National Science Challenges

HIGH-VALUE NUTRITION

Ko Ngā Kai Whai Painga

University of Otago

UNIVERSITY of OTAGO

Te Whare Wānanga o Otāgo

NEW ZEALAND

Bread **R**elated **E**ffect on Microbi**A**l **D**istribution (**BREAD**) Study

Project numbers:

Trial registration number:

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This study will be conducted in compliance with the protocol, Good Clinical Practice, and all other applicable regulatory requirements, including archiving essential documents.

Confidential information: No use or disclosure of this protocol is permitted without prior written authorisation from the Principal Investigators.

GUTS Gastrointestinal Unit for Translational Studies

National Science Challenges HIGH-VALUE NUTRITION Ko Ngā Kai Whai Painga

SYNOPSIS

Title

Bread Related Effect on Microbial Distribution (BREAD) Study

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- Dr Janine Cooney (Plant and Food Research)

Study Site

University of Otago, Christchurch, NZ

Study Objectives

To test the efficacy of Defatted Rice Bran (DRB) bread on the composition and gene abundance of the gut microbiota in healthy human adults with low dietary fibre (DF) intake.

To determine the effect of DRB on gut symptoms, physical and mental wellbeing in healthy human adults with low DF intake.

To determine the effect of DRB on whole gut motility and fermentation gas profile of different gut segments in healthy human adults with low DF intake.

Study Design

Two-armed, placebo-controlled, double blind, randomised, crossover study.

The study duration is a nominal total of 14 weeks: 2-week lead-in phase, 4-week intervention phase, 2-week washout phase and final 4-week intervention phase, and 2-week follow up phase.

Healthy volunteers with low DF intake will be recruited from the general population. For this study, low DF intake level is described as healthy individuals who consume below the NZ daily median DF intake (<18 g/d (female), <22 g/d (male)). The cut-off points are based on a validated habitual dietary fibre intake short food frequency questionnaire. Following informed consent, participants will be randomised and given either three (females)/ four (males) slices of DRB-fortified white toast bread, or three (females)/ four (males) slices of placebo white

toast bread per day for four weeks. After a washout phase of two weeks, the participants will cross over and receive the other intervention.

Before and after each intervention phase, participants will provide a stool and a blood sample, complete a three-day food diary and questionnaires in regards to their mental and physical health, general well-being, and clinical and demographic variables, and undergo anthropometry and blood pressure measurements.

A subgroup of participants will ingest a diagnostic device in capsule form (Atmo gas-sensing capsule) and blue food dye to measure gut segments' fermentation gas profiles and whole gut transit time, respectively.

Samples will be processed and stored appropriately and analysed for a variety of variables, such as the concentrations (absolute or relative) of proteins and metabolites in plasma and stool samples and the composition and gene abundance of the stool microbiota, to determine the differences between the placebo and DRB fortified breads.

Study Population

Sixty healthy male and female volunteers with low DF intake will be recruited from the general population.

Study Time Frame

The clinical phase of the study will be completed by the end of April 2023. All laboratory and data analyses will be completed by the end of June 2024.

Evaluation Methods

Evaluation will be composed of analyses of the clinical, demographic, and laboratory variables (see above), focusing on comparing the results between outcomes when consuming DRB fortified bread compared to the placebo white toast bread.

The methods used for evaluation will depend on the data type and will contain continuous and categorical data for demographic, clinical, and physical endpoints, stool metagenome datasets, and plasma/stool metabolome and known metabolite/protein datasets.

Statistical Considerations

This study is conducted as a superiority trial. The Guidelines of the Committee for Proprietary Medicinal Products (CPMP) require the use of "intention-to-treat" analysis (ITT) (1). ITT means that every enrolled participant is included in the analysis, despite dropout or non-compliance.

Categorical variables: Chi-squared tests (or Fisher's Exact tests for small samples). Continuous variables will be analysed using (parametric) t-tests and (non-parametric) Mann-Whitney tests for symmetrically and asymmetrically distributed data, respectively.

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ABBREVIATIONS AND DEFINITIONS

AX	Arabinoxylan
BM	Bowel Movement
BMI	Body Mass Index
CHL	Canterbury Health Laboratories
CPMP	Committee for Proprietary Medicinal Products
CT	Computer Tomography
DF	Dietary Fibre
DRB	Defatted Rice Bran
DNA	Deoxyribonucleic acid
eCRF	electronic Case Report Form
ELISA	Enzyme-linked Immunosorbent Assay
ELSI	Economic Living Standards Index
GC-MS	Gas Chromatography-Mass Spectrometry
GSRS	Gastrointestinal Symptom Rating Scale
HDL	High-Density Lipoprotein
HPLC	High-Performance Liquid Chromatography
IS	Internal Standard
ITT	Intention-to-Treat
LDL	Low-Density Lipoprotein
LC-MS	Liquid Chromatography-Mass Spectrometry
ModHNEHS	Modified Hunter New England Health Survey
NZ	New Zealand
PP	Per Protocol
PRO	Patient-Reported Outcomes
PROMIS-A-D	Patient-Reported Outcomes Measurement Information System – Anxiety – Depression
RNA	Ribonucleic acid
SCFA	Short-Chain Fatty Acid
SF12	Short form 12 Health Survey
hUOHEC	University of Otago Human Ethics Committee (Health)

STUDY SCHEDULE

Table 1: Study schedule overview

Day	**Screening**	**Enrolment** **-14**	**Baseline** **-7**	**Phase 1** **0-28**	**Washout** **29-41**	**Baseline** **42**	**Phase 2** **49-77**	**Washout** **78-90**	**Follow Up** **91**
Eligibility (inclusion/ exclusion criteria)	X								
Biochemical blood panel (fasting glucose and lipid) to CHL*	X		X	X		X	X		X
Living standard and lifestyle (ModHNES incl SF-12)		X							
Economic Living Standard (ELSI)		X							
Stool samples*			X	X		X	X		X
Blood samples (plasma)*			X	X		X	X		X
Anthropometry (BMI and waist circumference)*	X		X	X		X	X		X
Blood Pressure*	X		X	X		X	X		X
Atmo capsules*			X	X		X	X		
Blue dye*			X	X		X	X		
PROs*			X	X		X	X		X
Three Day Food diary*			X	X		X	X		X
Daily BM and bread diary app		X	X	X	X	X	X	X	X

* Following enrolment:

- Blood and stool samples, a 3-day food diary, and PROs will be collected during clinic visits (**days -7, 28, 42, 77 and 91**).
- Anthropometry, Blood pressure, Atmo capsules, and the blue dye will be done during clinic visits (**days -7, 28, 42, 77 and 91**).

1 INTRODUCTION AND STUDY RATIONALE

1.1 Background

Dietary fibre (DF) has an essential role in the human diet. As indicated in recent systematic reviews and meta-analyses, a DF intake of 25 g to 29 g (2) or an increase of 7 g of DF intake (3) is associated with a risk reduction in all-cause mortality, coronary heart disease mortality and incidence, (2) cancer, stroke, type 2 diabetes, and colorectal cancer. (2, 3) Most countries have recommended a daily DF intake of 25 g to 38 g based on optimal bowel function and chronic disease prevention. (4-11)

However, inadequate DF intake is a ubiquitous issue worldwide. Inadequate DF intake is described as the consumption below that of reference average daily DF intake level within an apparently healthy people. (11) In Japan, Singapore and Belgium, DF intakes in adults ranged between 11.9 g and 17.8 g per day, which were less than half of the recommended levels applicable to the respective countries. (12-14) Similarly, based on nutrition surveys carried out in Ireland, Australia, the Netherlands and the United Kingdom, daily DF intakes ranged between 17.8 g and 23 g, which were lower than the recommendation in each of these countries. (5, 7, 9, 15) These findings of low DF intake coincide with the New Zealand (NZ) population of 17.5 g to 22.1 g, and extend to all ethnicities, including the Māori (16.2 g /d to 21.5 g/d) and Pacific population (17.5 g/d to 21.4 g/d). (16) Encouraging the adult population to improve their DF intake for disease risk reduction and optimal health is warranted.

Food cost may be the principal factor in food purchasing decisions. (17-19) Many people are experiencing financial challenges during the current global economic recession that impact their ability to purchase healthy food, which are often expensive. (9, 19, 20) These data show that it is important to encourage adults to improve their DF intake by recommending consumption of food sources that are accessible and affordable.

Bread is the main food source of DF worldwide (7, 14, 16, 21) and is one of the oldest and most explored functional foods. (21) Bread is consumed either at home or in a restaurant, is commonly consumed by all cultures and ethnicities, and is a staple food for some individuals. Additionally, in terms of food costs, bread is considered one of the cheaper food products to purchase (20) and can be stored for long periods before consumption. (22) Further, bread is an ideal vehicle to incorporate ingredients such as cereal bran to increase DF content (22, 23) and ultimately improve DF intake in the adult population globally.

Dietary fibre intervention and the gut microbiome

Dietary intervention with plant glycans modulates bacterial species in the colon that interact to degrade this complex substrate. Members of the microbial community in the large intestine have distinct characteristics that allow them to specifically degrade plant glycans. (24)

A 2018 systematic review and meta-analysis of 58 intervention studies (of accepted and candidate prebiotics, general fibres) aiming to increase DF intake to modulate the gut microbiome composition in healthy adult participants showed that DF interventions (13 studies) did not affect the bacterial alpha-diversity of stool samples compared to samples from placebo-controlled/low-DF groups. (25) These findings align with other dietary interventions where diversity was unaffected but contrast with observational studies where alpha diversity positively correlated with DF intake or DF diversity.

The authors also showed that DF interventions resulted in a higher abundance of *Bifidobacterium* spp. (51 studies) and *Lactobacillus* spp. (24 studies), albeit considerable heterogeneity was noted. (25) Additionally, among a small number of studies eligible for meta-analysis, there were no differences in the abundance of bacterial taxa commonly measured, e.g., *Roseburia* spp., *Akkermansia muciniphila*, *Eubacterium hallii*, *Eubacterium rectale*, *Faecalibacterium prausnitzii* and *Ruminococcus bromii*. (25)

The authors also conducted subgroup analyses of fibre types (accepted prebiotic fibres, candidate prebiotics and general fibres) regarding the effects on the gut microbiota composition. Consuming accepted prebiotic fibres (fructans and galactose-oligosaccharides) and a broad range of prebiotic fibre types (candidate prebiotics such as polydextrose and resistant starches), but not general fibres (fibres that are neither accepted nor candidate prebiotics), resulted in a higher abundance of *Bifidobacterium* spp. (25) This analysis also showed that only accepted prebiotic fibre increased the abundances of *Lactobacillus* spp. (25) Nonetheless, it is noteworthy that the exclusion of many studies and their respective data due to the stringent eligibility criteria and the limited number of microbial taxa measured in the included studies are limitations of this analysis.

Rice bran intervention and the gut microbiome

Changing the quality of DF by using specific prebiotic fibres or intact cereal fibres to modulate the gut microbiota might be more feasible, as the effects of increased DF intake plateaued at 35 g per day. (26) Wheat fibre, or wheat bran, is the most studied cereal fibre concerning its impact on the gut microbiota. (26) Most studies with DF and specific prebiotic fibres focused on the modulation of species from the *Bifidobacterium* and *Lactobacillus* genera. However, intact cereal fibres which contain a diverse fibre structure would arguably lead to a more complex modulation of an adapted bacterial consortium (composite), with low abundance members mediating critical degradation steps to achieve an effective fibre breakdown.

Being one of the cheaper cereal by-products, (22) rice bran is gaining popularity due to its unique profile rich in nutrients and phytochemicals, including high DF content of 20-51%, which is double that of oat bran and exhibits apparent hypoallergenicity. (21, 27-30) Several studies have suggested considering the use of defatted rice bran (DRB) as a value-added food ingredient. (28, 31-33) Defatting increases the proportion of DF in rice bran by increasing its insoluble fibre content. (22) Only a few human studies have been undertaken on rice bran. (34-38) The focus of these studies has been on metabolic health, whole gut transit or modulation of the gut microbiota composition and function in healthy adults, (34, 37) participants with predominant-diarrhoea or mixed type irritable bowel syndrome, (36) colorectal cancer survivors or individuals with a high risk of colorectal cancer. (35, 38)

Among the few pilot studies focusing on the gut microbiota, either 30 g over two or four weeks (34, 35, 38) or 40 g over four weeks (37) of rice bran increased the abundance of taxa from several microbial genera when compared to baseline values; *Methanobrevibacter*, *Paraprevotella*, *Bifidobacterium*, *Ruminococcus*, *Bacteroides*, *Blautia*, Dialister, *Anaerostipes*, *Barnesiella*, and *Clostridium*. (34) In addition, an increase in taxa from the family *Veillonellaceae* was observed at 4-weeks post-intervention compared to baseline values. (37) The consumption of whole-grain brown rice flakes the abundance of *Blautia* spp. of stool samples from healthy adult volunteers. (39) A 24-week intervention with rice bran also increased the abundance of taxa from the genera *Bifidobacterium*, *Lactobacillus* and *Prevotella*_9 in the stool samples. (38)

Other measures of the microbial community based on the Firmicutes: Bacteroidetes ratio of human stool samples showed variable effects. Other studies did not provide data on these parameters. Heat stabilised rice bran intervention led to a lower Firmicutes: Bacteroidetes ratio over two weeks but not four weeks post-intervention (35) and tending to increase at 24 weeks. (38) when compared to baseline values. Consumption of whole-grain brown rice flakes (60g/d over four weeks) did not affect the Firmicutes: Bacteroidetes ratio in stool samples from healthy adult volunteers. (39)

Similarly, alpha-diversity of human stool samples showed variable responses to rice bran intervention. Studies with stabilised rice bran intervention increased the bacterial alpha-diversity of human stool samples at four weeks but not two weeks post-intervention (35) or did not change in alpha-diversity at 24 weeks post-intervention. (38) Other studies did not report the alpha-diversity. (34-37) In contrast, the consumption of whole-grain brown rice flakes (60 g/d over four weeks) increased bacterial diversity of stool samples from healthy adult volunteers. (39) As alpha-diversity is made up of two aspects, richness and evenness, a composite measure may be required to observe the relationship between diversity and health in clinical interventions. (40)

Shotgun metagenomic analyses of gut microbiomes revealed predicted differences in stool bacterial gene functional categories and showed that several microbial metabolic functions were enriched after two weeks but not four weeks of heat stabilised rice bran consumption. These enriched metabolic pathways included phenylpropanoid biosynthesis, other glycan degradation, starch and sucrose metabolism, streptomycin biosynthesis, and sphingolipid metabolism. (34) In addition, the intervention decreased the functional category of biosynthesis of unsaturated fatty acids. (34) Heat stabilised rice bran intervention (30 g over two or four weeks) also changed the concentrations of stool metabolites (butyrate, acetate, branched-chain fatty acids, amino acids and nucleosides, cholesterol and bile acids, phytochemicals and phenolics, lipids, and putative microbial metabolites). (34) Most of the studies with rice bran were not powered to compare the effect of rice bran intervention to the control or placebo intervention. (34-38) In addition, the rice bran interventions were administered in various forms (powder, rice-bran enriched meals and snacks, rice bran fraction vs whole grain), and the health status of the human volunteers was varied.

Arabinoxylan intervention and the gut microbiome

Like all cereal grains, rice bran also contains arabinoxylans (AX). (41) AX are non-starch polysaccharides found in plant cell walls. Their biochemical structures are distinct among cereals and within the cereal grain and impact their degradation and fermentation by the gut microbiota, with branched structures reportedly having a greater impact on the proliferation of microorganisms and increasing production of short chain fatty acids, at least *in vitro*. (42) Xylose, arabinose and ferulic acids are components of AXs, which have been associated with health-promoting effects. AXs are degraded along the large intestine, while other prebiotic fibres are degraded in the proximal part. (43) The structural diversity of AXs among cereals and whether the bran is pre-treated (which amplifies the naturally occurring structure diversity) are likely to contribute to variable outcomes in human intervention studies. (42)

Studies that supplemented wheat bran AX-oligosaccharide or wheat bran AX (0 to 10 g/day, bread, ready to eat cereals demonstrated an increased abundance of *Bifidobacterium* spp., (44-46) *Lactobacillus* spp. and increased butyrate concentrations (45) in stool samples of human volunteers. This highlights the degree of syntrophy or cross feeding that exists between microbes where acetate production is tied to increased butyrate production. (47) The bacterial alpha diversity of stool samples

was only increased with a dose of 15 g/d of AX from unspecified cereal source (43) Another study also demonstrated gut symptom improvement and immune modulatory effects (increased lymphocytes and NK cell activity) with 1 g modified AX rice bran (providing 2.5 g DF) in participants with predominant-diarrhoea or mixed type irritable bowel syndrome following intervention. (36) However, in this study, the immune modulation effects were unconvincing given that increased NK activity was not concomitant with increased neutrophil activity.

In light of the limited studies on rice bran and no studies on DRB, there is a need to investigate the effects of habitual consumption of bread fortified with DRB on microbial consortia composition and function relevant to glycan metabolism in healthy adults with low DF intake. As the recommended intake for DF differs between genders and female having potentially more gut symptoms with higher DF intake, this study will, therefore, assess these effects in healthy adults with low DF intake following the consumption of three (females)/ four (males) slices of DRB bread over 28 days.

1.2 Hypotheses

Several hypotheses will be tested in the main study. These are:

- That the consumption of three (females)/ four (males) slices of DRB fortified bread per day for 28 days compared to three (females)/four (males) slices of placebo white toast bread increases the relative abundance of a composite of selected key genera and species of gut microbiota known to be involved in plant glycan metabolism and/or known to be modulated by rice bran intervention. The composite microbiota will include genera from five phyla found in the gut microbiota of healthy human adults.
  - *Prevotella* and Barnesiella genera and *Bacteroides ovatus* and *Bacteroides xylanisolvens* from the Bacteriodetes phylum
  - *Roseburia*, *Anaerostipes*, *Blautia*, *Eubacterium*, *Ruminococcus*, *Faecalibacterium*, *Lactobacillus* genera from the Firmicutes (Bacillota) phylum
  - *Bifidobacterium* and *Eggerthella* genera from the Actinobacteria (Actinomycetota) phylum
  - *Akkermansia* genera from the Verrucomicrobiota phylum
  - M*ethanobrevibacter* genera from the Euryarcheaota phylum
- That the consumption of three (females)/ four (males) slices of DRB fortified bread per day for 28 days compared to three (females)/ four (males) slices of placebo white toast bread changes the profile of amino acids, bile acids and lipids in plasma and/or stool samples, and abundance of microbial genes encoding for xylan metabolism and relevant enzymatic activities in stool samples.
- That the consumption of three (females)/ four (males) slices of DRB fortified bread per day for 28 days improves bowel stool form, satisfaction, mental health, and wellbeing compared to three (females)/four (males) slices of placebo white toast bread.
- That the consumption of three (females)/ four (males) slices of DRB fortified bread per day for 28 days improves total DF intake compared to baseline in healthy adult volunteers with low DF intake.

Two hypotheses will be tested in the ATMO sub-study. These are:

- That the consumption of three (females)/ four (males) slices of DRB fortified bread per day for 28 days improves whole gut transit compared to baseline values.
- That the consumption of three (females)/ four (males) slices of DRB fortified bread per day for 28 days improves the gut segments' fermentation gas profiles as compared to baseline values.

2 STUDY AIMS AND OBJECTIVES

The primary aim is:

To determine the influence of three (females)/ four (males) slices of DRB fortified bread on the composition of selected genera and species of the lower gut microbiota known to be involved in plant glycan metabolism and/or known to be modulated by rice bran intervention using stool samples as a proxy and shotgun metagenomics sequencing.

Secondary aims include:

1. To investigate the influence of three (females)/four (males) slices of DRB fortified bread on
   a. Predictive function (gene abundances or frequencies) of the lower gut microbiota using stool samples as a proxy and shotgun metagenomics sequencing compared to the placebo white toast bread
   b. Stool and plasma metabolites/proteins using mass-spectrometry (MS) metabolomics and Gas Chromatography (GC) or Liquid Chromatography-Mass Spectrometry (LC-MS) methodologies compared to the placebo white toast bread
   c. Upper and lower gut comfort using Patient-Reported Outcome tools (PROs) compared to the placebo white toast bread
   d. Cardiovascular risk profile using blood pressure and lipid profile from blood samples compared to the placebo white toast bread
   e. Whole gut transit time using blue food dye compared to baseline values
   f. Gas profiles from gut segments generated by Atmo gas capsule compared to baseline values
2. To estimate DF intake during the consumption of three (females)/ four (males) slices of DRB fortified bread.

3 STUDY DESIGN

3.1 Study Overview

The study is a double blind, placebo-controlled, randomised, crossover study. The design and management of the clinical study will conform to the CONSORT guidelines. (48) Blinding, dietary compliance and data management will adhere to current international best practices. (49) This design accounts for the recognised variability in individuals and enables each participant to be their own control for their assigned interventions. (50, 51) Participants will be randomly selected (by using randomised permuted blocks, block size 4) to either begin the first study phase on the DRB intervention or the placebo (white toast bread) and will receive the opposite treatment after the washout phase.

The nominal study duration is 14 weeks: 14 days (two weeks) lead-in phase, 28 days (four weeks) intervention phase 1, 14 days (two weeks) washout phase, 28 days (four weeks) intervention phase 2, and 14 days (two weeks) follow-up phase. The 14-day washout period was selected based on studies that reported that the microbiota returns to baseline abundances within two weeks post-intervention. (52)

Prior to study commencement, a mutually agreed schedule will be discussed and set with each participant to ensure participants understand all expected clinic/visit times and the timeline of the study. However, if a participant cannot come to the clinic on the day a phase is ending, the participant's needs will be accommodated by allowing earlier visits to the clinic for up to three days before the end of the intervention phase. This step reduces the risk of participants exhausting intervention supplies before the visit and non-compliance or drop-out due to scheduling issues. Figure 1 shows the general and nominal study design.

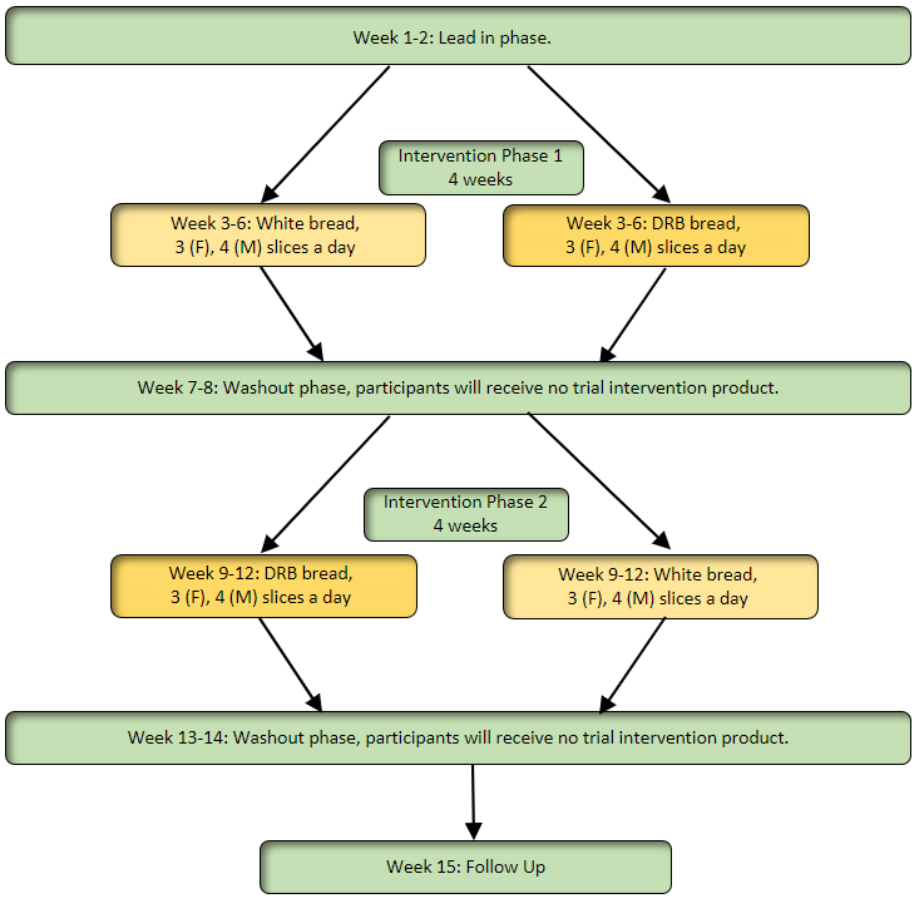

Figure 1: General and nominal study design

The intervention will consist of three (females)/ four (males) slices of DRB-fortified bread. The intervention will have an 18% replacement of the flour (cereal) weight used in a white toast bread and provide 9 g (females) and 12 g (males) of total DF. Of the 9 to 12 g of DF in three to four slices of DRB bread, 2.6 g (females), 3.4 g (males) will be fibre from wheat and 6.7 g (females), 8.9 g (males) will be from DRB.

The placebo will be a matched commercial white toast bread without the inclusion of DRB, providing 3 g to 4 g DF for females and males, respectively.

Table 2: Estimated nutrition information of both white toast and DRB fortified bread. Source: Preliminary data from Goodman Fielder Ltd (personal communication).

	Average Quantity per 100g of white toast bread		Average Quantity per 100g of DRB bread	
Energy	995.40	kJ	831.80	kJ
Protein	9.72	g	10.58	g
Fat, total	2.30	g	2.30	g
- saturated	0.52	g	0.48	g
Carbohydrate	43.18	g	38.60	g
- sugars	0.34	g	0.26	g
Dietary fibre*	2.80	g	8.20	g
• **Insoluble fibre**	2.42	g	7.50	g
• **Soluble fibre**	0.05	g	0.46	g
• **Resistant Starch**	0.01	g	0.29	g
Starch	50.39	g	54.80	g
Non-starch polysaccharides	0.00	g	0.00	g
Oligosaccharides	0.00	g	0.00	g
Inulin	0.01	g	0.01	g
Fructan	0.08	g	0.08	g
Beta glucan	0.06	g	0.34	g
Arabinoxylan	0.60	g	2.06	g
Phenolics	0.00	g	0.00	g
Sodium	394.50	mg	390.50	mg

*Dietary fibre (DF) is subtracted from the carbohydrate value and only includes insoluble fibre, soluble fibre and resistant starch.

There are differences in fibre types between the DRB fortified bread and placebo white toast bread. In terms of quantity and quality of fibre, as shown in Table 2, the DRB fortified bread has an estimated of thrice the DF and 3.4 times the AX content of a placebo white toast bread.

Participants will not be informed of the ingredients in the bread until study completion to maintain blinding. Analysts and researchers will be blinded to the order of treatment the participants receive and to which group the participants belong. The manufacturer of the bread will be responsible for labelling bread, A or B, to maintain blinding of research team members. To increase blinding, the manufacturer will add a food colouring to the placebo bread to make both breads visibly indistinguishable.

Specified members of the research team will be responsible for the randomisation, the handout of the interventions, and the management of the stock.

3.2 Study Plan

Healthy members of the public with low DF intake will be eligible for this study (see Section 4 Study Population for more details).

After initial contact and eligibility check, participants will be given the patient information sheet, including a consent form and sufficient time to consider participation in the study. They will also be given plenty of opportunities to ask questions and receive a full explanation of the study.

After written informed consent has been collected, the participant will receive a unique identifier to link the participant to data pertaining to them in a de-identified manner. The schedule for the participant will then be devised to ensure optimised timing for data and sample collection according to the overall study schedule.

Collected data will be stored and managed in a de-identified manner using REDCap (see section 9.3, Format of data) in a password protected customised database containing laboratory information, demographic information, and obtained questionnaire data. Diet data will be entered into FoodWorks10 for nutrient intake analyses. Atmo gas sensing capsule data will be transmitted in real-time to the phone app and the cloud for analysis. Analytical results will be uploaded to a secure, password-protected server.

Best practice will be the basis for collecting, processing, and storing all biological samples. Stool samples will be divided into six aliquots, three for the analysis of the microbiota and three for the analysis of other metabolites/proteins (untargeted metabolites, known metabolites/proteins, etc.). All samples will be stored at -80° Celsius. Blood plasma will be collected into multiple aliquots for metabolite/protein analyses (untargeted metabolites, known metabolites/proteins, etc.) before being frozen and stored according to standard operating procedures and methods.

For more details on the collection, processing, and analyses to be performed, please see Section 5: Measurements and sample methods.

3.3 Study Completion

The study will complete when all questionnaires and biological datasets will be collected from all enrolled participants after the follow-up phase, expected at the end of April 2023. The study aims to enrol a minimum of 60 participants.

3.4 Study Outcomes

3.4.1 Primary Outcome

Differences in relative abundance of a composite of selected key genera and species of the gut microbiota known to be involved in plant glycan metabolism and/or known to be modulated by DRB after intervention with three (females)/ four (males) slices of DRB fortified bread as measured from stool samples compared to three (females)/ four (males) slices of placebo white toast bread. The composite microbiota will include genera from five phyla found in the gut microbiota of healthy human adults.

- *Prevotella* and *Barnesiella* genera and *Bacteroides ovatus* and *Bacteroides xylanisolvens* from the Bacteriodetes phylum

- *Roseburia*, *Anaerostipes*, *Blautia*, *Eubacterium*, *Ruminococcus*, *Faecalibacterium*, *Lactobacillus* genera from the Firmicutes (Bacillota) phylum
- *Bifidobacterium* and *Eggerthella* genera from the Actinobacteria (Actinomycetota) phylum
- *Akkermansia* genera from the Verrucomicrobiota phylum
- M*ethanobrevibacter* genera from the Euryarcheaota phylum.

3.4.2 Secondary Outcomes

3.4.2.1 *Clinical outcomes*

Changes in clinical outcomes after intervention with three (females)/ four (males) slices of DRB-fortified bread compared to three (females)/ four (males) slices of placebo white toast bread:

- Gastrointestinal Symptom Rating Scale (GSRS) scores as an indication of digestive comfort
- Validated PROs detailing the subjective assessment of digestive health parameters, satisfaction, mood, and general well-being
- Stool form based on the Bristol Stool Chart
- Cardiovascular risk profile
- Total DF intake as measured via food diaries.

3.4.2.2 *Biological outcomes*

Changes in predictive function (gene abundances or frequencies) of the gut microbiome after DRB fortified bread as measured from stool samples compared to placebo white toast bread.

Changes in stool metabolome and known metabolites/proteins, plasma metabolome and known metabolites/proteins after DRB fortified bread compared to placebo white toast bread.

3.4.2.3 *Physiome outcomes*

Changes in whole gut transit time and gut segments' gas fermentation patterns as measured by blue food dye and Atmo gas sensing capsule, respectively compared to placebo white toast bread.

3.5 Study Organisation and Collaboration

Goodman Fielder Ltd, New Zealand, will provide DRB fortified and placebo white toast breads.

The Principal Investigators appointed to the study are Professor Nicole Roy from the Department of Human Nutrition, University of Otago, Dunedin, and Professor Richard Gearry from Otago Medical School, University of Otago, Christchurch. The clinical study will be conducted by the University of Otago, Department of Medicine, Gastrointestinal Unit for Translational Studies in Christchurch.

Standard laboratory tests will be conducted by Canterbury District Health Laboratories. Laboratories external to the clinical facility will complete the analyses related to the secondary outcomes, which include AgResearch, Plant & Food Research, and Riddet Institute.

4 STUDY POPULATION

4.1 Participants

The target population of this study is healthy adults with a low DF intake (<18 g/d (female), <22 g/d (male) (minimum of 60 participants).

Screening questionnaires for participant selection can be found in Appendix B: Screening Questionnaire and will enable the research team to determine the suitability of the participants during the screening process. This questionnaire is a validated habitual dietary fibre intake short food frequency questionnaire that was developed in NZ. (53) The questionnaire explores the frequency of consumption over the past year, and it can quickly and accurately classify individuals (low, moderate, high). The questionnaire uses cut off points of:

- low (cut off based on median dietary fibre intake in NZ: <18 g/d (female), <22 g/d (male))
- moderate (between low and high intakes: 18-24.9 g/d (female); 22-29.9 g/d (male))
- high (cut off based on the DF Adequate Intake in NZ: >25 g/d (female), >30 g/d (male))

Study participants will be recruited through a variety of methods such as local newspapers, posters, local newsletter advertisements, Facebook advertisements, Gastrointestinal Unit for Translational Studies online presence, local radio advertisement, translated Te Reo Māori advertisement, and word of mouth advertisements through Māori health nurses and GPs to attract Māori participants, etc.

All participants must fall within:

a. Adult (18-65 years). Female participants will need to declare the stage of the menstrual cycle during the different study phases, if applicable.
b. Good general health
c. A body mass index (BMI) between 18 and 35.

4.2 Inclusion Criteria

- Low baseline intake of DF (based on validated habitual dietary fibre intake short food frequency questionnaire)
  i. Males: under 22 g/day
  ii. Females: under 18 g/ day
- No history of bowel disease
- Non-smokers
- No fibre supplement consumption during the month prior to screening
- Regular bread consumption

4.3 Exclusion Criteria

- Inability to give informed consent
- Indication of inability to comply with the study procedures
- Antibiotic use within the last month
- Allergy or intolerance to wheat, rice or gluten
- Pregnant, breastfeeding or planning a pregnancy in the three months post selection/during the study period
- Alarm features associated with bowel habits such as recent changes in bowel habits (onset < three months), rectal bleeding, sudden weight loss, occult blood in stool, anaemia, anal fissures, bleeding haemorrhoids, and family history of gut cancer at an early age

- Known significant gut disorders and diseases: chronic constipation, diarrhoea, irritable bowel syndrome, inflammatory bowel disease, diverticulitis, coeliac disease, or previous bowel resection
- Chronic diseases such as cardiovascular disease, cancer, renal failure, previous upper or lower gut surgery other than cholecystectomy or appendectomy, neurological conditions such as multiple sclerosis, spinal cord injury, or stroke
- Known systemic conditions (heart disease, kidney disease, diabetes, metabolic syndrome, psychological disorder) that could influence the gut directly or through medication use such as diabetes, opiate, or non-steroidal anti-inflammatory drug use
- Fasting blood glucose ≥ 6.0 mmol/L
- Laxative, pre- and probiotic supplement use, and inability or unwillingness to stop using for the seven days before sample collections.

A

4.4 Number of Subjects

Using the GutFeelingKB cohort (54) the percentage abundance of the composite microbiome (incorporating 15 OTUs as outlined below) is estimated to be 28.3% with a standard deviation of 14%. Recruiting 60 participants into this crossover study and allowing for a dropout rate of approximately 15%, the study has >80% power to detect an absolute increase in the abundance of approximately 6%, as statistically significant (2-tailed α=0.05). An increase of 6% compared to the baseline level of 28% equates to a relative increase of approximately 22%.

The consumption of three (females)/ four (males) slices of DRB fortified bread per day for 28 days affects the individual and combined groups of the microbial taxa making up the composite.

The synthetic composite microbiota will include genera from five phyla found in the gut microbiota of healthy human adults.

- *Prevotella* and *Barnesiella* genera and *Bacteroides ovatus* and *Bacteroides xylanisolvens* from the Bacteriodetes phylum
- *Roseburia*, *Anaerostipes*, *Blautia*, *Eubacterium*, *Ruminococcus*, *Faecalibacterium*, *Lactobacill us* genera from the Firmicutes (Bacillota) phylum
- *Bifidobacterium* and *Eggerthella* genera from the Actinobacteria (Actinomycetota) phylum
- *Akkermansia* genera from the Verrucomicrobiota phylum
- *Methanobrevibacter* genera from the Euryarcheaota phylum

4.5 Participation and Withdrawal from Study

Participation in this study is voluntary, and participants may choose to withdraw at any time without explanation, as stated on the participant information sheet.

In case of the following occurrences, participants will be withdrawn:

- The participant requests withdrawal from the study. Participants can decide to end their involvement in the study for any reason and at any time during the study.
- The investigator can decide to withdraw a participant from the study if they consider it necessary. Examples include non-respect of at least one of the selection criteria after inclusion, non-compliance with the study protocol, gastroenteritis, or antibiotic use.
- The participant reporting allergic reactions or adverse effects from either intervention.

4.6 Compensation for Participation

The participants will receive compensation for taking part in this study. All individuals screened will receive a $20 MTA voucher. Participants entering the study will receive an additional $64 per visit to compensate for travel and time at the completion of the study in the form of New World Vouchers. This compensation will be a total value of $320 per participant. In addition, participants selected for the physiome sub-study will be given additional compensation of $100 for additional time and inconvenience.

In the unlikely event of a physical injury as a result of the intervention, compensation is available from the University of Otago in line with industry guidelines. The source of compensation is contingent on the type of injury and cause.

4.7 Termination Criteria for the Whole Study

In the case of serious safety concerns, the Principal Investigators can terminate or interrupt the study. If new information on the risk-to-benefit ratio of the intervention (including treatment and/or investigational processes) used in the study is obtained in the meantime, the Principal Investigators reserve the right to interrupt or terminate the project. Additionally, premature termination of the study is possible if the Principal Investigators notice that participant recruitment is insufficient and cannot be accelerated by appropriate measures.

5 MEASUREMENT AND SAMPLE METHODS

5.1 Screening Measurements

Potential participants will complete the following screening measurements to determine their eligibility for the study:

- Anthropometry and general health questions (height, weight, BMI, waist circumference).
- Blood pressure measurement
- Fasting blood sample
    - Fasting blood glucose
    - Lipid profile
    - C-reactive protein
    - Blood count
    - Liver function/ enzymes/ general health markers (the complete list of tests can be found in Appendix N: Canterbury District Health Laboratory Requirements)

5.2 Participant Measurements

Upon enrolment, participants will be invited to clinic visits at the beginning of the study period and the end of each intervention phase. This timeline is illustrated in Figure 2. The 14-week timeframe is nominal, and if a participant cannot come in at the end of each phase, an earlier or later date (+/-3 days) will be chosen to accommodate the participant.

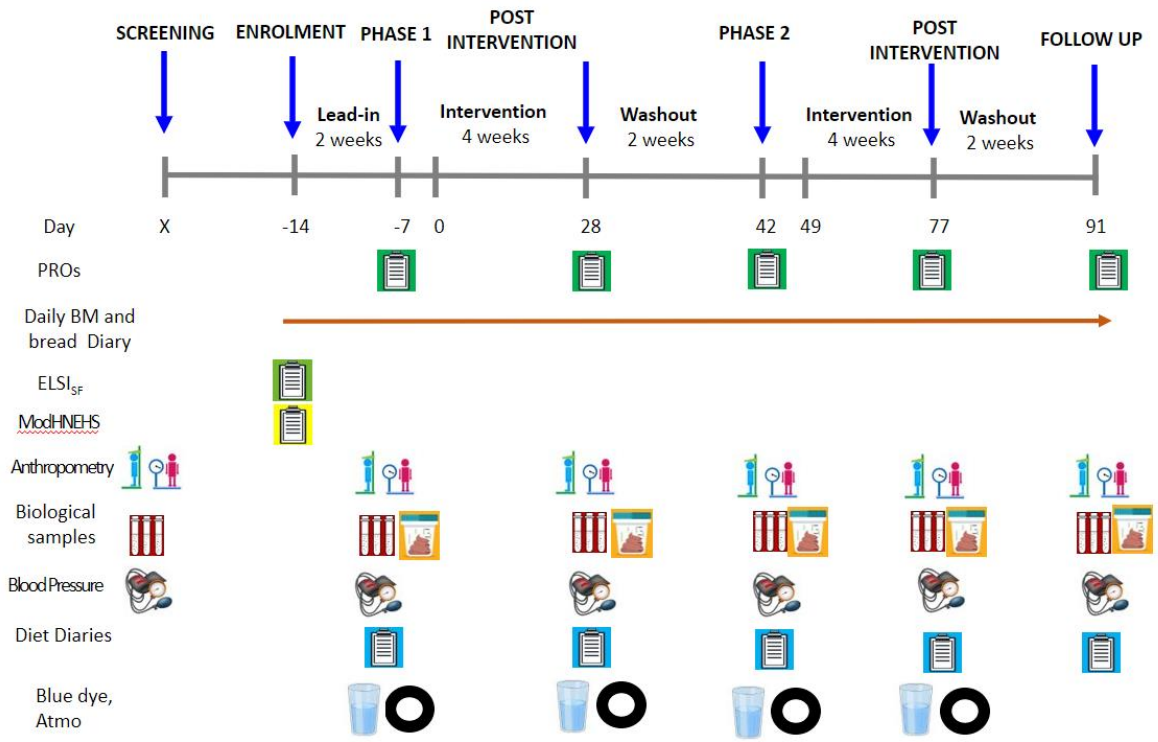

Figure 2: Study Schedule Flow Chart

At each visit, research staff will collect PROs, blood, and stool samples, and samples will be processed and stored. Additionally, members of the research team will hand out the DRB fortified bread or placebo white toast bread as allocated.

5.3 Outcome Measurement

5.3.1 Overview

Table 3: Overview of all study outcome measurements.

PRIMARY OUTCOME	• The changes in the relative abundance of selected key genera and species of the gut microbiota known to be involved in plant glycan metabolism and/or known to be modulated by rice bran intervention
SECONDARY OUTCOMES	
Clinical measurements	• Stool form • Cardiovascular risk • Anthropometry
PROs	• Digestive comfort • Food intake behaviour • Quality of life

	• Satisfaction • Psychological assessment of mood
Biology measurements	• Microbiota predictive function (gene abundances or frequencies) (*Stool*) • Metabolome (*Stool, Plasma)* • Known metabolites/proteins *(Stool, Plasma)*
Physiome measurements	• Whole gut transit (Blue dye and ATMO gas sensing capsule) • Gut segments' gas contents (ATMO gas sensing capsule)

5.3.2 Primary Measurement

Prior to the study visits (**days -7, 28, 42, 77, 91)**, participants will be asked to collect a stool sample using provided stool collection kits at home. They will be cooled with a provided ice pack and brought with them to the visit.

For microbiota-related analysis, 6 x 1 g of a stool sample will be collected and kept chilled until aliquoted in the laboratory. All aliquots will be snap-frozen in liquid nitrogen before storage at -80° C until analysis.

5.3.3 Secondary Measurements

5.3.3.1 *Clinical Measurements*

A. Anthropometry Measurements

- Height, weight, BMI, and waist circumference will be measured at each visit, i.e., screening, each phase at baseline, after the intervention, and follow up according to the procedures established by the Ministry of Health, New Zealand. (55)

B. Blood Pressure Measurements

- Blood pressure will be measured at each visit, i.e., during screening, each phase at baseline, after the intervention, and follow up, according to the recommendations established by the Australian Expert Consensus (56)

5.3.3.2 *PRO Measurements*

C. A range of additional measures will be made using questionnaires, as mentioned in Section 3.4 Study outcomes.

1. Modified Hunter New England Health Survey (ModHNES), collected at enrolment (Appendix E: Modified Hunter New England Health Survey (ModHNES) and SF-12v2® Health Survey)

    - Includes the validated Short Form 12v2® health survey (SF-12v2®), in addition to selected question domains from the new South Wales Population Health Survey for diabetes, smoking, alcohol consumption, and physical activity.
    - Assesses general physical and mental health in the last four weeks, specific health questions like diabetes, lifestyle questions, mental health during the last seven days, and demographic data.

- SF-12v2® assesses eight health domains (physical functioning, role-physical, bodily pain, general health, vitality, social functioning, role-emotional, and mental health).
- SF-12v2® data is used to get an overall view of each participant, to raise any issues of importance that may confound data. Only the SF-12 will be scored.

2. Economic Living Standard Index short form ($ELSI_{SF}$), developed and validated by the New Zealand Ministry of Social Development, collected at enrolment (Appendix F: Economic Living Standards Index- Short Form ($ELSI_{SF}$))

    - Assesses standard of living and socioeconomic class.
    - Provides data on the spread of symptoms and possible underlying factors.

3. The daily BM diary, accessible via smart phone app, will be collected every day after enrolment (Appendix C: Daily Bowel Habit Diary)

    - Frequency of BMs. Includes questions on spontaneity and completeness of bowel movement
    - Ease of defecation/level of straining
    - Stool form (Bristol Stool Scale)
    - Menstruation (if applicable)
    - Presence of blue dye (if applicable)
    - Will be filled out daily to provide a comprehensive record of bowel habits

4. The daily bread diary, accessible via smart phone app, will be collected every day after enrolment (Appendix D: Daily Bread Diary)

    - To check compliance with the consumption of interventions and to assess interest of bread consumption.
    - Used to determine if the DRB fortified and placebo white toast bread are toasted
    - Minutes of toasting (if applicable)
    - Additional bread consumed (if applicable, during washout)
    - Type, brand, and amount of additional bread consumed (if applicable)

5. GSRS, collected one day prior to each clinic visit at baseline, after the intervention, and follow up (Appendix G: Gastrointestinal Symptom Rating Scale (GSRS))

    - To assess GI comfort before and during interventions
    - The GSRS is a validated instrument with a 1-week recall that assesses symptom severity using a 7-grade Likert scale, ranging from 1 (“no discomfort at all”) to 7 (“very severe discomfort”).
    - The complete instrument consists of 15 primary items that are clustered into five domains: diarrhoea, constipation, reflux, abdominal pain, and indigestion.

6. Three-day food diary (1 weekend and 2 non-consecutive weekdays), started one week prior to baseline and after each intervention and follow up visit, will be collected at the clinic (Appendix H: Three-Day Food Diary)

    - To estimate usual food intake before and during interventions

- Will be used to link diet to biological samples

7. Patient-Reported Outcome Measurement Information System (PROMIS): Anxiety, Depression, collected one day prior to each clinic visit at baseline, after the intervention, and follow up (Appendix I: Patient-Reported Outcomes Measurement Information System (PROMIS) Survey)
    - PROMIS is a validated system with multiple domains, where specific domains can be chosen to be integrated into diverse data collection tools
    - Evaluates anxiety, and depression in the last seven days in detail
    - Mental symptoms will be rated by severity, from “not at all” to “very much” and from “never” to “always.”

8. World Health Organisation - Five question Well-Being Index (WHO-5), collected one day prior to each clinic visit at baseline, after the intervention, and follow up (Appendix J: WHO-5 Wellbeing Index (WHO-5))
    - A short self-reported rating scale of current wellbeing.
    - Consists of five statements in relation to the past two weeks, adjusted to one week (All of the time = 5; Most of the time = 4; More than half of the time = 3; Less than half of the time = 2; Some of the time = 1; At no time = 0).

9. The Warwick-Edinburgh Mental Wellbeing Scales (WEMWBS) will be collected one day prior to each clinic visit at baseline, after the intervention, and follow up (Appendix K: Warwick Edinburgh Mental Wellbeing Scale (WEMWBS))
    - To monitor mental health and wellbeing in relation to the past two weeks, adjusted to one week.
    - The 14-item scale WEMWBS has 5 response categories, summed to provide a single score. The items are all worded positively and cover both feeling and functioning aspects of mental wellbeing.
    - Covers key aspects of psychological functioning: optimism, autonomy, agency, curiosity, clarity of thought and positive relationships; and positive affect (feelings): confidence, feeling relaxed, cheerful, having the energy to spare (ranging from none of the time; rarely; some of the time; often; all of the time).

10. Multidimensional Fatigue Inventory Short Form (MFI-SF), collected one day prior to each clinic visit at baseline, after the intervention, and follow up (Appendix L: Multidimensional Fatigue Symptom Inventory Short Form (MFI-SF))
    - A 20-item self-report instrument designed to measure fatigue.
    - Covers the following dimensions: General Fatigue, Physical Fatigue, Mental Fatigue, Reduced Motivation and Reduced Activity.
    - A 7-point scale indicates to what extent each statement applies to the participant, ranging from “yes, that is true” to “no, that is not true”.

11. The Subjective Vitality Scale (SVS) (57, 58) will be collected one day prior to each clinic visit at baseline, after the intervention, and follow up (Appendix M: Subjective Vitality Scale (SVS))
    - To assess the state of feeling alive and alert, i.e., having energy available to oneself.

- Consists of 6 statements in relation to the current feeling on a scale of 1 to 7; 1= not at all true, 4= somewhat true, 7= very true

5.3.3.3 *Biological measurements*

Blood and stool samples will be collected to evaluate the biological response to habitual consumption of three/ four slices of DRB bread per day for 28 days. The collection of biological samples will be conducted according to established procedures. A researcher trained in phlebotomy will collect peripheral blood at each clinic visit, i.e., screening, baseline, post-intervention of each phase, and follow-up. Participants will collect a stool sample the day prior to baseline, post-intervention of each phase and follow-up visits.

5.3.3.3.1 Blood sample collection and processing

At **all-time points** (**screening, day -7, 28, 42, 77, 91)**, 1 x 6 mL blood will be taken into a lithium-heparin (green) vacutainer tube to measure fasting blood glucose, lipid panel, C reactive protein and liver function/enzymes / general health markers, and 1 x 4 mL blood into EDTA (purple) tube for a complete blood count. The collected blood sample will be kept at room temperature and delivered to the laboratory for analysis no longer than one hour after collection, according to instructions of the Canterbury District Health Laboratories (Appendix N: Canterbury District Health Laboratory Requirements).

At **time points (day -7, 28, 42, 77, 91)**, an additional of 6 mL blood in total will be collected into 1 x 4 mL lithium-heparin (green) vacutainer tubes. The 6 mL vacutainer will be kept on ice and processed within one hour.

The tubes will be centrifuged at 4°C for 5 minutes at 2000 x g, with high acceleration and slowest deceleration to separate plasma from cells.

For plasma metabolome and known metabolites/proteins, 4x 500 µL lithium-heparin plasma will be aliquoted and stored at - 80° C until analysis.

5.3.3.3.2 Stool sample collection and processing

See *Section 5.3.2.*

5.3.3.4 *Physiome measurements*

5.3.3.4.1 Atmo gas-sensing capsule

A subset of participants will complete an assessment by Atmo gas-sensing capsule. The Atmo gas-sensing capsule will be ingested at the start of each phase of baseline and end of post-intervention. The novel, indigestible electronic Atmo gas-sensing capsule will be used to accurately profile gases within the bowel (oxygen, hydrogen, carbon dioxide and methane) and determine general and localised gut transit time. During the passage, the data is collected in real-time on a smartphone app.

5.3.3.4.2 Blue food dye

Royal Blue Liqua-gel® (Chefmaster, USA) food colouring (12 drops/1.5g) will be ingested in water at the start of each phase of baseline and end of post-intervention, and intake time and date will be recorded. The dye is not fermented or absorbed, which allows for analysis of total bowel transit time

upon passing by visual confirmation. (59, 60) Visual confirmation will be recorded by the participant on the daily BM diary app.

6 STUDY VISITS

6.1 Screening Visit

Following first contact by phone or email by a member of the public answering to an advertisement, a participant information sheet and consent form will be sent to the respondent to read. If the respondent agrees, an online screening questionnaire will be sent to ensure they are eligible for the study (See Appendix B: Screening Questionnaire and Appendix A: Participant Information Sheet for details). If the respondent is eligible, an appointment will be made for a screening visit, and they will be advised to fast for 9 hours before the visit.

The screening visit will follow the HUI method to establish relationships with participants (61). At the screening visit, potential participants will have the time to ask questions, and the researcher will give a further explanation of the study. Participants will also be encouraged to bring their whānau or other support people with them. If the participant is still keen and willing, written informed consent will be obtained. A total of 10 mL of blood will be collected to determine blood glucose, cholesterol, lipid levels, and general health. Anthropometric data and blood pressure will also be taken. The participant will then receive a unique identifier for enrolment.

An electronic case report form record (eCRF) will be generated for all participants who have signed the consent form for the study, regardless of eligibility and/or continuing participation.

6.2 Enrolment

Once blood and screening survey results confirm eligibility, the participants will be informed of the enrolment and receive their enrolment questionnaires by email. The research team members will advise on and set a schedule for all the individualised study visits. Upon agreeing on the schedule, the participant will receive a personalised study calendar with all relevant information in regards to the schedule, as well as personalised access to the daily BM and bread diary app. Participants will be asked to complete a daily diary on BMs and bread consumption from enrolment until the completion of the study. (Appendix C: Daily Bowel Habit Diary, Appendix D: Daily Bread Diary).

Participants will be invited to the clinic at the beginning (baseline) and end of each intervention phase, and the end of the follow-up period. The week before each baseline and end of intervention visit, participants will be educated on how to complete a three-day food diary by a dietitian.

6.3 Baseline Visits/ days -7 and 42

The participant will be invited to the first baseline visit within two weeks of enrolment (day -7). They will receive a stool sample collection kit and a food diary.

The participants will be required to collect a stool sample into the provided container of their kit 24 hours prior to each visit, and the time of collection will be recorded in the BM app. They will be required to keep the stool sample refrigerated and transport the sample on ice to the study centre, or, if unable to produce a sample on time, collect and bring it as soon as possible.

On the evening prior to the baseline visit, participants will receive the necessary PRO by email to complete. Additionally, participants will be asked to fast for at least nine hours overnight before they come for their baseline visits.

At the visit, all participants will have 10-15 mL of blood (plasma and biochemical blood panel) drawn for all biological measures. Anthropometric and blood pressure measurements will be taken of all participants. They will also be asked to drink 200 mL of water containing blue food dye. The timing of the blue dye consumption will be recorded into REDCap.

A subset of participants will complete a specific informed consent form before the transit assessment by Atmo gas-sensing capsule. These participants will consume a standard cereal bar, followed by the Atmo gas-sensing capsule, and fitted with a transponder that the individual will wear until the exit of the capsule or for up to 5 days. They will be asked to enter additional daily BM and food diary information into the Atmo transponder.

A member of the research team will then provide the participants with their first set of respective interventions, or if they prefer, the complete supply of either DRB bread or white toast bread. The research team member will then advise the participant on how and when to start taking the interventions and provide the participant with another set of food diaries and a sample collection kit for the post-intervention visit. All samples will be taken immediately to the laboratory for processing and storage, as detailed in Section 5.3.3.3.

6.4 Pre-intervention / days 0 and 49

Participants will be asked to keep filling out the daily BM and bread diaries. The participant will inform the research team once they confirm the presence of blue dye or capsules (if applicable) in their stool. Participants will need to return to the clinic to drop off the transponder (if applicable). The data will be downloaded from the transponder and analysed using the manufacturers' software to determine whole gut transit, gastric, small intestinal and colonic gas concentrations.

6.5 Post-intervention Visits / days 28 and 77

Participants will be asked to keep taking their daily intervention and filling out the daily diary until the day of their visit.

The procedures of the day prior to and on the post-intervention visits are the same as outlined in 6.3.

6.6 Wash-out Phase (No visit)/ days 29 – 41 and days 78 to 90

Participants will be advised not to alter their diet during the washout and to keep filling out the daily diary during the washout phase. Participants will be asked to return to their usual brand of bread and to maintain a daily bread intake diary for the 2 weeks of washout period.

6.7 Follow Up Visit / day 91

Participants will be asked to keep filling out the daily diary until the day of their last visit. The participants will be required to collect a stool sample into the provided container of their kit prior to their last visit. They will be required to keep the stool sample refrigerated and transport the samples on ice to the study centre, or, if unable to produce a sample on time, collect and bring them as soon as possible.

On the evening prior to the follow-up visit, participants will receive their last PRO by email to complete. Additionally, participants will be asked to fast for at least nine hours overnight before they come for their visit.

At the visit, participants will have a 10-15 ml of blood (plasma and biochemical blood panel) drawn for all biological measures, including blood glucose, cholesterol, lipid profile, and general health measures. Anthropometric and blood pressure measurements will be taken. After thanking the participants for their involvement throughout the study, all participants will be given their compensation and invited to share their thoughts on the intervention given (their preferred bread, i.e. bread A or B; bread they think contains higher DF content). All samples will be taken immediately to the laboratory for processing and storage.

7 SAMPLE ANALYSES

7.1 Analyses of Blood Samples

7.1.1 Lipid Profile

Blood samples will be collected to analyse the complete lipid profile, including total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides. These analyses will be performed using standardised methods by Canterbury Health Laboratories (CHL) (Christchurch, NZ).

7.1.2 Glucose Profile

Fasting blood glucose will be measured and analysed using standardised methods by CHL (Christchurch, NZ).

7.1.3 Known Metabolites and Proteins

Modulation of the microbial balance in the large intestine through dietary intervention, particularly through the use of probiotics and fibre, can affect the production of organic acids such as short-chain fatty acids (SCFA). Organic acids are the end-products of microbial fermentation. Quantitative analysis of organic acids in plasma samples can monitor the efficacy of food interventions on some SCFA for gut health when stool samples cannot be collected. One aliquot of 200 µL of heparin plasma will be collected and sent to Plant and Food Research for analysis via LCMS. 14 linear and branched SCFAs (C1 SCFA through to C7 SCFA) will be derivatised with a probe using MS-probe and stable isotope techniques and measured using targeted high-resolution LCMS on a UHPLC-QQQ system. Labelled internal standards for each targeted SCFA will be used to ensure accurate quantitation.

Other proteins like digestive hormones and others will be measured using established methods.

7.1.4 Metabolome

Untargeted metabolite profiling (metabolomics) of plasma samples will be performed using high-resolution LC-MS on a Shimadzu Q-Tof 9030 equipped with electrospray ionisation. This analysis will provide more comprehensive profiling of lipids in plasma to support the traditional lipid profiled measured (see section 7.1.1). It will also allow to correlate the plasma profile of polar, semi-polar and

non-polar metabolites to those found in stool samples (see Section 7.2.2). Briefly, 10 µL plasma will be extracted for lipidomics using monophasic solvent extraction and chromatographed on a reverse-phase LC (RP-LC) column. (62) Polar and semi-polar metabolites will be extracted from 50 µL plasma. Polar metabolites will be resolved using hydrophilic interaction liquid chromatography (HILIC) (63), while semi-polar metabolites will be resolved using RP chromatography. (64)

7.1.5 Polyphenols

Phenolic acids are the most common phenolic compounds in cereal grains. The outer layer of the rice grain, rice bran, has a unique profile, as it is rich in different nutrients and phytochemicals. (31) The phytochemical ferulic acid, the dominant phenolic compounds in common cereals, is mainly bound to AX of the plant cell walls. (65) Up to a third of phenolic acids is thought to be absorbed in the small intestine and the remainder fraction is metabolised in the colon by the gut microbiota. Consumption of wheat bran increased concentration of ferulic acid in plasma of healthy adults (66, 67) and derived metabolites. (67) The ingestion of rice bran extract-fortified oatmeal porridge also increased ferulic acid concentrations in plasma of healthy individuals. (68) Plasma samples will be analysed for phenolic acids and their metabolites using HPLC according to the procedures described previously. (69) The identity of phenolic acids will be also confirmed using UPLC-MS based on the congruence of retention times and positively charged molecular ions using ESI positive mode.

7.2 Analyses of Stool Samples

7.2.1 Microbial Composition and Gene Abundances

Common approaches to investigating the human gut microbiota include high-throughput sequencing with subsequent correlative analyses. However, analysis of microbiota composition via 16S rRNA gene sequencing provides limited insights into microbial function. Shotgun metagenomics addressed some of the limitations of 16S sequencing by providing detailed taxonomic information and functional potential (gene abundances or frequencies).

Taxonomic composition and metagenome gene frequencies of the stool microbiome will be assessed by shotgun metagenomics using the Illumina HiSeq platform. Extracted DNA will be prepared using Illumina Nextera library preparation kits, while the sequencing will be performed using the NextSeq 500/550 PE 150. Sequence reads will be quality trimmed and filtered to remove low-quality reads and human reads. Sequences will be aligned against the NCBI NR database using DIAMOND and functional classifications assigned using the KEGG database and MEGAN. (70, 71) Taxonomic classifications will be assigned by extracting 16S rRNA reads from the metagenomic data using metaxa2 and aligning against the Silva 128 database. (72) Deep shotgun sequencing reads will be analysed using MetaPhlAn2 (73) for taxonomic profiling and HUMAnN2 (74) for profiling of metabolic pathways.

7.2.2 Metabolome

As mentioned in the Background section, heat stabilised rice bran intervention changed the concentrations of several stool metabolites (butyrate, acetate, branched-chain fatty acids, amino acids and nucleosides, cholesterol and bile acids, phytochemicals and phenolics, lipids, and putative microbial metabolites). (33) The relative abundance of these metabolites can be assessed in the polar, semi-polar and non-polar fractions of stool samples using untargeted metabolite profiling (metabolomics) of stool samples. This analysis will be carried out using high-resolution LC-MS on a

Shimadzu Q-Tof 9030 equipped with electrospray ionisation. 50 mg of lyophilised faecal samples will be extracted using a biphasic solvent mixture (methanol/water/methyl tert-butyl ether). (75) An aliquot of the aqueous layer will be taken and analysed using HILIC chromatography, (63) and semi-polar metabolites will be resolved using RP chromatography. (64) The organic phase will be analysed using the above lipidomic methodology. (62) All data will be collected using Data Independent Acquisition (DIA) mode to facilitate MS/MS spectral annotation and identification, and the resulting data files will be processed using MS-DIAL and the associated publicly available databases. (76)

7.2.3 Known Metabolites/Proteins

DF with fermentable characteristics, such as those in rice bran, are a substrate for the microbiota resident in the large intestine, stimulating the growth of specific species and increasing the production of a range of metabolites, including organic acids, in particular, the SCFA butyric acid. One aliquot of 1 g of stool samples will be collected and sent to Plant & Food Research for analysis to measure organic acids using an LCMS method. Fourteen linear and branched organic acids (C1 through to C7) will be derivatised with a probe using MS-probe and stable isotope techniques and measured using targeted LCMS on a UHPLC-QQQ system. Labelled internal standards for each targeted organic acid will be used to ensure accurate quantification.

Intervention with rice bran has changed the bile acid profile in stool samples (30). One hundred mg of freeze-dried stool samples will be spiked with 100 ng of d4-CA and extracted with 700 µL ice-cold 50% MeOH in Eppendorf tubes pre-filled with 4 mm ceramic beads to quantify the concentration of 23 bile acids and bile acid metabolites in stool samples using a SCIEX LCMS/MS QTRAP 6500+ system coupled to an ExionLC (SCIEX, Victoria, Australia). (77) Mass spectral detection will be performed in negative electrospray ionisation mode using multiple reaction monitoring for 23 bile acid compounds and the internal standard using electrospray ionisation.

As mentioned above, up to a third of phenolic acids is thought to be absorbed in the small intestine and the remainder fraction is metabolised in the colon by the gut microbiota. Phytochemicals (including ferulic acid and its derivatives) will be quantified in stool samples according to an established used in Plant and Food Research and modified for stool samples.

As mentioned earlier, AX is a significant component of cereal hemicelluloses linked to its prebiotic effects. The mechanisms of AX degradation involved various proteins including enzymes. (78). Briefly, the stool samples will be assessed for their microbial enzyme activities relevant to AX using a microplate reader according to published methods. (79)

7.3 Statistical Analysis

All statistical analyses will be completed by blinded researchers under the guidance of an independent biostatistician. Statistical analyses will describe the relationship between the consumption of DRB fortified bread and the gut microbiota parameters measured (see Sections 7.1 and 7.2).

This study is conducted as a superiority trial. The Guidelines of the CPMP require the use of Intention to Treat (ITT) analysis. (1) ITT means that every participant that is enrolled is included in the analysis, despite dropout or non-compliance.

Categorical variables will be applied to Chi-squared tests (or Fisher's exact tests for small samples). Continuous variables will be applied to (parametric) t-tests and (non-parametric) Mann-Whitney/Kruskal –Wallis tests for symmetrically and asymmetrically distributed data, respectively.

8 RISK ASSESSMENT

8.1 Possible Effects of Bread

Gluten in the bread can cause allergic reactions or intolerance in 1 to 2% of the world population. (80) Participants will be informed of this risk and the possible symptoms. Participants will be regular bread consumers, and individuals with known coeliac disease, gluten allergy or intolerance, or hypersensitivity to wheat or rice will be excluded from the study.

The excessive consumption of white toast bread may be associated with the risk of gaining weight and increase of blood glucose level. (81) In this study, participants will be asked to replace their regularly consumed bread with the study bread without increasing their daily consumption. The risk will be explained to the participants and blood glucose level will be checked for all the clinic visits.

The participants may experience abdominal discomfort, distension, and wind by consuming bread with high DF content. As the recommended intake of DF differs between males and females, we will be providing three slices and four slices of bread to females and males, respectively, to reduce the risk of gut issues during the 28 days intervention period. Participants will be informed that increasing DF intake stimulates the growth in gut microbe numbers, may increase gas production and that these symptoms will normalise. The participants will be asked to complete questionnaires on gut discomfort during the study. (82)

8.2 Possible Risks of Venepuncture

During the blood draw, slight pain may be experienced when the needle enters the arm, usually a prick or sting. Afterwards, there may be throbbing, excessive bleeding, bruising, fainting, or feeling light-headed, and in rare cases, infection.

To minimise risks, the researcher collecting the samples is trained in phlebotomy.

In case of negative experiences during venepuncture, the research team must be informed.

8.3 Possible Side Effects of Fasting for Venepuncture

Since the participants will be required to fast overnight (avoid all food and beverages except water for >9 hours) for the blood collection appointments, they may experience feelings of queasiness and shaking due to low blood sugar. We will provide snacks and beverages after sample collection to remedy this.

8.4 Possible Risk of Atmo Gas Sensing Capsule

Gut symptoms are not expected with the ingestion of the Atmo gas sensing capsule but may be possible (for example, include nausea, abdominal pain, and vomiting). Pill retention and bowel obstruction are unexpected adverse events. The capsules are expected to pass within five days of ingestion. If a capsule is not passed in five days, an x-ray may be required to confirm the location of the capsule, and laxatives may be administered to encourage passage. Bowel obstruction has rarely

been observed with the Atmo gas sensing capsule. Also, the experience from over two million capsule endoscopies indicates this is a very rare event (<2%). (83, 84)

8.5 Possible Risk of Blue Food Dye

Royal Blue Liqua-gel® (Chefmaster, USA) is a synthetic organic compound primarily used as a food colouring. It is poorly absorbed, and 95% of the ingested dye can be found in the stool. It is generally considered non-toxic and commonly used in medical settings. However, as a food additive, it can induce allergic reactions. Symptoms of allergic reactions may include rash, skin irritation, swelling, and difficulty breathing, which was not observed in an unpublished study by our research group (over 80 ingestions).

8.6 Possible Risk of Stool Sample Collection

The stool collection carries a small risk of infection for participants. Participants will be provided with stool collection kits containing gloves and biohazard bags to ensure the safe collection and storage of samples. Participants will be also instructed of good hygiene practices.

Participants may find the collection process embarrassing or difficult. The team will ensure that tikanga related to food is respected. We have sought guidance on ensuring Māori customs and protocols related to kai/ food are followed by all members of the study team. Research team members will explain the process and alleviate any potential concerns if such arise.

8.7 Possible Risk of COVID-19 Exposure

There is an increased risk of being exposed to COVID-19 during study visits. Research team members will screen participants prior to every visit and reschedule or postpone the visit if either participant or research team member tests positive for COVID-19 or feels unwell. Health and safety protocols for COVID-19 will be observed during the study.

8.8 Possible Risk of Data Entry Errors

Due to the large amount of data to be collected, there is an inherent risk of errors during the entry of the collected data into the databank. To minimise this risk, all paper-based data will be handled by blinded study personnel, and participants will be strongly encouraged to enter survey data directly into the database, by email links to the surveys and by the app.

9 DATA MANAGEMENT

9.1 Purpose of Data Collection:

Only data explicitly relevant to answering the research questions will be collected. There are no plans to link with any other datasets. Harm is not anticipated to occur with collecting this data beyond the risks of standard care and everyday life.

Related policies:

- National Ethical Standards: Health Data (https://neac.health.govt.nz/national-ethical-standards-health-and-disability-research-and-quality-improvement/part-two/12-health)
- Responsible Practice in Research -Code of Conduct (University of Otago): https://www.otago.ac.nz/administration/policies/otago003211.html

- Allegations of Misconduct in Research Procedures (University of Otago): https://www.otago.ac.nz/administration/policies/OTAGO028903.html
- Research Consultation with Māori Policy (University of Otago): https://www.otago.ac.nz/administration/policies/otago003272.html

9.2 Data Description

This study will generate quantitative including questionnaires, laboratory data, physiological measurements, and clinically relevant information regarding symptoms.

9.3 Format of Data

The data will be in the form of electronic files - excel spreadsheets, word documents, and data analysis files (R, SAS, Stata, or SPSS) with the potential for a small number of paper/posted questionnaires.

Identifying information provided upon enrolment will be kept in REDCap, a secure web application for creating and managing surveys and databases developed by Vanderbilt University. It is accessible only to the researchers to contact participants for planned data collection (for which consent has been received). All participants will have a unique identifying code which will be recorded in this file and used in lieu of any identifying information in all other data files, including labels on the samples and data collected during the study period. The identifying data is kept separate from all surveys, including the screening survey.

9.4 Data Collection, Storage, and Access

9.4.1 Data Collection and Storage by Researchers

- Training requirements of data collectors: The researchers collecting data are experienced in data collection, maintaining security and health-related confidentiality.
- Questionnaires and PROs: The majority of data collection will occur electronically via the REDcap data capture system, using the unique identifier allocated to the participants at the point of consent to the study. Participants who prefer paper data collection will be accommodated. Paper-based and telephone collected data will be entered directly into electronic data collection systems (using REDcap) as soon as it is received.
- Biological and physiome data will be analysed as per the above-mentioned measures. The data files will be stored in excel for raw data and as data analysis files (R, SAS, Stata, or SPSS). This data will only be identified by the unique code for the participant. Participants are identifiable to researchers only by their study code.
- Raw data collected in hard copy will be stored after electronic data entry as part of the CRF in a locked filing cabinet.
- All electronic data files generated in the study will be stored on a password-secured University of Otago server or Otago OneDrive cloud storage and will be accessed (and downloaded, as the need arises) to the password-protected computers of named investigators, stored on locked premises.

9.4.2 Data Access and Sharing

- As indicated above, a master file containing participants' personally identifying information will only be accessible to the researchers undertaking data collection.

- All named investigators will have access to the de-identified data via OneDrive or RedCap.
- The researchers (blind) will have only access to de-identified raw data files and will be responsible for the final data analysis.

9.5 Data Preservation Strategy

All raw data collected in hard copy will be held for 10 years in locked cabinets on locked premises of the University of Otago, after which time hard copy documents will be destroyed. Electronic files will also be retained for 10 years in a secure storage space provided by the University of Otago (as per the University of Otago Policy on the Responsible Practice in Research -Code of Conduct). Coded electronic 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.

9.6 Data Security and Confidentiality of Potentially Disclosing Information

National Health Index numbers will not be collected. However, the names, dates of birth, and contact details of all participants will be collected. All data will be stored on password-protected files, backed up to University of Otago servers and stored on computers on locked premises.

REDCap is a secure survey application which is web-based. The survey data is stored in the University of Otago data centres. Server backups are taken daily and are stored within the data centre. The server is secured with TLS Certificates, encrypting end-to-end communications. Security updates are applied to the server regularly. REDcap software is updated as security issues, and new features arise and dictate the need to update.

The Principal Investigators are accountable for complying with the requirement to maintain the privacy and confidentiality of participants' health data. Data monitoring will be undertaken by the research team (see 9.8). Any breaches of privacy and confidentiality, including unauthorised disclosure of health data, will be dealt with according to the approach outlined in the University of Otago Policy on Allegations of Misconduct in Research Procedures: https://www.otago.ac.nz/administration/policies/OTAGO028903.html; specifically raising the concern with the appropriate Divisional Pro-Vice-Chancellor, and following recommendations from a preliminary inquiry into the matter, including informing those affected by the disclosure and taking measures to mitigate any harm.

9.7 Māori Involvement in Governance of Data

High-Value Nutrition National Science Challenge (HVN), which is the governing body of this study, will provide guidance with respect to issues of Māori data sovereignty that are being implemented across its research programmes. The project team for this study includes an experienced Māori Senior Researcher, Dr Jane Mullaney, who co-led (with Professor Warren McNabb) the *in vitro* digestion and fermentation studies which provided results to select DRB for this intervention, participated in the study design (microbiome outcomes) and will be involved in the microbiome analyses. Their ongoing involvement in the study will ensure that Māori rights and interests in relation to data collection, storage, analysis, and dissemination are considered and prioritised (for details, see 9.4).

In addition, Māori research undertaken at the University of Otago, Christchurch requires reviewing by a Māori Research Advisor. This process is underway.

https://www.otago.ac.nz/christchurch/research/researchoffice/maoriconsultation/index.html

9.8 Data Monitoring

A formal Data Monitoring Committee will not be required as the intervention and control treatments in this study are considered a sufficiently low risk that no harm is anticipated to occur beyond the risks of standard care and everyday life.

Data Monitoring will occur through regular meetings of the research team. Procedures for recruitment and safety monitoring, and adverse events are detailed in this full study protocol. Concealment will be maintained by the unblinded research team. Ethical approval is sought from the University of Otago Human Ethics Committee (hUOHEC) along with locality authorisation for the study site prior to study commencement.

9.9 Case Report Forms

The eCRF was designed specifically for the needs of this study and is the data collection instrument for the study. Therefore, all data requested on the eCRF must be recorded, and missing data must be explained.

The eCRF is anonymous: all participants are identified by a unique five-digit identifier.

10 DISSEMINATION OF RESULTS

The Principal Investigators will be jointly responsible for disseminating results from this study.

After statistical analysis, the data reports will be compiled as lay summaries and sent to the participants, the human ethics committee, the Māori Research Advisor, and the funding bodies.

The findings of the study will be compiled and prepared for publication in an appropriate peer-reviewed journal, together with presentations at conferences and public HVN Webinars.

Upon publication, the compiled lay summaries of the study findings will also be sent to participants and the human ethics committee.

The research team will also provide seminars to stakeholders such as GPs, gut specialists, CDHB Māori Health workers, and researchers in the field and provide participants with lay summaries of the study using an email newsletter. The research team will also provide individual research data upon request by the individual participant.

We intend to disseminate results to Māori stakeholders in several ways. For example, email newsletters to Māori participants, Hui with Māori health care providers and nurses, and, if possible, presentation of Māori specific results at a health day at the Tuahiwi Marae.

In addition, interested participants are encouraged to sign up to the HVN Twitter feed or the HVN LinkedIn page to obtain additional information about HVN.

https://twitter.com/HVNutrition_NZ

https://www.linkedin.com/feed/update/urn%3Ali%3Aactivity%3A6777043462865989632/?actorCompanyId=18130412

11 ETHICAL, LEGAL, AND GENERAL CONSIDERATIONS

11.1 Ethical Conduct of Study

The study will be carried out in accordance with this protocol, International Conference of Harmonisation (ICH) guidelines, national and local requirements, and the ethical principles originating in the Declaration of Helsinki.

11.2 Ethics Approval and Registration

Ethical approval will be sought from the hUOHEC prior to the start of the study: https://www.otago.ac.nz/council/committees/committees/HumanEthicsCommittees.html

Approval by the University of Otago Christchurch Māori Research Advisor has been requested:

https://www.otago.ac.nz/christchurch/research/researchoffice/maoriconsultation/index.html

The study will be prospectively registered on the Australian New Zealand Clinical Trials Registry (www.anzctr.org).

11.3 Protocol Amendments

Any amendments to the study protocol will be reported to hUOHEC and all other local approval committees. Changes particularly pertaining to Māori (e.g., recruitment processes, analyses) will be reported to the Māori Research Advisor.

11.4 Consent

The researchers will invite eligible participants to consent to the study, emphasising that participation is voluntary and the decision to participate or not will not influence the quality of care they receive. Participants will be advised that they are welcome to take the time to consider consenting to the study and discussing participation with their friends, whānau, or other support persons. Māori participants will be informed of local Māori health advisors (e.g., local to each DHB) with whom they could discuss participation in the study.

If at any time the participant's circumstances change in ways that could affect ongoing consent to participate in the study, they will be advised to raise that with the researchers or to notify the research team if they wish to withdraw consent to participate.

11.5 Confidentiality

No identifying or identifiable information about participants will be reported in any way from this study, including names, dates of birth, images, or aspects of their circumstances that could identify them. Identifying information of participants will be gathered at enrolment, entered by researchers collecting data and accessible via REDcap only by these researchers.

For information about maintaining the confidentiality of health data (e.g., secure data storage), see section 9.4.

11.6 Funding Source

Funding for this study has come from the High-Value Nutrition (HVN) National Science Challenge (90%) and Goodman Fielder (10%). Goodman Fielder is also providing in-kind contributions.

11.7 Declaration of Interests

The Principal Investigators (Richard B Gearry, and Nicole Roy) have no declarations of interest in the proposed study. Goodman Fielder is a partial funder of the study and has had input into the study design. However, they are not involved in the analysis or interpretation of results.

11.8 Publication Policy

Data derived from this study will be the exclusive property of the Principal Investigators. No use and no transmission to a third party will be made possible without prior consent. Any publication or presentation related to the study will therefore be approved by the Principal Investigators. Clinical samples derived from this study may not be used by site investigators for research unrelated to this protocol without the prior approval of the Principal Investigators. Other legal aspects, including intellectual property, are covered by contracts between the University of Otago, the head contractor with HVN, and the subcontractors, Massey University (Riddet Institute), AgResearch, Plant & Food Research and industry/collaborator agreements with Goodman Fielder, and Atmo Biosciences®.

Authorship for each publication will be determined by the Principal Investigators of the study, in agreement with all research team members, according to the contribution made to the study, and in line with international journal standards.

The full protocol for this study will be published online in a peer-reviewed journal. Participant-level data will be available on request (de-identified). Some portions of the dataset may be published as supplementary files, as per journal specifications. Statistical code will not be published, given the conventional nature of the statistical methods planned. All analyses will be based on existing published methods.

11.9 Ethical Considerations

Participants being coerced or perceiving to be coerced to take part

- All participants will be advised verbally and in written form that participation is voluntary and will not impact on them in any way, now or in the future.
- The Patient Information Sheet and Consent Form state that participants are welcome to withdraw at any point without explanation by notifying the research team.

Participants may be concerned about the potential for breach of their privacy in the information shared

- Data will be gathered online using the REDcap data capture system. REDcap software will be stored in fully secured data centres secured with TLS Certificates and encrypted end-to-end communications.
- If any participants experience problems entering data using the REDcap application on their computers, they have the alternative of using paper versions of the questionnaire provided upon request by the researcher.
- All non-participant files within this project are stored and shared amongst relevant team members using the OneDrive cloud storage held in University of Otago servers.
- All devices on which study data are accessible will be password protected and stored in locked premises.
- No identifying data/ information will be shared with other researchers or collaborators in New Zealand and overseas.

Participants may experience side effects of the intervention/control or measures

- Participants will be provided with information about the management of possible side effects.
- Participants will be encouraged to contact their health care provider, followed by a researcher in the event of side effects.
- Participants may withdraw if they experience any side effects; participants will be encouraged to record these symptoms as they are clinically relevant.
- Any participants experiencing harm directly due to their involvement in the study will be withdrawn from the study immediately.

Time commitment

- Participation in this study will require multiple visits and multiple hours of commitment to complete outcome measures. The researcher will include this information in the participant information sheet and provide a schedule of visits and measures at the time of enrolment.

Return of bodily material samples to participants

Participants will be informed that they are given a choice to return remaining bodily material samples (such as blood and stool matter) after sample processing or that the remaining samples will be hygienically disposed of with appropriate karakia. Participants will be advised that bodily material samples sent for analysis to study collaborators cannot be returned.

12 KEY STAFF AND ROLES

Clinical coordinator

Dr Simone Bayer, University of Otago – Research Fellow; Research Coordinator, day-to-day clinical lead for the clinical phase and clinical data analysis and interpretation, co-author in publication.

PhD Fellows

Jasjot Maggo, University of Otago – PhD Fellow; day-to-day activities for the clinical phase and clinical data analysis and interpretation done in Christchurch and associated biological analyses done at other sites, co-author in publication.

Hwei Min Ng, University of Otago – PhD Fellow; day-to-day activities for the clinical phase and clinical data analysis and interpretation done in Christchurch and associated biological analyses done at other sites, co-author in publication.

Principal Investigators

Professor Nicole Roy, Department of Human Nutrition (University of Otago) – Professor of Human Nutrition and Physiology; Principal Investigator of systems nutrition analyses, support for data analysis and interpretation, co-author in publication.

Professor Richard Gearry, Department of Medicine (University of Otago) – Professor and Clinician; Principal Investigator of the clinical study and data analysis and interpretation, co-author in publication.

Team of other Investigators

Dr Catherine Wall, University of Otago – Research Fellow, Food dietary analysis and interpretation, co-author in publication.

Professor Warren McNabb, Riddet Institute (Massey University) – Professor Nutrition and Digestion; responsible for microbial enzymatic assays, support for data analysis and interpretation, co-author in publication.

Dr Jane Mullaney, AgResearch – Senior Scientist Microbiome and Molecular Biology; responsible for data analysis and interpretation of the stool metagenomics data, mentoring Jasjot Maggo and Hwei Min NG with these analyses and implementation of Vision Matauranga as relevant, co-author in publication.

Dr Karl Fraser, AgResearch – Senior Scientist analytical chemist and nutritional metabolomics; mentor of Dr Cabrera and responsible for data analysis and interpretation of plasma and stool metabolomics and bile acid data, co-author in publication.

Dr Diana Cabrera, AgResearch – Postdoctoral Researcher Nutritional Metabolomics; conduct analyses and interpretation of plasma and stool metabolomics and bile acid data, co-author in publication.

Dr Wayne Young, AgResearch – Senior Scientist Microbiome and Bioinformatics; support for bioinformatics data analysis and interpretation, co-author in publication.

Dr Janine Cooney, Plant and Food Research – Senior Scientist Analytical Chemistry; responsible for stool and plasma organic acid and phenolic acid analyses, data analysis and interpretation, co-author in publication.

International Collaboration

Professor Peter Gibson, Head of Luminal Gastroenterology Research in the Department of Gastroenterology at the Central Clinical School at Monash University, will act as an advisor for the physiome aspect of the proposed study.

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14 APPENDICES

Appendix A: Participant Information Sheet and Consent Form

Appendix B: Screening Questionnaire

Appendix C: Daily Bowel Habit Diary

Appendix D: Daily Bread Diary

Appendix E: Modified Hunter New England Health Survey (ModHNES) and SF-12v2® Health Survey

Appendix F: Economic Living Standards Index- Short Form ($ELSI_{SF}$)

Appendix G: Gastrointestinal Symptom Rating Scale (GSRS)

Appendix H: Three-Day Food Diary

Appendix I: Patient-Reported Outcomes Measurement Information System (PROMIS) Survey

Appendix J: WHO-5 Wellbeing Index (WHO-5)

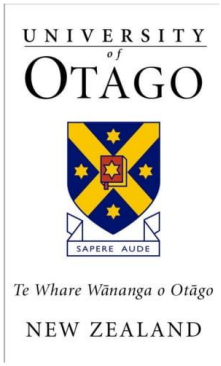
Appendix K: Warwick Edinburgh Mental Wellbeing Scale (WEMWBS)

Appendix L: Multidimensional Fatigue Symptom Inventory Short Form (MFI-SF)

Appendix M: Subjective Vitality Scale (SVS)

Appendix N: Canterbury District Health Laboratory Requirements

Appendix A: Participant Information Sheet and Consent Form



Participant Information Sheet

Study Title	**A flourishing biome for gut health – Promoting a diverse microbiome through bread (BREAD Study)**
Short Title	**B**read **R**elated **E**ffect on Microbi**A**l **D**istribution (BREAD Study)
Principal Investigator	Professor Richard Gearry Professor Nicole Roy
Locality	University of Otago, Christchurch
Ethics com. ref	TBA

You are invited to take part in a study on the effect of dietary fibre in bread on gut function, digestive health, and overall health and wellbeing. Whether or not you take part is your choice. If you don't want to take part, you don't have to give a reason, and it won't affect the care you receive. If you do want to take part now, but change your mind later, you can pull out of the study at any time.

This Participant Information Sheet will help you decide if you'd like to take part. It sets out why we are doing the study, what your participation would involve, what the benefits and risks to you might be, and what would happen after the study ends. We will go through 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 talk about the study with other people, such as family, whānau, friends, or healthcare providers. Feel free to do this.

If you agree to take part 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 both 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.

WHAT IS THE PURPOSE OF THE STUDY?

We are performing this study to understand more about effect of different types of dietary fibre on the gut microflora (bacteria/microorganism residing in gut) and its functions. The fibre in our diet is known to reduce the risk of constipation by increasing the bulk of stools. In addition to this, it is also essential in maintaining a healthy gut microflora. The gut bacteria break down fibre and produce a vast range of products (metabolites) which influence body functions like bowel movement, fight infections, modulate appetite and hunger, mood and wellbeing.

The average NZ diet lacks fibre, adult women consume 17g of DF on average instead of recommended 25 g per day and adult men consume 22 g instead of 30 g per day. The low intake of fibre can alter the gut microflora and result in dysfunction, and may contribute to the development of chronic diseases such as intestinal bowel disease, colorectal cancer, allergies, obesity, heart diseases, and type 2 diabetes. These diseases can, at least in part, be prevented by optimal fibre intake. An optimal fibre intake (25-30g) is associated with improvement of digestive function, general wellbeing, and decreased risk of chronic diseases.

Bread is the main food source of fibre in the NZ population. It is an ideal product to add ingredients to increase fibre content. In this study, we aim to find differences in the gut microbiota in individuals with inadequate fibre intake. As the recommended intake of fibre differs between genders, males will be consuming four slices, females will be consuming three slices of Bread A and Bread B, each for four weeks. The findings may allow us to better understand how different quantity and quality of fibre affects the microflora and to improve knowledge of the effects of fibre on gut health and general wellbeing.

For a deeper and more holistic understanding of the effects of fibre on the gut and body function, we would like to collect information from you by questionnaire, blue food dye to measure the time of digestion from beginning to end, as well as biological samples in the form of blood and stool. You may also be selected to swallow a diagnostic device, a pill-sized Atmo gas-sensing capsule. We will then analyse the samples and compare the results between Bread A and Bread B.

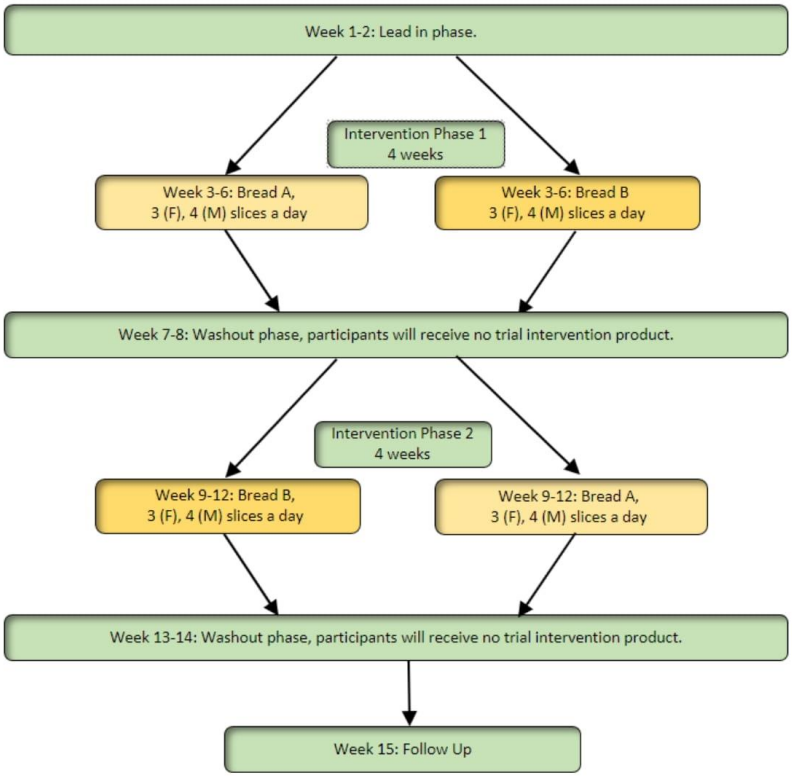
The study is being performed by researchers from the University of Otago, Department of Medicine, Gastrointestinal Unit for Translational Studies in Christchurch. The study is funded by the High-Value Nutrition National Science Challenge and Goodman Fielder Ltd. Goodman Fielder Ltd is providing both Bread A and Bread B. Laboratory studies will be performed by the Canterbury District Health Laboratories, AgResearch, Riddet Institute, Plant & Food Research, Teagasc, Agriculture and Food Development Authority, Ireland.

The study protocol has been reviewed by the University of Otago Human Ethics Committee (Health).

WHAT DOES THE STUDY INVOLVE?

The study is a double-blinded, placebo-controlled, cross-over study. This means that if you choose to take part, you will be randomly allocated to an intervention order by a researcher and you will consume Bread A and Bread B in random order. Each participant will receive both breads. You and the researchers will not know which intervention you are having. This is to ensure that there is no influence from the participants and researchers as to the effect of the interventions.

The trial will be a maximum of up to 15 weeks in total. A diagram of the study is shown below:



Participants: Study participants will be recruited through the general population with inadequate dietary fibre intake.

The age range of all participants is 18 to 65 years, and the BMI range is 18 to 35 kg/m^2 (BMI is the abbreviated term of body mass index, used to estimate a healthy

weight range for individuals based on weight and height. BMI is determined by your weight in kilograms divided by your height in metres squared).

All participants will need to be:

- Able to give informed consent and understand what is required of them during the course of this study.
- Free of any known significant gut disorder and diseases. This includes chronic constipation, diarrhoea, irritable bowel syndrome (IBS), inflammatory bowel disease (IBD) (Ulcerative colitis and Crohn's disease), diverticulitis, coeliac disease or previous bowel resection.
- Free of alarm features associated with bowel habit, such as recent changes in bowel habits (onset less than three months), rectal bleeding, sudden weight loss, occult (hidden) blood in stool, anaemia, anal fissures, bleeding haemorrhoids, and family history of gut cancer at a young age
- Free of systemic disease that could influence the gut directly or through medication use (e.g. diabetes, opiates or regular NSAID use (painkillers)
- Free of severe chronic disease or neurological conditions.
- Female participants who are **NOT** pregnant, breastfeeding or planning a pregnancy in the three months post-selection (study time frame).
- Free of known intolerance or allergy to wheat or rice.
- Free of antibiotic use within the last month
- Free of prebiotics, probiotics and fibre supplement use during month prior to screening
- Non-smokers
- Willing to stop laxative, pre- and probiotics or fibre supplement throughout the study.
- Able to comply with the study procedures.

The research in this project will be undertaken in a culturally sensitive manner, with all aspects of the trial explained in full to you in a manner most suitable to you. The research team will be available to answer questions throughout the study and will seek advice from appropriate advisory groups should it be necessary. You will be given access to interpreters at any time in the study should you require them. The opportunity for Whānau support is available at all times.

WHAT WILL MY PARTICIPATION IN THE STUDY INVOLVE?

You are invited to this study because you have indicated that you are interested in supporting our research.

If you choose to take part in the study, you will be expected to do the following:

Screening

You will be asked to complete a screening questionnaire to assess if this study is right for you. The questionnaire will include questions on your general health, bowel health and dietary history. You can use this link to assess your eligibility https://redcap.otago.ac.nz/surveys/?s=8WR7WCNRCETYTAC7

If you are eligible we will make an initial appointment for you to come either into 40 Stewart Street in central Christchurch, or to the Nicholls' Centre of Christchurch Public Hospital during the week at a mutually convenient time. At this appointment, you will have the time to ask questions, and we will give further explanation of the study. If you provide written informed consent, we will measure your height, weight, blood pressure and waist circumference.

You will also be asked to give a fasting blood sample. This means that you must have nothing to eat or drink except water from 10 pm the night before until you attend the clinic (9 hours fast) and have your blood taken. The blood sample will be done first thing in the morning, so we will not be asking you to go without food for long. We will also provide you with a light snack after your blood sample has been collected.

A researcher will take a blood sample (total amount of 10-15 mL, approximately one tablespoon) from a vein in your arm. Due to the nature of the analysis, we will not be able to return this blood sample to you once it has been collected. The following tests will be performed on your blood sample, which will give us information about your health. Canterbury Health Laboratories will perform the analysis. You will be given access to the results of these blood tests if you wish.

It is common that a test result falls just outside the normal range and is usually not concerning. Should any of your blood test results be clinically significant, we will inform you and recommend that you make an appointment with your medical practitioner. We will provide a copy of the test results for your GP.

If you fit all the eligibility criteria, you will be offered a place in the study.

TEST	**REASON**
Albumin	Liver function
Alkaline Phosphatase	Liver function
Alanine aminotransferase (ALT)	Liver function
Aspartate aminotransferase (AST)	Liver function
Blood Urea Nitrogen (BUN)	Kidney function
Calcium	Heart, Nerve, Kidney function
Chloride	Acid/base balance
Carbon dioxide	Acid/base balance
Creatinine	Kidney function
Glucose	Glucose metabolism
Potassium	Acid/base balance

Sodium	Acid/base balance
Total bilirubin	Liver function
Total protein	Liver function
C-reactive protein	Immune response
Blood count	Immune response, overall health
Lipid profile	Cardiovascular function

During the study

The study will require you to make five visits to the clinic, either at 40 Stewart Street or to the Nicholls' Centre of Christchurch Public Hospital. It is estimated that the visits will take a maximum of 30 minutes each time.

Due to the nature of the study and the outputs we are measuring, we would prefer that you stop taking any fibre supplements, prebiotics and probiotics you are currently taking for the duration of the study and not take any laxative in the week before your appointments.

Intervention: We will provide you with Bread A and Bread B during the study. The research staff will organise pick up with you, and instruct you on how the interventions are to be taken. Please let the research staff know when you run out, so we can provide more, if necessary.

Stool sample collection: At the baseline visit, you will be asked to provide us with stool samples. We ask you to collect the stool sample the day before you come and to bring the sample in with you. We will provide you with the appropriate gear to collect the samples hygienically. These samples will be frozen at -80°C and shipped to our New Zealand collaborators for analysis. Stool DNA (genetic code of the gut microflora) or RNA (DNA copy for protein production) extracted will also be shipped to a commercial service provider lab in Ireland for sequencing before these data is analysed by our research team. We will only analyse genetic code of the gut bacteria and microbes.

The stool will be used for several analyses. We will measure the concentration of a range of bacteria and other microbes that live in the gut and what they make with the fibre you are eating.

During the course of the study, you will be asked to provide further four stool samples at each of the following time points: end of treatment 1 (week 6), end of washout period 1 (week 8), end of treatment 2 (week 12), and end of washout period 2 (week 14). This is a total of five stool samples (including baseline visit).

Blood sample collection: At the baseline visit, you will be asked to provide us with a fasting blood sample. We will collect a total of 12 mL (approximately one

tablespoon). The blood will be split into different components and stored. Experiments will include metabolites of normal body processes (what your body makes from food), and lipid profile. The baseline measurement tells us the level before you start the trial, so we have a comparison.

During the course of the study, you will be asked to provide further fasting blood samples at the following time points: end of treatment 1 (week 6), end of washout period 1 (week 8), end of treatment 2 (week 12), and end of washout period 2 (week 14). This is a total of five blood samples (including baseline visit).

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

If you wish, we can arrange for the remainder of your blood and faecal samples to be returned to you on completion of the analysis or to dispose of them with appropriate karakia.

Gut Transit Measures: Over the course of the study, we will use various methods to measure gut function and motility.

- **Blue food dye:** at each visit (week 2, 6, 8 and 12) you will be asked to ingest Royal Blue Liqua-gel® food colouring in 200 mL water to measure how long it takes for food to travel through your body. You will be asked to record the passing of the dye in stool via the daily bowel movement app.
- **ATMO gas-sensing capsule:** Selected participants will be required to ingest an Atmo gas-sensing capsule at baseline visit (week 2), end of treatment 1 (week 6), end of washout period 1 (week 8), and end of treatment 2 (week 12) to measure transit time, temperature and various gases in the gut. We will also give you a standardised food bar before swallowing the capsules. This also means that you are asked to carry recorders on your body until the capsules have passed, to fill in additional details such as food, drink, and bowel movements into the recorders, and to drop the recorders off at our clinic at 40 Stewart Street once all capsules have passed.

Questionnaires: Over the course of the study, we will provide you with the following online questionnaires to complete. These questionnaires relate to your bread intake, bowel habits, health, socio-economic status, and how you are feeling, both mentally and physically. While many of the questions of these questionnaires are very similar, they do cover different aspects and details.

- **Gastrointestinal Symptoms Rating Score:** The primary interest of the study is your level of gut comfort. This questionnaire asks you to mark on a scale of

seven points how you are feeling. The questionnaire contains 15 questions and will take you about five minutes. There will be a total of five of these questionnaires over the duration of the study.

- **Daily Bowel Habit Diary (accessible via smartphone app):** We like to know how and if your bowel habits change in relation to the bread you eat. In order for us to assess this, we need you to fill out a record for each bowel movement you have in a day and its consistency (using the Bristol Stool Scale). This is done using a short questionnaire. The diary must be completed **EVERY DAY AFTER ENROLMENT OF THE STUDY**. There are 11 questions in total which require you to tick an answer, so it will not take very long. If the daily bowel habit diary is not completed regularly, you may have to be withdrawn from the study. If you have problems with the online version or cannot go online for a while, we can provide you with paper versions to cover that time if you wish.
- **Daily Bread Diary (accessible via smartphone app):** We like to know how you eat your bread (toasted/non-toasted) and if you eat extra slices of bread. In order for us to assess this, we need you to fill out a record for the bread you have in a day. This is done using a short questionnaire. The diary must be completed **EVERY DAY AFTER ENROLMENT OF THE STUDY**. There are 5 questions in total which require you to tick an answer, so it will not take very long. If the daily bread diary is not completed regularly, you may have to be withdrawn from the study. If you have problems with the online version or cannot go online for a while, we can provide you with paper versions to cover that time if you wish.
- **Patient-Reported Outcomes Measurement Information System:** We also want to know how your bowel habits affect your mental health and vice versa. This questionnaire contains 16 questions but should not take longer than 5 minutes to fill out. There will be a total of five questionnaires over the duration of the study.
- **World Health Organisation - Five Question Well-Being Index (WHO-5):** This questionnaire only contains five questions assessing how you have felt in the past week. You only have to fill it out five times over the duration of the study, and will not take you more than five minutes to complete.
- **Warwick-Edinburgh Mental Wellbeing Scale:** Alongside with other questionnaires that assess mental wellbeing, this questionnaire contains 14 questions that are all worded positively and cover both your feelings and functioning aspects of mental wellbeing. Similar to other questionnaires, you will only require to complete these five times over the duration of the study, which will take you no more than five minutes to complete.
- **Multidimensional Fatigue Inventory:** This questionnaire contains 20 questions designed to measure fatigue. It contains a seven point-scale to

indicate to what extent the particular statement applies to you. You will be asked to fill up this questionnaire three times over the duration of the study.

- **Subjective Vitality Scale:** This questionnaire contains 6 questions assessing your state of subjective vitality. You only have to fill it out five times over the duration of the study, and will not take you more than two minutes to complete.

- **Diet Records:** During the course of the study, we would like to get an idea of your usual dietary intake. There will be five food diaries to fill out. We ask you to record the type and amount of all the food and beverages you have consumed over a three-day period. The time points for these will be one week before the baseline of the study (week 1), end of treatment 1 (week 5), end of washout period 1 (week 7), end of treatment 2 (week 11) and end of washout period 2 (week 13). We ask you not to change your diet radically over the course of the study.

- **Modified Hunter New England Health Survey:** At the beginning of the study, you will be asked to fill out this questionnaire, which covers specific health, lifestyle and mental health questions, as well as some personal data. We want to get an overall view of you and to raise any issues that may affect the data. This questionnaire contains 11 questions, and you only have to fill it out once.

- **Economic Living Standard Index short form**: This questionnaire, which you only have to fill out once, allows us to understand your standard of living and your socioeconomic situation. It allows us to find out if symptoms or results are tied to specific issues in your life that have no obvious link to your bowels. It contains 25 questions and asks you to rate each by ticking a box. It should take you no more than 10 minutes to complete.

If you cannot complete the questionnaires during the study, you will have to be withdrawn from the study.

If you would like to switch from online to paper or from paper to fill out the questionnaires online at any time of the study, just tell us. We are happy to provide you with the necessary paperwork or send you the links via email.

WHAT ARE THE POSSIBLE BENEFITS AND RISKS OF THIS STUDY?

You may or may not benefit from taking part in this study. There is no guarantee that you will experience any changes in stool frequency or satisfaction from taking any of the study products. You will, however, gain knowledge regarding bowel health and be issued with bread for yourself during the study and your immediate family/whānau/fellow living companions after the study.

Additionally, if we are successful in understanding the impact of increasing quantity and quality of dietary fibre on gut microflora and on general health, we may be able to make further recommendation on fibre consumption.

Bread is a staple food for the majority of the population; however, a small percentage of the population has gluten allergy and is intolerant to various ingredients in bread. We recommend that those suffering from allergy or intolerance do not participate in this study. You may experience abdominal discomfort, distension and wind by consuming bread with high fibre content due to increased production of gas by your gut bacteria. These symptoms should settle by themselves in few days. We recommend participants to replace their regular bread with the study bread to avoid excessive consumption.

As with all blood tests, there may be some slight discomfort when the needle is inserted. You may also receive a bruise from the blood sampling. Should any serious adverse event related to the blood sampling procedure occur during the study period, you will be immediately withdrawn if you wish and asked to seek medical treatment.

There are minimal but possible risks associated with the use of Atmo gas-sensing capsules. There is a risk of the capsule becoming stuck on the way through the gut, but this has not yet been reported in healthy adults. For most people, the capsule is passed within five days of ingestion. Bowel obstruction is another possible serious risk but has not been reported with Atmo gas-sensing capsules.

Brilliant Blue food colouring is primarily used as a food colouring; it is not digested and can be found in the stool. It is non-toxic and commonly used in medical settings. However, it can induce allergic reactions. Should any adverse event related to the procedure occur, you will be immediately withdrawn and asked to access medical treatment.

There is a slightly increased risk of being exposed to COVID-19 during the study visits. We will screen participants prior to every visit and reschedule or postpone visits if either participant or researcher are positive for COVID-19 or develop COVID-19-like symptoms.

If you require it, we will return leftovers of your stool and blood samples to you after analysis. Otherwise, it will be disposed of hygienically (in accordance with NZS 4304:2002 “Healthcare Waste Management”) or with the appropriate karakia, if you wish.

Although necessary efforts (password protected files, secure database) will be made to protect your privacy, absolute confidentiality of your information cannot be guaranteed. Even with de-identified information, there is no guarantee that you cannot be identified. Your de-identified 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 which make decisions

about the use of your information. There is a risk that overseas researchers may work with information in a way that is not culturally appropriate for New Zealanders.

WHAT IF SOMETHING GOES WRONG?

Both breads are safe for consumption. If you are injured in this study, which is unlikely, you will be eligible for compensation from ACC just as you would be if you were injured in an accident at work or home. 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 will not affect your cover.

WHAT ELSE DO I NEED TO TELL YOU?

It is really important that you keep us informed on any health issues that may come up suddenly during the study, especially if they are related to your digestion. This includes a stomach bug or food poisoning, but also if you get ill and need to take antibiotics.

We need to know this to make sure the health issue is not related to the intervention we gave you, and to make sure it does not change the data we collect. Depending on the severity, we may need to withdraw you from the study in those cases.

YOUR PARTICIPATION AND COMPENSATION

Your participation in this study is completely voluntary. We are happy for you to bring along support persons to each of the clinic appointments if you like.

We will give you a $20 MTA voucher for the initial screening visit to compensate you for your travel and time. If you are accepted into the study, you will receive a further $60 in New World Vouchers each time you come in for your appointments. This will make a total of $320, which we will give you at the completion of the trial. If you are selected for the additional gas fermentation investigation with the ATMO capsule, you will be given an additional $100 compensation for your time and inconvenience.

If you decide to take part but later change your mind, you are free to withdraw at any time without having to give a reason. Your participation in the study will be withdrawn if it appears harmful to you in any way.

WHAT ARE MY RIGHTS?

Your participation in this study is voluntary, and you are free to decline participation or withdraw from the study at any time without compromising your medical care.

You have the right to access information about yourself that is collected as part of the study. If new information becomes available during the study that may have an impact on your health, you will be informed immediately.

At all times, your privacy will be maintained. No material that could personally identify you will be used in any reports on this study or closely related projects in the future. If the results of the trial are published, anonymity will be maintained. A code that identifies you to the research team will be used on all study documentation. The code will also be used for the faecal DNA or RNA that has to be sent to a commercial service provider lab in Ireland. The code is held on a database that is separate from the database being used to store your information. Both databases are securely housed on a University of Otago server and are password protected. This means only the Christchurch research team can link important results from the research to your identity so we can communicate these results to you, but other researchers analysing data cannot.

During the study, your physical file will be held in a locked filing cabinet when not in use. At the end of the study, your files will be kept for 10 years in secure document storage and then destroyed by shredding. The biological samples will be stored until publication of results has occurred, but not longer than 10 years, after which they will be destroyed hygienically (in accordance with NZS 4304:2002 "Healthcare Waste Management") or with the appropriate karakia, if you wish.

If you have any queries or concerns about your rights as a participant in this research study, you can contact an independent health and disability advocate. This is a free service provided under the Health and Disability Commissioner Act.

Telephone (NZ wide): 0800 555 050

Free Fax (NZ wide): 0800 2787 7678 (0800 2 SUPPORT)

Email (NZ wide): advocacy@hdc.org.nz

If you have any questions about the study at any time, please do not hesitate to call.

This study has been preliminary approved by the University of Otago Human Ethics Committee (Health).

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

Once the information and samples are collected, there are no further requirements with regard to participation in the study, you would be released from the study but advised to keen the copy of this participant information sheet. All information and biological samples will be stored in the University of Otago on password-protected servers and in secure research freezers. No identifying data is kept in the same place that could link results to you as an individual. Secure storage is the responsibility of the University of Otago and the other institutions where the research will be undertaken. The information and samples will be stored securely and be used for ongoing research into role of dietary fibre and gut microbiome.

The hard copy data will be destroyed 10 years after the commencement of the study. The biological samples will be stored until publication of results has occurred, but not longer than 5 years. 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.

Your coded information or tissue sample may be used for future research related to intervention or research question or outcomes. This future research may be conducted overseas. You will not be told when future research is undertaken using your coded information. Your coded information may be shared with other researchers or companies. Your coded information may also be added to information from other studies, to form much larger sets of data. You will not get reports or other information about any / some research that is done 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.

If you withdraw from the study after the samples and data have been collected, we will remove any data relevant to you or the samples that you have given from the study database. However, if the samples have already been processed and the data has been used for research purposes, then the data cannot be removed from scientific reports. If you were to die, your family will not be able to withdraw the data and samples from the study. Findings from this study will be communicated to participants who wish this by a newsletter.

WHO IS FUNDING THE STUDY?

Funding for this study has come from High Value Nutrition Science Challenge (The Ministry of Business Innovation and Enterprise) and Goodman Fielder Ltd. Goodman Fielder is also providing in-kind contribution

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

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

Research team:

Dr Simone Bayer, Jasjot Maggo, and Hwei Min Ng

HVN.GIstudies@gmail.com . 021 279 1519

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

Phone: 0800 555 050 Fax: 0800 2 SUPPORT (0800 2787 7678)

Email: advocacy@hdc.org.nz

For **Māori health support** please contact:

Nga Ratonga Hauora Christchurch Hospital

Tel 3640 640 (Ext 86160)

You can also contact the University of Otago Human Ethics Committee (Health) that approved this study on:

Phone: +64 3 479 8256 Email: gary.witte@otago.ac.nz

Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.

National SCIENCE Challenges

HIGH-VALUE NUTRITION

Ko Ngā Kai Whai Painga

Consent Form

UNIVERSITY of OTAGO

Te Whare Wānanga o Otāgo

NEW ZEALAND

If you need an interpreter, please tell us

I have read (or have had read to me) and understand the Participant Information Sheet.
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, whānau/ 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 taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without this affecting my medical care.
I consent to the research staff collecting and processing my information, including information about my health.
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.
I understand that this study involves five 30-minute visits with the research team at different locations.
I agree to my stool and blood samples being sent to New Zealand collaborators and I am aware that these samples will be disposed of using established guidelines for discarding biohazard waste.
I consent to use of de-identified data from this study to be used in future studies.
I consent to my de-identified data and faecal samples being sent overseas.
I understand that during the week before sample collection I must refrain from taking any laxative medication other than the rescue treatment offered by the research staff
I consent to my GP or current provider being informed about my participation in the

study in case of any significant abnormal results obtained during the study.

I agree to an approved auditor appointed by the University of Otago Human Ethics Committee (Health), or any relevant regulatory authority or their approved representative reviewing my relevant medical records for the sole purpose of checking 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 who to contact if I have any questions about the study in general.

I understand my responsibilities as a study participant.

I consent to be contacted by the researchers if there are other studies that I may be eligible to participate in.

I would like any remaining samples to be disposed of at the end of the study (please tick one):

Using standard disposal methods ☐ Disposed with appropriate karakia ☐

Be handed back to me ☐

I wish to receive a summary of the results from the study ☐

Declaration by participant:

I hereby consent to take part in this study.

Participant's name:

Signature: Date:

Declaration by member of research team:

I have given a verbal explanation of the research project to the participant, and have 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:

Participant Information Sheet
Wireless Motility Device

Study Title	**A flourishing biome for gut health – Promoting a diverse microbiome through bread (BREAD Study)**
Principal Investigator	Professor Richard Gearry Professor Nicole Roy
Locality	University of Otago, Christchurch
Ethics committee ref	

You have been selected to ingest the Atmo gas sensing capsule.

This Participant Information Sheet sets out why we are doing this part of the study, what your participation would involve, what the benefits and risks to you might be, and what would happen after the study ends.

This document is 6 pages long, including the consent form. Please make sure you have read and understood all the pages.

WHAT IS THE PURPOSE OF THE STUDY?

The Atmo gas sensing capsule is a medical device that tells us about the whole gut transit time food takes from ingestion to elimination. It also measures certain gases and transmits that to a receiver outside of the body. The Atmo gas sensing capsule is another way to tell us the rate at which things move through the gut and may also tell us about the activity of different gas-producing bacteria along the gut. The data collected from the Atmo gas sensing capsule will help us to understand your fermentation profile.

The study protocol has been reviewed by the University of Otago Ethics committee.

WHAT WILL MY PARTICIPATION IN THE STUDY INVOLVE?

You will be required to complete this investigation at 4 time points during the study period (day -7, 28, 43, 78), i.e. Phase1: baseline and post-intervention and Phase 2: baseline and post-intervention.

Pre- Visit Instructions

- You will be provided with list of food to consume and avoid the night before your visit (day -6, 27, 42, 77).
- You will be required to fast overnight for at least 9 hours prior to swallowing the capsule (water is allowed during this time)

Detailed instructions for each visit are as follows:

- A cereal bar and water will be provided on the day of investigation.
- The study investigator will wipe the ingestible capsule with an alcohol wipe.
- Swallow capsule immediately after the cereal bar (please do not bite the capsule!)
- Keep the data receiver within 1.5 m of the body until the capsule has been passed.
- The visit will take approximately 20 minutes and will be supervised by a study investigator.

Post visit instruction

- Refrain from eating for 6 hours after swallowing the capsule (but you'll be asked to consume small quantities of cold water at 30 min intervals for the first 2 h), then resume your diet as normal.
- The receiver must always be worn during the investigation, except when bathing and sleeping. Please keep receiver within 1.5 m of the body.
- Refrain from extreme sports and alcohol until the capsule is excreted.
- Keep a bowel movement and food diary on the receiver device.

The receiver can be removed when the passing of its corresponding capsule is confirmed. You will return the receivers to the study investigators at either 40 Stewart Street or University of Otago, Christchurch reception.

There are several ways to confirm the passing of these capsule:

- A loss of recording signal
- A Smartphone receiver device that will indicate its passing will be provided to you
- You can check visually whether the capsule has passed in your stools
- Study researchers can also confirm exit of the capsule once the receiver is returned

If there is still uncertainty whether the capsule has passed, please contact the research team. Please do not remove receiver until confirmation of capsule passing has been provided.

You should not feel any pain or discomfort when swallowing it or while the capsule moves through your gut. The capsule will be naturally passed during a bowel movement, usually within a few days.

Please notify the team if the capsule has not been passed after 5 days. In this event, the location of the capsule can be detected on anterior-posterior abdominal X-ray. Laxatives or other similar drugs can be employed to help clear the capsule from the body if necessary. Removal of the capsule can also be done through an endoscopy, but this step will only be undertaken if the other strategies have not worked. If any of these complications were to occur, the cost will be covered by study insurance.

WHAT ARE THE POSSIBLE BENEFITS AND RISKS OF THIS STUDY?

There is minimal risk associated with the Atmo gas sensing capsule. There may be a risk of the capsule passing through the gut and becoming stuck on the way. Bowel obstruction is another possible serious risk but has not been reported with the Atmo gas capsule. The experience from use of over 2 million Capsule Endoscopies indicates that this is an exceedingly rare event and only occurs in patients who have Crohn's disease who have narrowing (strictures) in the bowel. Most people do not experience gastrointestinal symptoms such as nausea, abdominal pain and vomiting during the test, but these symptoms are possible. The Atmo gas sensing capsules are standard capsule size, so most people will not have difficulty swallowing it.

What to do if you are adversely affected?

In the unlikely event of adverse effects, the medical staff of the Department of Medicine will be available to help. They can be contacted through the study researchers on 021 279 1519.

If the Atmo gas capsule has not been passed after 5 days of ingestion, please notify a member of the research team. In this event, the capsule may be identified by an x-ray at no cost.

WHAT ABOUT ANONYMITY AND CONFIDENTIALITY ?

All data collected as part of this study via the Atmo gas sensing capsule that can identify you will remain confidential. Your personal data will be re-identifiable using a code kept confidential by the researchers. Information linking your details to the code number and your personal details will be stored in locked filing cabinets in the Department of Medicine, University of Otago, Christchurch, or password protected on OneDrive. Access to these filing cabinets and OneDrive is restricted to the research staff. De-identified digital data from the Atmo gas sensing capsule is automatically stored in the password-protected cloud. Study investigators and Atmo Bioscience researchers in Australia will only have access to this de-identified data. We hold this information for a minimum of 10 years after publication of the results, after which hard copy documents will be shredded and digital data deleted.

De-identified data from this research may be used in closely related projects in the future. Any future research will be overseen by an appropriately constituted Human Research & Ethics Committee. As the data used for any future will be de-identified, results from these studies will not be made available to the participants. In any publication, information will be provided in a way that cannot identify you. If the results of the trial are published, anonymity will be kept.

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 which make decisions about the use of your information. There is a risk that overseas researchers may work with information in a for New Zealanders culturally inappropriate way.

WHAT ARE MY RIGHTS?

You can withdraw from the study at any stage. Please notify a member of the research team before withdrawing. This is to allow the researcher to inform you whether there are health risks or special requirements linked with withdrawing from the study.

Should you decide to leave the study, the researchers would like to keep the data collected to date. This is to ensure the results of the research can be measured properly. If you do not want them to do this, please inform them when leaving the study.

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

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

Research team:

Dr Simone Bayer, Jasjot Maggo, and Hwei Min Ng

HVN.GIstudies@gmail.com ☎ 021 279 1519

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

Phone: 0800 555 050 Fax: 0800 2 SUPPORT (0800 2787 7678)

Email: advocacy@hdc.org.nz

For **Māori health support** please contact:

Nga Ratonga Hauora Christchurch Hospital

Tel 3640 640 (Ext 86160)

You can also contact the University of Otago Human Ethics Committee (Health) that approved this study on:
Phone: +64 3 479 8256 Email: gary.witte@otago.ac.nz

National SCIENCE Challenges

HIGH-VALUE NUTRITION

Ko Ngā Kai Whai Painga

GUTS

Gastrointestinal Unit for Translational Studies

UNIVERSITY of OTAGO

Te Whare Wānanga o Otāgo

NEW ZEALAND

Consent Form

I have read (or have had read to me) and understand and the process involved in ingesting wireless medical device.
I understand the nature of the examination and the precautions necessary for this procedure.
I am satisfied with the answers I have been given about the procedure and I have a copy of this consent form and information sheet.
I consent to the use of de-identified data from this study for future studies.
I consent to my de-identified data being sent overseas.
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 who to contact if I have any questions about the study in general.

Declaration by participant:

I hereby consent to take part in this study.

Participant's name:

Signature: Date:

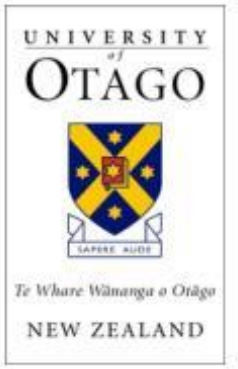
Declaration by member of research team:

I have given a verbal explanation of the research project to the participant, and have answered the participant's questions about it. I believe that the participant understands the study and has given informed consent to take part.

Researcher's Name:

Signature: Date:

Appendix B: Screening Questionnaire



Participant Screening Questionnaire

Bread Related Effect on MicrobiAl Distribution (BREAD Study)

Record ID: _____

Today's Date: _____

Date of Birth: _____

Please indicate the ethnicity that you **primarily** identify with:

☐ NZ European ☐ Māori ☐ Samoan ☐ Cook Island Māori ☐ Tongan ☐ Niuean

☐ Chinese ☐ Indian ☐ Other (e.g. Malaysian, Japanese): Please state _____

Height: _____

Weight: _____

Gender: ☐ Male ☐ Female ☐ Another gender ☐ Prefer not to say

Section A: Medical History

A1	Do you have any of the following conditions? Please select all that applies.	High blood pressure	☐
		Low blood pressure	☐
		Heart problems	☐
		Breathing problems	☐
		Cancer or tumour	☐
		Asthma	☐
		Migraines/ headaches	☐
		Diabetes type 1	☐
		Diabetes type 2	☐
		Kidney/ bladder problems	☐
		Hernia	☐
		Allergies	☐
		Blood disorder/ diseases	☐
		Neurological conditions e.g. multiple sclerosis, spinal cord injury, epilepsy	☐
		Chronic condition e.g. lupus, arthritis, hepatitis	☐
		Other (please identify) _____	☐
		Not applicable	☐
A1b	If so, **when** were you diagnosed with the condition and is it still ongoing?		

A2	Have you had any previous upper or lower GI surgery other than cholecystectomy or appendectomy?
	Yes / No
A2b	If so, **what** and **when** did you have this surgery?
A3	Do you have any alarm features associated with bowel habit such as recent changes in bowel habits (onset < three months), rectal bleeding, sudden weight loss, occult blood in stool, anaemia, anal fissures, bleeding haemorrhoids, and family history of gastrointestinal cancer at an early age?
	Yes / No
A3b	If so, **when** were you diagnosed with the condition and is it still ongoing?
A4	Do you have known systemic conditions (heart disease, kidney disease, diabetes, metabolic syndrome, psychological disorder) that could influence the gut directly or through medication use such as diabetes, opiate, or non-steroidal anti-inflammatory drug use?
	Yes / No
A4b	If so, **when** were you diagnosed with the condition and is it still ongoing?
A5	Please list any medications you are taking for your medical conditions. Please state the **dosage** and when you **started** taking this medication.
A6	Do you currently have any diagnosed gastrointestinal disorders and diseases such as chronic constipation, diarrhea, Irritable bowel syndrome, Inflammatory Bowel Disease (IBD), diverticulitis, coeliac disease, or previous bowel resection?
	Yes / No
A6b	If so, please specify:

A7	Have you had a COVID-19 diagnosis? If so, when were you diagnosed?		
	Yes / No		
A7b	Have you had a COVID-19 vaccination? If so, when did you receive it and what type of vaccination did you receive?		
	Yes / No		
A8	Are you **currently taking** laxatives? If yes, what/dose/frequency?		
	Yes / No		
A9	Are you **currently taking** any dietary and fibre supplements? This includes products such as Metamucil and Benefibre? If so, please state what type and how often.		
	Yes / No		
A10	Do you regularly consume probiotic yoghurt containing acidophilus such as Elivaě, Yakult or Activia? If yes, which brand and approximately how much do you consume/week?		
	Yes / No		
A11	Do you any have food sensitivities/allergies, in particular do you know if you are allergic to wheat, rice, latex, or blue food dye?		
	Yes / No		
A12	Do you smoke? *(Please tick one)*	Yes	☐
		No	☐
		Never	☐
A13	If you used to smoke, when did you quit smoking? *If you have never smoked, please tick Never in the previous question.*		
A14	Do you drink any alcohol? (please note that one standard drink equals one unit)?	Yes	☐
		No	☐
A15	If yes, approximately how many units/week?		

Section B: Dietary Fibre Intake

Fruit intake

On average, over the PAST YEAR, how many serves of FRUIT have you consumed?

The following are examples of 1 SERVE of FRUIT

1 medium piece of fruit- i.e. apple, banana, orange, tomato or pear OR
2 small pieces of fruit- i.e. apricot, kiwifruit or plum OR
1/2 cup fresh, frozen, tinned or stewed fruit- i.e. berries or tinned peaches OR
1 small handful of dried fruit- i.e. sultanas

	Never	Less than 1 serve per MONTH	1-3 serves per MONTH	1 serve per WEEK	2-4 serves per WEEK	5-6 serves per WEEK	1 serve per DAY	2 serves per DAY	3 serves per DAY	4 serves per DAY	5 serves per DAY	6 or more serves per DAY
Fruit	○	○	○	○	○	○	○	○	○	○	○	○

Vegetable intake

On average, over the PAST YEAR, how many serves of VEGETABLES have you consumed?

The following are examples of 1 SERVE of VEGETABLES

1 medium potato, kumara, yam, taro or carrot OR
1/2 cup cooked broccoli, green peas, corn, pumpkin or spinach OR
1 cup salad

	Never	Less than 1 serve per MONTH	1-3 serves per MONTH	1 serve per WEEK	2-4 serves per WEEK	5-6 serves per WEEK	1 serve per DAY	2 serves per DAY	3 serves per DAY	4 serves per DAY	5 serves per DAY	6 or more serves per DAY
Vegetables	○	○	○	○	○	○	○	○	○	○	○	○

Bread and Cereal intake

On average, over the PAST YEAR, how many serves of BREADS AND CEREALS have you consumed?

The following are examples of 1 SERVE of BREADS AND CEREALS

Wholegrain/wholemeal- 1 slice of bread, 1 small roll or 1 wrap OR
White- 2 slices of bread, 2 small rolls or 2 wraps OR
Rice/pasta- 1/2 cup cooked brown rice or wholemeal pasta or 1 cup cooked white rice or white pasta OR
Cereals- 1/2 cup cooked porridge or muesli, 1/3 cup All/Sultana/San Bran or 2 Weetbix

	Never	Less than 1 serve per MONTH	1-3 serves per MONTH	1 serve per WEEK	2-4 serves per WEEK	5-6 serves per WEEK	1 serve per DAY	2 serves per DAY	3 serves per DAY	4 serves per DAY	5 serves per DAY	6 or more serves per DAY
Breads and Cereals	○	○	○	○	○	○	○	○	○	○	○	○

Nut and Seed intake

On average, over the PAST YEAR, how many serves of NUTS AND SEEDS have you consumed?

The following are examples of 1 SERVE of NUTS AND SEEDS

2 tablespoons of peanut butter OR
1/3 cup (or a small handful) of nuts or seeds (e.g. cashew nuts, almonds, pistachio nuts, brazil nuts, macadamia nuts, hazel nuts, chia seeds, sunflower seeds, pumpkin seeds, sesame seeds)

	Never	Less than 1 serve per MONTH	1-3 serves per MONTH	1 serve per WEEK	2-4 serves per WEEK	5-6 serves per WEEK	1 serve per DAY	2 serves per DAY	3 serves per DAY	4 serves per DAY	5 serves per DAY	6 or more serves per DAY
Nuts and Seeds	○	○	○	○	○	○	○	○	○	○	○	○

Legume intake (e.g. beans, peas and lentils)

On average, over the PAST YEAR, how many serves of LEGUMES (e.g. BEANS, PEAS and LENTILS) have you consumed?

The following are examples of 1 SERVE of LEGUMES (e.g. BEANS, PEAS and LENTILS)

3/4 cup tofu OR
3/4 cup cooked legumes (e.g. kidney beans, chickpeas, green/brown/red lentils, hummus, baked beans, split peas, canned bean mix, broad beans, white/black beans)

	Never	Less than 1 serve per MONTH	1-3 serves per MONTH	1 serve per WEEK	2-4 serves per WEEK	5-6 serves per WEEK	1 serve per DAY	2 serves per DAY	3 serves per DAY	4 serves per DAY	5 serves per DAY	6 or more serves per DAY
Legumes-Beans, peas and lentils	○	○	○	○	○	○	○	○	○	○	○	○

Section C: Bread Intake

C1	Do you currently eat bread on a regular basis?		
	Yes / No		
C2	What type of bread, rolls or toast do you eat most of the time?	White	☐
		High Fibre White	☐
		Wholemeal (brown colour)	☐
		Light grain: Has some grains but soft to eat (e.g. Molenberg, Freya's, Ploughmans, And MacKenzie High Country)	☐
		Heavy grain – has some grains and a bit chewier (e.g. Vogels and Burgen)	☐
		Other (please specify):	
C3	Are you willing and able to consume provided intervention (in place of any usual bread) during the intervention period?		
	Yes / No		

Section D: Physical Activity

D1	Please indicate how much physical activity you participate in.	Inactive (little or no physical activity daily)	☐
		Active (Physically active for at least one hour per day. Noticeably increases the heart rate with a moderate amount of effort).	☐
		Intensely Physical (Includes at least 1 hour per day of physical activity which requires a large amount of effort and a substantial increase in heart rate)	☐

Section E: Other Information

E1	Is there any other information, not discussed, that you feel relevant regarding your health?		
E2	Are you post-menopausal? *(Please tick one)*	Not applicable (go to next page)	☐
		Yes	☐
		No	☐
		Other. Please specify: _____	
	If not, can you please state the date of your last menstrual period?		
E3	How would you describe your menstrual cycle?	Regular	☐
		Irregular	☐
E4	On average, can you please state the number of days in your cycle?		
E5	Is it possible that you may be currently or may wish to become pregnant in the next 24 weeks? *(Please tick one)*	Yes	☐
		No	☐
		Not Applicable	☐

E6	What is a preferred way for us to contact you?		
E7	How would you like to complete the questionnaires? This is so that we could either send you a link via email to complete online or send a paper format via post.	Electronically	☐
		Paper	☐

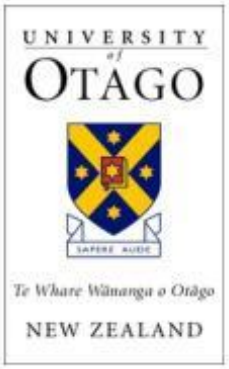
Declaration by participant:

I (print name) _____ have given true and complete information to the best of my knowledge.

Signature: _____ Date: _____

Researcher: _____ Date: _____

Appendix C: Daily Bowel Habit Diary



DAILY BOWEL HABIT DIARY

Participant ID

DATE: / /

TIME:

Thank you for completing the following questionnaire, which will ask you questions about your bowel movements.

Please complete the questionnaire in blue or black pen and mark the appropriate response as shown in the example on the next page.

The questionnaire should take you no more than 5 minutes to complete.

EXAMPLE	
1: Was the bowel movement complete? Did you feel that you had completely emptied your bowel when you had finished? That there was nothing left to pass.	Y / (N)

2. Was the bowel movement spontaneous? This means you went to the toilet naturally without taking any laxatives/medications or using any physical manoeuver to make you go.	Y / (N)
3: Did you use any manual manoeuvres to help you pass this bowel movement? This refers to pressing around your bottom or using something to help you pass the stool.	(Y) / N
4: Did you use any laxatives to help you pass the stool?	(Y) / N
5: If you did use laxatives can you please state which one?	**Metamucil**
6: How often did you use the laxatives?	**1**
7: Did you have to strain to pass this stool?	(Y) / N
8: Did you have a period/ menstruation today?	Y / N / (Not applicable)
9: Please rate your stool type according to the image shown: Please tick to specify the stool type.	

Type 1  Separate hard lumps, like sheep poo, hard to pass	**T1**	
Type 2  Cylindrical in shape, but lumpy	**T2**	
Type 3  Cylindrical-shape or snake like, but with cracks on the surface	**T3**	
Type 4  Cylindrical-shape or snake like, smooth and soft	**T4**	✓
Type 5  Soft blobs with clear-cut edges, easy to pass	**T5**	
Type 6  Fluffy pieces with ragged edges, mushy	**T6**	
Type 7  Watery, no solid pieces, entirely liquid	**T7**	

10: Did you see any blue dye in your stool?	Y / (N)

For the bowel movements you have had today, please answer the following questions.

1: Was the bowel movement complete? Did you feel that you had completely emptied your bowel when you had finished? That there was nothing left to pass.	Y / N	
2. Was the bowel movement spontaneous? This means you went to the toilet naturally without taking any laxatives/medications or using any physical manoeuver to make you go.	Y / N	
3: Did you use any manual manoeuvres to help you pass this bowel movement? This refers to pressing around your bottom or using something to help you pass the stool.	Y / N	
4: Did you use any laxatives to help you pass the stool?	Y / N	
5: If you did use laxatives can you please state which one?		
6: How often did you use the laxatives?		
7: Did you have to strain to pass this stool?	Y / N	
8: Did you have a period/ menstruation today?	Y / N / Not applicable	
9: Please rate your stool type according to the image shown: Please tick to specify the stool type.		

Type 1		Separate hard lumps, like sheep poo, hard to pass	T1
Type 2		Cylindrical in shape, but lumpy	T2
Type 3		Cylindrical-shape or snake like, but with cracks on the surface	T3
Type 4		Cylindrical-shape or snake like, smooth and soft	T4
Type 5		Soft blobs with clear-cut edges, easy to pass	T5
Type 6		Fluffy pieces with ragged edges, mushy	T6
Type 7		Watery, no solid pieces, entirely liquid	T7

10: Did you see any blue dye in your stool?	Y / N

Appendix D: Daily Bread Diary

UNIVERSITY of OTAGO
Te Whare Wānanga o Otāgo
NEW ZEALAND

GUTS
Gastrointestinal Unit for Translational Studies

National SCIENCE Challenges
HIGH-VALUE NUTRITION
Ko Ngā Kai Whai Painga

DAILY BREAD DIARY

Participant ID

DATE: / /

TIME:

Thank you for completing the following questionnaire, which will ask you questions about your bread consumption.

Please complete the questionnaire in blue or black pen and mark the appropriate response as shown in the example on the next page.

The questionnaire should take you no more than 3 minutes to complete.

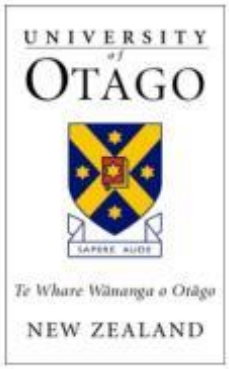
EXAMPLE			
1. Did you eat your assigned bread? Either Bread A or Bread B	○ **Y / N**		
If you did not, please state the reason.			
2. When and how many slices did you eat?	**Breakfast**	**X**	**3**
	Morning Tea		

	Lunch		
	Afternoon Snack		
	Dinner		
	Supper		
3. Did you toast your bread?	○ Y / N		
4. If yes, how long did you toast your bread?	< 1 min		
	2 to 3 mins	X	
	>3 mins		
5. Did you eat any extra commercially stored bought sliced bread?	○ Y / N		
If you did, please state: Type of bread (white, wholemeal, multigrain etc.)	White		
Brand of the bread (Vogel, Molenburg, Value etc.)	Value		
The number of extra sliced bread consumed	1		

For the bread you have had today, please answer the following questions.

1. Did you eat your assigned bread? Either Bread A or Bread B	**Y / N**		
If you did not, please state the reason.			
2. When did you eat your assigned bread?	**Breakfast**		
	Morning Tea		
	Lunch		
	Afternoon Snack		
	Dinner		
	Supper		
3. Did you toast your bread?	**Y / N**		
4. If yes, how long did you toast your bread?	**< 1 min**		
	2 to 3 mins		
	>3 mins		
5. Did you eat any extra commercially stored bought sliced bread?	**Y / N**		
If you did, please state: **Type of bread** (white, wholemeal, multigrain etc.)			
Brand of the bread (Vogel, Molenburg, Value etc.)			
The number of extra sliced bread consumed			

Appendix E: Modified Hunter New England Health Survey (ModHNES) and SF-12v2® Health Survey



Participant ID

MODIFIED HUNTER NEW ENGLAND HEALTH SURVEY

DATE / /

How to complete this survey

INSTRUCTIONS:

- Please use a black/blue pen or pencil.
- Please erase or correct mistakes.
- To answer each question you just need to tick ☑ the appropriate response box.

Example: **In general, would you say your health is:** *(choose one only)*

Excellent	☐
Very good	☐
Good	☑ You would tick this one if you think your health is good
Fair	☐
Poor	☐

If you have any questions or need help filling in this survey please call us on **021-279-1519** or email

HVN.GIStudies@gmail.com

Thank you for your help with this important research!

Section A.

Your Health and Well-Being

This questionnaire asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. ***Thank you for completing this questionnaire!***

For each of the following questions, please mark an ☒ in the one box that best describes your answer.

1. **In general, would you say your health is:**

Excellent	Very good	Good	Fair	Poor
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

2. **The following questions are about activities you might do during a typical day. Does <u>your health now limit you</u> in these activities? If so, how much?**

	Yes, limited a lot	Yes, limited a little	No, not limited at all
a. <u>Moderate activities</u>, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	☐ 1	☐ 2	☐ 3
b. Climbing <u>several</u> flights of stairs	☐ 1	☐ 2	☐ 3

(IQOLA SF-12v2 Standard, English (New Zealand), 7/03)

3. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Accomplished less than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. Were limited in the kind of work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

4. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Accomplished less than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. Did work or other activities less carefully than usual	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

5. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

Not at all	A little bit	Moderately	Quite a bit	Extremely
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

6. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks…

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Have you felt calm and peaceful?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. Did you have a lot of energy?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. Have you felt downhearted and depressed?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

7. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

All of the time	Most of the time	Some of the time	A little of the time	None of the time
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Thank you for completing these questions!

Section B.

Now we would like to ask you some specific questions about your health.

Please mark the appropriate response box with a tick (for example ☑).

B1	Have you ever been diagnosed with diabetes by a doctor? *(Please tick one box only)*	No (Please go to the question B5)	☐
		Yes	☐
B2	In addition to diet, what sort of treatment are you following for diabetes? *(Please tick one box only)*	Insulin injections only	☐
		Insulin injections and blood sugar lowering tablets	☐
		Blood sugar lowering tablets only	☐
		I control my diabetes by diet alone	☐
B3	How long have you had diabetes? _____ Years _____ months		
B4	How would you rate the control of your blood sugar levels in general? *(Please tick one box only)*	Very good	☐
		Good	☐
		Average	☐
		Poor	☐
		Very poor	☐
B5	In the last 2 WEEKS, have you walked for sport, recreation or fitness? *(Please tick one box only)*	No (Please go to B8)	☐
		Yes	☐
B6	How many times did you walk in the last 2 WEEKS? _____ times		
B7	What was the TOTAL AMOUNT of time you spent walking in the last 2 WEEKS?	Not applicable (Please go to B8)	☐
		_____ hours _____ minutes	
B8	In the last 2 WEEKS, did you do any exercise which caused a MODERATE increase in your heart rate or breathing, that is, MODERATE exercise?	No (Please go to B11)	☐
		Yes	☐
B9	How many times did you do any MODERATE exercise in the last 2 WEEKS?	Not applicable (Please go to B11)	☐
		_____ times	
B10	What was the TOTAL AMOUNT of time you spend doing MODERATE exercise in the last 2 WEEKS? _____ hours _____ minutes		
B11	In the last 2 WEEKS, did you do any OTHER exercise which caused a LARGE increase in your heart rate or breathing that is, VIGOROUS exercise? *(Please tick one box only)*	No (Please go to C1)	☐
		Yes	☐
B12	How many times did you do any VIGOROUS exercise in the last 2 WEEKS? _____ times		
B13	What was the TOTAL AMOUNT of time you spend doing VIGOROUS exercise in the last 2 WEEKS? _____ hours _____ minutes		

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Section C.

Now we would like to ask about your lifestyle.

These questions ask you about smoking and alcohol.

C1	Over your lifetime, would you have smoked at least 100 cigarettes or a similar amount of tobacco? *(Please tick one box only)*	No (Please got to C5)	☐
		Yes	☐
C2	How often do you NOW smoke cigarettes, cigars, pipes or other tobacco products? *(Please tick one box only)*	Not at all	☐
		Less often than weekly	☐
		At least weekly (not daily) Number per week _____	☐
		Daily Number per day _____	☐

C3 If you don't currently smoke, when did you finally stop **smoking**?

_____ weeks ago or _____ months ago or _____ years

C4 At what age did you first start smoking? _____ years old

C5	Which of the following best describes YOU? *(Please tick one box only)*	I am a life-long NON-drinker (Please got to D1)	☐
		I currently drink alcohol (Please go to C6)	☐
		I used to drink alcohol (Please go to D1)	☐

The next Question refers to a standard drink:

Beer 1 stubby or can (375ml or 12oz, or about 1 ½ "pots" or "middies")

Wine 1 medium glass (125ml or 4oz,)

Port or sherry 1 small glass (60ml or 2oz.)

Spirits/liqueur 1 nip (30 ml or 1 oz.)

C6 On how many DAYS in a typical week do you drink ANY alcohol? *(Please tick one box only)*

None ☐ Less than 1 day per week ☐ 1 day ☐

2 days ☐ 3 days ☐ 4 days ☐ 5 days ☐ 6 days ☐ 7 days ☐

C7 How many alcoholic drinks do you usually have each week? *(Please tick one box only)*

None ☐ Less than 1 ☐ 2-4 ☐

5-6 ☐ 7-13 ☐ 14-20 ☐ 21-27 ☐ 28 or more ☐

Section D.

Now we would like to finish off by asking you some general questions about yourself.

Please mark the appropriate response box with a tick (for example ☑).

D1	What gender do you identify with?		
		Prefer not to answer	☐

D2	What is your highest level of educational training, or equivalent?	Postgraduate qualifications	☐
		University graduate (3 years or more)	☐
		Completed Polytechnic or equivalent certificate/associate diploma, trades apprenticeship, or 2 years at university	☐
		NCEA Level 3 / University Bursary	☐
		Completed Year 11 (Fifth Form/School Certificate/NCEA level 1)	☐
		Some years at High School	☐
		Primary School only	☐

D3	May we contact you in the future about further research?	Yes	☐
		No	☐

D4	What is the date that you filled in this survey? *(Please write down)*
	_____/ Day_____/ month_____/ year

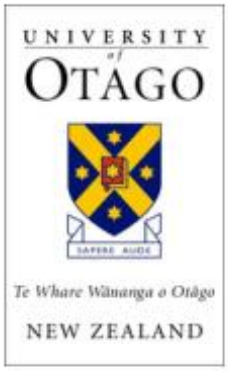
THANK YOU FOR TAKING THE TIME TO COMPLETE THIS SURVEY.

Your support is greatly appreciated.

Please bring your completed questionnaires with you when meeting the research assistants for sample collection.

Thank you!

Appendix F: Economic Living Standards Index- Short Form ($ELSI_{SF}$)



Economic Living Standards Index

Participant ID

DATE / /

Thank you for completing the following questionnaire, which will ask you questions about your Economic Living Standards.

Please complete the questionnaire in blue or black pen and mark the appropriate response with a tick ☑

The questionnaire should take you no more than 10 minutes to complete.

GSRS Survey – ANCIENT Study Page **51 of 7**

For the following items, please indicate whether you have (or have access to) the item or not by ticking one of the four options. Tick the first box if you have the item. Tick box 2 if you don't have the item because you don't want it. Tick box 3 if you don't have the item because of its cost. Tick box 4 if you don't have the item because of some reason other than not wanting it or cost.

		Yes-have it	No-because I don't want it	No-because of the cost	No-for some other reason
1	Telephone	☐	☐	☐	☐
2	Washing Machine	☐	☐	☐	☐
3	Heating available in all main rooms	☐	☐	☐	☐
4	A good pair of shoes	☐	☐	☐	☐
5	A best outfit for special occasions	☐	☐	☐	☐
6	Personal Computer	☐	☐	☐	☐
7	Home contents insurance	☐	☐	☐	☐

For the following activities, please indicate whether you do the activity or not by ticking one of the following options. Tick box 1 if you do the activity. Tick box 2 if you don't do the activity because you don't want to. Tick box 3 if you don't do the activity because of the cost. Tick box 4 if you don't do the activity because of some reason other than not wanting to or cost.

		Yes-do it	No-because I don't want to	No-because of the cost	No-for some other reason
8	Give presents to family or friends on birthdays, Christmas or other special occasions	☐	☐	☐	☐
9	Visit the hairdresser once every three months	☐	☐	☐	☐
10	Have holidays away from home every year	☐	☐	☐	☐
11	Enough room for family to stay the night	☐	☐	☐	☐
12	Have a holiday overseas at least every three years	☐	☐	☐	☐
13	Have a night out at least every fortnight	☐	☐	☐	☐
14	Have family or friends over for a meal at least once a month	☐	☐	☐	☐

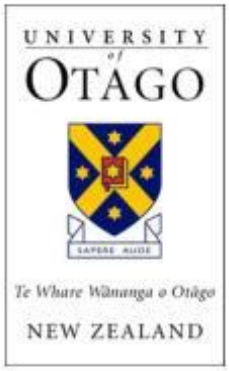
The following are a list of things some people do to help keep costs down. In the last 12 months, have you done any of these things not at all, a little, or a lot? Tick the answer that best applies.

		Not at all	A little	A lot
15	Gone without fresh fruit and vegetables to help keep down costs	☐	☐	☐
16	Continued wearing clothing that was worn out because you can't afford a replacement	☐	☐	☐
17	Put off by buying clothes for as long as possible to help keep down costs	☐	☐	☐
18	Stayed in bed longer to save on heating costs	☐	☐	☐
19	Postponed or put off visits to the doctor to help keep down costs	☐	☐	☐
20	NOT picked up prescription to help keep costs down	☐	☐	☐
21	Spent less time on hobbies than you would like to keep down costs	☐	☐	☐
22	Done without or cut down on trips to the shops or other local places to help keep down costs	☐	☐	☐

The following questions are about your material standard of living – the things that money can buy. Your material standard of living does NOT include your capacity to enjoy life.

23	Generally, how would you rate your material standard of living?	☐ High	☐ Fairly High	☐ Medium	☐ Fairly Low	☐ Low
24	Generally, how satisfied are you with your current material standard of living?	☐ Very satisfied	☐ Satisfied	☐ Neither satisfied nor dissatisfied	☐ Dissatisfied	☐ Very Dissatisfied
25	How well does you (and your partners combined) total income meet your everyday needs for such things as accommodation, food, clothing and other necessities? Would you say you have?	☐ More than enough	☐ Enough	☐ Just enough	☐ Not enough	

Appendix G: Gastrointestinal Symptom Rating Scale (GSRS)



Participant ID

GASTROINTESTINAL SYMPTOM RATING SCALE (GSRS)

DATE / /

INSTRUCTIONS:

- Please use a black/blue pen or pencil.
- Please erase or correct mistakes.
- To answer each question, you just need to tick ☑ the appropriate response box.

Example:

Have you been bothered by PAIN OR DISCOMFORT IN YOUR UPPER ABDOMEN OR THE PIT OF YOUR STOMACH during the past week?

- ☐ No discomfort at all
- ☐ Minor discomfort
- ☑ Mild discomfort
- ☐ Moderate discomfort
- ☐ Moderately severe discomfort
- ☐ Severe discomfort
- ☐ Very severe discomfort

If you have any questions or need help filling in this survey please call us on **021-279-1519** or email HVN.GIStudies@gmail.com

GSRS Survey – ANCIENT Study Page **57 of 7**

Thank you for your help with this important research!

THE GASTROINTESTINAL SYMPTOM RATING SCALE (GSRS)

Please read this first:

This survey contains questions about how you have been feeling and what it has been like **DURING THE PAST WEEK**. Mark the choice that best applies to you and your situation with a tick.

1. Have you been bothered by PAIN OR DISCOMFORT IN YOUR UPPER ABDOMEN OR THE PIT OF YOUR STOMACH during the past week?

- ☐ No discomfort at all
- ☐ Minor discomfort
- ☐ Mild discomfort
- ☐ Moderate discomfort
- ☐ Moderately severe discomfort
- ☐ Severe discomfort
- ☐ Very severe discomfort

2. Have you been bothered by HEARTBURN during the past week? (By heartburn we mean an unpleasant stinging or burning sensation in the chest.)

- ☐ No discomfort at all
- ☐ Minor discomfort
- ☐ Mild discomfort
- ☐ Moderate discomfort
- ☐ Moderately severe discomfort
- ☐ Severe discomfort
- ☐ Very severe discomfort

3. Have you been bothered by ACID REFLUX during the past week? (By acid reflux we mean the sensation of regurgitating small quantities of acid from the stomach up to the throat.)

- ☐ No discomfort at all
- ☐ Minor discomfort
- ☐ Mild discomfort
- ☐ Moderate discomfort
- ☐ Moderately severe discomfort
- ☐ Severe discomfort
- ☐ Very severe discomfort

4. Have you been bothered by HUNGER PAINS in the stomach during the past week? (This hollow feeling in the stomach is associated with the need to eat between meals.)

- ☐ No discomfort at all
- ☐ Minor discomfort
- ☐ Mild discomfort
- ☐ Moderate discomfort
- ☐ Moderately severe discomfort
- ☐ Severe discomfort
- ☐ Very severe discomfort

5. Have you been bothered by NAUSEA during the past week? (By nausea we mean a feeling of sickness that may lead to retching and vomiting.)

- ☐ No discomfort at all
- ☐ Minor discomfort
- ☐ Mild discomfort
- ☐ Moderate discomfort
- ☐ Moderately severe discomfort
- ☐ Severe discomfort
- ☐ Very severe discomfort

6. Have you been bothered by RUMBLING in your stomach during the past week? (Rumbling refers to vibrations or noise in the stomach.)

- ☐ No discomfort at all
- ☐ Minor discomfort
- ☐ Mild discomfort
- ☐ Moderate discomfort
- ☐ Moderately severe discomfort
- ☐ Severe discomfort
- ☐ Very severe discomfort

7. Has your stomach felt BLOATED during the past week? (Feeling bloated refers to swelling often associated with a sensation of gasses in the stomach.)

- ☐ No discomfort at all
- ☐ Minor discomfort
- ☐ Mild discomfort
- ☐ Moderate discomfort
- ☐ Moderately severe discomfort
- ☐ Severe discomfort
- ☐ Very severe discomfort

8. Have you been bothered by BELCHING during the past week? (Belching refers to the release of wind from the stomach via the mouth, often associated with easing a bloated feeling.)

- ☐ No discomfort at all
- ☐ Minor discomfort
- ☐ Mild discomfort
- ☐ Moderate discomfort
- ☐ Moderately severe discomfort
- ☐ Severe discomfort
- ☐ Very severe discomfort

9. Have you been bothered by BREAKING WIND during the past week? (Breaking wind refers to the need to release air or gas from the bowel, often associated with easing a bloated feeling.)

- ☐ No discomfort at all
- ☐ Minor discomfort
- ☐ Mild discomfort
- ☐ Moderate discomfort
- ☐ Moderately severe discomfort
- ☐ Severe discomfort
- ☐ Very severe discomfort

10. Have you been bothered by CONSTIPATION during the past week? (Constipation refers to a reduced ability to empty the bowels.)

- ☐ No discomfort at all
- ☐ Minor discomfort
- ☐ Mild discomfort
- ☐ Moderate discomfort
- ☐ Moderately severe discomfort
- ☐ Severe discomfort
- ☐ Very severe discomfort

11. Have you been bothered by DIARRHOEA during the past week? (Diarrhoea refers to a too frequent emptying of the bowels.)

- ☐ No discomfort at all
- ☐ Minor discomfort
- ☐ Mild discomfort
- ☐ Moderate discomfort
- ☐ Moderately severe discomfort
- ☐ Severe discomfort
- ☐ Very severe discomfort

12. Have you been bothered by LOOSE STOOLS during the past week? (If your stools (motions) have been alternately hard and loose, this question only refers to the extent you have been bothered by the stools being loose.)

- ☐ No discomfort at all
- ☐ Minor discomfort
- ☐ Mild discomfort
- ☐ Moderate discomfort
- ☐ Moderately severe discomfort
- ☐ Severe discomfort
- ☐ Very severe discomfort

13. Have you been bothered by HARD STOOLS during the past week? (If your stools (motions) have been alternately hard and loose, this question only refers to the extent you have been bothered by the stools being hard.)

- ☐ No discomfort at all
- ☐ Minor discomfort
- ☐ Mild discomfort
- ☐ Moderate discomfort
- ☐ Moderately severe discomfort
- ☐ Severe discomfort
- ☐ Very severe discomfort

14. Have you been bothered by an URGENT NEED TO PASS YOUR MOTIONS during the past week? (This urgent need to go to the toilet is often associated with a feeling that you are not in full control.)

- ☐ No discomfort at all
- ☐ Minor discomfort
- ☐ Mild discomfort
- ☐ Moderate discomfort
- ☐ Moderately severe discomfort
- ☐ Severe discomfort
- ☐ Very severe discomfort

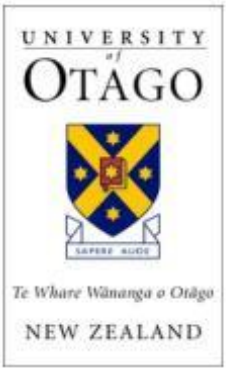
15. When going to the toilet during the past week, have you had the SENSATION OF NOT COMPLETELY EMPTYING THE BOWELS? (This feeling of incomplete emptying means that you still feel a need to pass your motions despite having exerted yourself to do so.)

- ☐ No discomfort at all
- ☐ Minor discomfort
- ☐ Mild discomfort
- ☐ Moderate discomfort
- ☐ Moderately severe discomfort
- ☐ Severe discomfort
- ☐ Very severe discomfort

PLEASE CHECK THAT ALL QUESTIONS HAVE BEEN ANSWERED!

THANK YOU FOR YOUR CO-OPERATION.

Appendix H: Three-Day Food Diary



Patient ID

BREAD Study

Food diary

Different eating patterns have an effect on people's health. To help us understand these eating patterns we would like you to complete this estimated food diary. You need record all food and drink that you consume on **3 non-consecutive days**.

If you have any questions regarding this food diary, please contact the Research Team at HVN.GIstudies@gmail.com or 021-279-1519.

Please start your diary on: _____

6

How to fill in your diary

How to fill in your diary

Below is a step-by-step guide on how to fill in your food diary. It is very important that you do not change what you normally eat or drink just because you are keeping a diary so that we get a true picture of what you eat and drink. Try to fill in the diary each time you have something to eat or drink rather than leaving it until the end of the day so that you dont forget anything.

Step 1: When

Write down the exact time you ate or drank something. So, for example, if you had breakfast at 7.30am, write in "7.30am".

Step 2: Where

Please record where you were when you ate something. The next column along in the food diary is for you to write in where you were when you ate or drank something. This could be:

At home – e.g. in the kitchen, in bed
Away – e.g. in the street, in the car/on a bus, at a friend's or relative's house,
In a café/ restaurant (please specify McDonalds, Pizza Hut, etc.),
At work – e.g. in canteen, in lunchroom, at your desk.

Step 3: Who with

In the next column in the food diary, please write down who you were with when you ate or drank something. For example, you might have been alone, with family or with friends. Experts have shown that by thinking who you were with during the day can help you to remember what you have eaten. We do not use this data in our research, it is just there to aid your memory.

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Step 4: Food and drink

The next step in the food diary is to describe what you ate or drank. The more details you are able to give about the food and drink you have consumed, the better we will be able to estimate your nutrient intake. Include any extras like sugar and milk in your tea or cereal, butter or other spreads on your bread and sauces such as tomato sauce and mayonnaise. Do not forget to include drinking water.

Step 5: Brand and details

It would also help us if you can write down the brand name of any foods or drinks if you know it (e.g Watties, Pams, Arnotts). If convenient, staple the wrapper to the back page of this book.

For breakfast cereals, as well as the brand name, please write down the name of the cereal (e.g. Coco Pops, Cornflakes, Sanitarium toasted museli: golden oats and fruit).

For sandwiches, please describe the type of bread used, how many slices of bread were used and give details of the filling.

For salad or mixed vegetables, please describe what is in it (eg. 1 lettuce leaf, half a tomato,6 slices of cucumber).

For pizza, please describe the topping (e.g. cheese and tomato, ham and pineapple).

Step 6: Preparation and cooking

If you know the cooking method used (e.g. roast, baked, boiled, fried) please write it down in this section.

Step 7: Quantity

In the next column, please write in the size of the portion of food or drink you had. For drinks, you can specify glass, cup, or mug or bottle/can size. Other descriptions include: packet (e.g. for crisps), number (e.g. for biscuits), slice (e.g. for cake, pizza), teaspoon (e.g. for sugar), tablespoon (e.g. for tomato sauce, peas), cupful (e.g. for cooked pasta or rice), handful (e.g. for nuts, grapes, berries), package weights (e.g. 150g Fresh and Fruity yoghurt). On the next page you will find some more information on how to describe the food and drink that you consume.

If you have **kitchen scales** it is helpful to weigh foods and record these amounts.

For **mixed food dishes and recipes** it may be easier to list the total ingredients, then describe the proportion of this recipe that you consumed.

e.g. 1/3 of recipe 1

Recipe example Creamy tuna pasta (recipe 1)

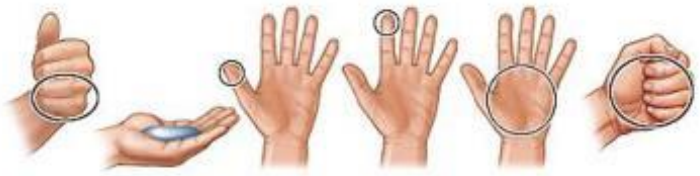
250g	Diamond spiral pasta
½ cup	Oxo chicken stock, pre-mixed with water
¼ cup	Chopped parsley
2 cups	Sliced button mushrooms
220g	John West tuna canned in oil, liquid drained
1 cup	Carnation evaporated skim milk
1 tablespoon	Parmesan cheese, dried
¼ teaspoon	Freshly ground black pepper

I had one third of this recipe

If you make your food from separate ingredients then you can write the recipes down in the recipe list at the back of this diary.

Please write down all the ingredients for each recipe (including brand names, amounts and preparation or cooking details). Indicate the proportion of the recipe you consumed.

Don't forget about any drinks that you have between meals e.g. tea, coffee, wine, beer, orange juice.



½ cup (125 mL)	1 oz (30 g)	1 Tbsp (15 mL)	1 tsp (5 mL)	2 ½ oz (75 g)	1 cup (250 mL)

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How to describe your food and drink using household measures

Below are some suggestions on how to describe certain food and drink items together with their household measures.

Food	Description of food or drink and brand	Household measure
Bacon	Shoulder or streaky; fried or grilled rashers, smoked or unsmoked	Number
Bread	Type of bread, eg. white, brown, wholemeal, granary, French stick, ciabatta, currant. Description of slice e.g. sandwich, toast	Number of slices
Canned drinks	Type, brand name For example: 335ml can Diet Coca Cola	Number or full or half can
Crisps	Type, brand name e.g. 30g Rashuns	Packet weight
Fruit	Type and size of fruit e.g. large Granny Smith apple For tinned fruit; slices/ halves etc in juice or syrup	Number of pieces or tablespoons
Jams	Type, brand name e.g. Pam's strawberry jam	Teaspoons, heaped or flat
Milk	Type; full cream, trim, semi-trim	Pints, glasses or cups
Oil	Type eg canola oil, sunflower oil, corn oil, olive oil Brand name e.g. Pam's olive oil	Tablespoons
Prepacked foods eg pies, biscuits, confectionery	Full name of product including brand name. For example: Bird's Eye fish fingers. Keep the package.	Number
Sandwiches	Describe fully if homemade or if bought; Full name, place of purchase and price, describe bread as above and note loaf size.	Number of slices of bread or number of rolls
Spreads on bread or toast	Type e.g. butter, low fat spread, rice bran oil spread, canola spread, reduced fat canola spread, Weightwatchers spread. Full description, and brand name Keep the package	Number of teaspoons or thinly, average or thickly spread
Sugar	Type e.g. caster, rich brown, white	Teaspoons, heaped or flat
Sweets, chocolate and snack bars	Name, size (weight) and price (if known) For example: king size Mars bar 99c Keep the wrapper	Weight of bar or number of sweets
Takeaways	Describe in full, give name of restaurant For example: One scoop chips, The High Street chip shop. Standard chicken chow mein, Kwang Chow	Portion size and price
Vegetables	Type; fresh, frozen, tinned or dried Brand name	Tablespoons, full or heaped

Adapted from NUGENOB study (www.nugenob.com)

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Sample record sheet

Please record all food and drink consumed during the whole day, including snacks and water.
Remember to report any additions to each food and drink, such as milk, sugar, salt, sauce or spreads.

Meal	When	Where	Who with	Food or Drink	Brand and details	Preparation/ Cooking	Quantity
A	8 am	In bed	alone	Gourmet muffin	New World – double chocolate	None	1
				Coffee Sugar Milk	Nescafe instant Sugar Green top	Hot water added	1 heaped teaspoon in a mug 1 heaped teaspoon 1/8th of a mug
B	10 am	Kitchen	Family	Tea	Twinings Peppermint	Hot water added	1 mug, no milk or sugar
				Biscuits	Tim Tam Double Chocolate	None	2
C	12pm			Creamy tuna pasta	Homemade recipe 1	Pasta boiled in water	1/3 recipe
				French bread stick	Bought–New World		6cm long
				Margarine	Pams–Canola low salt		1 level tsp
				Chicken breast	Skin and bone removed	Fried in olive oil	1 medium chicken breast
				Olive oil	Luppi	fried	½ tbsp
				Cherry tomatoes		raw	2
				Orange juice	McCoy, unsweetened		200ml
D	5.30pm	Mc Donalds	Son	Burger Fries Diet Coke	Mc Donalds Big Mac (no pickles)		1 Large Large
E	6.30pm	Home	Friends	Beer	Monteiths Radler		2 bottles

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				Toast	Vogels Rice and Rye	Toasted	2 slices
				Margarine	Pams–Canola low salt		1 level tsp

Please record **brand names** e.g. McCoy
Please use **household measures** to describe amounts of food such as margarine, butter and milk e.g. teaspoons (tsp), tablespoons (tbsp), cups

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Diet Diary Day 1 **Date**_____

Meal	When	Where	Who with	Food or Drink	Brand and details	Preparation/ Cooking	Quantity

Please state if you have **any sauce/ gravy, toppings on cakes/muffins/pizza etc.**
Please record **brand names**, and if possible, **staple/ attach wrappers of food/drink to the back page of this book**
Refer to **page 4 and 5** for more information on how to describe your food and drink using household measures.

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Diet Diary Day 1 continued **Date**_____

Meal	When	Where	Who with	Food or Drink	Brand and details	Preparation/ Cooking	Quantity

Please state if you have **any sauce/ gravy, toppings on cakes/muffins/pizza etc.**
Please record **brand names**, and if possible, **staple/ attach wrappers of food/drink to the back page of this book**
Refer to **page 4 and 5** for more information on how to describe your food and drink using household measures.

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Diet Diary Day 2 **Date**_____

Meal	When	Where	Who with	Food or Drink	Brand and details	Preparation/ Cooking	Quantity

Please state if you have **any sauce/ gravy, toppings on cakes/muffins/pizza etc.**
Please record **brand names**, and if possible, **staple/ attach wrappers of food/drink to the back page of this book**
Refer to **page 4 and 5** for more information on how to describe your food and drink using household measures.

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Diet Diary Day 2 continued **Date**_____

Meal	When	Where	Who with	Food or Drink	Brand and details	Preparation/ Cooking	Quantity

Please state if you have **any sauce/ gravy, toppings on cakes/muffins/pizza etc.**
Please record **brand names**, and if possible, **staple/ attach wrappers of food/drink to the back page of this book**
Refer to **page 4 and 5** for more information on how to describe your food and drink using household measures.

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Diet Diary Day 3 **Date**_____

Meal	When	Where	Who with	Food or Drink	Brand and details	Preparation/ Cooking	Quantity

Please state if you have **any sauce/ gravy, toppings on cakes/muffins/pizza etc.**
Please record **brand names**, and if possible, **staple/ attach wrappers of food/drink to the back page of this book**
Refer to **page 4 and 5** for more information on how to describe your food and drink using household measures.

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Day 3 continued **Date**_____

Meal	When	Where	Who with	Food or Drink	Brand and details	Preparation/ Cooking	Quantity

Please state if you have **any sauce/ gravy, toppings on cakes/muffins/pizza etc.**
Please record **brand names**, and if possible, **staple/ attach wrappers of food/drink to the back page of this book**
Refer to **page 4 and 5** for more information on how to describe your food and drink using household measures.

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Recipes

Please write down the ingredients of your recipes in this section.

Recipe Number	Food or Drink	Brand and Details	Quantity
Recipe number	**Food or Drink**	**Brand or Details**	**Quantity**

1. Are there any special reasons why this week may differ from 'normal' in terms of household food (for example a child's birthday party or other family celebration)? Please circle either Yes or No

No
Yes (Please state reason)

_____

_____

2. **Please let us know how you take your coffee or tea:**

☐ I don't drink tea or coffee

☐ Brand/strength eg. Maccona, medium _____

☐ With milk
Approx. _____ tablespoons;
and _____ brand

☐ Without milk

☐ With Sugar _____ teaspoons

☐ No sugar

☐ Other; please describe _____

Please check that you have **filled in your diary for all 2 non-consecutive weekdays and 1 weekend, including the date.**

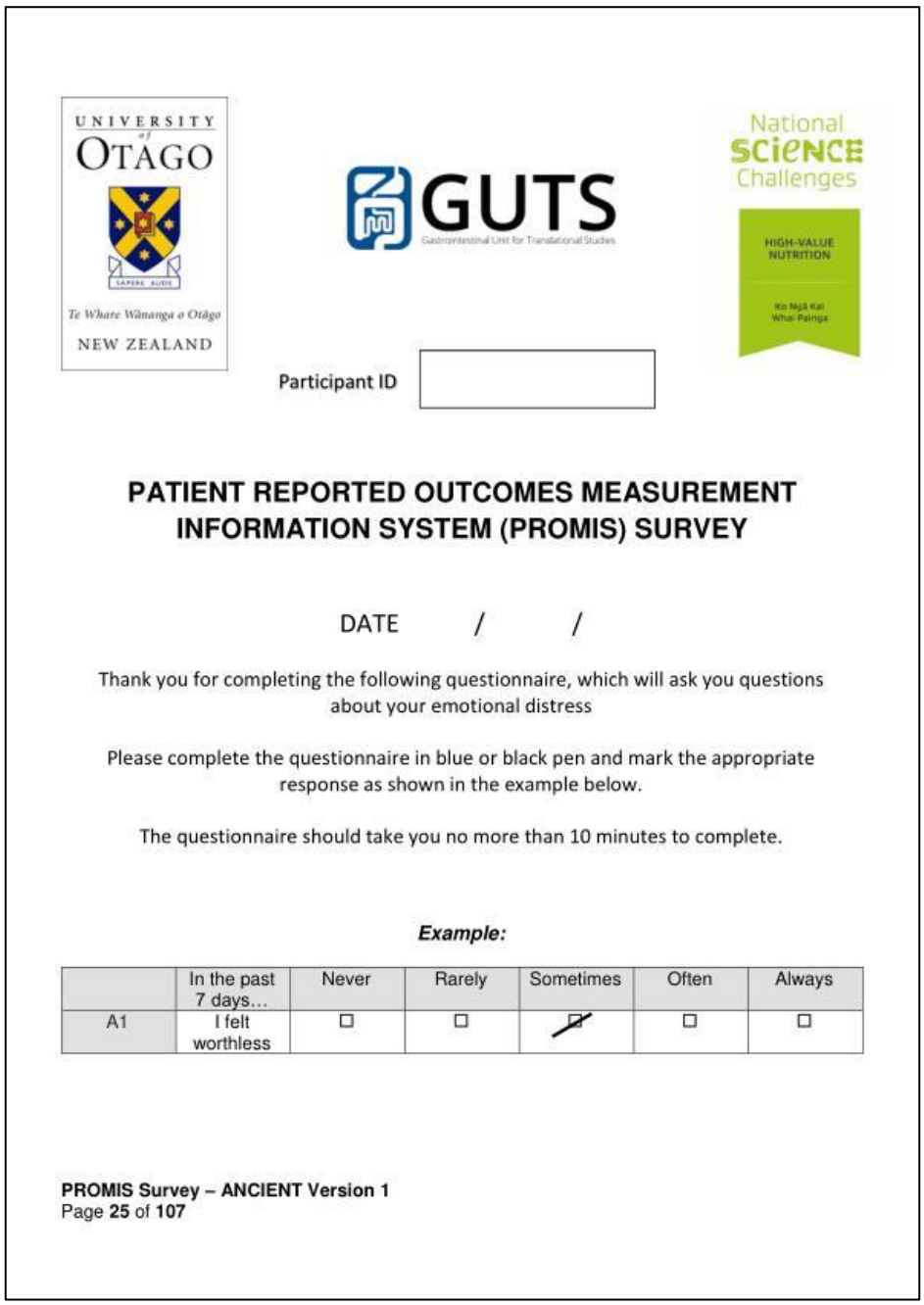
Don't forget to include any:

- **Drinks** e.g. tea, coffee, wine, beer, orange juice, soft drinks, water
- **Snacks between meals** e.g. biscuits, crisps, peanuts, slices, muffins
- **Lollies or sweets**

Please staple wrappers of food or drinks consumed here

Thank You!

Appendix I: Patient-Reported Outcomes Measurement Information System (PROMIS) Survey



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Gastrointestinal Unit for Translational Studies

National SCIENCE Challenges
HIGH-VALUE NUTRITION
Ko Ngā Kai Whai Painga

Participant ID

PATIENT REPORTED OUTCOMES MEASUREMENT INFORMATION SYSTEM (PROMIS) SURVEY

DATE / /

Thank you for completing the following questionnaire, which will ask you questions about your emotional distress

Please complete the questionnaire in blue or black pen and mark the appropriate response as shown in the example below.

The questionnaire should take you no more than 10 minutes to complete.

Example:

	In the past 7 days…	Never	Rarely	Sometimes	Often	Always
A1	I felt worthless	☐	☐	☒	☐	☐

PROMIS Survey – ANCIENT Version 1
Page **25** of **107**

Section A.

Emotional Distress

*In this section please respond to each option by **marking one box** per row on how you felt over the past 7 days.*

	In the past 7 days…	Never	Rarely	Sometimes	Often	Always
A1	I felt worthless	☐	☐	☐	☐	☐
A2	I felt fearful	☐	☐	☐	☐	☐
A3	I felt helpless	☐	☐	☐	☐	☐
A4	I found it hard to focus on anything other than my anxiety	☐	☐	☐	☐	☐
A5	I felt depressed	☐	☐	☐	☐	☐
A6	My worries overwhelmed me	☐	☐	☐	☐	☐
A7	I felt hopeless	☐	☐	☐	☐	☐
A8	I felt uneasy	☐	☐	☐	☐	☐
A9	I felt like a failure	☐	☐	☐	☐	☐
A10	I felt nervous	☐	☐	☐	☐	☐
A11	I felt unhappy	☐	☐	☐	☐	☐
A12	I felt like I needed help for my anxiety	☐	☐	☐	☐	☐
A13	I felt like I had nothing to look forward to	☐	☐	☐	☐	☐

WHO-5 Survey – BREAD Version 1

Page **1 of 2**

A14	I felt anxious	☐	☐	☐	☐	☐
A15	I felt that nothing could cheer me up	☐	☐	☐	☐	☐
A16	I felt tense	☐	☐	☐	☐	☐

Appendix J: WHO-5 Wellbeing Index (WHO-5)

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WORLD HEALTH ORGANISATION-5 (WHO-5) SURVEY

DATE / /

INSTRUCTIONS:

- Please use a black/blue pen or pencil.
- Please erase or correct mistakes.
- To answer each question, you just need to tick ☑ the appropriate response box.

Example: Please **select ONE option** to the item regarding how you felt **in the past week**.

		At no time	*Some of the time*	*Less than half the time*	*More than half the time*	*Most of the time*	*All of the time*
		0	1	2	3	4	5
1	I have felt cheerful and in good spirits.		V				

If you have any questions or need help filling in this survey please call us on **021-279-1519** or email HVN.GIStudies@gmail.com

Thank you for your help with this important research!

WHO-5 Well-being Index

INSTRUCTIONS: Please respond to each item regarding how you felt **in the past week**:

		At no time	*Some of the time*	*Less than half the time*	*More than half the time*	*Most of the time*	*All of the time*
		0	1	2	3	4	5
1	I have felt cheerful and in good spirits.						
2	I have felt calm and relaxed.						
3	I have felt active and vigorous.						
4	I woke up feeling fresh and rested						
5	My daily life has been filled with things that interest me.						

Appendix K: Warwick Edinburgh Mental Wellbeing Scale (WEMWBS)

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WARWICK-EDINBURGH M
WELLBEING SCALE (WEI

DATE / /

INSTRUCTIONS:

- Please use a black/blue pen or pencil.
- Please erase or correct mistakes.
- To answer each question, you just need to tick ☑ the appropriate response box.

Example: Please **select ONE option** that that best describes your experience of each **in the past week**.

	In the past week...	None of the time 1	Rarely 2	Some of the time 3	Often 4	All of the time 5
1	I've been feeling optimistic about the future		V			

If you have any questions or need help filling in this survey please call us on **021-279-1519** or email HVN.GIStudies@gmail.com

Thank you for your help with this important research!

Warwick-Edinburgh Mental Well-being Scale
Instructions: Below are some statements about feelings and thoughts. Please **select the option** that that best describes your experience of each **in the past week**.

	In the past week...	None of the time 1	Rarely 2	Some of the time 3	Often 4	All of the time 5
1	I've been feeling optimistic about the future					
2	I've been feeling useful					
3	I've been feeling relaxed					
4	I've been feeling interested in other people					

Page **1 of 2**

5	I've had energy to spare					
6	I've been dealing with problems well					
7	I've been thinking clearly					
8	I've been feeling good about myself					
9	I've been feeling close to other people					
10	I've been feeling confident					
11	I've been able to make up my own mind about things					
12	I've been feeling loved					
13	I've been interested in new things					
14	I've been feeling cheerful					

Appendix L: Multidimensional Fatigue Symptom Inventory Short Form (MFI-SF)

Participant ID

MULTI-DIMENSIONAL SYMPTOM INVENTORY (MFSI) SURVEY

DATE / /

INSTRUCTIONS:

- Please use a black/blue pen or pencil.
- Please erase or correct mistakes.
- To answer each question, you just need to tick ☑ the appropriate response box.

Example: Please **select ONE option** to the item which best describes how true each statement has been for you **in the past week**.

		0 Not at all	1 A little	2 Moderately	3 Quite a bit	4 Extremely
1	I have trouble remembering things			V		

If you have any questions or need help filling in this survey please call us on **021-279-1519** or email HVN.GIStudies@gmail.com

Thank you for your help with this important research!

Multidimensional Fatigue Symptom Inventory Short Form (MFSI-SF)

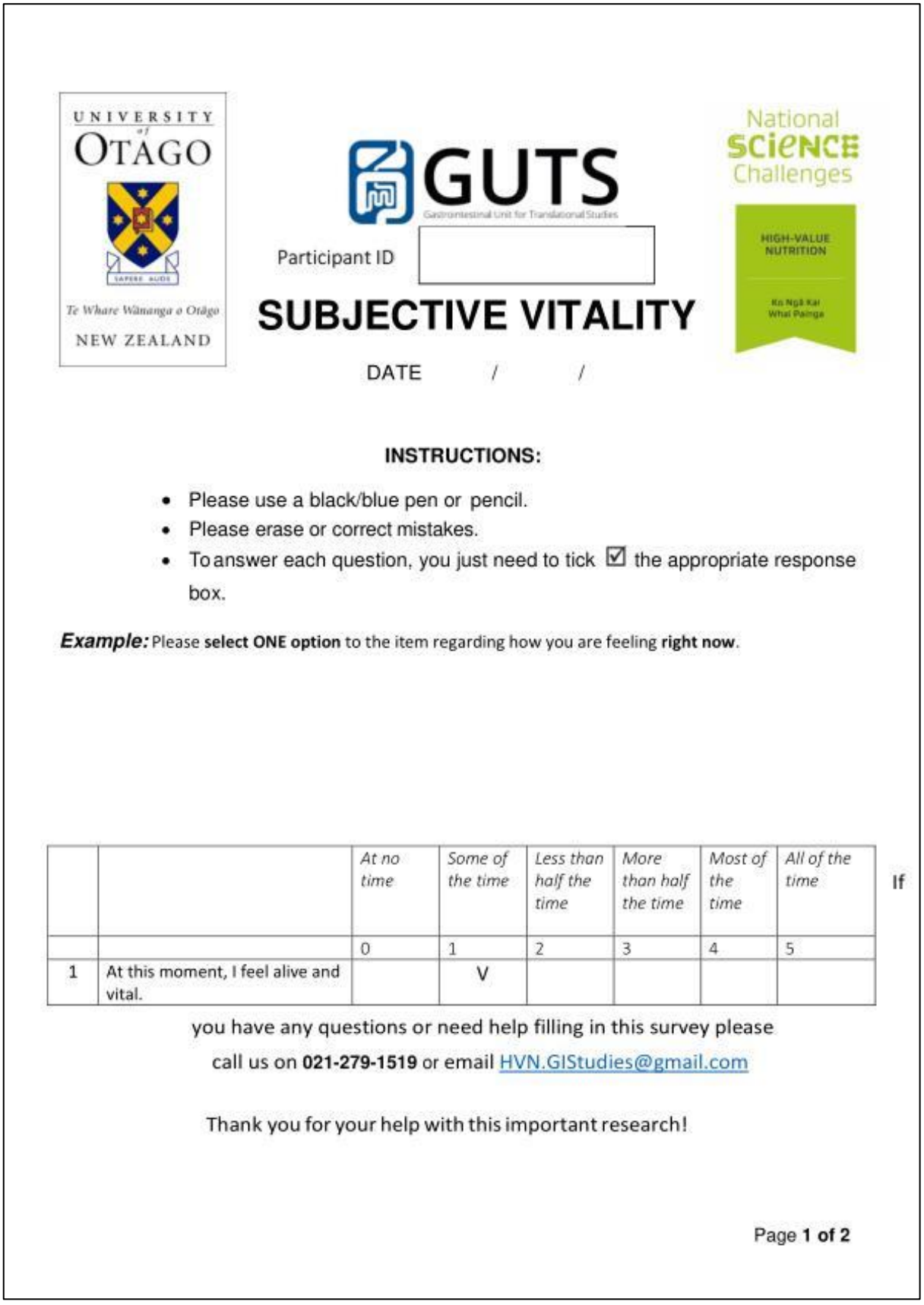
INSTRUCTIONS: Below is a list of statements that describe how people sometimes feel. Please read each item carefully, then select the one number next to each item which best describes how true each statement has been for you **in the past week**.

		0 Not at all	1 A little	2 Moderately	3 Quite a bit	4 Extremely
1	I have trouble remembering things					
2	My muscles ache					

Page **1 of 2**

3	I feel upset					
4	My legs feel weak					
5	I feel cheerful					
6	My head feels heavy					
7	I feel lively					
8	I feel nervous					
9	I feel relaxed					
10	I feel pooped					
11	I am confused					
12	I am worn out					
13	I feel sad					
14	I feel fatigued					
15	I have trouble paying attention					
16	My arms feel weak					
17	I feel sluggish					
18	I feel run down					
19	I ache all over					
20	I am unable to concentrate					
21	I feel depressed					
22	I feel refreshed					
23	I feel tense					
24	I feel energetic					
25	I make more mistakes than usual					
26	My body feels heavy all over					
27	I am forgetful					
28	I feel tired					
29	I feel calm					
30	I am distressed					

Appendix M: Subjective Vitality Scale (SVS)



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SUBJECTIVE VITALITY

DATE / /

INSTRUCTIONS:

- Please use a black/blue pen or pencil.
- Please erase or correct mistakes.
- To answer each question, you just need to tick ☑ the appropriate response box.

Example: Please **select ONE option** to the item regarding how you are feeling **right now**.

		At no time	*Some of the time*	*Less than half the time*	*More than half the time*	*Most of the time*	*All of the time*
		0	1	2	3	4	5
1	At this moment, I feel alive and vital.		V				

If you have any questions or need help filling in this survey please call us on **021-279-1519** or email HVN.GIStudies@gmail.com

Thank you for your help with this important research!

Page **1 of 2**

Vitality Scale

INSTRUCTIONS: Please respond to each of the following statements in terms of how you are feeling **right now**. Indicate how true each statement is for you at this time, using the following scale:

	Not at all true			*Somewhat true*		
	1	2	3	4	5	6
At this moment, I feel alive and vital.						
Currently I feel so alive I just want to burst.						
At this time, I have energy and spirit.						
I am looking forward to each new day.						
At this moment, I feel alert and awake.						
I feel energized right now.						

Appendix N: Canterbury District Health Laboratory Requirements

Test	Tube	Code (CDHL)
Anion gap (electrolytes)	Lithium Heparin	AGAP
Liver function	Lithium Heparin	LFTS
Creatinine	Lithium Heparin	CRN
Calcium	Lithium Heparin	CA
Blood Urea Nitrogen	Lithium Heparin	UREA
C reactive protein	Lithium Heparin	hsCRP
Fasting Glucose	Lithium Heparin	GLU
Complete blood count and differential	EDTA	CBCD
Lipid profile	Lithium Heparin	LIPS
Total	At room temperature	**two 6ml Lithium-Heparin collection tube one 4ml EDTA collection tube**

Page **1 of 2**