

For this visit you will be asked to attend the Deakin University research facility after a two hour fast. You will be asked to complete general health, physical activity, and medical history questionnaires. Your resting heart rate, blood pressure and ankle brachial index will be measured. You will then be shown how to use the exercise treadmill (used for a subsequent exercise testing session) and how we use the ultrasound machine to measure your vascular health and function. During this visit you will be encouraged to ask any questions you have about the study and what is involved.

Exercise testing sessions (2 sessions) (~ 2.5 h each)

For the exercise testing sessions, you will be asked to attend the Deakin University research facility after a two hour fast and an intravenous cannula (a very small plastic tube) will be inserted into each arm for blood collection and intravenous infusions. Your resting heart rate and blood pressure will be measured, and a blood sample will be taken (approximately 10 ml). Baseline vascular assessments of the heart, brain and skeletal muscle will also be done. The intravenous infusion of antioxidants/saline will then begin, and you will rest quietly on a bed for 60 minutes. After 60 minutes rest, blood samples and vascular assessments will be repeated. You will then be asked to undergo an exercise test on a treadmill and your heart rate, blood pressure, cerebral blood flow and energy expenditure will be measured throughout. A blood sample and vascular measures will be completed again immediately post exercise and 30 minutes post exercise, and an additional blood sample will be taken 15 minutes post exercise (10 ml for each blood sample). Before, during, and 30 minutes after the exercise test an approved ultrasound contrast agent solution will be intravenously infused to allow us to measure blood flow in the muscle of your leg.

Procedures involved in this study

- **Exercise test on a treadmill.** The exercise test will be performed on a treadmill. The exercise test will start after a 5-minute period of rest. The test will begin at a walking pace, which will be maintained throughout the test. At first, you will feel that the exercise is very easy. The test will start at an incline of 0% for the first 5 minutes and then increase by 3.5% every three minutes thereafter until you are unable to exercise any further (exercise until exhaustion). The duration of the test will depend on your fitness level, but usually doesn't last any longer than 20 minutes. Before, during, and after the exercise test we will analyse your oxygen consumption (you will be asked to breathe into a silicone face mask), and we will take ultrasound measurements of your thigh, chest and head to measure cardiac function and blood flow in the brain and body. You will also be monitored by an electrocardiography (ECG) to assess your heart rhythm.
- **Blood sampling.** Research staff qualified to perform cannulation (thin plastic tube) will collect blood samples via an intravenous catheter. Catheters are used when several blood samples are needed from one site over a brief duration such as to be used here. Once the catheter is in place, it is a simple and painless procedure to remove further blood samples. It is possible that some minor bruising may occur around the site of cannulation. The estimated volume of blood collected at each visit is far less than the amount provided during a single blood donation (approximately 500 mL). Venous blood samples will be analysed for markers of oxidative stress, vascular function and cardiometabolic health (e.g., markers of inflammation and bone, fat, and glucose metabolism), and exercise metabolism (e.g., lactate, pH, and oxygen saturation).
- **Intravenous infusions.** Similar to blood sampling, an intravenous infusion of a solution requires a small plastic cannula (thin tube) to be inserted into a vein on your forearm using a needle. The insertion of the needle can be uncomfortable (similar to receiving an injection or donating blood). However, once the catheter is in place the needle is removed and the infusion procedure is painless.

- **Ultrasound measurements.** We will use a specific ultrasound technique to measure blood flowing through the small blood vessels in the muscle of your thigh. This will require infusing an approved ultrasound contrast agent (called Definity) into one of your veins so that pictures of these small blood vessels can be taken. An ultrasound probe will then be placed on top of the skin on your thigh to capture videos and images. We will also measure how well your heart is pumping blood through the body, and how much blood is flowing to your brain, by placing a non-invasive ultrasound probe on the surface of the skin of your chest (to image the heart), temple (to image the arteries in the brain) and the thigh (to image the large arteries within the leg). These ultrasound techniques use sound waves to measure blood flow which you will not be able to feel or notice.
- **Indirect calorimetry.** To measure how much oxygen your body is consuming and how much carbon dioxide you are producing during and after the exercise test, we will ask you to wear a breathable silicone face mask during the exercise in each visit. The mask can be easily removed at any time.
- **Blood pressure and heart rate.** Your blood pressure will be monitored periodically throughout the visit via an automated blood pressure cuff. Your heart rate will be continuously recorded by a heart rate strap.

5 Do I have to take part in this research project?

Participation in any research project is voluntary. If you do not wish to take part, you do not have to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with Deakin University.

Before you make your decision, a member of the research team will be available to answer any questions you have about the research project. You can ask for any information you want. Sign the Consent Form only after you have had a chance to ask your questions and have received satisfactory answers.

If you decide to withdraw from this project, please notify a member of the research team. This notice will allow that person or the research supervisor to inform you if there are any health risks or special requirements linked to withdrawing.

6 What are the possible benefits of taking part?

We cannot guarantee or promise that you will receive any direct benefits from this research; however, possible benefits may include gaining a better understanding of your general health including your fitness level and your vascular health.

7 What are the possible risks and disadvantages of taking part?

Before you volunteer to be part of this study, there are some important things to understand:

- It is important that you do not have one or more of the following conditions;
 - i. Previous heart attack or cardiac arrest.
 - ii. Exercise test that resulted in chest pain, or chest pain at any other time in the past 6 months related to exertion.
 - iii. Current muscle and joint pain (e.g., arthritis) and/or nerve pain that will prevent comfortable participation in exercise.
 - iv. Current and unstable intermittent claudication or severe limb ischemia.
- All exercise activity carries a risk of injury and, in extreme cases, risks of suffering a heart attack or stroke. We will take all reasonable precautions when performing the exercise test

on the treadmill. All exercise tests and contrast agent infusions will be supervised by qualified and experienced research staff.

- There is the rare possibility that the glutathione intravenous infusion could result in gastrointestinal reactions such as stomach or intestinal gas, nausea, vomiting, and diarrhoea and non-gastrointestinal reactions such as sleepiness, metallic taste, light-headedness, redness of eye, face or hands, runny nose, and coughing. If you exhibit any of these symptoms, please immediately contact the research team for further advice.

Other possible risks of this study include:

- Blood collection may cause some discomfort, bruising, minor infection, or bleeding. If this happens, it can be easily treated.
- A small number of people may exhibit side-effects during the infusion of the contrast agent during ultrasound imaging which may include back pain, chest pain, headache, dizziness, nausea, or flushing. These symptoms usually go away within 30 minutes once the infusion has stopped.
- When the face mask is on to measure oxygen consumption you may initially experience hyperventilation (quicker rate of breathing). There is no physical danger involved with this measurement. If you feel uncomfortable at any time, the face mask can easily be removed.
- During the exercise tests, you may experience some muscle or other soft tissue soreness. In this case, you will be treated immediately using appropriate sports first aid (e.g., ice treatment and compression). If an injury persists, or the injury needs medical evaluation and/or treatment, a research team member will discuss the injury/conditions with you and refer you to an appropriate medical or allied health practitioner. You will not be able to return to the study until cleared to do so by the treating practitioner.
- It is important that women participating in this study are not pregnant. It is important to let the researchers know if you think you might be pregnant. If you think you might be pregnant then we cannot enrol you into the study.
- There may be additional unforeseen or unknown risks.

We will use every possible safety measure to protect you while performing the activities in this research:

- In the case of medical emergencies, a call to 000 will be made. The researchers will commence appropriate resuscitation methods or other appropriate procedures while waiting for an emergency team to arrive. In the event of emergencies, you will need to undergo an additional medical review and consent process before you will be permitted to return to the study.
- Although rare, some participants may experience anaphylaxis in response to the infusion of the contrast agent. Anaphylaxis response will be managed by the qualified researcher who will be present during the infusion of the contrast agent.
- For all other adverse events of a physical nature, exercise will be terminated immediately, you will be consulted by a research team member and if necessary, recommended to see a medical practitioner or appropriate health professional external to the research project.
- In the case of any reportable (e.g., more serious than just muscle soreness in response to new exercise) adverse event (whether described above or not), a research team medical doctor will be informed as soon as practical, and upon review make a decision regarding the safety of continued participation in the study.

If you suffer an injury as a result of participating in this research project, a research team medical practitioner or specialist will discuss the injury/conditions with you at no extra cost to you. Depending on the injury/condition you may be asked to see an appropriate medical or allied health

practitioner external to the research project for consultation and treatment where costs may be incurred depending on your level of public or private healthcare cover.

8 What will happen to my test samples?

The collection of venous blood is essential to the study and will be utilised for both medical health screening and research purposes. Blood samples and subsequent data will be coded with a re-identifiable ID number and stored for a minimum of 15 years at Deakin University (Burwood Campus). Blood will be analysed for markers of oxidative stress, cardiometabolic and vascular health.

9 Can I have other treatments during this research project?

Whilst you are participating in this research project, you may not be able to take some or all of the medications or supplements you have been taking for your condition or for other reasons. It is important to tell the research staff about any treatments or medications you may be taking, including over-the-counter medications, vitamins or herbal remedies, acupuncture, or other alternative treatments. You should also tell the researchers about any changes to these during your participation in the research project. The researcher will explain to you which treatments or medications may need to be stopped for the time you are involved in the research project or you may be excluded from participation.

10 What if I withdraw from this research project?

You can withdraw from the study at any time. If you decide to withdraw from the project, please notify a member of the research team.

With your permission, the information already collected will be retained to ensure that the results of the research project can be measured properly and to comply with law. You should be aware that data collected by the research team up to the time you withdraw will form part of the research project results unless you request to have this data withdrawn.

11 What happens when the research project ends?

Following participation in the research project you will be provided with a health report detailing some of the results that have been collected. However, many of the main findings of the research project will not be available until completion of the research project and data analysis of all participants has been completed. Upon finalisation of the results, you will be notified of the main findings of the research project.

We hope that results from this study will be published in a physiology or medical journal. Information will be reported as non-identifiable data and therefore you will not be individually identified. We will also be happy to provide you, upon request, with the results of the project and/or relevant publications.

12 What will happen to information about me?

By signing the consent form, you consent for the relevant research staff to collect and use personal information about you in the research project. Any information obtained in connection with this research project that can identify you will remain confidential. All information is re-identifiable (coded). Only the research staff will have the code and access to the data. Your information will only be used for the purpose of this research project, and it will only be disclosed with your permission, except as required by law. We will also ask your permission to store your blood samples and other data collected from this study for the potential use in future research that is closely related to this research project. This future use may include comparison of your data to data from other studies, and the future analysis of blood samples for proteins, enzymes and other factors that are related to cardiovascular and metabolic health.

Information about you may be obtained from your health records held at this and other health services for the purpose of this research. By signing the consent form, you agree to the study team accessing health records if they are relevant to your participation in this research project.

It is anticipated that the results of this research project will be published and/or presented in a variety of forums. In any publication and/or presentation, information will be provided in such a way that you cannot be identified, except with your permission. All data will be presented as the average of the group or non-identifiable raw values (values not associated with your identity or unique study code). It is possible that data may be presented in a publicly available database, as per policy of many academic journals. In this case it will be stored as non-identifiable data. It is important to know that the information in this study will only be used in ways that will not reveal who you are. You will not be identified in any publication from this study or in any data files shared with other researchers. Your participation in this study is confidential.

In accordance with relevant Australian and/or Victoria privacy and other relevant laws, you have the right to request access to your information collected and stored by the research team. Please contact the study team member named at the end of this document if you would like to access your information.

13 Who is organising and funding the research?

This research project is being conducted and funded by Deakin University and the Institute for Physical Activity and Nutrition (IPAN).

14 Who has reviewed the research project and is it approved?

All research in Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this research project have been approved by the Deakin University Human Research Ethics Committee (DUHREC). This project will be carried out according to the principles of ICH Good Clinical Practice and the National Statement of Ethical Conduct in Human Research (2007) produced by the National Health and Medical Research Council (NHMRC). This statement has been developed to protect the interests of people who agree to participate in human research studies.

15 Further information and who to contact

If you require further information or if you have any problems concerning this project, you can contact the principal researchers:

- Dr Hannah Thomas: h.thomas@deakin.edu.au
- Dr Lewan Parker: +61 3 9246 8740, lewan.parker@deakin.edu.au
- A/Prof. Michelle Keske: +61 3 9246 8850, Michelle.Keske@deakin.edu.au
- Dr Andrew Garnham (Research medical Doctor): +61 4 1624 6911, Andrew.garnham@deakin.edu.au

16 Complaints

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about your rights as a research participant, then you may contact:

The Human Research Ethics Office,
**The Manager, Office of Research Integrity, Deakin University, 221 Burwood Highway,
Burwood Victoria 3125.**

Telephone: 03 9251 7129

Facsimile: 9244 6581

Email: research-ethics@deakin.edu.au.

Please quote the project protocol number: **xxxxx**.

Plain Language Statement and Consent Form

To: Participants

Consent Form

Project Title	The effects of antioxidants on vascular function and exercise capacity in peripheral artery disease.
Protocol Number	2022-XXX
Primary Investigator	Dr Hannah Thomas
Associate Investigators	Dr Lewan Parker A/Prof Michelle keske Dr Andrew Garnham

I have read and I understand the attached Plain Language Statement.
 I freely agree to participate in this project according to the conditions in the Plain Language Statement.
 I have been given a copy of the Plain Language Statement and Consent Form to keep.
 The researcher has agreed not to reveal my identity and personal details, including where information about this project is published, or presented in any public form.

Participant's Name (printed)

Signature Date

Dr Hannah Thomas h.thomas@deakin.edu.au
Dr Lewan Paker lewan.parker@deakin.edu.au
A/Profr Michelle Keske michelle.keske@deakin.edu.au (03) 9246 8850
Dr Andrew Garnham Andrew.garnham@deakin.edu.au

Institute for Physical Activity and Nutrition
School of Exercise and Nutrition Sciences
Deakin University
221 Burwood highway, Burwood
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Plain Language Statement and Consent Form

To: Participants

Consent Form for Tissue Sample Storage and Use

Project Title	The effects of antioxidants on vascular function and exercise capacity in peripheral artery disease.
Protocol Number	2022-XXX
Primary Investigator	Dr Hannah Thomas
Associate Investigators	Dr Lewan Parker A/Prof Michelle keske Dr Andrew Garnham

I consent to the storage and use of blood and tissue samples taken from me for use in further closely aligned research as described in this Plain Language Statement by Dr Hannah Thomas.

Participant's name (printed).....

Signature Date

Dr Hannah Thomas h.thomas@deakin.edu.au
Dr Lewan Paker lewan.parker@deakin.edu.au
A/Profr Michelle Keske michelle.keske@deakin.edu.au (03) 9246 8850
Dr Andrew Garnham Andrew.garnham@deakin.edu.au

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Plain Language Statement and Consent Form

To: Participants

Withdrawal of Consent Form

Project Title	The effects of antioxidants on vascular function and exercise capacity in peripheral artery disease.
Protocol Number	2022-XXX
Primary Investigator	Dr Hannah Thomas
Associate Investigators	Dr Lewan Parker A/Prof Michelle keske Dr Andrew Garnham

I hereby wish to WITHDRAW my consent to participate in the above research project and understand that such withdrawal will not jeopardise my relationship with Deakin University

Participant’s Name (printed)

Signature Date

I wish to have all of my data withdrawn from the study (please circle) – **yes/no**

Please mail or return this form to:
 Dr Hannah Thomas
 Institute for Physical Activity and Nutrition
 School of Exercise and Nutrition Sciences
 Deakin University
 221 Burwood highway, Burwood
 Victoria
 3125