

Participant Information Sheet

The DIPO Study: Combining Dietary Protein Sources to Improve Amino-Acid Digestibility and Net Protein Balance in Older Adults



Formal Study Title: *How does the Digestible Indispensable Amino Acid Score (DIAAS) influence Protein Net Balance in Older Adults? An Evaluation of the Health Efficacy Potential of Combinatorial Proteins.*

Sponsor: Riddet Institute, Massey University

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Lead Researcher: Professor David S. Rowlands

Study Site: Massey University, Albany

Contact phone number: 0272099383, email: d.s.rowlands@massey.ac.nz

Ethics committee ref.: 19566

This is the first clinical trial of the digestible indispensable amino acid score (DIAAS) on protein turnover in healthy people. You may not get any health benefits from the study of nutritional intervention. The approach to evaluating dietary protein has been used previously to quantify whole-body protein turnover in humans and has been shown to be safe. There are risks of injury or illness with your involvement in the study.

You are invited to participate in a study investigating the influence of the digestible indispensable amino acid score (DIAAS) on whole-body protein turnover, which pertains to protein synthesis, protein degradation, and net protein balance. It is your choice if you want to participate or not. You do not have to give a reason if you do not want to participate. If you want to participate now but change your mind later, you can withdraw from the study anytime without experiencing any disadvantages.

This Participant Information Sheet will help you decide if you want to participate. It sets out why we are doing the study, what your participation would involve, the benefits and risks to you, and what would happen after the study ends. We will review this information with you and answer any questions you may have. You do not have to decide today whether you will participate in this study. Before you decide, you may want to discuss the study with others, such as family, whānau, friends, or healthcare providers. Feel free to do this.

If you agree to participate in this study, you will be asked to sign the Consent Form on the last page of this document. You will be given a copy of the Participant Information Sheet and the Consent Form to keep.

This document is 15 pages long, including **the Consent Form**. Please make sure you have read and understood all the pages.

VOLUNTARY PARTICIPATION AND WITHDRAWAL FROM THIS STUDY

Participation in the study is voluntary, and you are free to decline to participate or withdraw from the research/study at any time without experiencing any disadvantage.

WHAT IS THE PURPOSE AND BACKGROUND OF THE STUDY?

The DIPO study aims to understand how different dietary protein sources affect our body's use of protein in older age. We want to see how protein quality, measured using the Digestible Indispensable Amino Acids Score (DIAAS), influences how our body processes protein when we eat different kinds of food. The DIAAS is a new way to measure protein quality, and it was introduced in 2013. It is more objective than the traditional method because it gives a more accurate assessment of how well our body can use the protein we consume.

The DIAAS measures the true digestibility of each amino acid in a protein by using data from growing pigs. This method has been shown to predict how well humans can digest protein. Unlike the old method, the DIAAS does not limit the protein quality score to a maximum of 1.0 (100%). This means it can provide a more accurate score for protein quality and help us identify which proteins work best together. It also allows us to evaluate protein quality from plant sources and find ways to replace animal-based protein with more sustainable options.

The DIAAS score falls into three categories: less than 75 (low quality), 75-99 (high quality), and 100 or more (excellent quality). A DIAAS score of 100 or more means the protein source provides all the essential amino acids we need.

Despite our growing knowledge about protein quality and the DIAAS, we still need to understand how it relates to how our body uses protein in real-life situations, like when we eat a mix of different foods. This information is crucial because protein plays a vital role in maintaining good health and preventing diseases in older adults. The DIAAS helps us measure the protein that our body can use.

Integrating the DIAAS is important, as older adults tend to lose muscle, strength, and function. Slowing down or preventing this muscle loss (called sarcopenia) is crucial for maintaining or improving the quality of life as we age. One way to help with this is by making sure older adults get enough high-quality protein in their diets. Protein helps the body build and repair muscles, and certain types of amino acids (IAAs) are especially important for this process. However, in older adults, their bodies might not respond as well to protein, so we need to pay extra attention to the DIAAS to make sure they get the right amino acids they need to keep their muscles healthy.

The main goal of this study is to compare how different levels of DIAAS affect the processes of protein synthesis, protein breakdown, and net protein balance in our body. We have chosen specific combinations of protein sources based on research suggesting animal-based proteins are better for our overall protein use. However, we believe that combining certain plant-based proteins can achieve the same level of protein quality as animal-based proteins. We hope that the results of this research will provide useful information for creating dietary recommendations that can help us optimise protein intake and improve our health as we age.

HOW IS THE STUDY DESIGNED?

The design is a 3-arm randomised crossover conducted in healthy men and women aged 67 to 77.

If you choose to volunteer in each arm of the study, you will ingest a meal comprising a different combination of proteins, each with a different digestibility score defined by DIAAS. You will visit the lab 4 times: 1 for introduction/screening and signed consent, and 3 times for each arm of the study, representing the test days.

You will be asked to refrain from alcohol, caffeinated foods, and drinks the day prior from 6 pm the night before. Upon waking, you will be asked to go to the toilet, drink 300 ml of water, and have no food before entering the research lab. Other requirements will be to refrain from hard exercise (<45 min light aerobic only; no hard exercise, weight-lifting, eccentric exercise such as running downhill) two days before visits 1-3.

After coming to the lab at an agreed time between 6:00 am and 9:00 am, catheters for blood sampling and infusion will be placed in veins at the top of your non-dominant hand and forearm on the other side. The non-dominant hand will be placed into a heated-hand box to arterialise the blood flow through the hand. Administration of stable isotope amino acid tracers will occur continuously, and blood sampling will occur at regular time points over 5.5h. After 1.5 h, you will ingest the pre-heated test meal comprising whole foods of combinatorial protein (foods are commercially bought) and a stable isotope amino acid tracer (Figure 1).

Blood will be collected from a hand vein at specified intervals for another 4 hours after meal ingestion (Figure 1). During this time, you will remain rested either fully or semi-reclined on a research bed or chair during sampling and may do (1-handed) computer work, watch TV, or relax. You will drink a glass of water each hour: 133 ml/hour if male or 115 ml/hour if female.

There will be a minimum 1-week washout between test days to eliminate any carry-over effects.

Blood plasma will be stored and later analysed using mass spectrometry at Massey University for amino acids and tracer/tracee concentration.

Data will be analysed to determine how much and how fast the combinatorial protein has affected the whole-body protein net balance. Results will inform how protein quality is related to whole-body protein turnover determined by the meal's DIAAS and provide the first data on how dietary protein quality defined by DIAAS affects protein metabolism in older adults.

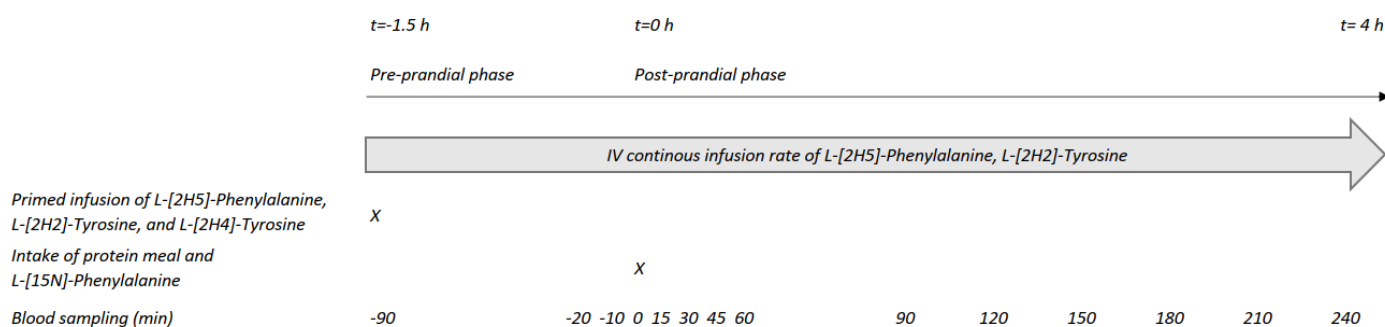


Figure 1. Overview of the experimental protocol and timeline.

WHO CAN TAKE PART IN THE STUDY?

Healthy men and women aged 67 to 77 with a body mass index (BMI) of 23-30 kg/m² who pass the general health questionnaire, are sedentary to moderately active, have an HbA1c range below 40 mmol/mol (non-diabetic), and provide written consent may volunteer for the study.

Important *exclusion criteria* include:

- Planning on leaving the city or proximity that would affect the ability or willingness (we may be able to support travel costs) to participate in all 3 study arms for the entire study duration.
- Other foreseen factors that may prevent the completion of the study.
- Criteria-defined sedentary due to a precluding disability
- Missing hands (for arterialised-venous blood sampling)
- Active malignancy (cancer) within the past six months.
- Unwilling to ingest meat and meat alternatives.
- Allergy to experimental foods (i.e., gluten, lectin, and allergens).
- Any gastrointestinal disease or disorder that may affect the study outcomes.
- Gastrointestinal bypass surgery or congenital gastrointestinal issues.
- Chronic inflammatory disease (rheumatoid arthritis, psoriasis, psoriatic arthritis, Crohn's disease, ulcerative colitis, and ankylosing spondylitis).
- Taking medications that may interfere with the study outcomes.
- Vigorously physically active.
- Currently participating or having participated in another clinical study during the last four weeks prior to the beginning of this study that may affect results.

WHAT WILL MY PARTICIPATION IN THE STUDY INVOLVE?

The timeline for your participation in the experiment is shown in Figure 2.

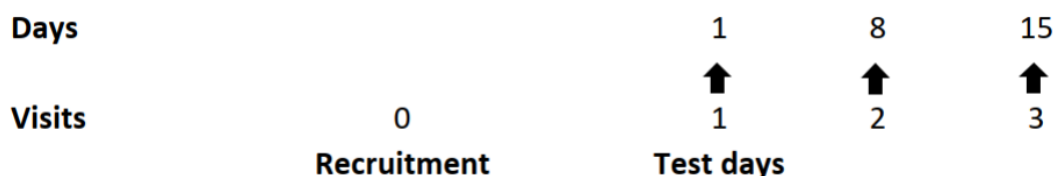


Figure 2. Study plan showing the four lab visits. Days 1 through 15 assuming weekly testing.

Flow Chart Timeline of the Study Procedures (see also Figure 1).

Visit 0. Introduction

- Following initial contact, a meeting will be arranged at Massey University to review the study details and protocols and to respond to any inquiries you might have.
- If you agree to participate in the study (with the option to reconsider later if you do not feel ready), we will conduct a Health questionnaire screening and invite you to sign the Consent form if everything is in order.
- During the first meeting day, you will be asked to answer three questionnaires regarding habitual protein intake, views towards consumption of meat and meat alternatives, and healthy dietary patterns and environmental behaviours. These can be answered at home and must be completed before visit 1.
- Following this, baseline parameters (age, height, body weight, fat and lean mass, and blood sample - HbA1c) will be recorded. Bioelectrical impedance scales will be used to measure total, fat, and lean mass by passing a low-level electrical current through the body and measuring how well it travels through different tissues.
- You will have to consume an evening meal and snack the day before each experimental day, which we will provide on visit 0.

Visit 1. Main trial day. The total duration is estimated to be about six hours.

Will comprise:

- After waking, please drink 300 ml of water. Before entering the lab, do not eat or drink tea, coffee, or other beverages.
- You will travel to the lab, arriving between 6.00 and 9.00 a.m., at a consistent time across the study, and commute by bus or car.
- You will go to the toilet to urinate prior to beginning the experiment.
- You will then be asked to be seated or semi-reclined in a comfortable chair or clinic bed.
- Your non-dominant hand of choice will be placed into a heated hand box to warm up the hand. A trained person will then place a line into a hand vein for repeated blood sample collection over the next 5.5 hours. These lines are the same as

would be placed in a hospital and should not be painful or sore after they have been placed.

- A catheter for infusion of stable isotope amino acids will be placed in the opposite arm (see Figure 3). A trained person will place a line into your arm vein to continuously infuse stable amino acid isotopes for the next 5.5 hours.
- These lines are also the same as would be placed in a hospital and should not be painful or sore after they have been placed.
- During the experiment, you may work (one hand only available), watch movies, read, and relax.
- At time=0, you will ingest the experimental combinatorial protein-rich meal, containing ~22g protein, carbohydrates, and fat, for a total of about 900 kcal. The meals will contain some or all of the following proteins: bread, quinoa, Quorn, chickpeas, tofu, and L-[15N]-phenylalanine.
- During the experiment, you will be asked questions about the taste and feelings of meals and gut comfort at time 0 (before meal ingestion) and 30, 90, and 240 min after eating the meal.
- During the experiment, you will be allowed to drink 133 ml/hour if you are male or 115 ml/hour if you are female.
- After the test is completed, the line will be removed, and the access site will be covered with a band-aid.

The time between the next trial (washout period)

- There will be at least one week before the next trial to eliminate any carry-over effects.

Visits 2-3 (Study arms 2 through 3; days approx. 8 to 15).

- These visits will occur every week for three weeks. Please discuss the most suitable testing dates with the researchers.
- In the following 2 study arms, you will repeat all the procedures from day 1, Visit 1, as listed above, but ingesting a different meal.

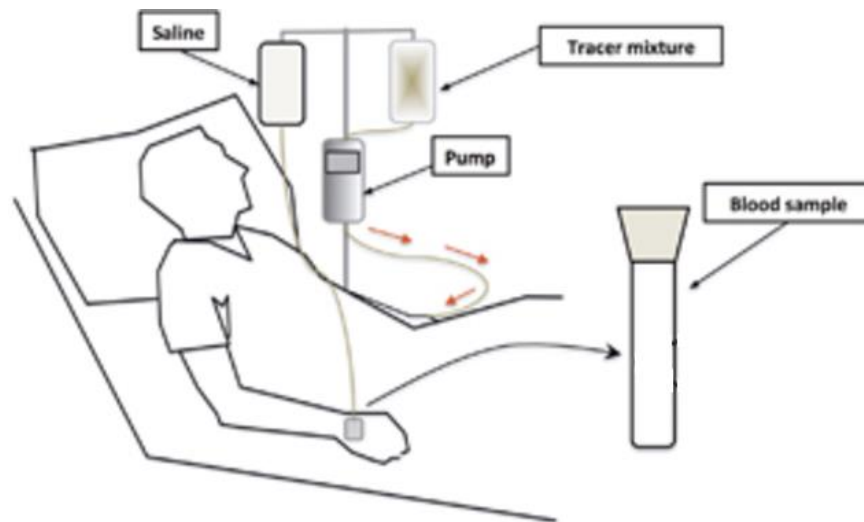


Figure 3. Picture of the experimental setup.

WHAT WILL HAPPEN TO MY BLOOD SAMPLES?

Blood will be collected from a hand vein using the heated-hand method to improve blood sample quality. Four samples will be collected at times: -90 min, -20 min, -10 min, and 0 min (before ingesting the meal) and in the following period after ingesting the meal at 15-, 30-, 45-, 60-, 90-, 120-, 150-, 180-, 210-, and 240-min. Total of 14 samples. The sample volume will be 70-100 ml in total. The blood samples will be used to analyse stable isotope tracers, amino acids, glucose, and insulin levels. Your samples may also be measured for gut or blood hormones and metabolite concentrations.

Blood samples will be measured at Massey University. Additionally, some blood samples may be analysed overseas (T&M University, Texas, USA; University of Copenhagen, Denmark; or University of Maastricht, Netherlands). Samples will be stored for ten years. You have the right to have the *unused* portion of the sample returned before analysis (see below). If you wish to enact this right, please inform the researchers at the start of the study.

You may hold beliefs about a sacred and shared value of all or any tissue samples removed. The cultural issues associated with sending your samples overseas and/or storing your tissue should be discussed with your family/whānau. There is a range of views held by Māori around these issues; some Iwi disagree with the storage of samples, citing whakapapa and advising their people to consult before participating in research where this occurs. However, it is acknowledged that individuals have the right to choose.

WHAT ARE THE POSSIBLE RISKS OF THIS STUDY?

The primary risks associated with the study are those associated with blood sampling and infusion. You may experience a pinprick sensation when placing the catheters. There is a small chance of bruising and a remote chance of hematoma (bruising), thrombosis (clotting), or site infection risk. The catheter is placed by a trained nurse (Miss Ayla Blaxell) or phlebotomist (Prof. David Rowlands, Mr. Owen Mugridge, Mr. Robin D. Nielsen, Miss Anja Zoellner), and full sterile procedures are followed according to standard operational procedures. The doses of stable isotope tracer substances that are used for this clinical study appear safe and without any adverse effect.

You will be told of any new information about adverse effects related to the study if such information becomes available during the study.

WHAT ARE THE POSSIBLE BENEFITS OF THIS STUDY?

You will not directly benefit from the study. However, you may learn how dietary protein quality, measured by the DIAAS, is related to whole-body protein turnover, i.e., protein synthesis, protein degradation, and net protein balance. Another learning outcome will be learning which combinations of proteins are needed to increase the dietary protein quality of non-animal foods. You may enjoy participating in nutrition and health research for altruistic or other reasons.

WILL ANY COSTS BE REIMBURSED?

Participation will not incur direct costs except travel costs (e.g., petrol or bus). To compensate for costs in part, as koha, you will be reimbursed gift vouchers of your choice (usually MTA or supermarket) of \$300, which will be provided upon completion of all three arms of the study.

WHAT IF SOMETHING GOES WRONG?

If you were injured in this study, you would be eligible to apply for compensation from ACC just as you would be if you were injured in an accident at work or at home. This does not mean that your claim will automatically be accepted. You will have to lodge a claim with ACC, which may take some time to assess. If your claim is accepted, you will receive funding to assist in your recovery.

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.

WHAT WILL HAPPEN TO MY INFORMATION?

The researchers, nurses, and other site staff will record information about you and your study participation during this study. This includes the results of any study assessments. You cannot participate in this study without consent to collect this information.

Identifiable Information

Identifiable information is any data that could identify you (e.g., your name, date of birth, or address). Only researchers will have access to your identifiable information. The following groups may have access to your identifiable information:

- Massey staff (to complete study assessments).
- The Riddet Institute (internal funder and Sponsor), ethics committees, or government agencies from New Zealand or overseas if the study or site is audited. Audits ensure that participants are protected, the study is run properly, and the data collected is correct.
- Your usual doctor (your GP or specialist) if a study test gives an unexpected result that could be important for your health or well-being. This allows appropriate follow-up to be arranged.
- Rarely, it may be necessary for the principal investigator to share your information with other people – for example, if there is a serious threat to public health or safety, or the life or health of you or another person, or if the information is required in certain legal situations.

De-identified (Coded) Information

To ensure your personal information is kept confidential, information that identifies you will not be included in any report generated by the researchers, or any study information sent to the Sponsor. Instead, you will be identified by a code. The researchers will keep a list linking your code with your name to identify you by your coded data if needed.

The following groups may have access to your coded information:

- The Sponsor (Riddet Institute) for this study.
- People and companies working with or for the Sponsor for the purposes of this study (this may include 2-20 people).
- Regulatory or other governmental agencies worldwide.

The study's results may be published or presented, but not in a form that would reasonably be expected to identify you.

Future Research Using Your Information.

If you agree, your coded information may be used for future research related to whole-body protein turnover or dietary protein interventions.

This future research may be conducted overseas. You will not be told when future research will be undertaken using your information. Your information may be shared widely with other researchers or companies. Your information may also be added to information from other studies to form much larger data sets.

You will not get reports or other information about any research using your information.

Your information may be used indefinitely for future research unless you withdraw your consent. However, it may be extremely difficult or impossible to access your information, or withdraw consent for its use, once your information has been shared for future research.

Security and Storage of Your Information.

Your identifiable information is held at Massey University during the study. After the study, it is transferred to a secure archiving site, stored for 10 years, and then destroyed. Your coded information will be entered into electronic case report forms and sent through a secure server to the Sponsor. Coded study information will be kept by the Sponsor in secure, cloud-based storage indefinitely. All storage will comply with local and/or international data security guidelines.

Risks.

Although efforts will be made to protect your privacy, absolute confidentiality of your information cannot be guaranteed. Even with coded and anonymised information, there is no guarantee that you cannot be identified. The risk of people accessing and misusing your information (e.g., making it harder to get or keep a job or health insurance) is very small. However, it may increase in the future as people find new ways of tracing information.

Your coded information is being sent overseas. Other countries may have lower levels of data protection than New Zealand. There may be no New Zealand representation on overseas organisations that make decisions about using your information. There is a risk that overseas researchers may work with information in a way that is not culturally appropriate for New Zealanders.

Rights to Access Your Information.

You have the right to request access to your information held by the research team. You also have the right to request that any information you disagree with is corrected.

Please ask if you would like to access the results of your screening and safety tests during the study.

If you have any questions about the collection and use of information about you, you should ask Professor David Rowlands.

Rights to Withdraw Your Information.

You may withdraw your consent for collecting and using your information at any time by informing the researchers.

If you withdraw your consent, your study participation will end, and the study team will stop collecting your information.

Information collected until your withdrawal from the study will continue to be used and included in the study. This is to protect the quality of the study.

Ownership Rights.

Information from this study may lead to discoveries and inventions or the development of a commercial product. The rights to these will belong to Riddet Institute. You and your family will not receive any financial benefits or compensation, nor have any rights in any developments, inventions, or other discoveries that might come from this information.

Māori Data Sovereignty

Māori data sovereignty protects information or knowledge about (or comes from) Māori people. We recognise the taonga of the data collected for this study. To help protect this taonga:

- We have consulted with Dr. Bevan Erueti (Taranaki, Ngāti Tūwharetoa, Te Atihaunui-a-Pāpārangī), Associate Dean Māori, School of Health Sciences, Massey University about the collection, ownership, and use of study data.
- While the study focuses on the general population of Aotearoa, New Zealand, it will collect health data relevant to Māori. It may add to the growing body of research required to inform government and policymakers.
- Consideration is given to the term *taonga*, which refers to both tangible and intangible possessions/assets. Hence, bodily fluid as a tangible taonga needs to be regarded and valued in scientific research as a gift/prized possession. Accordingly, the blood and urine samples will be treated with respect, and you have the right to have the used or unused portion of the sample returned prior to analysis. If you wish to enact this right, please inform the researchers at the start of the study.
- The signing of consent also comprises reciprocal *whakaute* - a responsibility of the individual to make informed choices, which may require consultation with whanau and iwi prior to consent to be involved in the study – the *whakaute* relates to the considerable financial (~\$8500/participant) and time investment made to collect the samples by all involved in the research process from the sponsors, ethics committee, other participants, the Universities. A request to return *unused* blood samples would result in withdrawal from the study. Accordingly, *kōtua* – if in doubt about the storage and use of the blood samples, you should, in the first instance, not participate in the study; in the second instance, you should consult as above or with the researchers for resolution. If you exercise your right to change your mind during the study, any request should be made as soon as possible, so a replacement participant may be found if resources permit.
- A karakia is available if requested up to the end of the study's protein net balance, insulin and glucose, and amino acid analysis point. Please inform the researchers if you would like this.
- A karakia will be available up to final tissue destruction.
- If you have any questions, please discuss them with the researchers, your whanau or iwi, Dr. Erueti (see below), or other contacts (see below).

WHAT HAPPENS AFTER THE STUDY OR IF I CHANGE MY MIND?

If you wish to withdraw from the study, you should contact the researcher in the team whom you are most in contact with by email or call Professor David Rowlands. Unless you request a return of unused samples, the sample may be processed, and the data used in the study analysis may be processed. No study intervention material will be available to any participants, irrespective of completion status, after the study completion.

CAN I FIND OUT THE RESULTS OF THE STUDY?

Participants will be provided with a plain English summary of study results, if requested, within 1 year of study completion. The study will be registered in the Australian New Zealand Clinical Trials Registry, which is publicly accessible.

WHO IS FUNDING THE STUDY?

The Riddet Institute, Centre of Research Excellence (CoRE) research program, Palmerston North, New Zealand, funds the study.

The Riddet Institute is a premier national centre for fundamental and strategic scientific research in food. Its area of expertise is at the intersection of food material science, novel food processing, gastrointestinal biology, and human nutrition. See for details: <https://riddet.ac.nz/>.

The research team includes:

Massey University

Professor David Rowlands, lead investigator, School of Sport, Exercise, and Nutrition

Mr. Robin D. Nielsen, doctoral student, School of Sport, Exercise, and Nutrition

Professor Rozanne Kruger, sub-investigator, School of Sport, Exercise, and Nutrition

Assoc. Professor John Harrison, sub-investigator, Chemistry

Riddet Institute

Dr. Suzanne Hodgkinson, sub-investigator, Riddet Institute, Massey University, Palmerston North

Dr. Carlos Montoya, sub-investigator, Ag Research, Massey University, Palmerston North

WHO HAS APPROVED THE STUDY?

This study has been approved by an independent group of people called the Health and Disability Ethics Committee (HDEC), which check that studies meet established ethical standards. The Northern Committee has approved this study.

WHO DO I CONTACT FOR MORE INFORMATION OR IF I HAVE CONCERNS?

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

Mr. Robin D. Nielsen, doctoral student
P: 021 2874 100
E: R.Nielsen@massey.ac.nz

Professor David Rowlands, principal investigator
P: 027 2099 383
E: D.S.Rowlands@massey.ac.nz

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

P: 0800 555 050
F: 0800 2 SUPPORT (0800 2787 7678)
E: advocacy@advocacy.org.nz
W: <https://www.advocacy.org.nz/>

For Māori cultural support, please contact:

Dr. Bevan Erueti, Associate Dean Māori, College of Health, Massey University
P: +64 6 356 9099 ext. 83087
E: B.Erueti@massey.ac.nz

For Pacific cultural support, please contact:

Jack Scanlan, Lecturer, School of Social Work, College of Health, Massey University
P: +64 921 363 53
E: J.Scanlan@massey.ac.nz

You can also contact the Health and Disability Ethics Committee (HDEC) that approved this study on:

P: 0800 4 ETHIC
E: hdecs@health.govt.nz

Consent Form

The CPs Study



An interpreter for Te Reo, most Pacific languages, and Chinese is available on request.

Please tick in the optional box to indicate your consent for the listed item; for all other items, signing the consent entails agreement with the study procedures.

I have read the Participant Information Sheet or had it read to me in a language I understand and fully comprehend what it says.

I have been given sufficient time to consider whether or not to participate in this study.

I have had the opportunity to use a legal representative, whanau/ family support, or a friend to help me ask questions and understand the study.

I am satisfied with the answers I have been given regarding the study, and I have a copy of this consent form and information sheet.

I understand that participating in this study is voluntary (my choice) and that I may withdraw from the study at any time without affecting my medical care.

I consent to the research staff collecting and processing my information, including information about my health.

I consent to my information being sent overseas.

If I decide to withdraw from the study, I agree that the information collected about me up to the point when I withdraw may continue to be processed. Yes No

I consent to my GP or current provider being informed about my participation in the study and of any significant abnormal results obtained during the study.

I agree to an approved auditor appointed by the New Zealand Health and Disability Ethics Committees or any relevant regulatory authority or their approved representative reviewing my relevant medical records to check the accuracy of the information recorded for the study.

I understand that my participation in this study is confidential and that no material, which could identify me personally, will be used in any reports on this study.

I understand the compensation provisions in case of injury during the study.

I know whom to contact if I have any questions about the study in general.

I agree to my de-identified data being deposited in an online database associated with publishing the results in a scientific journal.

I understand my responsibilities as a study participant.

I wish to receive a summary of the results of the study. Yes No

Declaration by participant:

I hereby consent to take part in this study.

Participant's name: _____

Signature: _____

Date: _____

Declaration by a member of the research team:

I verbally explained the research project to the participant and answered the participant's questions about it.

I believe that the participant understands the study and has given informed consent to participate.

Researcher's name: _____

Signature: _____

Date: _____