**CONFIDENTIAL**

**Title: Tūhauora ka tahi: Effects of kawakawa containing beverage on the energy metabolism and physiology in healthy human volunteers**

**Short Title: Impact of kawakawa on energy metabolism and human physiology**

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**Protocol Number:** Tūhauora ka tahi \_V2

**Trial:** Nutrition Intervention

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

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

**Investigators:** Dr Jennifer Miles Chan (University of Auckland)

Dr Farha Ramzan (University of Auckland)

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Dr Meika Foster (Edible Research)

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**Version:** 2.0

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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:**  | **Tūhauora ka tahi: Effects of kawakawa containing beverage on the energy metabolism and physiology in healthy human volunteers** |
| **Short Title** | Impact of kawakawa on energy metabolism and human physiology |
| **Principal Investigator** | Dr Chris Pook (University of Auckland) |
| **Co-Principal Investigators** | Dr Farha Ramzan (University of Auckland)Dr Jennifer Miles Chan (University of Auckland)  |
| **Clinical Project team** | Prof Richard Mithen (University of Auckland)Dr Meika Foster (Edible Research, University of Auckland)Ramya Jayaprakash (PhD student) |
| **Trial** | Nutrition Intervention |
| **Objectives** | To investigate the impact of acute consumption of a beverage containing kawakawa on measures of energy metabolism including energy expenditure and respiratory quotient in healthy human volunteers |
| **Methodology** | Single blinded, randomised, four-period, four-arm crossover study |
| **Number of Subjects** | 20 |
| **Main Criteria for Inclusion** | Healthy volunteers (males and females) , 18-45 years |
| **Dose and Mode of Administration** | Acute ingestion of beverage containing aqueous kawakawa extract |
| **Study Treatments** | **Treatment-1:** 15 mL base beverage formulation containing Livaux gold kiwifruit powder, lemon juice, ginger, turmeric. Washed down with 235 mL water**.** **Treatment-2:** 15 mL base formulation + aqueous kawakawa infusion equivalent to tea made with 16 g kawakawa per litre of hot water. Washed down with 235 mL water.**Treatment-3:** Aqueous kawakawa infusion equivalent to tea made with 16 g kawakawa per litre of hot water**.** **Treatment-4:** 250ml of water as a control**.** |
| **Duration of Intervention** | Total 5 visits 1. One screening visit (0.5 hour)
2. Four acute intervention visits (4 hrs each, separated by at least 48 hrs washout)
 |
| **Study Design & Visit Schedule:** | Randomised, Single blind, Four-arm, Four-period crossover trialThe study requires 5 visits: one for screening and four acute intervention visits separated by at least 48 hrs washout. |
| **Study data:** | **Primary endpoints**To examine the effect of including kawakawa in the beverage formulation upon metabolic rate (energy expenditure) and respiratory quotient (relative substrate utilisation). **Secondary endpoints**To examine the impact of including kawakawa in the beverage formulation on the plasma and urine metabolic profiles including metabolic and hormonal biomarkersTo examine the impact of including kawakawa in the beverage formulation on the cardiovascular profile (heart rate, blood pressure). To examine the impact of including kawakawa in the beverage formulation on body temperature.To examine the impact of including kawakawa in the beverage formulation on the changes of visual analogue scale (VAS) scores of hunger, comfort, nausea; appetite and satiety assessed via an *ad lib* meal.To quantify the bioactive compounds in test beverages.To quantify the impact of including kawakawa in the beverage formulation on the metabolic- and inflammatory-related gene expression. |
| **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 Investigators 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 Investigators. Appropriate reports including a report on the progress of the study by the Principal Investigators 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 Investigators may withdraw a subject if, in their 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 Investigators 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

Dr Chris Pook (University of Auckland) is the Principal Investigator for this study and will have overall responsibility for the conduct of the study, including adherence to established ethical standards.

Dr Farha Ramzan (University of Auckland) and Dr Jennifer Miles-Chan (University of Auckland) will be Co-Principal Investigators. Dr Ramzan will be responsible for the day-to-day running of the study. Dr Ramzan will work closely with the research team, who will take the lead role in running the study visits and ensuring appropriate sample collection. Dr Miles-Chan will take the lead role in training and running of indirect calorimetry technique.

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

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**Clinical Project Team**

Prof Richard Mithen (University of Auckland)

Dr Meika Foster (Edible Research, University of Auckland)

Ramya Jayaprakash

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

# INTRODUCTION

## Background

The way our bodies allocate resources and sequester metabolic reserves is labile and can be influenced at the molecular and cellular level by elements of our diet. While the underlying mechanisms for these effects are often not fully understood, one goal of nutritional research is to assist with the development of new foods and beverages that promote desirable metabolic health outcomes (1,2). An example of a treatment and prevention strategies is the stimulation of the postprandial thermogenic cascade (3,4) through consumption of TVRP1 receptor agonists, such as capsaicin, which has been reported to alter the profile of lipolysis and visceral fat remodelling (5).

Kawakawa (*Piper excelsum*) is endemic to Aotearoa New Zealand and, as a taonga, is of great cultural importance to Māori. For example, kawakawa is used extensively in rongoā Māori (traditional Māori healing). Kawakawa is reported to contain a number of pharmacologically active compounds, demonstrated to influence pathways related to thermogenesis (6–8). For example, piperine and its analogues are shown to influence sympathetic nervous system, increases energy expenditure and fat oxidation, and alters intestinal fat and glucose absorption (7). *Trans*-pellitorin, an analogue of capsaicin and TRPV1 agonist which may inhibit adipogenesis, induce satiety, activate brown adipose tissue, and modulate intestinal hormones and the microbiome (7).

Despite considerable mātauranga Māori of the therapeutic benefits of Kawakawa, research into the pharmacology of kawakawa has progressed little beyond identification of the chemical profile. Limited research using cellular and/or animal models has confirmed that aqueous extracts are safe for consumption. Given the small number of studies conducted knowledge of the metabolic effects of pharmacologically active compounds is often derived from their study in isolation. However synergistic effects between these compounds are likely. Indeed, piperine is a well-known bioenhancer recognised to increase intestinal absorption and the bioavailability of a large number of micronutrients and drugs (9). Hence it is necessary to consider the metabolic effects of kawakawa per se. As kawakawa is used locally in both traditional Maori tonics and commercially-manufactured foods and beverages, this study presents a unique opportunity to conduct such research. Therefore, we aim to examine the effects of kawakawa containing beverage on postprandial thermogenesis using a human randomized controlled intervention.

## Study Rationale

### Rationale for subject selection

Kawakawa is commercially available in markets and has been consumed by Māori since historical times. It has been reported to contain metabolically-functional compounds that are demonstrated to influence pathways related to thermogenesis. However, due to lack of experimental scientific data in human participants to validate these findings, it is empirical to conduct human clinical trials to understand these effects. We hypothesise that consumption of kawakawa containing beverage would promote lipolysis and visceral fat remodelling on postprandial thermogenesis and substrate utilisation. Therefore, to test our hypothesis this study aims to quantify the effect of kawakawa containing beverage formulation on metabolic rate (energy expenditure) and respiratory quotient (relative substrate utilisation) in 20 healthy human volunteers. Since lean mass is a key determinant of resting metabolic rate we aim to evenly recruit both normal and overweight participants in the BMI range of 18-30 Kg/m2. To account for potential sex differences even numbers of men and women will be recruited.

### Rationale for duration of treatment

This study will examine the postprandial thermogenic and substrate utilisation effect of kawakawa containing beverage. As previously reported, the postprandial state lasts for approximately 4-5 h post-meal period (10). 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 3 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. Participants will be provided with a standardised meal to eat the evening before each study visit to reduce variability in the fasting substrate oxidation profile. This meal will be of identical macronutrient composition and energy content within- and between-participants. Participants will be advised to abstain from intense exercise, caffeinated or herbal drinks, spices and alcoholic drinks in the 24 hrs prior to the study visits. Participants will be asked to arrive at the CRU in the morning, following a 12 h fast.

### Rationale for intervention dose and washout period

Based on the safe dose limits of kawakawa for human consumption, the beverages will contain an aqueous extract of kawakawa equivalent to tea prepared from 16 grams dry kawakawa per litre of water as previously reported by Butts *et al* (6). Further, based on the preliminary data from 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 (16 g/L hot water) prior to the meal, with no recorded adverse effects in participants.

Furthermore, we have observed that the plasma and urine metabolites reach to baseline levels in around 24 hrs in our pilot study preliminary data. Therefore, to minimise the effect of any carry-over metabolites significantly, we aim to achieve at least a wash-out period of around 48 hrs for this study.

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

To examine the effect of beverage formulation on metabolic rate (energy expenditure) and respiratory quotient (relative substrate utilisation).

* + 1. **Secondary objectives of the project**
1. To examine the impact of beverage formulation on the plasma and urine metabolic profiles including metabolic and hormonal biomarkers.
2. To examine the impact of beverage formulation on the cardiovascular profile (heart rate, blood pressure).
3. To examine the impact of beverage formulation on body temperature.
4. To examine the impact of beverage formulation on the changes of visual analogue scale (VAS) scores of hunger, comfort, nausea; appetite and satiety assessed via an *ad lib* meal.
5. To quantify the bioactive compounds in test beverages.
6. To quantify the impact of beverage formulation on the metabolic and inflammatory related gene expression.
	* 1. **Hypotheses**

The primary hypothesis is that consumption of kawakawa containing beverage would promote postprandial thermogenesis and fat oxidation.

The secondary hypothesis is that kawakawa consumption would impact the biochemical, inflammatory and cardiovascular 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 Randomised, Single blind, Four-arm, Four-period crossover trial. The study will be conducted at the CRU at the Liggins Institute, University of Auckland.

## 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 all the intervention visits.
* Age: 18-45 yr.
* BMI: 18-30kg/m2
* Non-smokers
* Self-reported not consuming dietary supplements
* Self-reported healthy

### 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
* Are Claustrophobic
* Have recently gained or lost around >5% body weight

### Interventions

The study will involve four randomised treatments served at room temperature including:

**Treatment-1**: 15 mL base beverage formulation containing Livaux gold kiwifruit powder, lemon juice, ginger, turmeric. Washed down with 235 mL water.

**Treatment-2:** 15 mL base formulation + aqueous kawakawa infusion equivalent to tea made with 16 g kawakawa per litre of hot water. Washed down with 235 mL water.

**Treatment-3**: Aqueous kawakawa infusion equivalent to tea made with 16 g kawakawa per litre of hot water.

**Treatment-4:** 250ml of water as a control

### Outcomes:

**Primary endpoints**

To examine the effect of beverage formulation on metabolic rate (energy expenditure) and respiratory quotient (relative substrate utilisation).

**Secondary endpoints**

To examine the impact of beverage formulation on the plasma and urine metabolic profiles including metabolic and hormonal biomarkers.

To examine the impact of beverage formulation on the cardiovascular profile (heart rate, blood pressure).

To examine the impact of beverage formulation on body temperature.

To examine the impact of beverage formulation on the changes of visual analogue scale (VAS) scores of hunger, comfort, nausea; appetite and satiety assessed via an *ad lib* meal.

To quantify the bioactive compounds in test beverages.

To quantify the impact of beverage formulation on the metabolic and inflammatory related gene expression.

## Sample size

### Power calculation

Based on the a-priori sample size calculation specific to the intervention, the required sample size was estimated as the number of participants required to detect a “physiologically meaningful” increase in metabolic rate (the primary variable) of 5%, a type I error (α) of 0.05 and a desired power (1-β) of 0.90, and assuming a within-participant variability in resting metabolic rate of 0.04 kcal/min. This calculation suggested a sample size of 7 participants per group, however we aim to increase recruitment to 10 per group to maintain statistical power in the event of participant drop-outs. As lean mass is a key determinant of resting metabolic rate and to account for potential sex differences in the thermic effect of food, we will recruit 10 men and 10 women for this study, evenly divided between normal- and overweight (by body mass index); i.e., a total of 20 participants. Owing to the repeated-measures, cross-over design and a low level of intra-individual variability relative to inter-individual variability in indirect calorimetry studies (11) , this comparatively small sample size will still provide sufficient statistical power to detect a physiologically meaningful effect on metabolic rate.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Waitangi day | Liggins Research day  | 1 |  | 1 | 8th-11th Feb |
| 1 | 2 | 1 | 2 |  | 14-18th Feb |
|  |  |  |  |  | 21st -25th Feb |
| FoodOmics | Foodomics |  |  |  | 28th -4th March |
|  |  |  |  |  | 7th -11th March |
|  |  |  |  |  | 14th -18th March |
|  |  |  |  |  | 21st to 25th March |
|  |  |  |  |  | 28th to 1st April |
|  |  |  |  |  | 4th -8th April |
|  |  |  |  |  | 11th to 15th April |

### 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 *ad libitum* lunch, and a $50 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 5 visits (Screening, 4 intervention visits separated by at least 48 hrs washout (Table 1)

#### Informed Consent (Screening and Body composition visit)

This screening visit will take place 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. Demographics (age and ethnicity) and anthropometry (height, body weight, BMI) will be recorded. Participant will also be asked to complete screening questionnaire.

Prior to their intervention visits, participants will have their body composition assessed by dual Energy X-Ray Absorptiometry (iDXA, GE-Lunar) at the Clinical Research Unit (CRU) of the Liggins Institute. DXA is based on the 3-compartment model of body composition, and uses two x-ray energies to measure body fat mass, lean mass, and bone mineral. The participant is required to lie recumbent on the open scanner bed for ~10 minutes. Body composition comprising total body fat, fat-free soft tissue and bone mineral content as well as regional fat deposition will be determined from DXA whole-body and segmental scans. Fat-free mass is a main determinant of resting energy expenditure, and hence this information will enable us to mathematically adjust our metabolic rate measurements accordingly.

Participants will be provided with a standardised meal to eat the evening before each study visit to reduce variability in the fasting substrate oxidation profile. This meal will be of identical macronutrient composition and energy content within- and between-participants. Participants will be advised to abstain from intense exercise, caffeinated or herbal drinks, spices and alcoholic drinks in the 24 hrs prior to the study visits.

|  |  |  |
| --- | --- | --- |
| Timeline | What will happen | Duration |
| Screening (Visit-1) | Description & consent, DXA scan |  1hr |
| Intervention (Visit-2) | Height, weight ,waist circumference,Resting blood pressure, Blood & Urine samples, Indirect calorimetry, Haemodynamic monitoring, thermometry, and VAS | 4 hrs |
| Intervention (Visit-3) |  Weight, Resting blood pressure, Blood & Urine samples, Indirect calorimetry, Haemodynamic monitoring, thermometry, and VAS | 4 hrs |
| Intervention (Visit-4) | Weight, Resting blood pressure, Blood & Urine samples, Indirect calorimetry, Haemodynamic monitoring, thermometry, and VAS | 4 hrs |
| Intervention (Visit -5) | Weight, Resting blood pressure, Blood & Urine samples, Indirect calorimetry, Haemodynamic monitoring, thermometry, and VAS | 4 hrs |

Table 1**.** Timeline of the intervention

#### Intervention Visit (4 per participants)

Subjects will be asked to attend the Clinical Research Unit (CRU) of the Liggins Institute on 4 occasions.

Participants will be provided with a simple, standardised meal to eat the evening before each study visit to reduced variability in fasting substrate oxidation profile. This meal will be of identical macronutrient composition and energy content within- and between-participants, and is to be consumed between 7:30 and 8pm. Participants will also be advised to abstain from intense exercise, caffeinated and alcoholic drinks in the 24hrs prior to the study visits. Participants are required to come in an overnight (12 h) fasted state.

Upon arrival, the participant’s anthropometric measurements (height, weight) will be taken and a spot urine sample collected. Participants will then be asked to sit in a comfortable seat, in a quiet, temperature-controlled (20-22°C) laboratory. Body temperature will be measured using a tympanic thermometer to exclude the presence of fever. A peripheral venous cannula will be inserted by the Research Nurse for repeated blood sampling. A 16mL fasting baseline blood sample will be collected, and baseline Visual Analogue Scale (VAS) ratings of hunger, comfort and nausea will be measured using standard VAS methods. Participants will then be connected to equipment for continuous physiological monitoring. The respiratory gas exchange will be measured non-invasively by indirect calorimetry. Continuous, non-invasive, haemodynamic monitoring will also be undertaken. Participants will also be instrumented with autonomous wireless temperature sensors to measure changes in skin temperature. Following 3 h of postprandial monitoring, participants will be invited to eat a controlled lunch meal *ad libitum* to objectively assess appetite and satiety.

Participants will be permitted to watch calm documentaries or films during cardio-metabolic monitoring. After 30 mins of baseline measurements by indirect calorimetry using an open-circuit ventilated hood system, the hood will be removed and participants will be provided with the randomised test beverage (t= 0 mins) and requested to consume this in entirety within 10 mins prior to recommencing indirect calorimetry measurements. Throughout the morning, a total of 6x blood samples will be collected after the test beverage at the following time-points: t= 15, 30, 60, 90, 120, and 180. A total volume of 70mL will be taken at each study visit (Figure 1).



***Figure 1: Timeline of Study Visits***

#### 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.

### Analyses

#### Blood and Plasma Analyses

Venous blood samples will be drawn from an arm vein by an experienced phlebotomist/ Registered nurse. Metabolic responses will be assessed both at fasting and over the postprandial period. These include:

**Indirect calorimetry**: Respiratory gas exchange will be measured non-invasively by indirect calorimetry using an open-circuit ventilated hood system (Quark, Cosmed srl, Italy). Energy expenditure (EE) and respiratory quotient (RQ) are calculated from the rates of oxygen consumption (VO2) and carbon dioxide (VCO2) production.

**Cardiovascular Monitoring:** Continuous heart rate monitoring will be monitored. Blood pressure will be monitored at regular time points, first at the baseline and then postprandially every hour until the end of each intervention visit.

**Thermometry**: Participants will be instrumented with autonomous wireless temperature sensors (Thermochron iButton model DS1922H, Maxim) in 12 locations: forehead, upper-back, lower-back, chest, abdomen, bicep, forearm, hand, quadriceps, hamstring, front calf and back calf. A weighted-mean skin temperature will be calculated.

**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 expression of genes involved in metabolic homeostasis (such as CPT1A, FAS,UCP) and also 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 and postprandial plasma lipids will be analysed by Roche Cobas c311 autoanalyser by enzymatic colorimetric assay.

**Metabolomics Analysis:** Test beverage bioactive compounds along withplasmaand urinemetabolites will be assayed using liquid chromatography with mass spectrometry (LC-MS) techniques.

#### VAS analysis:

VAS will be used following the methodology of Blundell et al (12). Participants will mark their responses by placing a vertical line across the 100-mm scale according to their subjective feelings (Figure 3). For example:



#### 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.

#### Urinary Collection

Urine samples at fasting and then at 180 min will be collected on the days of acute interventions (Visit 2, 3, 4 and 5) 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

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 and co-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 the subject’s willingness to participate in the study. If a serious discrepancy resulting from error, fraud or misconduct e.g. failure to obtain informed consent or a breach in randomisation procedures, it will be assessed as a major violation and in such circumstances all IRB’s will be notified.

### Harm

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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