**Improving function of the pancreas and decreasing diabetes risk using a plant-polyphenol, rutin**

**PARTICIPANT INFORMATION SHEET**

We invite you to participate in a clinical study to investigate the beneficial effects of a polyphenol plant extract rutin on blood glucose and prevention of diabetes.

To help understand these effects, the trial will be conducted in two parts:

1. DOSE STUDY: The first study will compare the absorption of 2 doses of rutin (250 mg and 500 mg); each of these doses will be taken in a capsule and a food product (yogurt) on a single occasion.
2. INTERVENTION STUDY: The second study will investigate whether taking rutin (500mg) for 3 months will decrease your risk of developing diabetes.

Rutin is found in many foods including apple skins, grape skins, some berries, figs and also is a component of buckwheat (soba noodles). It is also commercially available at 500 mg as a supplement in many Health Food stores. Therefore, we are investigating the effects of rutin at this dose.

Your participation is entirely voluntary (your choice). If you do agree to take part, you are free to withdraw from the study at any time, without having to give a reason. You may take as much time as you need to consider whether or not you would like to participate.

**Who can take part?**

You can participate if you are male or female, between 18-65 years of age, with body mass index (BMI=weight/height2) between 23-35kg/m2 , and are healthy. You should not have a diagnosis of diabetes or any other significant disease, including cardiovascular disease, pancreatic disease, cancer and digestive diseases. Your inclusion into the study will also be based on your fasting blood glucose levels that we will assess during your Screening Visit. To take part in the DOSE STUDY you must have normal glucose levels; to take part in the INTERVENTION STUDY you must have high glucose levels (but not have diagnosed diabetes).

**Background to the study**

Type 2 diabetes typically occurs when secretion of the hormone insulin from your pancreas decreases, or if its action is no longer sufficient to keep blood glucose at normal healthy levels. Gaining weight and an unhealthy lifestyle commonly effect insulin and increase the risk of developing diabetes. Interestingly, however, there may be another reason why enough insulin is not secreted by the pancreas. Another hormone called amylin has been shown to be slowly deposited in the pancreas in some people. This affects the ability of the pancreas to produce sufficient insulin which again leads to increased blood glucose levels and high risk of diabetes.

A number of studies in animals have shown that dietary intake of the plant polyphenol rutin may prevent the accumulation of amylin in the pancreas, and so help to maintain healthy levels of insulin and glucose. Rutin is a natural antioxidant that is found in the skin of fruits (apple, figs, berries, grapes), raw onion, green and black tea and also in buckwheat and its products such as soba noodles. Rutin is also commonly available in health food stores in the form of capsules (500mg) and also found in commercially sold foods and beverages.

Our research team is conducting clinical studies to assess whether rutin, at a dose of 500 mg per day, can help prevent the problems that occur with amylin and insulin in the pancreas, and so possibly help to delay or prevent diabetes. We also want to test if the same effect occurs when rutin is taken as a supplement in a capsule and also in a food (yogurt). Importantly, also to see if there are any differences in rutin absorption when taken in these two forms.

**Who designed the study?**

This trial is part of the New Zealand National Science Challenge - High Value Nutrition (NSC-HVN) program. This is a research program being run all across New Zealand, with many scientists and members of the public involved in different studies. To know more about the High Value Nutrition program, visit [www.highvaluenutrition.co.nz](http://www.highvaluenutrition.co.nz). The study is designed by the Research Staff at the Human Nutrition Unit (HNU), University of Auckland. Data from the study will also contribute to the PhD research program of Mr. Wilson Yip.

**What is the aim of the study?**

The aims of the study are to assess:

1. The absorption of 2 doses of rutin; 250 mg and 500mg, taken in a capsule and in a food (yogurt), and measured over 24 hours
2. The effect of longer term rutin (500mg) intake, for 3 months, on blood markers of T2D risk.

**Your time on the study**

*STUDY 1 (DOSE STUDY):* Will compare absorption of 2 doses; 250 and 500 mg, of rutin; taken as a capsule and in a food (yogurt), over 24 hours (Fig. 1); with 2 week washout between treatments.

You will be randomised to receive either:

* 2 x rutin capsule OR
* 2 x rutin yogurt OR
* All 4 treatments.



**Figure 1:** Study design with 4 randomised cross-over treatments.

If you were to choose to participate in Study 1; you must be available to attend the HNU on 2 study visits (or a total of 4 study visits), each visit 2 weeks apart, and stay for 24 hours. *As this study is for a whole day, you will need to arrive at the HNU at 7.00 am in the morning and stay through the day and overnight until 9.00 am the following morning.*

*Study 2 (INTERVENTION STUDY):* Will compare the effect of 500 mg rutin (once per day) taken over 3 months, in a randomised 3-arm placebo controlled parallel study. You will be randomised to receive either: the placebo (a control yogurt + a control capsule), or yogurt containing 500 mg rutin + a control capsule, or a capsule containing 500 mg rutin + a control yogurt (see Fig.2). As this is a randomised study, the treatment that you receive will be selected by chance, and you cannot choose which you will receive.



**Figure 2:** Study design with 3 randomised parallel treatments.

If you choose to participate in Study 2, you will be enrolled in the study for 3 months. We would ask you to come to a clinic visit every month, which you must attend. In total there would be 4 visits, which will take place at the Human Nutrition Unit (HNU) in Mt Eden, Auckland. *These study visits will take approximately 2-3 hours (8.00am to 11.00am) of your time.*

**What happens if you decide to take part?**

You will need to attend a **screening visit** at the Human Nutrition Unit (HNU) in Mt Eden, Auckland. You should be fasted (approximately 10 hours overnight), i.e. not have anything to eat after your dinner the previous night (you may however drink some water). At your screening visit, we will explain the study to you and you will have a chance to ask us any questions you may have. If you would like to participate, we will then ask you to sign a Consent form that says that you agree to do the study. By signing the consent form you will be giving permission for the researchers to enrol you into the study and conduct the various measures described below. We will also gather your personal information such as contact details, demographic and anthropometric data (age, gender, ethnicity, height, weight, waist and hip circumference, body mass index (BMI) and blood pressure). You will also be asked for some diet information to calculate your usual dietary rutin intake. You may stop the interview at any time if you are not comfortable answering the questions.

We will then collect a small amount of blood for a simple blood glucose test to assess whether you are suitable for the study. If you are eligible to participate, we will ask you to maintain a low rutin (and another polyphenol quercetin) diet and you will receive both verbal as well as written instruction for this.

We will then schedule you to attend the HNU for your **study visits**.

*Study 1*: On each study visit you will arrive at the HNU at 7.00 am following an overnight fast (nothing to eat or drink, water only is acceptable if required) and remain at the facility all day and overnight, until 9.00 am the following day. Upon arrival, you will be given a glass of water and we will record your body weight. Following this, our Research Nurse will insert a cannula (small plastic tube) into your arm. This is to facilitate blood collection throughout the study session so that you will feel the needle prick just once. Cannulas are often used in hospitals, and once inserted do not usually cause more than a little discomfort. You will be supervised/monitored by Research Staff over the 24 hour study period. Following this, we will collect a fasting blood sample (approximately 3 mL). Additionally, we will also get you to collect a fasting urine sample; instructions for this will be given to you.

After we collect a fasted blood sample, at 8.00 am during each study visit, we will be give you 1 of the 4 treatments: *either* (i) 250 mg rutin capsule, (ii) 500 mg rutin capsule, (iii) 250 mg rutin enriched yogurt, or (iv) 500 mg rutin enriched yogurt. You will have 10 minutes to finish these completely.

After this, we will collect small samples (approximately 3 ml) of blood from the cannula at 5, 10, 20, 40, 60, 90, 120 minutes and thereafter at 3h, 4h, 5h, 6h, 8h, 10h and 12h and then every 4 h, i.e. 16h, 20h and 24 h. We will collect a total of 54 ml of blood over the whole day and night. This volume is far less than collected when you do a routine blood donation.

We will also ask you to collect urine samples during the 24h period after consuming the treatment. These will be as pooled samples, i.e. collected over time intervals: 0-2, 2-4, 4-6, 6-8, 8-10, 10-12 h after dosing and then at 12-16, 16-20 and 20-24 h.

**Table 1:** Summary table of procedure during study visits

|  |  |  |
| --- | --- | --- |
| **Sample collection time points (minutes/hours)** | **Blood timings**  | **Urine timings** |
| Baseline  | 7.40 am | 7.30 am |
| 5 minutes | 8.05 am | 8.00 am – 10 am |
| 10 minutes | 8.10 am |
| 20 minutes | 8.20 am |
| 40 minutes | 8.40 am |
| 1 hour | 9.00 am |
| 1½ hours | 9.30 am |
| 2 hours | 10.00 am | 10.00 am – 12.00 pm |
| 3 hours | 11.00 am |
| 4 hours | ~11.55 am  | 12.00 pm – 2.00 pm |
| 5 hours | 1.00 pm |
| 6 hours | 2.00 pm | 2.00 pm – 4.00 pm |
| 8 hours | 4.00 pm | 4.00 pm – 6.00 pm |
| 10 hours | ~5.55 pm | 6.00 pm – 8.00 pm |
| 12 hours | 8.00 pm | 8.00 pm – Midnight  |
| 16 hours | Midnight  | Midnight – 4.00 am |
| 20 hours | 4.00 am | 4.00 am – 8.00 am |
| 24 hours | 8.00 am |  |

We will provide and monitor all of your food and drinks throughout your study visit and will give you a glass of water (250mL) at 10.00 am, a standardised lunch meal at 12 pm, dinner meal at 6 pm. From 2 hours after the treatment (capsule or yoghurt), you can have as much water as you want throughout the day however no other drinks will be allowed.

Once you have completed your study visit, we will be in touch with you by telephone for a 1 week.

*Study 2*: At the start of the study (week 0) and at end of the study (week 12) (Fig. 3), you will arrive at the HNU between 8.00 - 9.00 am after an overnight fast (nothing to eat or drink, water only is acceptable if required). Upon arrival, you will be given a glass of water (250mL) and your weight will be recorded.After this our Research Nurse will insert a cannula (small plastic tube) into your arm. We will then collect a fasting blood sample (approximately 5 mL; t=0) and give you a 75g glucose drink to consume. This is a routine test used clinically called the oral glucose tolerance test (OGTT) and is conducted over 2h. We will collect small amounts of blood (approximately 5 ml) at 30 min (t=30), 60 min (t=60), 90 min (t=90) and 120 min (t=120) after you have the glucose drink. We will also ask you to complete a 4 day food record while you are at the HNU.

You will also be asked to collect a fasted urine sample at HNU (verbal as well as written instructions will be given on how to do this). You will also be asked to bring with you the faecal sample in the kit that we provide you with at your screening visit (we will give you verbal and written instructions for this at your screening visit).

Once you have completed the OGTT we will give you with breakfast at HNU and after this you will then go for a DXA scan at the Auckland City Hospital.

You will additionally come to the HNU every 4 weeks from the start of the study - at week 4 and week 8 (Fig. 3) At these visits we will record your weight and only collect a fasting blood sample (approximately 5 ml). We will also ask you a few questions regarding your general health and if you have changed any medication etc. since your previous study visit. Additionally at these study visits you will receive your assigned intervention that you will need to consume during the next 4 week period.

CID 1

CID 2

CID 3

CID 4

WEEK 0

WEEK 4

WEEK 8

WEEK 12

**Longer visit (approximately 3 hours)**

At HNU: Body weight measured

 2 hour Oral Glucose Tolerance Test (x5 blood samples)

 Urine sample collected (x1)

 Bring faecal sample that you collected in kit given to you at your screening visit

 Complete 4 day food record diary

At Auckland City Hospital: DXA scan

**Shorter visit (approximately 30 minutes)**

At HNU: Body weight measured

 Blood sample collected (x1)

 Questions about general health

 Collect assigned intervention for following 4 weeks

**Figure 3:** Summary of study visits at HNU on Intervention Study

Your compliance will be assessed at each study visit. You will be asked to retain the lids from the yogurt pottles as well as any remaining capsule with the bottle they are contained in. You will need to bring these with you at each of your study visits.

**What will my blood samples be collected for?**

During your screening visit, a small (3mL) blood sample will be collected to test for iron status and fasting blood glucose to see if you are suitable for this study.

At each study visit, we will test for known biomarkers of diabetes risk; including HbA1c, glucose, lipids and liver function. We will also measure rutin (and its metabolites) in your blood and urine samples. A blood sample will also be stored at the University of Auckland to look for new markers of diabetes, if funding is available. This will include a method known as metabolomics – measuring lots of small circulating metabolites in your blood - which will be done in our collaborators research laboratory at AgResearch, in Palmerston North. As this study is part of a long PhD program, we will store the blood samples for up to 4 years. Samples will only be stored in NZ and used for the purpose of this research. Any leftover samples will be destroyed following standard methods according to University policy (all biological waste is placed in yellow biohazard bags and is disposed of by incineration by a commercial company, Interwaste).

Participants who would like to perform a karakia (blessing) at the time of blood collection are encouraged to do so. Once your blood has been collected, it is sent for storage and then analysed as a group with all other participants. Any remaining samples will be destroyed at the end of the study. If you would like to request a specific tikanga (Māori custom) process, please feel free to talk with the research team.

**What is Dual energy X-ray Absorptiometry (DXA)?**

DXA is a scanning method, to measure body composition (bone, fat, muscle). The scan takes about 10 min and is not unpleasant. You will need to lie quietly, without moving, on an open bed and a scanning arm passes quickly over the top of you. As the scanning arm passes over you it emits 2 types of very low dose X-ray, similar to the radiation dose that you would receive if you took a 1 hour flight – perhaps between Auckland and Wellington. The DXA then measures the density of the different tissues in your body. Bone is very dense so it appears bright white on the scan. Muscle is less dense and so it is less white, and fat even less dense and so it is the least white of all. At the end of the 10 minute scan we will print a picture of you showing an image of the bone, fat and lean tissue in your body for you to take home with you.

**What are my faecal samples collected for – what is the microbiome?**

Our gut contains millions of bacteria (gut bugs) which recent research have shown to be associated with good or poor health - with being either lean or overweight, and healthy or diabetic. These bacteria are known as the ‘microbiome’. Differences in the composition of microbiome may function as early diagnostic markers for the development of T2D in high risk patients. The test can be done on a very small sample. At your screen visit, we will give you a home faecal sample collection kit with verbal and written instructions on how to collect the sample. You will need to freeze your sample before you bring it to the HNU at your scheduled first study visit to prevent contamination. Alternatively, you may also collect your sample at the HNU, during your study visit, and we will immediately store the container in the -20$℃$ freezer at the HNU. We will then store your faecal sample in the -80$℃$ freezers at the University of Auckland until the end of the study, when all of the the microbiome analyses will be run. Any leftover faecal samples will be destroyed following standard methods according to University policy (all biological waste is placed in yellow biohazard bags and is disposed of by incineration by a commercial company, Interwaste).

 **The risk and benefits of the research**

There is low risk associated with taking part in this research study. There are no known risks associated with consuming 500 mg rutin (once a day). Rutin is commercially available at this dose in the form of supplements and in food and beverage products.

Some individual may experience discomfort during cannulation. Research staff will monitor you during the treatments. The research will be stopped should any harmful effects appear or if research staff feel that it is not in your best interest to continue. You should promptly inform the research staff if any condition arises during the study visits.

The dose of X-ray involved in the DXA scan, is similar to the radiation exposure on a flight from Auckland to Wellington.

You will receive results of some indices of health such as your weight, BMI, blood pressure and DXA scan at your study visits. At the end of the study you will also receive results from your blood tests such as your blood glucose, insulin, HbA1c, lipid profile and liver function tests. In this results letter we will explain to you whether your risk of diabetes has changed during the study. There may be some delay in receiving the end of study results as we must wait until all participants have completed the study, and the blood samples have all been analysed.

**Compensation**

If you were injured in this study, which is unlikely, 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.

**Confidentiality**

All data will be de-identified using unique study ID. Research files and all other information that you provide will remain strictly confidential. No material that could personally identify you will be used in any reports on this research. Upon completion of the research, your records will be stored for 10 years at HNU, after the study ends and be accessible only to members of HNU research team. All computer records will be password protected.

**Trial Payments**

You will receive $20 voucher after the completion of your screening visit as travel expenses. You will receive $150 voucher after the completion of each 24 hour study visit as gratuity for your time and travel expenses for Study 1. You will receive $50 voucher after the completion of each of the 4 study visits, plus $100 upon completion of the full study, as gratuity for your time and travel expenses for Study 2.

**Finally**

Thank you for considering taking part in this study.

For more information, please contact:

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This research has received Ethical Approval from The Central Health and Disabilities Ethics Committee (HDEC) 18/CEN/52.

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***Please keep this information sheet for your records.***

**Improving function of the pancreas and decreasing diabetes risk using a plant-polyphenol, rutin**

# Consent Form

I have read and I understand the Participant Information Sheet dated \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ and wish to take part in the research entitled “*Improving function of the pancreas and decreasing diabetes risk using polyphenol rutin*”.

I have had the opportunity to discuss this research with the investigator. I am satisfied with the answers I have been given.

1. I have had the opportunity to use support from a family (whanau) member or a friend to help me ask questions and understand the research.
2. I understand that taking part in this research is voluntary (my choice), and that I may withdraw from the research at any time and this will in no way affect my future or continuing health care.
3. I understand that my participation in this research is confidential and that no material which could identify me will be used in any reports on this research. I understand that the sponsor of the research, others working on the sponsor’s behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current research and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published.
4. I understand that the treatment, or investigation, will be stopped if it should appear harmful to myself.
5. I understand the compensation provisions for this research.
6. I have had time to consider whether to take part.
7. I know whom to contact if I have any side effects from the research.
8. I know whom to contact if I have any questions about the research.
9. I agree not to restrict the use of any data or results that arise from this research provided such a use is only for scientific purposes.

|  |  |
| --- | --- |
| ***Participant to complete:*** *Please circle as appropriate* | Participant Signature: |
| I consent to participate in the Study 1: comparing the absorption of 250 mg and 500mg rutin. | Yes | No |  |
| I consent to participate in the Study 2: investigating if 500 mg rutin decreases risk of developing T2D | Yes | No |  |
| I consent to having my blood samples collected  | Yes | No |  |
| I consent to having my urine and faecal samples collected | Yes | No |  |
| I consent to having a DXA scan. | Yes | No |  |
| I wish to receive a copy of the results, when published. I understand that there may be a delay between data collection and the publication of the research results. | Yes | No |  |
| I consent for research staff at HNU to contact me later if there are future studies for which I am eligible. | Yes | No |  |

# INFORMED Consent Form

***Participant to complete:***

I \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Print full name

of \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Print address

 \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

 \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ hereby consent to take part in this research which is designed to investigate the efficacy of polyphenol rutin on improving pancreatic function and preventing progression to T2D .

 \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Signature of Participant

 \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date

***Research Personnel to complete:***

 \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Project explained by

 (on behalf of the Principal Investigator)

 \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Signature

 \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Project Role

 \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date

***A copy of this consent form is to be given to the participant***

***and a copy to be kept in their research file by the Investigator.***