**CONFIDENTIAL**

**Impact of acute kawakawa tea ingestion on postprandial glucose metabolism in healthy human volunteers (TOAST)**

**Document Type:** Clinical Study Protocol

**Protocol Number:** 1

**Trial:** Nutrition Intervention

**Sponsor:** University of Auckland, Auckland, NZ

**Study Sites:** Liggins Institute, University of Auckland, Auckland, NZ

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**Version:** 1.0

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# PROTOCOL SYNOPSIS

The following synopsis is provided as an overview of the study. The protocol text and appendices should be referred to for a comprehensive description.

|  |  |
| --- | --- |
| **Title of Study:**  | **Impact of acute kawakawa tea ingestion on postprandial glucose metabolism in healthy human volunteers** |
| **Principal Investigator****Co-Investigators** | Prof Richard Mithen (University of Auckland)Dr Farha Ramzan (University of Auckland)Dr Chris Pook (University of Auckland)Dr Jennifer Miles Chan (The University of Auckland)Dr Meika Foster (Edible Research)Ramya Jayaprakash (PhD student) |
| **Trial** | Nutrition Intervention |
| **Objectives** | To investigate the effect of acute kawakawa tea ingestion on postprandial glucose metabolism in healthy human volunteers |
| **Methodology** | Open-Label, Randomised, Two-period, Two-arm crossover study |
| **Number of Subjects** | 30 |
| **Main Criteria for Inclusion** | Healthy Volunteers (Males and Females) , 18-45 years |
| **Dose and Mode of Administration** | Acute ingestion of kawakawa tea  |
| **Study Treatments** | Treatment 1- Hot water -250ml (1cup)Treatment 2- Kawakawa Tea infusion-4g/250ml (1cup) |
| **Duration of Intervention** | Total 3 visits over 2weeks1. One screening visit (0.5 hour)
2. Two acute intervention visits (4hour each)
 |
| **Study Design & Visit Schedule:** | Randomised, Open-label, Two-arm, Two-period crossover trialThe study requires 3 visits (screening (x1), Acute intervention visits (x2) over a minimum of 1 week. |
| **Study data:** | **Primary endpoints**To examine the effects of kawakawa tea intake prior to a consumption of high glycemic meal on postprandial glucose metabolism in healthy individuals. **Secondary endpoints*** Change in inflammatory biomarkers

To measure the impact of kawakawa ingestion on the inflammatory cytokines using PBMC gene expression.* Change in blood biochemistry

To measure the impact of kawakawa ingestion on plasma insulin, total cholesterol, HDL, LDL, Triglycerides. |
| **Safety:** | Adverse events will be recorded and coded using the MedDRA coding system. Subjects presenting with adverse events during the trial will be treated as per standard clinical practice by their local primary career, health provider or other appropriate medical facility. |
| **Statistical Methods:** | Differences in the primary endpoints will be compared between treatment groups using Repeated measure ANOVA or non-parametric tests where appropriate and followed-up with post-hoc tests. The relationship between secondary end-points will be assessed using multiple regression analysis.  |

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# ETHICS

## Institutional Review Board / Ethics Committee (IRB/EC)

The Principal Investigator agrees to provide the IRB/EC with all appropriate material, including the participant information sheet and the informed consent document. The trial will not be initiated until appropriate IRB/EC approval of the protocol and the informed consent document have been obtained in writing by the Investigator. Appropriate reports including a report on the progress of the study by the Principal Investigator will be submitted, if required, to the IRB/EC, in accordance with applicable government regulations.

## Informed consent

Properly executed written informed consent, in compliance with the International Conference on Harmonization (ICH) guidelines, shall be obtained from each subject before the subject is entered into the trial or before any unusual or non-routine procedure is performed that involves risk to the subject.

A signed and dated copy of the informed consent document must be provided to the subject. If new information related to the study arises, subjects will be asked to sign a revised informed consent document. If applicable, the informed consent document will be provided in a certified translation of the local language. Signed consent forms must remain in each subject’s medical record and must be available for verification by study monitors if required.

## Study discontinuation

A subject may withdraw consent for participation in the study at any time without prejudice. Additionally, the Investigator may withdraw a subject if, in his/her clinical judgment, it is in the best interest of the subject or if the subject cannot comply with the protocol. Whenever possible, the tests and evaluations listed for the End of Study (EOS) visit should be carried out at the time of subject withdrawal or whenever the Investigator feels that the subject will be unable to make any further visits. A genuine effort must be made to determine the reason(s) why subjects fail to return for the necessary study visits.

## Roles and Responsibilities

Prof Richard Mithen (The University of Auckland) is the PI for this study, and will have overall responsibility for the conduct of the study, including adherence to established ethical standards.

Dr Farha Ramzan (The University of Auckland) will be responsible for the day-to-day running of the study, and will work closely with the research team, who will take the lead role in running the study visits and ensuring appropriate sample collection.

Ramya Jayaprakash will conduct the study and relevant analysis and will be responsible for day-to-day study management and reporting to Dr Farha Ramzan.

# INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

## Investigators

**Principal Investigator:** Prof Richard Mithen

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**Clinical Project Team**

Dr Jennifer Miles Chan (The University of Auckland)

Dr Chris Pook (The University of Auckland)

Dr Meika Foster (Edible Research)

Additional members will include other researchers, students, and technicians conducting the trial.

# INTRODUCTION

## Background

Kawakawa (*Piper excelsum)* is a New Zealand (NZ) endemic plant species, with particular traditional, and medicinal importance to the indigenous Māori. Kawakawa contains a number of biologically active compounds with both known and unknown functions (1). Among various active compounds in kawakawa phenylpropanoids: myristicin, elemicin, piperine; lignans: diayangambin; and amides: piperchabamide are shown to have antibacterial, anti-carcinogenic, and anti-inflammatory properties (2–4). Despite the traditional use of kawakawa and its use in commercial products, there is a significant gap in the scientific literature regarding the potential biological and functional effects of kawakawa on the human metabolic health profile, particularly glucose metabolism.

Elevation of postprandial glucose is shown to be related to the development of type 2 diabetes (T2DM) and cardiovascular disease (CVDs) (5). Studies have reported green tea containing beverage consumption to reduce the risk of diabetes by influencing the postprandial glucose metabolism (6). Since kawakawa leaf is used locally in both traditional Māori tonics and commercially-manufactured foods and beverages, no studies have been conducted to examine whether these beverages would have similar effects to that of green tea on the postprandial glucose metabolism. Therefore, we aim to examine the effects of kawakawa tea ingestion on postprandial glucose flux using a human randomized controlled intervention.

## Study Rationale

### Rationale for subject selection

Kawakawa tea is commercially available in markets and has been consumed by Māori since historical times. However, there is no evidence supporting its impact on the metabolic health parameters in human subjects. In our previous pilot study (**20/CEN/69**) we observed a decrease in the extent of post prandial glucose flux from a high glycaemic meal in individuals who had consumed a high dose of kawakawa (4g/250ml hot water) prior to the meal in comparison to just hot water. We hypothesise that consumption of high dose kawakawa would have an impact on the postprandial glucose flux. Therefore to test our hypothesis and replicate our findings from the pilot study, this study aim to quantify post prandial glycaemic response. We will adopt the same methodology as used in the pilot study but increase the number of participants to enhance the power of the study, and also will include males and females. This study will provide new insights for identifying impact of kawakawa consumption on human postprandial glycaemic response.

### Rationale for duration of treatment

This study will examine the postprandial glycaemic response after an acute (single) ingestion of either water or kawakawa tea (4g per 250ml of hot water). As previously reported, the postprandial state lasts for approximately 4-5 h post-meal period (7). Thus, the study will require participants to attend the Clinical research unit (CRU), of the Liggins Institute on each intervention visit for a period of 4 hours. Participants will complete a screening assessment (Visit 1) and if eligible for entry into the trial will be required to visit Liggins Institute in fasted condition to consume either a kawakawa tea infusion (4g per 250ml of hot water) or only hot water. Following the tea or hot water ingestion, a high glycaemic breakfast containing two slices of white bread, along with 15g of fruit jam and 250ml of rice milk will be provided after 30 minutes and participants will be asked to finish within 10 minutes. Blood will be sampled at regular intervals following their visit in fasting state at 0 min and then postprandial following tea or hot water at 30, 45, 60, 90, 120, 180 mins from a cannula inserted into an arm vein. Urine samples will be collected in fasting state at 0 min and then 0-4hrs urine will be collected postprandial following tea or hot water consumption (Figure-1).



***Figure-1: Study design.***

### Rationale for intervention dose

Kawakawa Tea will be prepared based on the safe dose limits of consumption and recommendation of 4 cups/day (4g/250mL) as previously reported by Butts *et al* (1).

* 1. **STUDY OBJECTIVES & HYPOTHESES**
		1. **Primary objective of the project**

The primary objective of the study is to examine the effects of kawakawa tea intake prior to a consumption of high glycaemic meal on postprandial glucose metabolism in healthy individuals.

* + 1. **Secondary objectives of the project**

The secondary objectives include:

* 1. To measure the impact of kawakawa tea ingestion on the inflammatory cytokines using PBMC gene expression.
	2. To measure the impact of kawakawa tea ingestion on plasma insulin, total cholesterol, HDL, LDL, triglycerides.
		1. **Hypotheses**

The primary hypothesis is that consumption of kawakawa tea before a high glycaemic meal consumption will result in a decreased postprandial plasma glucose response compared to the consumption of hot water.

The secondary hypothesis is that kawakawa consumption would impact the biochemical and inflammatory profiles related to metabolic pathways of these individuals.

# INVESTIGATIONAL PLAN

## Study design

This clinical study was designed by researchers at the Liggins Institute, University of Auckland. The study will be Open-Label, Randomised, Two-period, Two-arm crossover design. The study will be conducted at the Liggins Institute, clinical research unit at the University of Auckland.



Participants will be recruited via local advertising in the Auckland area. Included participants will be assigned to consume hot water or kawakawa tea (4g per 250ml of hot water) on separate mornings along with a high glycaemic meal breakfast.

## Subject selection

### Inclusion Criteria:

Participants will be eligible to participate if:

* Gender: both males and females. To control for menstruation cycle variation in results, female participants would be required to come in the same phase of their cycle for both the intervention visits.
* Age: 18-45 yr.
* BMI: 18-25 kg/m2
* Non-smokers
* Self-reported not consuming dietary supplements
* No medical conditions

### Exclusion criteria

Participants will be excluded from participation if they:

* Are taking dietary supplements or herbal remedies which may affect the study outcome
* Are allergic to pepper, nutmeg or similar spices
* Are diagnosed with gastrointestinal disease (i.e. celiac, Crohn’s, colitis, etc.) or pre-existing metabolic disease
* Are currently taking medications expected to interfere with normal digestive or metabolic processes including proton pump inhibitors, laxatives, etc.
* Have used antibiotics within the previous one month or were on long-term antibiotic therapy.
* Have a medical history precluding a healthy state: history of myocardial infarction, angina, stroke, cancer or pre-existing diabetes.

### Interventions

The intervention will include a commercially available kawakawa leaf extract tea consumed acutely. 4 grams of loose tea leaves will be brewed for 10 min in 250ml of hot water and filtered before serving to the participants. Participants will be expected to finish within a set period of 10 min. Representative aliquots of each intervention for each participant will be frozen at -80 degrees for further analysis.

### Outcomes:

**Primary endpoints**

To measure change in the postprandial glucose absorption following kawakawa tea intake prior to a high glycemic meal.

**Secondary endpoints**

* Change in inflammatory biomarkers

To measure the impact of kawakawa ingestion on the inflammatory cytokines using PBMC gene expression.

* Change in blood biochemistry

To measure the impact of kawakawa ingestion on plasma insulin, total cholesterol, HDL, LDL, Triglycerides.

## Sample size

### Power calculation

The power calculation was performed based on the results obtained from our pilot study. The required sample size was estimated as the number of participants required to detect a “physiologically meaningful” decrease in the glucose absorption (the primary variable) of 5%, a type I error (α) of 0.05 and a desired power (1-β) of 0.90 (26, 27). This calculation suggested a sample size of 26 participants per group, however we aim to increase recruitment to 30 per group to maintain statistical power in the event of participant drop-outs; 15 men and 15 women will therefore be recruited.

### Recruitment and retention

Participants will be recruited through electronic and print advertising circulated through the University of Auckland, social media, and local newspapers.

Participants will have access to video entertainment and free Wi-Fi during their visits to the Clinical research unit (CRU), of the Liggins Institute and will be provided with a high glycaemic meal breakfast, lunch, and dinner, a $30 gift voucher as compensation for their time on each intervention day, and complimentary parking on each visit. Participants will be given the option to receive summary information, and their individual blood results for routine blood work at the conclusion of the trial.

## Study methods

### Visits

The study design includes 3 visits over the course of a minimum 1 week period (Figure 1).

#### Informed Consent (Screening)

This screening visit will take place not more than 1 week prior to the commencement of the intervention period. The subject will be asked to attend the Clinical Research Unit (CRU) of the Liggins Institute for a 30 minute visit. Written and verbal description of the study will be provided and informed consent obtained from eligible participants. Subjects who meet the inclusion/exclusion criteria will then be registered into the trial.

After consenting, subjects will be assessed for habitual diet patterns. Subjects will be instructed to avoid taking any spices and herbs with their diets in the 24hrs preceding each Intervention Visit. Subjects will be asked to collect a diet record for 1 day immediately preceding the Intervention Visit for assessment of habitual nutrient intake.

|  |  |  |
| --- | --- | --- |
| Timeline | What will happen | Duration |
| Screening (Visit-1) | Description & consent | < 1hr |
| Intervention (Visit-2) | Height, weight & waist circumference,Resting blood pressure. Blood & Urine samples | 4 hrs |
| Intervention (Visit-3) | Height, weight & waist circumferenceResting blood pressure. Blood & Urine samples | 4 hrs |

Table 1**.** Timeline of the intervention

#### Intervention Visit

Subjects will be asked to attend the Clinical Research Unit (CRU) of the Liggins Institute on 3 occasion.

Following an overnight fast, a venous cannula will be inserted and a fasting blood sample will be collected. A fasting urine sample will also be collected. Subjects will be provided a cup of either hot water or tea infused with Kawakawa (4g per 250ml of hot water) followed by a high glycaemic meal breakfast on different visits. Blood samples will be collected at 0, 30, 45, 60, 90, 120, 180 mins from an arm vein. Urine samples will be collected in fasting state at 0 min and 0-4hrs urine will be collected and pooled postprandially following each intervention.

#### Modifications in trial proposal in light of Covid-19 or similar situation

Depending on the presence of Covid-19 or any other unprecedented happening during the trial procedure, appropriate measures will be adopted. Since a broad age group and BMI will be used for screening of the participants, if required, participants can be screened online. Also during the physical visits to the CRU, appropriate working practices will be followed and all the necessary SOP’s will be put in place, with all the questionnaires to be completed online by the participants. Furthermore, if physical visit to CRU is not possible, assigned researchers could visit the participant’s at their home to complete all the required measurements including anthropometric and blood collections.

### Analyses

#### Blood and Plasma Analyses

Venous blood samples will be drawn from an arm vein by an experienced phlebotomist. Metabolic responses will be assessed over the postprandial period. These include:

**Plasma glucose and insulin Analysis:** Plasma glucose will be measured using a Roche Cobas c311 autoanalyser by enzymatic colorimetric assay. Plasma insulin will be measured on a Roche Cobas e411 by electro-chemiluminescence immunoassay.

**Blood Chemistry**: including whole blood count to evaluate changes in white blood cell populations in response to intervention ingestion as a measure of immune activation. These will be obtained at fasting, and after 1 & 2 hours. Plasma and peripheral blood mononuclear cell (PBMCs) gene expression will be performed following total RNA extraction for measurement of changes in inflammatory gene expression (such as TNF-α, MCP-1, IL-1β and microRNAs). These will be assessed by either RT-PCR from fasting samples and at 1 & 2 hours following intervention consumption. Fasting plasma lipids will be analysed by Roche Cobas c311 autoanalyser by enzymatic colorimetric assay.

**Metabolomics Analysis:** Plasmaand urine **m**etabolites will be assayed using liquid/ gas chromatography-mass spectrometry (LC/G- MS) techniques.

#### Anthropometric analyses

Height will be measured at baseline by stadiometer. Weight will be measured at each Intervention Visit by clinical body scale. Waist circumference will be measured at each Intervention Visit by tape measure. Resting blood pressure will be measured using an arm cuff.

#### Urinary Collection

Urine samples collected both on the day of acute interventions (Visit 2, and 3) and will be analysed for kawakawa metabolites using both targeted and untargeted metabolic profiling approaches. The analysis will be performed at the Mass Spectrometry Unit, Liggins Institute, University of Auckland.

### Statistical methods

Sample size was calculated on basis of the attainment of the primary endpoint in our previous phase 1 clinical trial ((**20/CEN/69**)). Differences in the primary endpoints will be compared between treatment groups using Repeated measure ANOVA or non-parametric tests where appropriate and followed-up with post-hoc tests. The relationship between secondary end-points will be assessed using multiple regression analysis.

# DATA MANAGEMENT AND MONITORING

## Data management

Data will be collected prospectively using a web-based secure database. Two-pass verification will be used for any data that is not collected electronically. Electronic data will be password protected on secure servers accessible only to the research team. Hard copies will be secured in locked filing cabinets.

## Confidentiality

All personal information collected will be stored securing in electronic databases or locked filing cabinets at the Liggins Institute. Only the research team will have access to personal information at any point. Participants enrolled in the study will be identified on records by a unique de-identified code to protect confidentiality. Records with personal information will be stored separately from data records with de-identified coding.

## Access to Data

Final dataset access will be managed by the Liggins Institute. The principal investigators will have access to final data sets, which will remain password protected. Any data sets distributed for use will be blinded of any personal identifying information.

## Monitoring

### Data monitoring

Data monitoring will be conducted internally. All incidents or deviations from the protocol will be documented. A major violation is defined as one which may impact subject safety, affect integrity of study data and/or affect subject’s willingness to participate in the study. This is a serious discrepancy resulting from error, fraud or misconduct e.g. failure to obtain informed consent or a breach in randomisation procedures. Should a major violation occur, all IRB will be notified.

### Harms

Adverse events will be collected by observing and interviewing the subject during the study. All adverse events (serious and non-serious) will be recorded on the appropriate CRF/record and will be coded using the MedDRA coding system.

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