PARTICIPANT INFORMATION SHEET

Polyphenol-rich drink for gut and brain health (LINK Study)

Invitation

You are invited to take part in this study which aims to understand the effects of a blackcurrant-based beverage on markers of the gut-brain axis. As a volunteer, it is important for you to understand why we are doing this research, and to understand what will be involved if you decide to participate.

This participant information sheet will help you decide if you would like to take part. It describes why we are doing the study, what your participation would involve, what the benefits and risks to you might be, and what would happen after the study ends. Please take time to read this information sheet carefully, and contact us if anything is not clear or you would like further information. Please also talk to other people like family/whānau, friends, or healthcare providers to help you decide whether you would like to take part in the study. You will have the opportunity discuss the information presented here with the study team, who can answer any questions you might have.

If you agree to take part in this study, you will be asked to find the Consent Form attached at the end of this document. You will be given a copy of this information sheet and the signed consent forms.

What is the LINK study?

The gut microbiome (gut bacteria) plays an important role in many aspects of our health, particularly our mood and mental wellbeing. We are carrying out the LINK study to find out whether a blackcurrant-based beverage (Ārepa) can provide benefits to markers of the gut-brain axis, including the gut microbiome, neurocognitive responses (cognition, mood, sleep), and related markers in the blood. We will also explore whether baseline diet or the gut microbiome mediates the effects of the blackcurrant drink on study outcomes.

The LINK study will recruit 40 healthy female adults to participate in a 3-month long intervention. The study period includes four weeks on one arm of the intervention (Ārepa performance beverage or placebo beverage), four weeks of a ‘wash-out’ period, and then four weeks on the second arm of the intervention. The order of intervention will be randomised, and there will be 5 study visits in total. The first will be a screening visit and take approximately 1-hour, the following four will be study visits conducted in the morning which take approximately 45-minutes. We will be collecting blood and stool (poo) samples, and you will also complete questionnaires and cognitive tasks during the study.

What is the purpose of the LINK study?

There is an increasing number of people with functional gastrointestinal disorders, like irritable bowel syndrome. These conditions are thought to be driven by altered communication along the gut-brain axis. Strategies which improve microbiota-gut-brain interactions are thought to be important in optimising gastrointestinal health and preventing the onset of functional gastrointestinal disorders. Translational to practical solutions are still needed though.
Several dietary components might have positive or negative impacts on aspects of microbiota-gut-brain interactions. The New Zealand blackcurrant cultivar Black Adder is rich in anthocyanins, a class of plant-based compounds called polyphenols. Previous data in animals and humans has shown a prebiotic effect of anthocyanins, leading to the growth of health-promoting bacterial species in the gut. Supplementation with the Black Adder cultivar has also been shown to favourably impact brain physiology and behaviour. Integrative studies which simultaneously measure the microbiota, neurocognitive responses, and related blood biomarkers are needed to better understand the potential of polyphenols.

The Ārepa beverage is rich in anthocyanins and other plant-based ingredients known to provide neurocognitive benefits (L-theanine, pine-bark extract). We hypothesize that this unique formula will enhance microbiota-gut-brain interactions through dual effects on the gut microbiota and neurocognitive responses.

Who are the researchers?

This study involves a team of researchers from the University of Auckland (Dr Nicola Gillies, Dr Tommi Vatanen, Dr Andrea Braakhuis), the University of Otago (Prof Nicole Roy) and Swinburne University of Technology (Prof Andrew Scholey). These are the researchers who have designed this study. Dr Nicola Gillies is the Principal Investigator and will be managing the study. The research team includes registered dietitians, neuroscientists, and specialists in research on the gut microbiota.

Who is funding the study?

The LINK study is funded by grants from the Ministry of Business and Innovation (MBIE) and AlphaGen Ltd through the National Science Challenges – High Value Nutrition (www.highvaluenutrition.co.nz).

Who can participate in the LINK study?

This study may be suitable for you if you are a healthy female, and aged between 18-45 years at the time of enrolment.

We cannot include people who have taken antibiotics in the four weeks prior to the study starting, or who are diagnosed with gastrointestinal disorders (e.g., Coeliac Disease, Inflammatory Bowel Disease), who have been treated for anxiety, depression or psychiatric disorders within the past two years, or who have a history of neurological disorders (e.g., Epilepsy, serious head trauma) or cognitive impairment. This is because these conditions might impact our study outcomes.

If you are taking prebiotic, probiotic or other supplements which might impact our study outcomes we ask that you stop taking these four weeks prior to the study starting and for the duration of the study period. You can ask us if you’re sure about which supplements might be relevant here.

No one has to take part in the LINK study, and it is completely up to you to decide whether or not to take part in the study. If you do decide to enrol in the study, you can withdraw at any time without giving a reason.
What will my participation in the LINK study involve?

**Online screening:** If you are interested in participating, you will complete an online screening questionnaire to make sure that you meet the criteria for taking part. If you are eligible according to this questionnaire, you will be invited to attend a screening visit at the Grafton Clinical Research Centre (University of Auckland).

**Screening visit:** If you agree to attend this study visit, researchers will review this participant information sheet with you in person, explaining the study in detail and answering any questions you may ask about participation. If you are satisfied and agree to take part, we will ask you to sign the consent form which can be found at the end of this document. At this point you will be officially enrolled into the LINK study.

After enrolment, you will be familiarised with the computerised multi-tasking programme we use in the study to trigger cognitive stress. You will also complete some questionnaires at this visit, which are repeated online during the study. These questionnaires will give us information on your mood, sleep quality, dietary intake, and physical activity. At the end, you will be given instructions and materials for stool (poo) sample collection which allows us to measure your gut bacteria. This screening visit will take around 1 hour in total.

**Study period:** Approximately two weeks after your first study visit you will start the study, which takes three months in total. This involves four weeks on the first study beverage (“arm 1”) where you will consume one 300mL beverage daily, four weeks in a ‘wash-out’ period where you do not consume any study beverage, and then four weeks on the second study beverage (“arm 2”) where will you will consume the other 300mL beverage daily. You will be supplied with all the beverages needed for your participation in this study at no cost.

This trial is ‘blinded’, which means that neither you nor the researchers will know whether you are having the study treatment (Ārepa) or the taste- and colour-matched placebo beverage. This information is only known to someone unconnected with the trial. The order in which you consume the study beverages will be randomly assigned after you are enrolled in the study. This study design helps to make sure that the researchers interpret the results in a fair and appropriate way, and avoids researchers or participants jumping to conclusions.

Both beverages are manufactured in a registered facility that complies with Food Standards Australia and New Zealand guidelines, with respect to manufacturing standards and compliance with food safety requirements.

**Data collection:** There are 4 visits during the study period, where data will be collected in person. These happen at the start and end of each intervention arm (i.e., every four weeks), and are expected to take around 45 minutes each. Each study visit will have similar testing procedures. All data will be collected at the Clinical Research Centre in Grafton, Auckland. You will be asked to avoid alcohol the day before your study visit, caffeine in the 12h prior to your appointment, and to not eat after 10pm. Except for water, you will fast overnight and attend your scheduled study visit in the morning.
You will provide researchers with a stool (poo) sample which was collected at home, in the 24h before your study visit using the kit and instructions provided at previous study visits.

Height and weight will be taken, and blood samples will then be collected. You will be asked to rest on a bed or chair, and a small needle will be placed into your arm vain. This can be slightly painful, and can cause discomfort. The researcher will then take approximately 15mL of blood which will be used to measure inflammatory markers (circulating cytokines), neurocognitive markers (monoamine oxidase B, brain derived neurotropic factor), and amino acids and their metabolites (serum tryptophan, kynurenine).

You will then complete questionnaires which evaluate your mood in the present moment. After this, you will complete the cognitive stressor which takes approximately 20 minutes. Finally, you will repeat the questionnaires on your mood. This allows us to measure your mood under conditions of stress or no stress.

You will complete online questionnaires at fortnightly intervals during the study which provide information on your diet, mood, sleep, and physical activity. These will take approximately 15 minutes of your time or less. At weekly intervals you will need to confirm that you have consumed the study beverage through a quick online questionnaire.
## What will happen to my blood and stool samples?

**Blood samples:** Your blood will be used to analyse inflammatory markers, neurocognitive markers, and amino acids and their metabolites. These markers are related to the gut and/or brain and provide insights into whether there are differences in the response to the intervention or placebo beverage, and how the intervention might be achieving these outcomes.

Blood samples will be collected, prepared, and stored at the Nutrition Department Laboratory (Faculty of Medical and Health Sciences, University of Auckland). Analysis of blood samples will take place at the Liggins Institute Laboratory (University of Auckland) or Plant and Food Research (Palmerston North, New Zealand). After these analyses have been performed, it will not be possible to return any unused samples to you. You can request the return of your blood prior to any analysis; this would mean we would not use your information in the study.

**Stool samples:** We will measure changes in gut microbiota (through identifying and classifying the types of gut bacteria present), microbiota diversity, and the predictive function of your gut bacteria (through measuring the genetic material of the gut bacteria) from your stool samples. These samples will be processed at the University of Auckland. A small sample will be sent to an overseas laboratory (Beijing, China), frozen on dry ice, for expert analysis of gut bacteria that cannot take place in New Zealand.

**Transport and storage:** All blood and stool samples will be transported locally, nationally, and internationally according to international guidelines for the transport of human tissue. All samples will be labelled with your LINK study ID number and not your name, to maintain confidentiality.

Iwi, hapu, and whānau might disagree with transport of tissue samples due to issues with the loss of rights to your whakapapa. It is acknowledged that individuals have the right to choose, and these concerns might also apply to non-Māori. We encourage you to consult with your whānau before agreeing to participate. You can also have a support person contact us or attend your screening visit if you have any questions.

All samples will be labelled with your LINK study ID and will be stored in secure freezers in an access-restricted area at the University of Auckland until analysis. Any unused samples collected in this study will be kept for a total of 10 years. At the end of this time, a medical waste contractor will dispose of your tissue. If you would like a karakia said at this time, please indicate so in the consent portion of this form. Any samples for disposal by karakia will be clearly marked. It is possible that the entire sample will be used for analysis, in that case there will be no need for disposal and a karakia will not be possible. Karakia ceremonies take place through the Auckland District Health Board. **Stored tissue will not be used for any future unspecified research purposes.**

**Detection of abnormalities:** We will advise you of any abnormal test results found as part of the study which might have implications for your future health, including results from questionnaires, blood samples, and stool samples. These findings will be provided to you, along with a letter for your doctor. We will contact your doctor or an appropriate specialist if you agree.

If you do not wish to be informed of any results indicating a possible medical concern, you cannot participate in this study.
What are the benefits or risks of taking part in the LINK study?

Risks: There are no major risks associated with taking part in this study. You do need to be aware that this study will involve collection of a blood sample. This will be performed by an experienced researcher. A blood sample may hurt a little, and some people get a small bruise where the needle goes in. Occasionally, this can become infected but this is very rare and most people have no problems. If you ever faint with blood samples or when you see blood, please let the researchers know beforehand. That way, we can be prepared for this and take the sample while you are lying down.

There is minimal risk in other procedures associated with the trial, but acknowledge that the multi-tasking activity and questionnaires can bring about disturbances in mood which people might find uncomfortable. Psychological assessments will be checked weekly by the research team. If any questionnaires raise concerns, these will be reviewed by a neuroscientist in the research team, who may suggest referral to counselling services if needed with your permission.

Researchers will check in on your safety regularly through the trial. Any adverse events (e.g. reaction to the beverages such as gastrointestinal upset) or serious adverse events that emerge or worsen relative to your usual state will be recorded and reported to the Health and Disability Ethics Committee.

Benefits: There are many benefits to being involved in this research. You will have several assessments which are not usually available through standard care, receive information about how you respond to an experimental stressor, and receive information about your gut microbiota. You will be acknowledged in publications (anonymously), and be provided with the study findings. Your involvement in this study is of great value to the researchers and will help to advance understanding on how dietary components affect the gut-brain axis, thank you for considering taking part.

How will my confidentiality be protected?

Your confidentiality and protection of personal information will be treated very seriously, and individual results from this study will be kept strictly confidential. On entering the study, you will be given a unique study identification number/ID, which will be used on all forms, questionnaires, measurements, and blood/stool samples. Researchers will remove any personal information provided by you, and there is no risk that you will be able to be identified if samples are sent nationally or overseas. It is important to note that privacy protections in other countries may be different to those offered in New Zealand, but using your study ID will protect your identity. Researchers will analyse the whole study group’s data and report on averages in any scientific publications and presentations and no person will be identifiable.

Any hard-copy documents, including paper copies of questionnaires, data collection forms or measurements will be stored in a locked filing cabinet in a secure swipe-access area at the University of Auckland, where only the research team has access. Any information on electronic files will only be accessible by the research team. The LINK study researchers responsible for data collection and management have been trained by an independent committee at the Greenlane Coordinating Centre (GLCC), who ensure the study is carried out according to guidelines for Good Clinical Practice.
What happens if I suffer harm, injury, or complications because of the study?

It is unlikely that you will suffer any harm or complications because of this study. If you were injured as a result of treatment given as part of this study, you won’t be eligible for compensation from ACC. However, Dr Nicola Gillies has satisfied the XXX Health and Disability Ethics Committee that approved this study that it has up-to-date insurance for providing participants with compensation if they are injured as a result of taking part in the study.

New Zealand ethical standards require compensation for injury to be at least ACC equivalent. Compensation should be appropriate to the nature, severity and persistence of your injury and should be no less than would be awarded for similar injuries by New Zealand’s ACC scheme. Some sponsors voluntarily commit to providing compensation in accordance with guidelines that they have agreed between themselves, called the Medicines New Zealand Guidelines (Industry Guidelines). These are often referred to for information on compensation for commercial clinical trials. There are some important points to know about the Industry Guidelines:

- On their own they are not legally enforceable and may not provide ACC equivalent compensation.
- There are limitations on when compensation is available, for example compensation may be available for more serious, enduring injuries, and not for temporary pain or discomfort or less serious or curable complaints.
- Unlike ACC, the guidelines do not provide compensation on a no-fault basis. The Sponsor may not accept the compensation claim if:
  - Your injury was caused by the investigators, or;
  - There was a deviation from the proposed research plan, or;
  - Your injury was caused solely by you.

An initial decision whether to compensate you would be made the by the sponsor and/or its insurers. If they decide not to compensate you, you may be able to take action through the Courts but it could be expensive and lengthy, and you might require legal representation. You would need to be able to show that your injury was caused by participation in the trial. You are strongly advised to read the Industry Guidelines and ask questions if you are unsure about what they mean for you.

If you have private health or life insurance, you may wish to check with your insurer that taking part in this study won’t affect your cover.

You may have your friend, family, or whānau support help you understand the risks and/or benefits of this study or any other explanation you require. You are also welcome to have a friend or whānau support with you during every session.
What will happen to my information?

All data will be stored electronically in password-protected files on a secure network drive managed by the University of Auckland. With your consent, your study records will be stored securely for 10 years after the study is completed and then destroyed. Your blood and stool samples will also be stored for 10 years after the study ends, which will be stored securely in the Nutrition Department freezers at the University of Auckland. If you decide to withdraw from the study, you may request that your samples are disposed of.

What happens with the results of the study?

If you give us your permission by signing the Consent Form, findings from the study will be used in internal reports, scientific/professional conference presentations, and scientific journal. The findings may also be featured in the media. You will not be identified in any presentations or publications.

At the end of the study, we will provide you with a summary of results from the study. Please note that there may be a delay between your study visit and publication of the results.

What happens if I change my mind?

You have the right to withdraw from the study at any time. Your contribution is entirely voluntary. If you decide to withdraw from the study, data that has already been obtained may be kept and used to contribute to the overall results. However, you can request that any data or information relating to you can be destroyed and we will ensure that this happens.

Will taking part in the LINK study cost me anything, and will I be reimbursed?

You will not incur any costs for taking part in this study except for your time, for which we thank you. All study treatments and data collection will be paid for by the study funders.

We appreciate that taking part in this study involves 5 visits to our research centre, and approximately 4-5h of your time. All participants will receive a koha (gift) in the form of vouchers as an expression of thanks for dedicating time to this research. Vouchers will be provided at completion of each arm of the intervention (study visits 3 and 5). When visiting the Grafton Campus for research visits reserved parking will be arranged for you by the study team. Please let the study team know if there is a problem getting to your appointment, as arrangements can be made.
Who do I contact for more information or if I have concerns?

Once you have read this information, a member of the study team will discuss it with you and answer any questions you may have.

If you have any questions, concerns, or complaints about the study at any stage you can contact:

Dr Nicola Gillies, Principal Investigator  
Discipline of Nutrition,  
Faculty of Medical and Health Sciences  
The University of Auckland, New Zealand  
Email: n.gillies@auckland.ac.nz

If you want to talk to someone who isn’t involved with the study, you can contact an independent health and disability advocate on:

Phone: 0800 555 050  
Fax: 0800 2 SUPPORT (0800 2787 7678)  
Email: advocacy@advocacy.org.nz  
Website: https://www.advocacy.org.nz/

For Māori cultural support, talk to your whānau in the first instance. Alternatively, you may contact the administrator for He Kamaka Waiora (Māori Health Team) by telephoning 09 486 8324 ext 2324

You may also contact the health and disability ethics committee (HDEC) that has approved this study:

Phone: 0800 4 ETHICS  
Email: hdecs@moh.govt.nz

APPROVED BY THE HEALTHY AND DISABILITY ETHICS COMMITTEE ON 27/05/2022, Reference Number (2022 EXP 12513)