**ABSTRACT**

**Introduction:** The commensal gut microbiota have been shown to have an impact on human health as aberrant gut microbiota have been linked to disease. Dietary constituents are influential in shaping the gut microbiota. Diet-specific therapeutic strategies may therefore play a role in optimising human health via beneficial manipulation of the gut microbiota. Research has suggested that an individual’s baseline gut microbiota composition may influence how the gut microbiota respond to a dietary intervention and individuals with differing habitual dietary intakes appear to have distinct baseline gut microbiota compositions. The responsiveness of the gut microbiota may therefore be influenced by habitual dietary intakes. This study aims to investigate what influence differing habitual dietary fibre intakes have on the responsiveness of the gut microbiota to a prebiotic intervention.

**Methods and analysis:** In this randomised, double-blind, placebo-controlled, cross-over, single-centre study, 20 low dietary fibre (dietary fibre intake <18 g/day for females and <22 g/day for males) and 20 high dietary fibre (dietary fibre intake ≥25 g/day for females and ≥30 g/day for males) consumers will be recruited. Participants will be randomised to a placebo (Glucidex 30 Premium) or a prebiotic (Synergy 1) intervention for 3 weeks with a 3-week washout followed by 3 weeks of the alternative intervention. Outcome measures of gut microbiota composition (using 16S rRNA gene sequencing) and functional capacity (faecal short chain fatty acid concentrations and Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt)) as well as appetite (visual analogue scale appetite questionnaire) will be assessed at the beginning and end of each intervention phase.

**Ethics and dissemination:** The Massey University Human Ethics Committee approved this study (Massey University HEC: Southern A application—15/34). Results will be disseminated through peer-review journal publications, conference presentations and a summary of findings will be distributed to participants.

**Strengths and limitations of this study**

- This study will be the first to provide robust data on the influence habitual dietary fibre intake has on how an individual and their gut microbiota respond to a prebiotic intervention.
- Very few studies have used 16S rRNA gene sequencing to conduct detailed taxonomy characterisation of specific bacterial changes secondary to a prebiotic intervention.
- A challenge of this study is the reliance on the dietary fibre intake food frequency questionnaire to accurately classify participants as low, moderate and high dietary fibre consumers.

**Trial registration number:** ACTRN12615000922572; Pre-results.

**INTRODUCTION**

Humans and their gut microbiota have, over time, established a symbiotic relationship. The human host provides a steady supply of nutrients within an environment which favours microbial growth while the gut microbiota protect their human host against enteropathogenic organisms, extract nutrients from undigested dietary components, modulate the immune system and synthesise essential vitamins. It has been hypothesised that an aberrant host–microbe relationship is associated with a number of disease states including obesity, type 2 diabetes and inflammatory bowel disease. There are a number of factors which can influence the composition and therefore balance of the gut microbiota including diet, genetics, life stage, and so on. The commensal gut microbiota have been shown to have an impact on human health as aberrant gut microbiota have been linked to disease. Dietary constituents are influential in shaping the gut microbiota. Diet-specific therapeutic strategies may therefore play a role in optimising human health via beneficial manipulation of the gut microbiota. Research has suggested that an individual’s baseline gut microbiota composition may influence how the gut microbiota respond to a dietary intervention and individuals with differing habitual dietary intakes appear to have distinct baseline gut microbiota compositions. The responsiveness of the gut microbiota may therefore be influenced by habitual dietary intakes. This study aims to investigate what influence differing habitual dietary fibre intakes have on the responsiveness of the gut microbiota to a prebiotic intervention.
Research is investigating ways of targeting beneficial gut microbiota to enhance human health. Since dietary components are particularly influential in shaping the gut microbiota, diet-specific therapeutic strategies could help optimise human health and wellbeing via their influence on the community structure and function of the gut microbiota. Prebiotics are ‘selectively fermented ingredients that result in specific changes in composition and/or activity in the gastrointestinal microbiota, thus conferring benefit(s) upon human health’.14 Established prebiotics, such as galacto-oligosaccharides, lactulose and inulin-type fructans (eg, inulin, oligofructose and fructo-oligosaccharides), have been shown to be effective in eliciting beneficial alterations in gut microbiota composition and function (ie, short chain fatty acid (SCFA) production)15–21 and regulating appetite.22–24 Generally, the target of prebiotic interventions is the enhancement of Bifidobacteria and Lactobacillus species; however, other beneficial bacteria such as Roseburia intestinalis, Ruminooccus bromii and Faecalibacterium prausnitzii are emerging as bacteria associated with good health.25

Principal coordinate analysis data have revealed that gut microbiota samples cluster by participant rather than by dietary intervention, suggesting that an individual’s gut microbiota do not respond in a consistent manner to a particular dietary intervention.26,27 Baseline Bifidobacteria concentrations appear to have an impact on how effective a dietary intervention is in modifying the gut microbiota. Individuals with low baseline Bifidobacteria levels have been shown to have greater increases in Bifidobacteria concentrations after prebiotic intervention than individuals with high baseline Bifidobacteria levels.16,28 One study collated the results of three separate dietary intervention and gut microbiota studies to determine whether the composition of the gut microbiota may prove informative in predicting how the gut microbiota will respond to a dietary intervention.29 They established from these studies that individuals with lower baseline Bifidobacteria or particularly low or high abundance of Eubacterium ruminantium and Clostridium felsineum had a gut microbiota community which was more responsive to a dietary intervention than individuals with higher baseline abundance of Bifidobacteria and moderate abundance of E. ruminantium and C. felsineum. They also demonstrated that the dietary intervention needed to elicit changes in the gut microbiota to have an influence on lowering serum cholesterol, which suggests that there is a connection between the gut microbiota and human host responsiveness. They did not, however, determine whether there were any habitual dietary intake differences between responders and non-responders to the intervention. Baseline microbial gene count (MGC) has also been shown to affect host responsiveness in overweight and obese individuals. Individuals with low MGC had higher levels of insulin resistance and fasting triglycerides than individuals with high MGC. Dietary differences were demonstrated between the low and high MGC groups, with the low MGC group consuming lower quantities of fruit, vegetables and fish with a trend towards lower dietary fibre intakes. After a weight loss promoting, energy restricted diet, individuals with high MGC at baseline had a more significant improvement in inflammatory markers, insulin resistance and triglycerides than individuals with low MGC.30 MGC may therefore help predict how effective a dietary intervention may be on host outcomes.

An individual’s habitual dietary intake has been shown to influence baseline gut microbiota composition.30–35 It is therefore plausible that an individual’s habitual dietary intake, particularly dietary fibre intake, may influence how their gut microbiota respond to a particular dietary intervention. The impact of palm date intake was studied in a group of healthy participants to test its prebiotic potential. Fluorescence in situ hybridisation analysis showed that there were no significant differences in any of the bacterial groups after palm date consumption when compared with the control. The researchers then grouped participants as high dietary fibre consumers and low dietary fibre consumers and found that at baseline their Bacteroides concentrations were significantly different. Palm date consumption led to a significant increase in total bacteria, Lactobacillus/Enterococcus group, Bacteroides, C. coccoides–E. rectale group, R. bromii+R. flavefaciens group and Roseburia+E. rectale group in the low dietary fibre group however there was no change in any of the bacterial groups analysed in the high dietary fibre group.36 Although these studies suggest that baseline gut microbiota composition and habitual diet may influence the responsiveness of the gut microbiota to a dietary intervention, no studies have specifically investigated whether differing habitual dietary intakes lead to gut microbiota which respond to a dietary intervention in a distinct manner. Given the limited research undertaken in this area until now, the primary objective of this study is to investigate whether differing habitual dietary fibre intakes influence how the gut microbiota respond to a prebiotic intervention.

METHODS AND ANALYSIS

Study design
This is a randomised, double-blind, placebo-controlled, cross-over, single-centre study in healthy individuals with differing habitual dietary fibre intakes. The study will investigate whether low versus high habitual dietary fibre intake influences bacterial relative abundance, diversity and faecal SCFA concentrations and appetite in a distinct manner in response to a prebiotic intervention.

Primary objective
To determine the effect of low versus high habitual intakes of dietary fibre on the way in which the
composition (bacterial relative abundance) and diversity (α and β diversity) of the gut microbiota change in response to a prebiotic as measured by 16S rRNA gene sequencing.

**Secondary objectives**

To determine the effect of low versus high habitual intakes of dietary fibre on the way in which the functional capacity of the gut microbiota change in response to a prebiotic as predicted by Phylogenetic Investigation of Communities by Recon (PICRUSt) and faecal SCFA concentrations.

To determine whether baseline gut microbiota relative abundance, bacterial diversity, predicted relative abundance of bacterial gene functions and SCFA concentrations differ between individuals with low versus high dietary fibre intakes.

To determine whether low versus high habitual dietary fibre intakes alter the efficacy of a prebiotic to change appetite scores.

**Primary hypothesis**

The bacterial relative abundance and diversity of individuals with a low habitual dietary fibre intake will change more significantly in response to a prebiotic than individuals with a high habitual dietary fibre intake.

**Secondary hypotheses**

The predicted relative abundance of bacterial gene function and SCFA production of individuals with a low habitual dietary fibre intake will change more significantly in response to a prebiotic than those of individuals with a high habitual dietary fibre intake.

Individuals with low habitual dietary fibre intake will have baseline bacterial relative abundance, diversity and predicted relative abundance of bacterial gene function and SCFA concentrations which are distinctive from individuals with high dietary fibre intakes.

The efficacy of a prebiotic to influence appetite will be more pronounced in individuals with a low habitual dietary fibre intake than in individuals with a high habitual dietary fibre intake.

**Study setting**

The study will be undertaken at the Massey University Human Nutrition Research Unit (HNRU) which is located in Palmerston North, New Zealand.

**Exclusion criteria**

- Less than 19 or >65 years of age;
- Taken antibiotics within the past 6 months;
- Taken laxatives, gastric motility medications, prebiotic or probiotic containing foods or supplements within the past month;
- Medical history of clinically significant disease, that is, cancer, gastrointestinal disorders (irritable bowel syndrome, inflammatory bowel disease, coeliac disease, constipation, diarrhoea, excessive bloating), autoimmune disorders, diabetes, heart disease, renal failure or previous gastrointestinal surgery;
- Body mass index of <18.5 or >30 kg/m²;
- Significant weight loss or weight gain (>5% of total body weight) within the past year;
- Significant dietary change within the past year (ie, has become vegetarian, removed gluten from their diet, actively trying to lose weight, etc);
- Pregnant, breast feeding or planning a pregnancy in the next 3 months;
- Food intolerance causing gastrointestinal symptoms (ie, lactose intolerance, gluten sensitivity);
- Smokers;
- High alcohol consumers (>15 standard drinks per week for males and >10 standard drinks per week for females AND fewer than two alcohol-free days per week—New Zealand Ministry of Health guidelines).

If a potential participant is found to be ineligible to participate in the study because they are taking prebiotic or probiotic containing foods or supplements, but are otherwise eligible, they can be included in the study if they are willing to discontinue the probiotic and prebiotic containing foods and supplements for a month prior to starting the study and during the study period.

**Study duration**

The study length is 10 weeks and will be split into four separate study phases:

- Screening phase (weeks −1 to 0);
- Intervention phase 1 (weeks 0 to 3);
- Washout phase (weeks 4 to 6);
- Intervention phase 2 (weeks 7 to 9).

A summary of the four separate study phases is provided in figure 1.

**Sample size calculations**

In order to detect a significant difference in responsiveness of the key phylum and genera (ie, Actinobacteria, Ruminococcus, Faecalibacterium, Bifidobacteria, etc) to the prebiotic intervention (difference of 3% in bacterial composition with a variance of 9% between and within individuals) between the low and high dietary fibre intake groups (with a power of 80% and significance of 5%), 17 participants per dietary fibre intake group need to be recruited. To allow for participant withdrawal, a total of 40 participants will be recruited: 20 with low dietary fibre intakes and 20 with high dietary fibre intakes.

**Participant recruitment**

Study participants will be recruited via a number of avenues including email, radio and newspaper advertising and flyer displays around the Palmerston North area.

**Participant screening**

Screening questionnaire

Eligibility will initially be assessed using a screening questionnaire. Once participants have provided written consent, they will be screened for eligibility using a screening questionnaire. Participants will be excluded if they meet any of the exclusion criteria.

**Participant outcomes**

The study outcomes will be assessed at the end of each study phase. The outcomes will be measured using a combination of questionnaires, blood tests, faecal samples and dietary diaries.

**Participant follow-up**

Participants will be followed up for 6 months after the end of the study to assess any long-term effects of the prebiotic intervention.

**Participant withdrawal**

Participants are free to withdraw at any time without giving a reason. All data collected from withdrawn participants will be included in the analysis.

**Data protection**

All data will be stored in a secure location and will be accessible only to the principal investigator and the study team.

The study has been approved by the Massey University Ethics Committee and all participants will provide written informed consent before participating in the study.

**Confidentiality**

All data will be kept confidential and will only be used for the purposes of the study.

**Funding**

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consent to the lead researcher to participate in the study, a link to an online screening questionnaire will be provided. Each participant will be asked to complete the screening questionnaire which will collect information relating to the participant’s gender, health status, age, weight, height, medication use, prebiotic and probiotic intake, recent weight and dietary changes, habitual dietary fibre intake, drinking patterns, smoking status and food intolerances. If potential participants are considered eligible to participate in the study, then on the basis of the screening questionnaire, they will be invited to attend the screening phase visit (first research unit visit).

**Habitual dietary fibre intake screening**
An online dietary fibre intake food frequency questionnaire (FFQ) will be used to assess the habitual dietary fibre intake of participants. This is a component of the screening questionnaire. Only participants assessed as having low (dietary fibre intake <18 g/day for females and <22 g/day for males) or high (dietary fibre intake ≥25 g/day for females and ≥30 g/day for males) dietary fibre intakes will be eligible for inclusion in the study. The high dietary fibre intake cut-offs were chosen to reflect the New Zealand recommended dietary fibre intake which is ≥25 g/day for females and ≥30 g/day for males. The low dietary fibre intake cut-offs were

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**Figure 1** Flow diagram summarising the four separate study phases including the two possible intervention orders. The intervention orders may not be as described within the figure as they are blinded to the lead researcher, analysts and participants.
chosen as the median dietary fibre intake in New Zealand is 17.5 g/day for females and 22.1 g/day for males, which is below recommended amounts.38

Screening phase visit

Once a participant is assessed as being eligible to take part in the study, then on the basis of the online screening questionnaire, they will be invited to attend a screening phase visit which will take place at the HNRU. At the screening phase visit, participants will provide a blood sample for baseline health screening (liver and kidney function tests, blood glucose levels, electrolytes, complete blood count, calcium and C reactive protein) and will have their weight, height and body composition, using air displacement plethysmography (BodPod), measured. Participants will also be provided with written and oral instructions on how to collect a faecal sample. The 3-day diet record and appetite questionnaire will be completed during the 3 days leading up to the start of the intervention phase 1 (IP1) visit (second visit). The FI-FFQ will be completed and a faecal sample collected on the day before the start of the IP1 visit. The results of the blood test will be received and interpreted (any abnormal blood results will be reviewed by the research clinician) prior to the start of the IP1 visit, as individuals with blood results which may suggest chronic disease (ie, liver disease, kidney disease, diabetes) will be excluded from the study. The screening visit will take place around 1 week prior to the start of the IP1 visit and will provide a short run-in period.

Interventions

Each participant will consume 16 g (as two 8 g doses; 8 g 30 min before breakfast and 8 g 30 min before dinner) of powdered fructan prebiotic (Beneo Orafti Synergy 1–50:50 inulin to fructo-oligosaccharide mix) each day for 3 weeks. Participants will also consume 16 g (as two 8 g doses; 8 g 30 min before breakfast and 8 g 30 min before dinner) of powdered placebo (Roquette Glucidex 29 Premium) each day for 3 weeks. The prebiotic and placebo are low in calories and provide 17 and 31 kcal, respectively, per dose. The prebiotic and placebo will be mixed into hot or cold beverages that the participants regularly consume. There will be a 3-week washout phase between the two intervention phases. Previous research has shown that a 3-week intervention provides sufficient time for the gut microbiota to respond to a fructan prebiotic15 and that a 3-week washout provides sufficient time for the gut microbiota to revert back to a baseline composition.40 Participants will be asked to continue their usual food intake and physical activities throughout the duration of the study.

Randomisation and intervention concealment

Participants will be randomly allocated one of two intervention orders: intervention order A or B (figure 1). The intervention order will be randomised using a computer-based pregenerated intervention order as participants will be recruited one at a time over a number of months. Randomisation will be the responsibility of the research unit manager who will not be involved in administering the intervention to participants, assessing the outcomes or analysing the data. Randomisation will be blinded from the lead researcher, analysts and participants. Unblinding may be permitted if medically relevant. The placebo and prebiotic will be in opaque sachets within sealed paper bags and are similar in appearance and taste.

Start of the intervention phase 1 visit

Eligible participants will visit the HNRU ~1 week after their screening visit for their start of the IP1 visit. Participants will provide a completed 3-day diet record and appetite questionnaire, FI-FFQ and a faecal sample at this visit. Body weight will be measured and 1 week of either the placebo or prebiotic will be provided to each participant. The remaining placebo or prebiotic allocation will be mailed to the participants on a weekly basis. The participants will also be asked to complete a daily diary over the following three intervention weeks to help assess compliance to the intervention and to report any adverse gastrointestinal symptoms that may develop.

End of the intervention phase 1 visit

Three weeks after the start of the IP1 visit, participants will be invited back to the HNRU for the end of the IP1 visit. Another completed 3-day diet record and appetite questionnaire and FI-FFQ will be collected along with an end of the IP1 faecal sample. Body weight will again be measured and the completed daily diaries will be collected. Participants will then enter the 3-week washout phase where they will continue their usual food intake and physical activities but will not be taking either of the interventions or completing a daily diary.

Start of the intervention phase 2 visit

At the end of the 3-week washout phase, participants will be invited back to the HNRU to attend the start of the intervention phase 2 (IP2) visit. At this visit, participants will provide a completed 3-day diet record and appetite questionnaire, FI-FFQ as well as a start of the IP2 faecal sample. Body weight will be measured and each participant will be provided with 1 week of either the placebo or prebiotic at this visit. The remaining placebo or prebiotic allocation will be mailed to the participants on a weekly basis. The participants will be asked to complete a daily diary over the following three intervention weeks.

End of the intervention phase 2 visit

Three weeks after the start of the IP2 visit, participants will attend their final HNRU visit. Participants will provide the last 3-day diet record and appetite questionnaire, FI-FFQ, faecal sample, daily diaries and body weight measurement. Figure 2 provides an illustration of the participant flow.
through the study including the measurements, questionnaires and samples that will be obtained.

**Intervention compliance**

Intervention adherence will be assessed at the end of each IP visit (end of IP1 visit and end of IP2 visit). Participants will be asked to bring back any unused sachets to each end of IP visit. A daily diary will be completed by the participants which allows them to report whether they had taken all of the allocated morning and afternoon interventions. If participants experience significant gastrointestinal symptoms that prevent them from complying with the treatment as instructed, they will be withdrawn from the study.

**Adverse symptom monitoring**

High intakes of inulin and fructo-oligosaccharides have been associated with mild gastrointestinal symptoms including flatulence, diarrhoea, borborygm and bloating. Adverse symptoms relating to the consumption of the prebiotic and placebo will be monitored using the daily diary. Participants will be asked to report daily on whether they have experienced nausea, diarrhoea, flatulence, stomach rumbling, bloating or abdominal cramps or pain over the past 24 hours. For each symptom, the participants will be asked to indicate whether the symptom was absent, mild (nagging or annoying), moderate (strong negative influence on their daily living) or severe (disabling).

**Outcome measures**

**Primary outcome measure**

The primary outcome measure is gut microbiota composition after prebiotic intervention in individuals with low and habitual high dietary fibre intakes.

Compositional changes in gut microbiota will be analysed in the faecal samples collected before and after the prebiotic intervention. 16S rRNA gene sequencing methodology (Illumina MiSeq) and Quantitative Insights Into Microbial Ecology (QIIME) software will be used to analyse changes in bacterial relative abundance and α and β diversity.

**Secondary outcome measures**

A secondary outcome is the functional changes in gut microbiota, as assessed by bacterial SCFA concentrations and relative abundance of bacterial gene function, after the prebiotic intervention in individuals with low and high dietary fibre intakes. 16S rRNA gene sequencing data will be further analysed using PICRUSt software to predict the relative abundance of bacterial gene function. Bacterial metabolic functionality will be determined by measuring faecal SCFA concentrations using gas chromatography.
The differences in baseline gut microbiota composition (bacterial relative abundance and diversity) and function (faecal SCFA concentrations and relative abundance of bacterial gene function) between individuals with low versus high dietary fibre intakes will be assessed as secondary outcomes. Compositional and functional differences in gut microbiota at baseline will be analysed in the faecal sample collected at the start of the IPI (week 0) visit. 16S rRNA gene sequencing data and faecal SCFA concentrations will be analysed (methods outlined above).

Appetite will be assessed as a secondary outcome to determine whether the participant’s habitual dietary fibre intake (low vs high) alters the efficacy of a prebiotic to influence appetite. A validated 100 mm anchored visual analogue scale appetite questionnaire will be used in conjunction with weight and dietary intake (assessed using the 3-day diet records) information.

**Statistical analysis**

Bacterial relative abundance and relative abundance of bacterial gene function differences (at baseline and between groups postprebiotic intervention) will be analysed using non-parametric Mann-Whitney tests. A Wilcoxon matched-pairs test will be used to analyse the bacterial relative abundance and relative abundance of bacterial gene function differences between the placebo and prebiotic intervention phases for the low and high dietary fibre groups. Bacterial diversity differences will be analysed using a non-parametric two-sample t-test. Analysis of variance (ANOVA) and discriminant analysis tests will be used to analyse the differences in SCFA concentrations. ANOVA tests will also be used to analyse differences in appetite ratings, dietary intake and weight measurements and a p value <0.05 will be considered significant.

**ETHICS AND DISSEMINATION**

**Research ethics approval**

A human ethics application was submitted.

Participants will provide signed informed consent before participating in the study. Participants are able to withdraw from the study at any point with no reason for withdrawal required.

**Dissemination**

The results of the study will be disseminated through a number of avenues including peer-reviewed journal publications, conference presentations and a summary of findings provided to participants.

**DISCUSSION**

Habitual dietary intake plays a significant role in shaping the community of microbes which reside in the gastrointestinal tract; however, it is still unknown what impact distinctive habitual dietary intakes have on how responsive the gut microbiota are to a dietary intervention. This intervention study has been designed to investigate how differing habitual dietary fibre intakes influence how the gut microbiota respond to a prebiotic. This information may be invaluable as currently it is difficult to predict how an individual or their gut microbiota will respond to a prebiotic intervention. The high diversity of habitual dietary fibre intakes between individuals may assist in explaining why there is no consistent response by the gut microbiota to a particular prebiotic intervention. If our hypothesis is shown to be correct, researchers may need to take into consideration the habitual dietary fibre intake of their participants at baseline to ensure that potentially confounding factors are controlled for or eliminated in dietary intervention studies which aim to target the gut microbiota.

A limitation of this study is the reliance on the dietary fibre intake FFQ to accurately classify individuals as having low, moderate or high dietary fibre intakes. To verify that the participants have been classified correctly based on the dietary fibre intake FFQ, their first 3-day diet record (collected at the STP1 visit) will be analysed to ensure that each participant meets the low or high dietary fibre intake criteria.

The results generated from this study will provide information for future interventional studies which aim to beneficially modulate the gut microbiota, to help ensure that they are robustly designed so that the true efficacy of a dietary intervention can be determined.

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**Contributors**

GH was involved in the conception, design, writing and editing of the protocol. JC, CB, RM and LB were involved in the conception, design and editing of the protocol. KW was involved in the editing of the protocol. All authors read and approved the manuscript.

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**Competing interests**

None declared.

**Patient consent**

Obtained.

**Ethics approval**

Massey University Human Ethics Committee in July 2015 (Massey University HEC: Southern A, Application 15/34).

**Provenance and peer review**

Not commissioned; externally peer reviewed.

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**Correction notice**

This article has been corrected since it was first published as the correspondence author name is changed from Gene Healey to Gene Lunken.