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The influence of habitual dietary intake on the responsiveness of the gut microbiota to a dietary intervention

A thesis presented in partial fulfilment of the requirements for the degree

of

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Abstract

Preliminary evidence suggests that inter-individual variability in gut microbiota response to a dietary intervention is influenced by baseline gut microbiota composition. Differing habitual dietary intakes lead to distinctions in baseline gut microbiota composition making it plausible that habitual dietary intake may also influence gut microbiota response. Prior to conducting this research no studies had been undertaken to determine whether habitual dietary intake has an impact on gut microbiota responsiveness. Therefore, the aim of this research was to investigate the influence habitual dietary intake has on gut microbiota response to a dietary intervention.

Initially, secondary data analysis was conducted to determine whether there was any support for the hypothesis that individuals with differing habitual dietary intakes would have gut microbiota that respond in a distinctive manner to a dietary intervention. The secondary data analysis results demonstrated that dietary groups rich in dietary fibre had the greatest impact on gut microbiota responsiveness. An *in vitro* three-stage colonic model system study was conducted to determine whether media with differing fermentable carbohydrate (i.e. dietary fibre) contents influenced gut microbiota response to an inulin-type fructan prebiotic. It was demonstrated that differing prebiotic driven changes in organic acids and bacterial taxa occurred between the low (LFC) and high fermentable carbohydrate (HFC) content media. The results of the secondary data analysis and *in vitro* study provided evidence to suggest that a human intervention study was warranted. A randomised, double-blind, placebo-controlled, cross-over, human intervention study in 34 healthy participants was undertaken to determine whether habitual dietary fibre intake influenced gut microbiota response to an inulin-type fructan prebiotic. The results of the human intervention study demonstrated that the low habitual

dietary fibre (LDF) group harboured gut microbiota that were less responsive to the inulin-type fructan prebiotic than the high habitual dietary fibre (HDF) group.

Future studies which aim to modulate the gut microbiota via dietary change or to determine the prebiotic potential of a novel fermentable substrate should take habitual dietary fibre intakes into consideration when recruiting participants or analysing the data. This will help reduce the confounding influence of inter-individual variability in gut microbiota responsiveness and ensure the true efficacy of a dietary intervention is demonstrated.

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List of Abbreviations

AA	African Americans
AC	African children
AMER	Typical American style dietary pattern
ANOVA	Analysis of variance
BMI	Body mass index
BodPod	Air displacement plethysmography
CD	Crohn's disease
CHO	Carbohydrate
CNAQ	Comprehensive nutrition assessment questionnaire
CRON	Plant-rich, calorie-restricted diet with optimal nutrient composition
D	Donor
DFI-FFQ	Dietary fibre intake short food frequency questionnaire
DNA	Deoxyribonucleic acid
EC	European children
EI:BMR	Energy intake: basal metabolic rate
FFQ	Food frequency questionnaire
FI-FFQ	Fructan intake food frequency questionnaire
FISH	Fluorescence *in situ* hybridisation
FMP	Fermented milk product
FODMAP	Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols
GC	Gas chromatography
GI	Gastrointestinal
GLP-1	Glucagon-like peptide-1
HFC	High fermentable carbohydrate medium
HDF	High habitual dietary fibre
HMP	Human Microbiome Project
HNRU	Human Nutrition Research Unit
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
IP1	Intervention phase 1

IP2	Intervention phase 2
LFC	Low fermentable carbohydrate medium
LDF	Low habitual dietary fibre
LPS	Lipopolysaccharide
MetaHIT	Metagenomics of the Human Intestinal Tract
MGC	Microbial gene count
MoH	Ministry of Health
NA	Native Africans
NCD	Non-communicable disease
NS	Not specified
NZ	New Zealand
OTU	Operational taxonomic units
PANDASeq	Paired-end assembler for DNA sequencing
PCR	Polymerase chain reaction
PICRUSt	Phylogenetic investigation of communities by reconstruction of unobserved states
PS	Prudent style
PYY	Peptide YY
QIIME	Quantitative Insights Into Microbial Ecology
qPCR	Quantitative real-time polymerase chain reaction
SCFA	Short-chain fatty acid
SD	Standard deviation
T2DM	Type 2 diabetes mellitus
TE	Total energy
UC	Ulcerative colitis
VAS	Visual analogue scales
WS	Western style

CHAPTER ONE

INTRODUCTION

THE INFLUENCE OF HABITUAL DIETARY INTAKE ON THE RESPONSIVENESS OF THE GUT MICROBIOTA TO A DIETARY INTERVENTION

Chapter 1 Introduction

1.1 Aim and objectives

1.1.1 Aim

The aim of my PhD research was to investigate the influence that habitual dietary intake has on gut microbiota response to a dietary intervention.

1.1.2 Primary objectives

- To conduct secondary data analysis to determine whether distinctive habitual dietary intakes influence gut microbiota response to high-dose Actazin™.
- To conduct an *in vitro* three-stage colonic model system study to demonstrate what influence two media with distinctive fermentable carbohydrate contents have on the responsiveness of the gut microbiota to an inulin-type fructan prebiotic.
- To conduct a randomised, double-blind, placebo-controlled, cross-over, human intervention study to determine the effect of low (LDF) *versus* high habitual dietary fibre (HDF) intakes on gut microbiota response to an inulin-type fructan prebiotic.

1.1.3 Secondary objectives

- To conduct secondary data analysis to determine which nutrients and/or food groups have the greatest influence on gut microbiota response to high-dose Actazin™.
- To determine whether baseline differences in gut microbiota composition, diversity and predicted functional capacity exist between individuals with distinctive habitual dietary intakes.

- To demonstrate what influence differing habitual dietary intakes have on prebiotic driven changes in the predicted functional capacity of the gut microbiota.
- To conduct a randomised, double-blind, placebo-controlled, cross-over, human intervention study to investigate whether LDF *versus* HDF intake influences appetite rating scores (host outcome) in response to an inulin-type fructan prebiotic.

1.2 Hypotheses

1.2.1 Primary hypotheses

- Secondary data analysis will show that individuals with differing habitual dietary intakes will harbour gut microbiota that will respond distinctively to high-dose Actazin™.
- The *in vitro* three-stage colonic model system study will demonstrate that the two media with differing fermentable carbohydrate contents used in an *in vitro* three-stage colonic model system will lead to distinctive gut microbiota responses to an inulin-type fructan prebiotic.
- The randomised, double-blind, placebo-controlled, cross-over, human intervention study will show that LDF consumers harbour gut microbiota that have a more pronounced response to the inulin-type fructan prebiotic than HDF consumers.

1.2.2 Secondary hypotheses

- Secondary data analysis will demonstrate that dietary groups rich in dietary fibre will have the largest impact on gut microbiota response to high-dose Actazin™.
- Baseline differences in gut microbiota composition, diversity and predicted function will exist between individuals with distinctive habitual dietary intakes.
- Individuals with distinctive habitual dietary intakes will harbour gut microbiota that have differing prebiotic driven changes in the predicted functional capacity of the gut microbiota.
- The randomised, double-blind, placebo-controlled, cross-over, human intervention study will show that LDF consumers will have a more significant improvement in appetite due to the inulin-type fructan prebiotic than HDF consumers.

1.3 Thesis structure

This introduction chapter is followed by a literature review (Chapter 2) that provides an overview of gut microbiota composition and function, bacterial enumeration techniques, factors that influence gut microbiota composition, *in vitro* human gastrointestinal (GI) tract model systems and the influence of gut microbiota on appetite regulation. The literature review also explores the influence that baseline gut microbiota composition and habitual dietary intake have on gut microbiota and host response to a given dietary intervention. The secondary data analysis, conducted to determine whether distinctive habitual dietary intakes influence gut microbiota response to high-dose Actazin™, is discussed in Chapter 3. Secondary data analysis demonstrated that dietary groups rich in dietary fibre had the greatest impact on gut microbiota responsiveness to high-dose Actazin™. Based on the results of secondary data analysis, an *in vitro* three-stage colonic model system study was undertaken to test the hypothesis that habitual dietary fibre (i.e. fermentable carbohydrate) intake influences gut microbiota response to an inulin-type fructan prebiotic. The *in vitro* three-stage colonic model system study is presented in Chapter 4. The *in vitro* three-stage colonic model system results demonstrated that there was validity to the hypothesis that the two differing fermentable carbohydrate content media will lead to distinctive gut microbiota responses to an inulin-type fructan prebiotic. As a result, a randomised, double-blind, placebo-controlled, cross-over, human intervention study was planned. Chapter 5 discusses the study that was conducted to validate a habitual dietary fibre intake food frequency questionnaire. This study was conducted as no suitable questionnaires were available to assess whether an individual has a low, moderate or high habitual dietary fibre intake. Such a questionnaire was required during the screening phase of the human intervention study to ensure only LDF and HDF consumers were recruited. The study protocol for the randomised, double-

blind, placebo-controlled, cross-over, human intervention study is given in Chapter 6. Chapter 7 outlines the study design and reports the results from the randomised, double-blind, placebo-controlled, cross-over, human intervention study. The discussion, conclusion and areas of future research are outlined in Chapter 8.

CHAPTER TWO

LITERATURE REVIEW

INTER-INDIVIDUAL VARIABILITY IN GUT MICROBIOTA AND HOST RESPONSE TO DIETARY INTERVENTIONS: THE INFLUENCE OF BASELINE GUT MICROBIOTA COMPOSITION AND HABITUAL DIETARY INTAKE

This chapter, in part, has been accepted for publication in Nutrition Reviews.

The literature review presented in this thesis has been altered from the accepted manuscript to include additional background information relating to "healthy" gut microbiota (Section 2.3), metabolic functionality of the gut microbiota (Section 2.4), analysing gut microbiota communities (Section 2.5), factors that influence gut microbiota composition (Sections 2.61, 2.62 and 2.63), influential nutrients (Section 2.6.5.1), *in vitro* human GI tract model systems (Section 2.7) and the influence of gut microbiota on appetite regulation (Section 2.8).

Evidence of permission to use figure 2-1, 2-2 and 2-4 is available as appendix 2-1, 2-2 and 2-3; respectively.

Chapter 2 Literature review

2.1 Abstract

Dysbiosis is linked to human disease, therefore, gut microbiota modulation strategies provide an attractive means of correcting microbial imbalance to enhance human health. As diet has a major influence on the composition, diversity and metabolic capacity of the gut microbiota, numerous dietary intervention studies have been conducted to manipulate the gut microbiota to improve host outcomes and reduce disease risk. Emerging evidence suggests that inter-individual variability in gut microbiota and host responsiveness exists making it difficult to predict gut microbiota and host response to a given dietary intervention. This may in turn have implications on the consistency of results between studies, making it problematic to assess the true efficacy of a dietary intervention in eliciting beneficial changes on the gut microbiota and human health.

2.2 Introduction

Non-communicable diseases (NCD), such as cardiovascular disease, type 2 diabetes mellitus (T2DM), chronic respiratory diseases and cancer, contribute to over half of all deaths worldwide[1]. Major modifiable NCD risk factors include smoking, unhealthy dietary intakes (i.e. high saturated fat, trans fat, salt and sugar and low wholegrain, fruit and vegetable intakes), physical inactivity and excessive alcohol consumption[2]. It has been postulated that the commensal microbes that reside within the GI tract are implicated in human disease. Dysbiotic gut microbiota have been associated with obesity[3], inflammatory bowel disease (IBD)[4], T2DM[5] and colon cancers[6]. It is difficult to disentangle whether a dysbiotic gut microbiota is causally linked to these diseases, or is the result of these diseases or the dietary patterns and medications associated with certain disease states.

High-throughput sequencing technology has revolutionised how microbes that colonise the gastrointestinal (GI) tract are analysed. The main bacterial phyla that are detected in faeces are Bacteroidetes and Firmicutes[7]. The key genera within the Bacteroidetes phyla include *Alistipes, Bacteroides* and *Prevotella* and key genera within the Firmicutes phyla include *Enterococcus, Lactobacillus, Coprococcus, Dorea, Blautia, Roseburia, Ruminococcus* and *Faecalibacterium*. Other lower abundance phyla that reside in the human GI tract include Actinobacteria (*Bifidobacterium*), Verrucomicrobia (*Akkermansia*) and Proteobacteria (*Escherichia*). The predominant intestinal bacteria appear to be relatively stable over time in adults[8]. However, observational studies have demonstrated intra-individual variability in gut microbiota composition which may occur secondary to external factors such as age[9–11], gender[12,13], antibiotics[14,15], disease[3–6] and diet[16].

Numerous human studies have established that diet has a major influence on the structural and functional capacity of the gut microbiota[16–20] but emerging research suggests that the gut microbiota response to dietary interventions is highly variable among individuals[21–23]. It has been established that individuals harbour microbial taxa that are either responsive or resilient to dietary change making it very difficult to predict how an individual's gut microbiota may respond to a given dietary intervention and ultimately how the individual will benefit from the dietary intervention. Previous research suggests that baseline gut microbiota composition[20,24,25] and habitual dietary intake[26,27] may influence gut microbiota and host response. However, dietary intervention studies which aim to modulate the gut microbiota do not currently take baseline gut microbiota composition and habitual dietary intake into consideration. A greater understanding of the factors implicated in gut microbiota and host responsiveness is needed to establish successful gut microbiota and host outcome modulation strategies.

This review discusses the evidence for the link between gut microbiota and human disease. This review also explores the role diet has to play in modulating the gut microbiota and highlights whether heterogeneous gut microbiota and host response to a given dietary intervention could be influenced by differing baseline gut microbiota composition and habitual dietary intake.

2.3. "Healthy" gut microbiota

Humans harbour hundreds of trillions (10^{14}) of microbiota that contain more genes than present within the human genome[28,29]. Microbes readily colonise human surfaces exposed to the external environment including the skin, vagina, respiratory and GI tracts[30–32]. Of the microbes which reside in and on the human body, the GI tract contains the majority, with the distal colon harbouring the largest amount ($\geq 10^{12}$ per gram contents) (Figure 2-1)[33]. The majority of the gut associated microbiota are anaerobic and outnumber the facultative anaerobes and aerobes by 2-3 times[34].

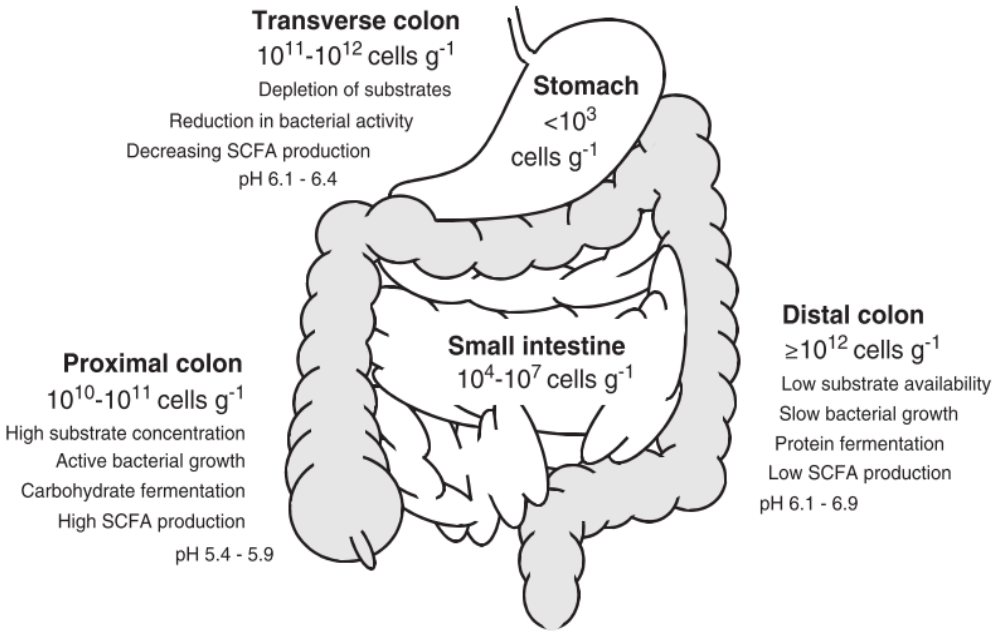

Figure 2-1. Representation of the microbiota content (cells/g) of a healthy human GI tract including the pH, nutrient utilisation and fermentation products associated with each section (figure from reference[35])

Deoxyribonucleic acid (DNA) sequencing has enabled researchers to demonstrate that there is considerable diversity within the gut microbiota of humans[12]. There, however, appears to be a "core" microbiota in adults at the phylum level which is dominated by Firmicutes and Bacteroidetes[36] and it appears that intra-individual microbial composition remains relatively stable over time[8,37,38]. One study demonstrated that around 60% of the bacterial strains continued to be stable over a 5 year period[8].

Members of the Bacteroidetes and Actinobacteria phyla appeared to be more resilient to perturbations whereas Proteobacteria and Firmicutes were relatively less constant. This study also revealed that a low calorie diet, which lead to a 10% reduction in body weight in a sub-sample of participants, resulted in one of the largest perturbations in gut microbiota composition over the 5 year time period.

Even though the gut microbiota of each individual generally appear to have a distinct and diverse profile, especially at the genera and species level, the bacterial genes (microbiome) within the community seem to be shared amongst individuals which may suggest there is functional redundancy[28,39]. The microbial capacity to metabolise and extract energy from substrates would be advantageous in a diverse community of gut microbiota as changes to the microbiota composition would not necessarily lead to changes in metabolic function. Greater microbial diversity may in turn lead to a microbiota profile which is more resilient to change[40].

The European Metagenomics of the Human Intestinal Tract (MetaHIT)[28] and the US Human Microbiome Project (HMP)[29] are large scale projects which are characterising human associated microbiota across various sites on and in the body, including the GI tract. The sequencing work these projects have undertaken have helped to demonstrate the significant variability in microbial ecology amongst individuals including those with certain disease states. Diseases such as obesity[3], IBD[4], T2DM[5] and colon cancers[6] have been associated with a disruption in "healthy" gut microbiota composition commonly referred to as dysbiosis. Relationships between the gut microbiota, host health and disease are convoluted and multi-factorial prompting further investigation in spite of the advances in our knowledge largely driven by these projects.

Researchers are highly motivated to discover what depicts a “healthy” gut microbiota profile as variations from the norm may constitute disease or disease risk. This knowledge may also help in the design of diet specific therapeutic strategies to alleviate dysbiosis. Variation in gut microbiota composition between and within individuals complicates our understanding of a “healthy” gut microbiota.

2.4 Metabolic functionality of the gut microbiota

The metabolic activity of the commensal bacteria has a huge influence on the human host. Humans are able to digest and absorb certain disaccharides but are unable to break down most plant cell wall derived complex carbohydrates. Gut bacteria synthesise enzymes that have the capacity to digest polysaccharides making them available to their host as a source of energy[41,42]. The study of germ-free mice has demonstrated that without the presence of microorganisms within the GI tract, 30% more energy is required from the diet as a result of a lack of microbiota assisted energy harvesting. These animals excrete twice as much energy in their urine and faeces as conventional mice consuming the same diet[43]. This helps provide evidence to suggest that gut microbiota have the ability to salvage energy from food which would otherwise be unavailable to their host. There is limited direct evidence of the role of gut microbiota in energy harvest in humans[44]. Schwiertz and co-authors[45] have, however, demonstrated that there is indirect evidence to suggest that human associated gut microbiota have energy harvesting abilities.

The majority of the energy extracted from indigestible carbohydrates is thought to occur as a consequence of bacterial fermentation resulting in short-chain fatty acid (SCFA) production (acetate, propionate and butyrate)[46,47]. Butyrate is utilised by the colonic epithelial cells and the peripheral tissue make use of acetate and propionate as energy substrates[48]. The microbial ecology of the GI tract and substrate availability influence the metabolic capacity of the gut microbiota community[49,50]. It remains unclear what role certain gut-associated bacteria have to play in carbohydrate fermentation. Dietary substrates do have a major influence on the composition of the microbiota and subsequently the metabolites produced by the community (Figure 2-2)[51].

As well as providing additional energy which is utilised by the host, SCFA have an integral role in maintaining intestinal epithelial integrity[46,47]. SCFA have also been shown to influence the production of gut hormones involved in appetite regulation (peptide YY [PYY] and glucagon-like peptide 1 [GLP-1]), affect gut motility and have anti-inflammatory effects[52–54]. As SCFA have multiple links to human physiological function it is prudent to gain a better understanding of the factors which influence SCFA production.

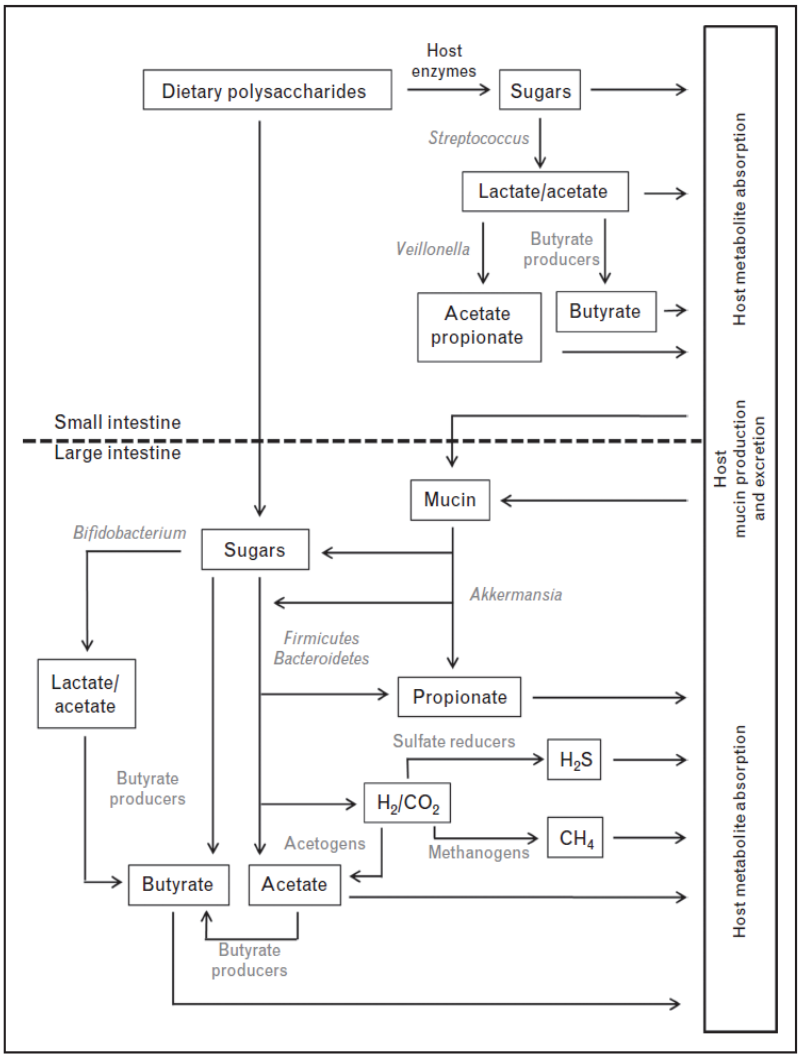

Figure 2-2. An overview of the microbial groups (indicated in grey) and by-products formed as a result of dietary polysaccharide fermentation within the human GI tract (figure from reference[55])

2.5 Analysing gut microbiota communities

2.5.1 Culture-based methods

Traditionally, observations of microbial communities were undertaken using culture-dependent methods which involved culturing microbes using specific medium within the laboratory[56,57]. Cultivation of single strains of bacteria and subsequent microscope observation were the first techniques utilised to observe gut microbiota ecology. These techniques provided inconsistent results relating to the type and number of bacteria present. Gram staining and subsequent microscope observations suggested that gram-positive bacteria account for 2-45% of the microbes observed, however, culture-based techniques have identified that 16-84% of the cells are gram-positive[58].

There are a number of benefits associated with culture-based methods such as cost-effectiveness and reproducibility[59]. However, as a result of the technological advances in molecular analysis a number of pitfalls in culture dependent methods have been demonstrated. Around 80% of the predominant gut-associated microbes are uncultured[7]. Important growth requirements such as preferred carbon sources for a number of gut microbiota are unknown[60]. Additionally, the majority of gut microbiota are strict anaerobes making culturing particularly difficult, therefore, easily isolated populations which flourish in standard laboratory conditions were the predominant bacteria studied[61].

2.5.2 Culture-independent methods

The repertoire of known gut microbes increased extensively once commercial sequencing methods became available[62,63]. Molecular analysis techniques have increased the precision of gut microbiota community observations. The 16S rRNA bacterial gene, which is approximately 1,500 base pairs in length and present in all bacteria, is commonly targeted in phylogenetic studies as it is a relatively small gene containing a

good balance of variable and conserved regions. The variable regions provide enough distinction to help in the identification of differing species and strains but enough similarity to identify bacterial phylum and genus within a sample[64]. The Ribosomal Database Project, Greengenes and Silva are reference databases which contain sequence data relating to a huge number of differing bacteria[65]. These databases provide invaluable information to enable identification of the various microbes present within a particular sample.

2.5.2.1 Next-generation sequencing

Molecular analysis techniques available for the analysis of the bacterial genome vary widely. However, for the purposes of this review only next-generation sequencing will be discussed. Bacterial gene sequencing has been available since the 1970s[66] but had long been out of reach for most of the scientific community secondary to its expensive, labour intensive and time consuming method. In 2005, the first commercially available next-generation sequencer became available[67]. Instead of amplifying the full length of the 16S rRNA bacterial gene (Sanger sequencing) this new technology amplified only select highly variable regions within the gene. The processing speed and capacity were much higher and the costs associated were, therefore, much lower than Sanger sequencing[59]. The fields of molecular and genomic research were revolutionised.

A number of next-generation sequencers have been developed including the Roche 454 pyrosequencer, Illumina/Solexa Genome Analyzer, SOiLD, HeliScope Single Molecule Sequencer and Single Molecule Real Time technology[64]. The majority of sequencing research is undertaken using either 454 pyrosequencing or Illumina sequencing. Up until recently 454 pyrosequencing was preferred, however, improvements in Illumina sequencing technology have increased the read length and this combined with an increase in read numbers per run have led to a reduction in the costs

associated with this next-generation sequencer, increasing its popularity [68]. Next-generation sequencing enables the identification of the types of bacteria present, their relative abundance, diversity and the phylogenetic relationships within a certain microbial community. This technology is more effective at providing taxonomic assignment for phylum, classes, orders, families and genus. Identification at the species or strain level are less clear[60,64].

2.5.2.2 "-Omics"

The functional capacity of the gut microbiota can be analysed using a number of techniques such as metagenomics, metatranscriptomics and metabolomics, amongst others. Metagenomics involves analysing the entire microbial communities' genome using shotgun sequencing to derive the functional capacity of the microbiota. This information helps to demonstrate whether certain genes, involved in carbohydrate degradation for example, within a microbial community differ between individuals. Metabolomics and metatranscriptomics can provide insight into the metabolites produced and the genes expressed by a particular microbial community[59]. Next-generation sequencing data (16S rRNA bacterial gene) can also be used to predict the functional capacity of a bacterial community. This technique is called Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt)[69].

Phylogeny and metagenomic approaches complement each other as the combination of these techniques provide useful information relating to the dominant phylogenetic groups within a microbial community as well as their functional capacity.

2.6 Factors that influence gut microbiota composition

Much research has been conducted to identify factors that influence the composition of the gut microbiota to help explain why there is such high variability between individuals[7,70]. It appears that the gut microbiota of a particular individual are relatively stable over time[71,72]; however, significant external factors such as genetics, age, gender, antibiotics, disease and diet can shape the composition of the gut microbiota, with diet appearing to be particularly influential.

2.6.1 Genetics

It appears that host genotype may help shape the composition of the gut microbiota. Genetically dissimilar strains of mice appear to exhibit gut microbiota which comprise of divergent communities independent of external factors such as diet and housing environment[73]. To date the majority of gut microbiota and genetic research conducted has utilised murine species, however, human research focusing on the microbial ecology of monozygotic and dizygotic twins has suggested that it is more likely that the environment, including the fact that twins share a mother, rather than genetics which play a significant role in shaping human gut microbiota[74]. Even though mother-daughter pairs and twins have a gut microbiota composition that is more similar than unrelated individuals, monozygotic and dizygotic twins appear to have equally similar microbial profiles. This is surprising as it had previously been suggested that more genetically similar monozygotic twins would have a microbiota profile which is less distinctive then dizygotic twins[39].

2.6.2 Life stage and gender

While it appears the gut microbiota is relatively stable over time within adults, there are stages throughout the human lifecycle which lend themselves to dramatic compositional and functional alterations. During the first two to three years of life, the gut microbiota

composition increases in stability and diversity[12,75]. Initially the infant gut is colonised predominately by *Enterococcus* and *Enterobacteriaceae*, however, as the oxygen supply within the colon depletes anaerobes such as *Bifidobacterium, Bacteroides* and *Clostridium* appear[9]. Factors such as mode of delivery, feeding type (breastfeeding vs. infant formula feeding) and introduction of complementary foods also influence early life microbial composition[9,76,77]. By the age of two to three years, the appearance of the microbiota is very similar to that of the adult microbial community. It is generally accepted that throughout later life, the structure of the microbiota remains relatively stable. Contradictory research has, however, demonstrated that adolescents have a higher abundance of *Clostridium* spp. and *Bifidobacterium* spp. compared to adults[10]. Furthermore, it appears that a last "shift" in the microbiota is then experienced in elderly individuals resulting in an increase in *Bacteroides* and an alteration in the *Clostridium* genera[78]. It has been suggested that these changes are brought about due to chronic low-grade GI tract inflammation and changes in dietary intake because of institutional living[78].

Gender has been explored as another potentially influential factor involved in shaping the ecology of the GI tract[12,13]. Males have been shown to have a higher abundance of the *Bacteroides-Prevotella* group compared to females but significant differences in other microbial groups were not exhibited[13].

2.6.3 Antibiotics

Clinicians commonly use broad-range antibiotics to treat a number of ailments. As this treatment option does not often specifically target particular bacterial strains, commensal gut associated bacteria may be perturbed. Research has shown that antibiotics do influence the composition of the gut microbiota[14,15]. Antibiotics generally reduce bacterial diversity but the effect antibiotics have on the taxonomic composition varies

between individuals. It appears that the resilience of the gut microbiota to antibiotic treatment also varies between individuals[15]. In some individuals antibiotic treatment has been shown to have an influence on the microbial composition for as long as 2 years[14]. Microbial genes associated with antibiotic resistance can also persist within the microbiome for a number of years[79]. Interestingly, it appears that alteration of the gut microbiota via targeted antibiotic treatment may be emerging as a beneficial therapeutic option to alleviate dysbiosis associated with disease[80].

2.6.4 Human disease

The human GI tract harbours a complex ecosystem of bacteria. A symbiotic host-microbe relationship is believed to benefit human health as commensal gut microbiota protect their human host against enteropathogenic organism colonisation[81,82], modulate the innate and adaptive immune system[82], extract nutrients from undigested dietary components[42,82] and synthesise essential vitamins[46]. In contrast, intestinal dysbiosis has been suggested to promote or aggravate certain disease states.

2.6.4.1 Early-life gut microbiota development

Development of the gut microbiota in early-life, during a critical period of time, is thought to play a significant role in the maturation of the immune-system and may have an influence on the incidence of atopic, auto-immune and inflammatory diseases. Modern medical interventions implemented to reduce infant mortality such as Caesarean sections, formula feeding and antibiotic treatment may contribute to the rise in immune-mediated diseases observed in economically-developed countries. It has been suggested that gut microbiota development is influenced by mode of delivery[83], maternal microbe transfer during gestation[84], environmental microbe exposure[85], use of antibiotics[86], breast or formula feeding[87] and complementary feeding practices[86]. Breastfed infants have been shown to have lower concentrations of opportunistic bacteria such as *Escherichia coli*

and *Clostridium difficile*[87] but higher *Bifidobacterium* spp. concentrations[88,89] compared to formula-fed infants. Jimenez and co-authors[84] utilised a murine model to investigate whether maternal gut microbiota transfer to the fetus *in utero*. Pregnant mice were given milk that contained genetically-labelled *Enterococcus faecium*. After sterile Caesarean section, microbes within the pups' meconium were analysed. Genetically-labelled *E. faecium* was only cultured from the meconium of pups born to mothers that consumed the genetically-labelled milk not from the control pups. This result suggests that maternal microbiota transmission does occur in mammals. Human studies have suggested that maternal microbiota transmission may occur[90] but to date the most convincing evidence comes from animal studies. Antibiotic use in the first month of life has been shown to reduce the concentrations of bifidobacteria and *Bacteroides fragilis*. However, antibiotic use by the mother during pregnancy did not appear to have an influence on the gut microbiota composition of the offspring[86]. Early-life exposure to antibiotics is also associated with childhood overweight and obesity which suggests that antibiotic driven perturbation of the microbial balance at this critical time point may have implications on host metabolic functionality[91,92]. In one study the negative association between antibiotic exposure in infancy and childhood overweight was only observed in children born to normal weight mothers. Children born to overweight mothers experienced a slight reduction in childhood overweight if exposed to antibiotics in infancy[91]. This is an interesting finding as it suggests that early-life antibiotic exposure in offspring of overweight women may reduce the abundance of potentially obesogenic microbiota that may have been passed on via maternal microbiota transmission.

2.6.4.2 Obesity

Utilisation of gnotobiotic mice models has demonstrated that germ-free mice have a reduced adiposity, lower circulating leptin, insulin and glucose levels and an enhanced

glucose tolerance compared to conventional mice that harbour gut microbiota[93]. An association between the gut microbiota and metabolic disease was further strengthened when it was discovered that germ-free mice fed a Western-style diet did not develop diet-related obesity[94]. Additionally, faecal microbiota transfer from obese human donors into germ-free mice led to greater body fat accretion than in germ-free mice colonised with the microbiota of lean human donors further suggesting that gut microbiota are linked to host metabolism[95]. Several mechanisms have been proposed to explain the association between the gut microbiota and host metabolism, including (1) increased energy extraction from fermentable substrates due to the production of SCFA[96], (2) altered sensory perception of nutrients such as dietary fat[97] and sucrose[98] and (3) high-fat dietary intakes leading to an increase production of bacterial lipopolysaccharide (LPS) and, therefore, high concentrations of circulating LPS leading to insulin resistance, endotoxaemia and chronic low-grade inflammation[99,100]. The mechanisms which underlie the link between gut microbiota and host metabolism are incompletely understood and need to be explored in greater detail. In humans, obese individuals have a gut microbiota profile that is distinct from normal weight individuals. Some studies have demonstrated that obese individuals have a decreased abundance of Bacteroidetes and an increased abundance of Firmicutes[3,39]. A study conducted in 98 individuals (30 lean [BMI 18.5-25 kg/m^2], 35 overweight [BMI 25.1- 30 kg/m^2] and 33 obese [BMI >30 kg/m^2]) demonstrated contrary results[45]. Obese and overweight individuals had Firmicutes:Bacteroidetes ratios that favoured Bacteroidetes, highlighting the controversy surrounding obesity and the Firmicutes:Bacteroidetes ratio[45]. The same study demonstrated that total SCFA and propionate concentrations in overweight and obese individuals were more than 20% higher than that of lean individuals. A number of known propionate producers belong to the phylum Bacteroidetes (i.e. *Bacteroides* and

Prevotella) which were shown to be significantly higher in overweight and obese individuals[45]. Another study demonstrated that overweight and obese individuals (n = 37) produced higher amounts of total SCFA, propionate, butyrate and acetate compared to lean individuals (n = 52). Firmicutes:Bacteroidetes ratio did not differ between the two groups but the ratio was shown to significantly correlate with total SCFA concentrations suggesting that they may be inter-related[101]. Microbial diversity also appears to be lower in obese compared to lean individuals[39] with lower microbial diversity being shown to be associated with disease risk[102]. Even though fairly convincing evidence exists to link dysbiosis with an obese state, uncertainty continues to exist around whether dysbiosis is a cause or consequence of obesity.

2.6.4.3 Type 2 diabetes mellitus

Paralleling obesity, rates of T2DM continue to rise worldwide. It is predicted by 2035 that the number of individuals with T2DM will rise from 382 million (prevalence in 2013) to 592 million[103]. Emerging evidence suggests that the microbiota residing within the GI tract influence T2DM risk. It appears that dysbiosis is associated with T2DM with a depletion in the abundance of several butyrate-producing bacteria (i.e. *Roseburia* spp. *Clostridium* spp. *Eubacterium rectale* and *Faecalibacterium prausnitzii*) observed in T2DM individuals[104–107]. A number of bacterial groups have been shown to be positively correlated with plasma glucose concentrations including the ratio of Bacteroidetes to Firmicutes and *Bacteroides-Prevotella* to *Clostridium coccoide-E.rectale* ratio[104]. Gut microbiota shown to be higher in abundance in individuals with T2DM include Betaproteobacteria, *Desulfovibrio* and a number of *Lactobacillus* spp[104,105,107]. In a study of 123 non-obese and 169 obese individuals a phenotype characterised by metabolic disturbance (i.e. increased serum leptin, decreased HDL-cholesterol, marked inflammation, insulin resistance and hyperinsulinaemia) was observed in individuals

with lower microbial gene richness[102]. Therefore, a reduced microbial diversity could be a result of insulin resistance and chronic low-grade inflammation leading to an increased metabolic disease risk. It has been suggested that LPS produced by bacteria stimulate Toll-like receptors (TLR) resulting in a pro-inflammatory response. In mice, increasing the circulating levels of LPS (also known as metabolic endotoxaemia) through continuous subcutaneous infusion led to an increase in glycaemia, insulinaemia and weight gain comparable to the degree observed in mice fed a high-fat diet[99]. In humans, metabolic endotoxaemia has been observed to increase inflammatory markers and insulin resistance[108]. The metabolic functionality of gut bacteria may also be implicated in glucose tolerance. De Mello and co-authors[109] recruited individuals with impaired glucose tolerance and followed them up over a 15 year period to determine whether they developed T2DM. In participants that did not develop T2DM, serum metabolite profiles, particularly 3-indolepropionic acid (a neuroprotective antioxidant) and lipid metabolites, were positively linked to improved insulin production and sensitivity and negatively linked to low-grade inflammation. The researchers suggested that 3-indolepropionic acid could be utilised as a biomarker of T2DM risk. It has been proposed that SCFAs produced by gut microbiota also affect insulin sensitivity and glucose homeostasis[110]. A number of mechanisms of action have been proposed including activation of intestinal gluconeogenesis by butyrate and propionate[111] and an increased secretion of gut hormones from intestinal enteroendocrine cells, such as GLP-1 and PYY, which enhances satiety and glucose control[110]. The mechanisms underlying the effect SCFAs have on T2DM development generally remain unknown.

It is worth noting that recent research has highlighted that gut microbiota analysis of T2DM patients may be confounded by anti-diabetic drugs such as metformin[112]. Metformin treatment has been shown to be associated with a significant increase in

Escherichia spp. and a reduction in *Intestinibacter* abundance. Therefore, it is important to separate the effect a specific disease has from the effect a drug taken to help control a specific disease has on the gut microbiota[112].

2.6.4.4 Inflammatory bowel disease

The incidence and prevalence of IBD in economically-developed countries, such as the United States of America, United Kingdom, Canada and New Zealand, continue to rise[113]. IBD pathogenesis is not fully understood; however, genetic and environmental factors are believed to be involved. Recent work has indicated that interactions between the gut microbiota and host may be involved in the development and exacerbation of IBD[114]. It has been hypothesised that IBD may develop due to a dysfunctional immune response which targets the commensal gut microbiota in hosts with genetic susceptibility. In healthy individuals, the interaction between the gut microbiota, mucosal barrier and immune system are thought to be in equilibrium. In IBD this balance is disturbed, leading to dysbiosis, a leaky gut (i.e. increased intestinal permeability) and inflammation. Ulcerative colitis (UC) and Crohn's disease (CD) are the two major types of IBD. In individuals with UC the disease is isolated to the colon, whereas, Crohn's disease can occur anywhere along the GI tract. Microbial dysbiosis is regularly reported in individuals suffering from IBD, particularly in individuals with CD[114]. It is, however, unclear whether the changes in gut microbiota are a cause or consequence of the disease. CD is also characterised by a lower microbial diversity, a reduction in *F. prausnitzii, Ruminococcus gnavus* and *Bifidobacterium adolescentis* and an increase in *E. coli*[115]. UC has been associated with lower microbial diversity[116] but gut microbiota profiles in UC tend to be more similar to healthy individuals than individuals with CD[117]. A number of studies have demonstrated that bacterial species from the butyrate-producing *Clostridium* cluster IV and XIVa (i.e. *F. prausnitzii* and *Roseburia hominis*) are reduced in abundance

in individuals with IBD[115,118,119]. A number of bacterial genera shown to be in low abundance in individuals with IBD (i.e. *Bifidobacterium, Lactobacillus* and *Faecalibacterium*) are known to enhance immune system tolerance and reduce GI inflammation by stimulating $CD4^+$ regulatory T cells activity[120], down-regulating inflammatory cytokines (such as tumor necrosis factor alpha)[121] and up-regulating anti-inflammatory cytokines (such as interleukin-10)[122]. It has been reported that a large proportion of individuals with IBD do not respond adequately to current medical treatments, such as immune-modulating therapies. As the gut microbiota is implicated in IBD pathogenesis, novel microbiota-based therapeutic strategies (i.e. prebiotics and probiotics) have the potential to reduce disease incidence and severity.

2.6.4.5 Colorectal cancer

Gut microbiota have been recognised as an important component of colon carcinogenesis, therefore it has been hypothesised that colorectal cancer can be prevented through modulation of the gut microbiota. Studies have shown that individuals with colorectal cancer have a dysbiotic gut microbiota profile compared to healthy individuals[6]. Butyrate-producing species have been demonstrated to be under-represented in patients with colorectal cancer[123]. Ohigashi and co-authors[6] established that colorectal cancer patients (n = 93) also had lower total bacteria, *C. coccoides* group, *Clostridium leptum* subgroup, *B. fragilis* group, *Bifidobacterium*, *Atopobium* cluster, total SCFA, acetate, butyrate and propionate concentrations than in healthy individuals (n = 49). Faecal pH was also higher in patients with colorectal cancer compared to healthy individuals with no adenoma[6]. Another study provided evidence to suggest that colorectal cancer risk may be mediated by health-promoting (i.e. butyrate) and potentially carcinogenic (i.e. secondary bile acids) bacterial fermentation metabolites. The microbiota composition and metabolic activity of high colorectal cancer risk African

Americans (n = 12) were compared to matched low colorectal cancer risk native Africans (n = 12). The high risk group was found to have a higher abundance of *Bacteroides* and potentially pathogenic Proteobacteria and a lower abundance of *Prevotella*, *Faecalibacterium*, *Succinivibrio* and *Oscillospira*. The high risk group had faecal primary and secondary bile acid concentrations that were significantly higher compared to the low risk group. There were also SCFA production distinctions between the two groups with the high risk group having lower concentrations of acetate, propionate and butyrate[124]. The mechanisms hypothesised to be implicated in the carcinogenic potential of dysbiotic gut microbiota link to the metabolic by-products they produce. Intestinal bacteria provide their human host with a supply of folate, biotin and butyrate. Folate is involved in DNA repair, replication and methylation, biotin is involved in gene repression and DNA repair and butyrate has potent anti-inflammatory and anti-neoplastic properties. These bacterial metabolites are known to regulate epithelial proliferation, therefore, they have the potential to facilitate colorectal cancer risk[125].

As compositional, diversity and functional distinctions in gut microbiota exist between healthy individuals and individuals with disease, microbial modulation strategies provide an attractive target to enhance human health and wellbeing.

2.6.5 Dietary intake

Dietary intake has been shown to have a major influence on the structural and functional capacity of the gut microbiota. In mice, dietary change (high fat diet) explains 57% of the variability in gut microbiota composition, with genetics being much less influential[126]. It has been hypothesised that the shifts that occur in gut microbiota composition, due to dietary change, occur as the saccharolytic capacity of the bacterial species that reside within the GI tract vary depending on their genetic potential. Additionally, the GI environment (i.e. pH, substrate availability, bile salt concentrations)

will have an influence on bacterial species tolerance and survival. As the gut microbiota play a significant role in human health and disease, modulation of the gut microbiota offers a novel target for nutrition and health strategies.

2.6.5.1 Influential nutrients

Fermentable dietary substrates have a major influence on the composition of the gut microbiota and are likely to be responsible, at least in part, for the large inter-individual variation in gut microbiota communities. It is necessary to have a broad understanding of how dietary components influence bacterial ecosystems within the GI tract. A deeper understanding of the influence specific nutrients have on the gut microbiota will help determine how diet shapes the microbiota and whether specific dietary components can be utilised as therapeutic tools to beneficially manipulate the microbial community to improve host health. The composition of the diet, particularly carbohydrates, protein and fat, influence the gut microbiota composition greatly[80]. It is estimated that 25-70 g of fermentable substrates are delivered to the commensal gut microbiota daily[127]. The majority of these substrates are indigestible carbohydrates, however some protein and fat escape digestion and absorption in the small intestine adding to the fermentable substrates available to the gut microbiota[127].

Indigestible carbohydrates provide major carbon and energy sources to the gut microbiota. Within the colon there is intense competition for substrates and the ability of the microbiota to compete for substrates relies on their specialised profile of enzymes which are able to break down carbohydrates inaccessible to the host[128]. The differing chemical structures of the indigestible carbohydrates influence how and what bacteria utilise them. Simple structured carbohydrates include fructo-oligosaccharides, starch, β-glucan and inulin. More complex structured carbohydrates usually contain branched-chain configurations and include pectin and hemi-cellulose[129]. Carbohydrates can either

promote the growth of certain bacteria in a direct manner or via cross-feeding interactions (indirect) between bacterial species[130]. Bacterial species shown to have saccharolytic capabilities include *Roseburia intestinalis, Lactobacillus* spp., *B. adolescentis, E. rectale, F. prausnitzii, Bacteroides thetaiotaomicron* and *C. coccoides* to name a few[131–133].

It is likely that gut microbiota utilise substrates in a hierarchical manner. This was demonstrated when the response of *B. thetaiotaomicron* to a number of differing carbohydrate substrates within an *in vitro* system was investigated. It was shown that *B. thetaiotaomicron* preferentially ferments certain substrates over other substrates in a process named "metabolic hierarchy"[134]. It is therefore plausible that bacteria within the GI tract respond in a similar way. A better understanding of the substrates which are preferentially fermented by the "good" bacteria within the gut could help increase the relative abundance of these "good" bacteria via selective carbohydrate manipulation interventions.

Dietary fat has also been shown to influence the microbial community within the GI tract. Fat is not believed to reach the colon in large amounts as it is usually effectively digested and absorbed in the small bowel. The main role of fat in modulating gut microbiota composition is thought to be indirect as a consequence of bile acid excretion mediated by dietary fat intake. The higher the fat intake the higher the presence of bile acids within the colon as a result of reduced reabsorption[135]. Colonic microbiota produce secondary bile acids via the breakdown of primary bile acids. These secondary bile acids may be carcinogenic and therefore could be connected to colon cancer and/or other GI disorders[136]. High fat diets are also thought to increase the circulation of lipopolysaccharides, a component of the cell wall of Gram negative bacteria, which have been linked to host inflammatory agents[137]. Bile acids usually have an inhibitory

influence on gut microbiota, however, some species have bile salt hydrolase enzymes which reduce their inhibitory influence. Bacterial groups known to thrive in a high fat environment include Firmicutes (particularly *Clostridium*) and Proteobacteria (particularly *Desulfovibrio*). The Bacteroidetes phyla (particularly *Bacteroides)* is also particularly sensitive to the presence of fat within the colon, however, their numbers usually reduce as a result of a high fat intake[96,138]. Relatively little is known about the amount of bile acids which reach the colon in response to a high fat diet or what influence the small amount of fat which reaches the colon may have on the microbial community and therefore host health.

As well as providing the gut microbiota with an additional energy source, dietary protein also serves as a major source of nitrogen, a key component for bacterial growth. Like dietary fat, dietary protein is highly susceptible to digestion and absorption in the small bowel, however, high intakes can result in protein being delivered to the colon making it accessible to the colonic microbiota as a fermentable substrate. Digesta transitioning through the colon progressively lose carbohydrate content as this substrate is utilised by the proximal colonic microbiota. The reduced abundance of carbohydrate substrates increase the importance of protein fermentation as the digesta progresses through the GI tract towards the distal colon (Figure 2-1)[139]. The distal colon provides a more favourable neutral pH environment for putrefying, proteolytic bacteria such as *Bacteroides* spp., *Clostridium, Eubacterium*, *Enterococcus* spp.*, Bifidobacterium* spp. and *Propionibacterium* spp[50,140,141]. Protein fermentation results in the generation of a large number of by-products including branched-chain fatty acids, ammonia, phenols and hydrogen sulphide[142]. Protein fermentation may improve the health of the GI tract via branched-chain fatty acid production but may also cause harm as a result of potentially carcinogenic end-products such as ammonia, phenols and hydrogen sulphides[142]. A large

proportion of human studies investigating protein fermentation by gut microbiota do so by detecting end products within the faeces rather than determining how protein elicits changes to the gut microbiota community directly[143].

2.6.5.2 Human observational studies

Traditional populations have been studied to investigate the influence modern lifestyles, in economically-developed countries, have on the structural and functional capacity of the gut microbiota[12,17,124,144,145]. De Filippo and co-authors[17] demonstrated that European children (EC; n = 15) had a higher abundance of Firmicutes and Proteobacteria while children from a rural African village (AC; n = 14) had a higher abundance of Actinobacteria and Bacteroidetes. *Xylanibacter, Prevotella, Butyrivibrio* and *Treponema*. These bacteria are capable of fermenting indigestible carbohydrates and were also found exclusively in the AC. Large distinctions in dietary patterns were observed between the AC and EC which led the researchers to hypothesise that the microbiota of the AC were adapted to the high indigestible carbohydrate content of their diet leading to the higher abundance of saccharolytic bacteria. The gut microbiota of EC were also shown to be lower in microbial diversity and were less functionally active compared with those of the AC. It is possible that the lower intake of indigestible carbohydrates led to the lower SCFA concentrations observed in EC[17]. Similar results were demonstrated when structural and functional distinctions in gut microbiota were analysed in African Americans (AA; n = 12) and rural native Africans (NA; n = 12). The dietary differences between the two groups were also similar to what was observed in the de Filippo study; with AA consuming more animal protein and fat and less dietary fibre compared to NA. The most prominent difference in gut microbiota observed was a higher abundance of *Prevotella* in the NA group and a higher abundance of *Bacteroides* in the AA group[124]. These results correspond with the pioneering enterotype study which

established that individuals could be classified based on three predominant bacterial groups - *Prevotella, Bacteroides* and *Ruminococcus*[146]. A study published in the same year observed that enterotypes were strongly linked with habitual dietary intakes, with dietary differences being the primary reason for the distinction in enterotypes. High intakes of animal protein and saturated fat were correlated with a *Bacteroides* enterotype, whereas, carbohydrates and simple sugars were correlated with a *Prevotella* enterotype[16].

Other distinctive habitual dietary patterns (i.e. omnivorous *versus* vegetarian/vegan diets) are also characterised by differing bacterial profiles and metabolomes[18,147–149]. Matijasic and co-authors[18] showed there were a number of compositional differences in the gut microbiota of lacto-vegetarians/vegans (n = 31) and omnivores (n = 29) living in Slovenia. Lacto-vegetarians/vegans had a higher relative quantity of *Bacteroides-Prevotella, Bacteroides thetaiotaomicron, Clostridium clostridioforme* and *Faecalibacterium prausnitzii* and a reduced relative quantity of *Clostridium* cluster XIVa compared to omnivores. The large variance in microbial composition was suggested to be related to the consumption of differing dietary components. Another study, conducted in the United States of America, reported very few gut microbiota composition differences between vegans (n = 15) and omnivores (n = 16) but they did demonstrate that plasma metabolome differences existed, with 25% of the metabolites tested differing between vegans and omnivores. Interestingly, diet appeared to be more predictive of metabolome production than gut microbiota composition in this cohort[149].

As previously discussed, commensal microbes that reside within the GI tract are implicated in human disease. Therefore, as habitual dietary intakes have a major impact on the composition of the gut microbiota it is plausible that dietary interventions could be utilised to modulate the gut microbiota to reduce disease risk.

2.6.5.3 Human dietary intervention studies

Dietary interventions which aim to elicit changes in the gut microbiota provide an exciting prospect for disease prevention. Extensive research has demonstrated that dietary interventions can elicit significant changes in gut microbiota composition and function. Pronounced short-term changes in macronutrient intake have been shown to result in a dramatic shift in gut microbiota composition within 24 hours. David and co-authors[19] initiated a 5-day dietary intervention consisting of either a plant-based diet of grains, legumes, fruits and vegetables or an animal-based diet of meat, eggs and cheese in ten healthy individuals. The animal-based diet had the most significant impact on the bacterial taxa particularly for bile-tolerant bacterial groups (i.e. *Bilophila wadsworthia, Alistipes putredinis* and *Bacteroides* spp.) and amino acid fermentation specific SCFA production (i.e. isovalerate and isobutyrate). A positive correlation between saccharolytic bacteria (i.e. *Roseburia* spp., *E. rectale* and *F. prausnitzii*) and carbohydrate fermentation by-products (i.e. acetate and butyrate) was demonstrated during the plant-based diet.

Dietary fibre are non-digestible plant oligo- and poly-saccharides, such as non-starch polysaccharides, resistant starch, β-glucan and inulin-type fructans, which are found in wholegrains, legumes, nuts and seeds, fruits and vegetables. A high fibre containing diet has been shown to have a number of health benefits[150]. The exact mechanisms are not fully understood, however, research suggests that the benefits of dietary fibre on human pathophysiology may be mediated by the gut microbiota[151,152]. In germ-free mice, inoculated with human microbiota, dietary fibre deficiency led to microbial utilisation of host-secreted mucin as a source of energy, as diet-derived fermentable substrates were unavailable. Colonic mucin degradation led to intestinal barrier dysfunction which promoted microbial induced colitis[153]. Long-term low-fibre

consumption in mice has also been shown to lead to inter-generational loss of certain bacterial species that are not restored by increasing the fibre-content of the diet in future generations suggesting that "extinction" of potentially beneficial microbes may occur when the GI environment is depleted of fermentable carbohydrates[154]. A study conducted in 82 individuals demonstrated that in women total fibre and fibre from fruit, vegetables and grains were associated with overall gut microbiota composition. Whereas in men, the only association that existed was for bean fibre and gut microbiota composition. Additionally, intakes of fibre from fruit and vegetables was shown to cluster with the class Clostridia, and members of the phylum Actinobacteria clustered with fibre from bean intake[151]. Low indigestible short-chain carbohydrate diets, also known as a low Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols (FODMAP) diet, have been utilised as a dietary therapy for individuals with irritable bowel syndrome (IBS). A reduction in the FODMAP content of the diet is believed to reduce the production of microbial fermentation by-products, such as gas, helping to alleviate common IBS symptoms (i.e. flatulence and bloating). Microbial production of SCFA are thought to help reduce diarrhoea, another common symptom of IBS, as SCFA help stimulate fluid absorption in the colon[155]. A number of low FODMAP dietary intervention studies in individuals with IBS have been conducted to demonstrate whether the reduction in symptoms is associated with changes in gut microbiota. Considerable changes in gut microbiota have been shown after a low FODMAP diet[156–158]. Halmos and co-authors[156] observed lower total bacteria concentrations, *Clostridium* cluster XIVa and *Akkermansia muciniphila* abundance in individuals consuming a low FODMAP diet compared to a typical Australian diet. Stool pH was higher after the low FODMAP diet but there were no differences in stool SCFA concentrations between the two diet types. The low FODMAP diet reduced GI symptoms irrespective of changes in stool SCFA

concentrations[159]. Conversely, increases in dietary fibre intake in healthy individuals have led to significant changes in the intestinal microbiota. Five-day supplementation with 10 or 40 g/day of dietary fibre in a group of 19 healthy individuals led to modest differences in gut microbiota composition between the low and high dietary fibre supplemented groups[20]. Over-representation of microbial genes associated with glycan and lipid metabolism and a down-regulation of genes associated with mucin degradation were seen in individuals whose diets were supplemented with 40 g/day of dietary fibre[20]. These results suggest that dietary fibre associated changes in the functional capacity of the microbiota may occur irrespective of changes in microbiota community structure.

A prebiotic is "a substrate that is selectively utilized by host microorganisms conferring a health benefit"[160]. Prebiotics have the potential to be used to increase beneficial bacteria that are low in abundance in individuals with increased disease risk. Established prebiotics include inulin-type fructans (e.g. inulin, oligofructose and fructo-oligosaccharides), galacto-oligosaccharides and lactulose. Having the ability to target specific bacterial taxa requires an understanding of what constituents a beneficial bacterial profile as well as a broad appreciation of the substrates utilised by beneficial bacteria. A number of prebiotic intervention studies in humans have been conducted to demonstrate the influence a given fermentable substrate (i.e. prebiotic) has on gut microbiota composition and host outcomes. A large proportion of prebiotic intervention studies have focused on eliciting changes in bifidobacteria as high levels of these bacteria are considered to represent a healthy microbial consortia[161–164]. Other bacterial groups have also been shown to increase as a result of prebiotic interventions, including *Lactobacillus*[163] and *Faecalibacterium*[165] but very few studies have demonstrated what influence prebiotics have on the entire community. Holscher and co-authors[166] utilised high-throughput sequencing technology to determine the impact 5 or 7.5 g/day of agave

inulin had on the entire microbial community of 29 healthy adults. As well as observing a significant increase in *Bifidobacterium* relative abundance, a reduction in *Ruminococcus* and *Desulfovibrio* relative abundances were demonstrated providing a clearer picture of the entire microbial communities' response to agave inulin.

A number of studies which have demonstrated that a given dietary intervention elicits significant changes in the structure and functional capacity of the gut microbiota have also shown that gut microbiota changes are associated with improvements in host health outcomes. A study conducted in 81 metabolically healthy individuals aged between 40-65 years determined whether a whole grain rich diet was able to change the gut microbiota and improve host immune response[167]. Participants were instructed to consume a Western-style diet high in refined grains for 2 weeks to help minimise the influence their habitual diet had on the results. For the following 6 weeks, half of the participants continued on the high refined grain diet (8 g of dietary fibre per 1000 kcal) and the other half commenced a diet rich in whole grains (16 g of dietary fibre per 1000 kcal). The calorie content of the diets were personalised to ensure each participant's weight was stable throughout the trial. Individuals consuming the high whole grain diet experienced an increase in stool weight and frequency. A small but significant increase in total SCFA and acetate was observed in the high whole grain group compared to the high refined grain group. A modest increase in the acetate producing genera *Lachnospira* and a reduction in the pro-inflammatory genera *Enterobacteriaceae* was also observed. The high whole grain diet also had an influence on effector memory T cells and the acute innate immune response[167]. Dewulf and co-authors[168] demonstrated that a 3-month inulin-type fructan prebiotic intervention in 30 obese women led to significant increases in *Bifidobacterium* and *F. prausnitzii* concentrations. The increases in these bacterial groups were shown to be negatively associated with changes in LPS, a pro-inflammatory

by-product of bacterial fermentation. This result is interesting as high plasma concentrations of LPS are thought to be associated with the chronic low-grade inflammation characteristic of obesity[99]. The prebiotic also appeared to reduce fat mass and led to a significant reduction in post oral glucose tolerance test glycaemia compared to the placebo[168].

2.7 *In vitro* human GI tract model systems

The most robust method of determining what influence dietary components have on the gut microbiota is via *in vivo* human studies. *In vivo* human studies are, however, expensive, and resource and labour intensive. Additionally, there are inherent limitations to *in vivo* human studies including ability to take samples from within the human GI tract, ethical restrictions and difficulties controlling potentially confounding factors such as immune parameters, dietary non-compliance and medication use. *In vivo* human studies rely on the analysis of the gut microbiota from faeces alone, however, *in vitro* GI tract models provide an opportunity to take samples from different regions within the gut so changes in gut microbiota can be observed along the GI tract[169]. There are some limitations to *in vitro* GI tract models, with the main limitation relating to the physiological relevance of the models. Some models may not be very accurate due to a lack of endogenous substrates, epithelial mucosa and interaction with the hosts' immune system. A number of *in vitro* GI tract models have, however, been validated against the GI contents of sudden death victims to support their physiological relevance[170].

There are a diverse range of *in vitro* GI tract models available from single vessel batch culture models to sophisticated multiple-vessel continuous culture models. Single vessel batch culture models generally consist of a single vessel kept anaerobic, under pH control, at physiologically relevant temperature with a basal medium. Batch culture studies are often only run for 24-48 hours as the substrates within the medium are quickly utilised leading to nutrient deficiencies and build-up of metabolites produced as due to bacterial fermentation[171,172]. The batch culture model generally represents the distal colon due to the specific pH used (generally 6.8-7) and the lower substrate availability. This model is relatively simple to run and is often employed before running more time and resource consuming multiple-vessel continuous culture models. One example of a

multiple-vessel continuous culture model is the *in vitro* three-stage continuous colonic model system. This system has been validated against the colonic contents of four sudden death victims to simulate the physiologically relevant conditions found in the human colon[170]. The *in vitro* three-stage continuous colonic model system consists of three vessels connected in series, which mimic the proximal (V1), transverse (V2) and distal (V3) colon. The temperature of the vessels are maintained at 37^{o}C, with a pH of 5.4–5.6 (V1), 6.1–6.3 (V2) and 6.7–6.9 (V3) and are kept anaerobic by sparging with oxygen free nitrogen gas. The contents within each vessel are stirred to keep them homogenous. Media flows from a 5 L media vessel into V1 via peristaltic pump with V2 and V3 being fed from the overflow of V1 and V2, respectively. Media which overflows from V3 passes into a waste bottle. The transit time for the model is generally around 36 hours (flow rate of 25 mL/hr) (Figure 2-3). The *in vitro* three-stage continuous colonic model system cannot be used to study host-microbe interactions, however, it can be used to model functional capacity and compositional changes in gut microbiota, elicited by fermentable substrate interventions, along the length of the colon.

Various *in vitro* GI tract model studies have been conducted to determine what influence various fermentable substrates have on the composition and function of the gut microbiota. A study conducted by Shen and co-authors[173] used an *in vitro* three-stage continuous colonic model system to demonstrate what impact increasing the total fibre content of the medium would have on the metabolic activity of the gut microbiota. The found that the increase in fibre led to a significant increase in *Bifidobacterium, Lactobacillus-Enterococcus* group, *Ruminococcus* group and an increase in clostridial cluster XIVa and *F. prausnitzii* in vessel 1 (mimicking the proximal colon). Total SCFA concentrations also increased as a result of the fibre enriched medium. The results of this study provides insight into the positive effects an increase in dietary fibre on gut

microbiota composition and function suggesting that high intake of fibre may have a similar effect in *in vivo* human studies. Other studies have utilised *in vitro* GI tract models to demonstrate the prebiotic potential of a novel fermentable substrates. In one such study, the bifidogenic potential of a novel prebiotic-fortified juice was investigated using an *in vitro* three-stage continuous colonic model system. It was demonstrated that the novel prebiotic-fortified juice elicited a significant increase in bifidobacteria in all vessels within the model. There was also a significant increase in acetate and butyrate in vessel 1 of the model due to the novel prebiotic-fortified juice. The researchers concluded that as the prebiotic-fortified juice induced positive changes in gut microbiota composition and function *in vitro* the product has the potential to improve GI health, however, future *in vivo* research is required to ensure the bifidogenic response remains evident in humans[174].

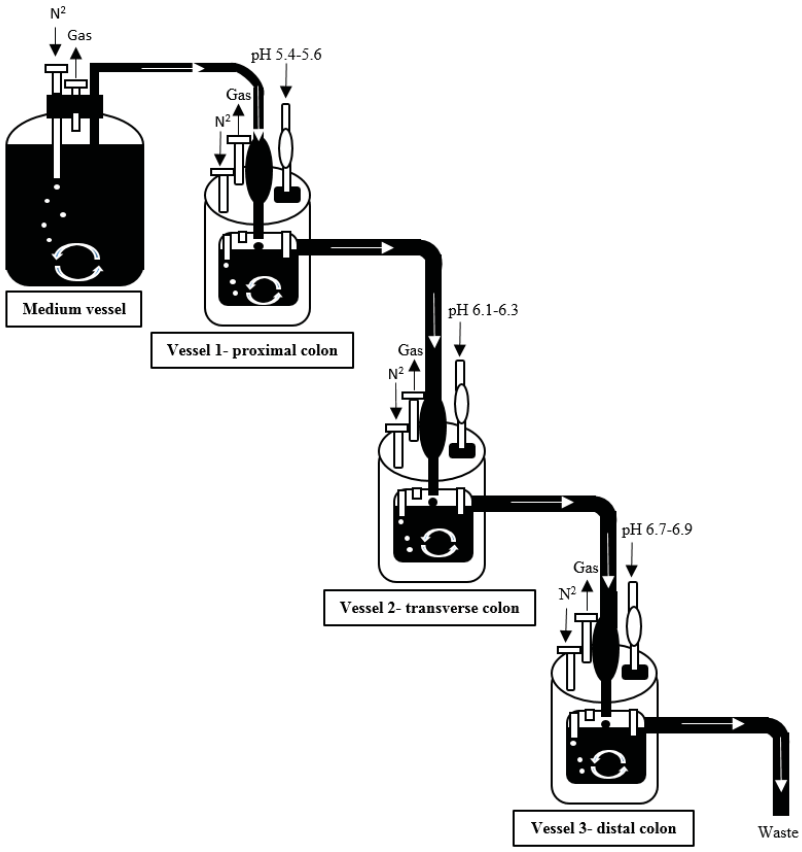

Figure 2-3. *In vitro* three-stage continuous colonic model system

Before making a decision to conduct an *in vitro* GI tract model study consideration needs to be given to the fact that *in vitro* GI tract models only provide a representation of what may occur within an *in vivo* system. Additionally, faecal donors selection, the specific *in vitro* GI tract model to use and the potential for the model contamination also need to be considered[35]. However, the development and utilisation of *in vitro* GI tract models provides an opportunity to test human gut microbiota specific research hypotheses within a controlled environment before progressing to *in vivo* human studies.

2.8 The influence of gut microbiota on appetite regulation

The worldwide prevalence of obesity continues to rise making it a major public health concern. The healthcare costs associated with obesity have now been shown to exceed the costs associated with smoking[175]. Traditional medical management of obesity has not proven to be successful in curbing the rising rates of obesity. Therefore, novel strategies to prevent and manage obesity are in high demand. Appetite regulation plays a key role in the homeostatic processes associated with energy balance and weight management. There are a number of hormonal, neural and psychological mechanisms involved in appetite regulation. Appetite regulation and subsequent energy balance is co-ordinated via neural and hormonal signals received by the hypothalamus and brainstem. The gut-brain axis is involved in regulating short-term energy intake by transmitting hormonal signals from the GI tract to the brain via the vagus nerve. Key satiety and appetite regulatory hormones produced in the GI tract include GLP-1, PYY, pancreatic polypeptide, oxyntomodulin, cholecystokinin and ghrelin. Longer-term energy storage is signalled by leptin which is produced in adipose tissue[176].

Appetite regulation cannot be measured directly, therefore, appetite regulation is commonly measured using eating pattern or food intake information, appetite rating questionnaires and appetite-related biomarkers[177]. Appetite rating questionnaires contain primary scales to demonstrate a person's perceived state of hunger, fullness, satisfaction, desire to eat and prospective quantity of consumption[178]. Appetite regulation questionnaires often comprise of visual analogue scales (VAS)[179] or category scales[180]. VAS were developed from scales used to assess pain and are the most common scale used to assess subjective appetite ratings[178]. Visual analogue scales prompt participants to place a mark on an anchored line measuring 100-150 mm in length. Each end of the line is anchored by opposing statements such as "not at all full" and "as full as I have

ever felt". Visual analogue scale appetite rating questionnaires are often completed before and after meals[181,182], however, they can also be completed every 30 minutes to an hour in test meal trials[183]. Category scales use the same questions as VAS, however, instead of having a continuous line anchored with opposing statements they have a scale split into several categories. Visual analogue scales and category scales are simple for participants to use, easy to explain and do not require labour intensive data management. There are some limitations to using appetite rating questionnaires including participant errors in completing the questionnaires leading to missing data values and the time consuming nature of tabulated and analysing the data from appetite rating questionnaires that have been completed via pen and paper[184]. That being said, appetite rating questionnaires are commonly used to help demonstrate whether an intervention has elicited positive changes in appetite; often but not always in combination with appetite-related biomarkers. Proposed biomarkers of appetite regulation include some of the gut hormones previously mentioned, i.e. GLP-1, PYY, oxyntomodulin and ghrelin. Research has shown that changes in the gut peptide ghrelin are associated with subjective appetite ratings. Additionally, higher circulating levels of ghrelin have been linked to increases in appetite and food intake[185]. GLP-1 is also thought to modulate appetite as it influences GI motility. GLP-1 infusions have been shown to decrease appetite and reduce food intake[186]. PYY and oxyntomodulin are released in the intestine in response to a meal. In mice, administration of these hormones has been shown to reduce food intake, body weight and adiposity. Preliminary research in humans has also shown promising appetite regulation results[187]. Limitations of biochemical analysis of gut hormones are the expense of the assays and the burden on participants.

An emerging concept suggests that gut microbiota have the ability to regulate appetite via the gut-brain axis. One proposed mechanism of action is via SCFA triggered

release of gut hormones leading to enhanced in appetite control[188]. Short chain fatty acids are produced as metabolic by-products of bacterial fermentation of indigestible substrates. Novel dietary strategies, which facilitate changes in appetite regulation and weight management via increased bacterial production of SCFA, are therefore of great interest. Most of the evidence that suggests SCFA play a role in appetite regulation comes from studies in animals[188]. Numerous human intervention studies have been conducted to determine what effect fermentable substrates have on appetite regulation and weight management, however, the majority of human appetite regulation studies have not analysed bacterial composition or SCFA production. Inconsistencies in results between human studies have also been demonstrated. A study conducted in 88 overweight individuals demonstrated that a 14 day galacto-oligosaccharide intervention lead to a reduction in appetite and food intake[189]. However, a systematic review of the influence fructan-based prebiotics have on appetite, energy intake and body weight concluded that the evidence for an effect was very limited[190]. Only one study (n = 10) demonstrated significant differences in appetite scoring and energy intake between intervention and control groups[191]. All other reviewed studies showed no beneficial effect of fructan-based prebiotics on appetite, body weight or energy intake in normal weight individuals. The reasons for the heterogeneity in results between studies may be related to the fermentable substrate doses used[192], participant characteristic differences and/or the method used to assess appetite regulation. Lastly, no human studies have been conducted to demonstrate whether baseline gut microbiota composition or habitual diets influence fermentable substrate driven appetite regulation. Therefore, further research in this area is required.

2.9 Inter-individual variability in responsiveness to dietary interventions

Even though dietary manipulation has been shown to prompt significant changes in both host outcomes and the gut microbiota, mounting evidence suggests that individuals are heterogeneous when it comes to gut microbiota responsiveness to a given dietary intervention. It is possible that gut microbiota resilience to dietary change may be related to inter-individual variability in baseline gut microbiota composition and habitual dietary intake. Individualised gut microbiota resilience may actually play a more significant role in gut microbiota response than dietary change itself[21]. It appears that the responsive bacterial taxa differ between individuals[23,131,193,194]. Presently, it is very difficult to predict how the gut microbiota will respond to a given dietary intervention. Individualised gut microbiota responsiveness has the potential to influence study results and have an impact on reproducibility between studies. Identification and a greater understanding of the factors that influence individualised responsiveness may help ensure the true efficacy of a given dietary intervention is established.

2.9.1 Gut microbiota response to dietary change

2.9.1.1 Baseline gut microbiota composition

The emerging concept of individualised gut microbiota responsiveness indicates there is a link between baseline gut microbiota composition and response to a dietary intervention. Certain baseline gut microbiota profiles may express an inherent resistance to change and increased resilience towards dietary modification. Table 2-1 summarises a sample of human intervention studies that have observed varying gut microbiota responses based on differing baseline gut microbiota profiles or habitual dietary intakes. The first studies to observe individualised gut microbiota responses established that baseline bifidobacteria concentrations affected the magnitude of change that occurs in bifidobacteria after a prebiotic intervention. Tuohy and co-authors[195] were the first

researchers to demonstrate that individuals with lower baseline bifidobacteria concentrations experience a more pronounced increase in bifidobacteria after an inulin intervention. This result has been replicated in a number of prebiotic intervention studies[162,196–198]. One study, conducted in 31 healthy individuals, demonstrated that biscuits containing fructo-oligosaccharides (6.6 g/day) and guar gum (3.4 g/day) stimulated bifidobacteria growth only in individuals with a baseline bifidobacteria concentration of less than 9.3 $\log_{10}$ cells/g[24]. Contrary to these results one study demonstrated that baseline bifidobacteria populations between individuals varied considerably (i.e. below detectable levels to 4.6% of total bacterial genes) but no correlation existed between baseline bifidobacteria and change in bifidobacteria after an inulin-type fructan prebiotic intervention[199]. The heterogeneity in results seen between this study and the studies previously discussed may be related to the small sample size used (n = 12) or that the relative proportion of bifidobacteria (i.e. % of total bacterial genes) rather than absolute bifidobacteria concentrations were reported.

As well as being associated with metabolic and immune-mediated inflammatory disease risk[102] it also appears that microbial diversity and gene richness are associated with individualised gut microbiota response[20,21,193]. A study conducted in 14 overweight men classified participants as responders and non-responders based on microbial community stability during three dietary interventions; resistant starch, non-starch polysaccharide and weight loss interventions. Principal co-ordinate analysis data identified responders as having gut microbiota communities that were unstable and non-responders as having gut microbiota communities that were more stable in response to the dietary interventions. It was demonstrated that responders had significantly lower baseline alpha diversity scores (bacterial diversity within a sample; inverse Shannon index) compared to non-responders[21]. Tap and co-authors demonstrated in 19 healthy

individuals, consuming a low (10 g/day) *versus* high (40 g/day) dietary fibre intervention, that individuals with higher alpha diversity (species richness) at baseline had gut microbiota that were more resilient to change (Jensen Shannon Distances metrics) during the high dietary fibre intervention phase [20]. Microbial gene richness has also been shown to influence gut microbiota responsiveness. Cotillard and co-authors[200] conducted a calorie-restriction study in 45 overweight and obese participants and identified that individuals with high bacterial gene richness (HGR) were less likely to experience a change in gene richness but low bacterial gene richness (LGR) individuals had a significant increase in gene richness in response to the dietary intervention. Shoaie and co-authors[193] demonstrated, using the same participant cohort as Cotillard and co-authors[200], that significant baseline differences in *B. adolescentis, F. prausnitzii* and *E. rectale* existed between the LGR and HGR groups. Moreover, different bacterial taxa significantly responded to the dietary intervention between the LGR and HGR groups. The HGR group experienced a significant increase in *B. thetaitaomicron* and a decrease in *Lactobacillus reuteri* and *F. prausnitzii*, whereas, the LGR groups only experienced a significant decrease in *L. reuteri* in response to the calorie-restriction diet. These studies highlight that greater microbial diversity and gene richness may in turn lead to a gut microbiota profile that is more resilient to dietary change. Additionally, individuals with differing bacterial gene richness also appear to have differing baseline gut microbiota communities that respond distinctively to a given dietary intervention.

Inter-individual variability in gut microbiota response may also be evident in probiotic intervention studies. Zhang and co-authors[25] conducted a study to determine whether any of the probiotic strains found in a fermented milk product (FMP) persisted after the probiotic intervention was stopped. It was reported that persistence of *Lactococcus lactis* (probiotic strain found in the FMP) was reliant upon whether a

participant was a *Lactococcus* carrier or non-carrier during the washout phase. *Lactococcus* non-carriers appeared to shed *L. lactis* whereas *Lactococcus* carriers appeared to retain *L. lactis* after FMP administration. Baseline differences in gut microbiota composition existed between carriers and non-carrier with carriers having a higher abundance of *Barnesiellaceae, Odoribacteraceae* and *Clostridiaceae* compared to non-carriers. Carriers also experienced a greater change in beta diversity (bacterial diversity between samples; weighted UniFrac distances) due to the probiotic intervention compared to non-carriers which suggests that the gut microbiota of *Lactococcus* carriers were more responsive to the probiotic intervention. It is, therefore, possible that *Lactococcus* carriers harboured endogenous bacteria that were unable to successfully compete with *L. lactis* and/or had a colonic environment which allowed *L. lactis* to thrive (i.e. optimal pH and substrate availability).

A number of preliminary studies have demonstrated that baseline gut microbiota differences exist between individuals who have gut microbiota that are responsive or resilient to a given dietary intervention. Very few studies have, however, been conducted with the primary aim of determining what constitutes a resilient gut microbiota profile. It is highly likely that gut microbiota resilience will differ depending on the dietary intervention being studied and the host characteristics, such as age, gender and habitual dietary intakes, of the study cohort.

2.9.1.2 Habitual dietary intakes

The type and amount of fermentable substrates (i.e. indigestible carbohydrates or fibre) normally presented to colonic microbiota will vary considerably depending on the host's habitual dietary intake. Individuals consuming a high fibre diet will present their gut microbiota consortium with a large range of fermentable substrates available for utilisation as key energy sources. A low fibre diet deficient in fermentable substrates will

deprive the gut microbiota of external energy sources resulting in reliance on endogenous fermentable substrates such as mucin. Variability in the types and amounts of fermentable substrates available to the gut microbiota has a major impact on their composition and functional capacity. Gut microbiota distinctions exist between individuals with differing habitual dietary intakes[16]. It is, therefore, plausible that the responsiveness of the gut microbiota to an increase in fermentable substrates, i.e. prebiotic supplementation, may also be influenced by an individual's habitual dietary intake. Figure 2-4 provides a hypothetical example of the influence differing habitual dietary fibre intakes have on gut microbiota response to a prebiotic intervention. In this example, individuals with low habitual intakes of fibre are expected to have more responsive gut microbiota as the prebiotic supplement increases the amount of fermentable substrates available leading to a proliferation of previously low abundance bacterial taxa that are well equipped to utilise fermentable substrates but had earlier been deprived of them. An alternative scenario may be that bacterial taxa metabolically capable of utilising fermentable substrates are lost in individuals with low habitual dietary fibre intakes due to chronic fermentable carbohydrate deficiency. Therefore, individuals with higher habitual dietary fibre intakes may harbour a gut microbiota consortium that are better equipped to utilise the additional fermentable substrates leading to a greater microbial response.

Very little is known about the influence habitual dietary intake has on gut microbiota response. Preliminary research has shown that habitual diet may influence gut microbiota responsiveness (Table 2-1). A recent study, conducted using germ-free mice colonised with human gut microbiota from donors with two varying dietary patterns (typical American style dietary pattern [AMER] or a plant-rich, calorie-restricted diet with optimal nutrient composition [CRON]), demonstrated that mice inoculated with

AMER microbiota were less responsive to the plant-based diet when compared to mice inoculated with CRON microbiota. When AMER-colonised mice were cohoused with CRON-colonised mice it appeared that CRON-associated bacteria were exchanged (via coprophagia) between the two mouse types as the AMER microbiota had an improved response to the plant-based diet[26]. In humans, a 21-day palm date intervention in 22 healthy individuals did not lead to significant changes in select bacterial taxa[27]. Secondary analysis demonstrated that participants with low dietary fibre (LDF) intakes had a significant change in a number of bacterial taxa, including total bacteria, *Lactobacillus/Enterococcus* and *Roseburia + E. rectale,* whereas, high dietary fibre (HDF) participants had no change in bacterial taxa. These results suggest that HDF participants harboured gut microbiota that were more resilient and LDF participants harboured gut microbiota that were more responsive to the palm date intervention[27]. Holscher and co-authors demonstrated that an agave inulin supplement (7.5 g/day) only led to a greater than 1% increase in *Bifidobacterium* relative abundance in 15 of the 29 participants. A trend towards a positive correlation between *Bifidobacterium* relative abundance and grams of dietary fibre consumed per kcal was observed. Additionally, a positive correlation between butyrate production and total dietary fibre intakes was established. These results suggest that habitual dietary fibre intakes influence butyrate production and bifidogenic response[166]. Some studies have shown that individuals with gut microbiota that are more resilient to dietary change have a higher diversity of vegetable intake[20] and higher vegetable, fruit and fish intakes[200]. Other studies have found no significant differences in habitual dietary intake[21] or dietary fibre intakes[22] between individuals with responsive or permissive gut microbiota indicating that inconsistencies in study results exist. Heterogeneous results may exist due to considerable differences in the dietary interventions and dietary assessment methods

used, and in participant characteristics between studies. Additionally, in these studies only post-hoc analysis has been conducted to determine whether dietary differences existed between responders and non-responders. Up until recently no studies had been conducted with the primary aim of determining whether habitual dietary intakes influence gut microbiota responsiveness to dietary interventions.

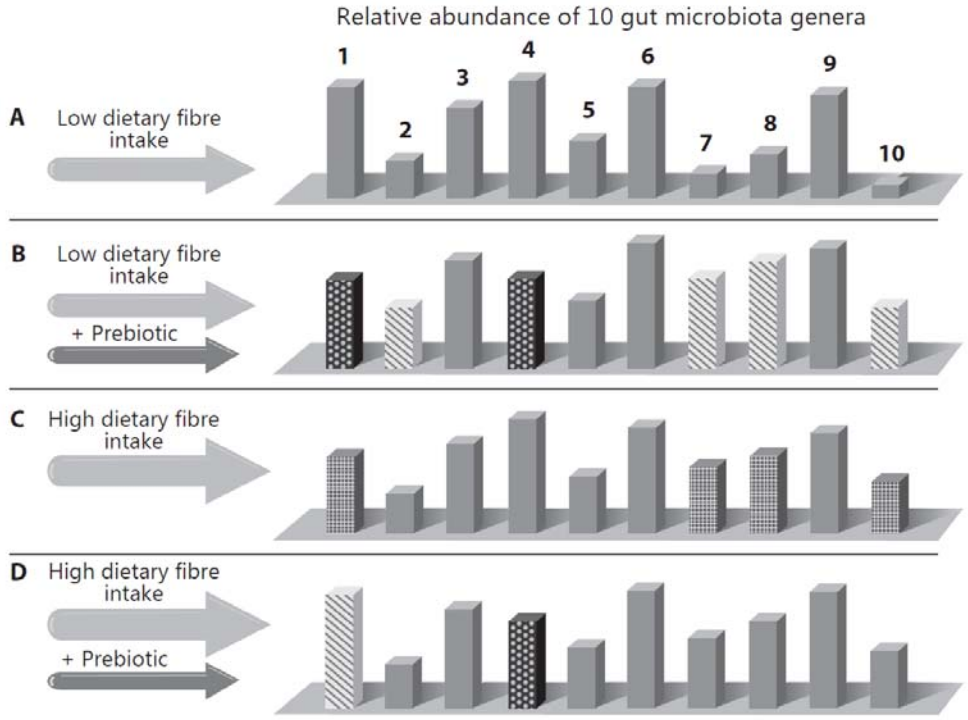

Figure 2-4. Hypothetical response in relative abundance of a number of gut microbiota genera (represented by vertical bars) to differences in habitual dietary fibre intake (A+C) and the addition of a prebiotic in the presence of the differing habitual dietary fibre intakes (B+D). This figure depicts that the abundance of certain bacterial genera will significantly differ between individuals with low versus high dietary fibre intakes (the ▦ bars indicate a significant difference in bacterial relative abundance between low and high dietary fibre consumers) (A+C). The figure also illustrates that certain bacterial genera may respond in a distinctive manner to a prebiotic as a result of differing baseline gut microbiota profiles and habitual dietary fibre intakes (B+D). Hypothetically individuals with low habitual dietary fibre intakes (B) may have a higher proportion of gut microbiota genera which significantly change in relative abundance in response to a prebiotic (the ▨ bars indicate a significant increase in relative abundance from baseline and the ▩ bars indicate a significant decrease from baseline) when compared to individuals with high habitual dietary fibre intakes (D) (adapted from reference[201]).

To help fill this knowledge gap we conducted a study that recruited participants with distinctive habitual dietary fibre intakes to determine whether low *versus* high habitual dietary fibre influenced gut microbiota response to an inulin-type fructan

prebiotic (n = 34) (under review). We established that the LDF group had bacterial taxa that were less influenced by dietary change compared to the HDF group. The only bacterial genus that significantly changed due to the inulin-type fructan prebiotic in the LDF group was *Bifidobacterium* but in the HDF group significant prebiotic driven changes in *Bifidobacterium, Faecalibacterium, Coprococcus, Dorea* and *Ruminococcus* were observed. These results differ from the palm date intervention study previously discussed[27]. In the case of the palm date intervention study the LDF rather than HDF group appeared to have gut microbiota that were more responsive to the palm date. The dietary fibre intake criteria used to classify participants as LDF and HDF consumers did differ markedly between studies. The average dietary fibre intake of the LDF (6 g/d *versus* 18 g/d) and HDF (18.5 g/d *versus* 38.6 g/d) groups were much lower in the palm date intervention study. The discrepancy in responsiveness between the two studies may also be related to the different dietary interventions studied. This suggests that a palm date intervention may have more of an influence on the gut microbiota of LDF consumers and an inulin-type fructan intervention may have more of an influence on the gut microbiota of HDF consumers. Additional research is required to provide further clarification on the influence habitual dietary fibre intake has on gut microbiota responsiveness to certain dietary interventions.

Table 2-1. The observed differences in baseline gut microbiota composition and function and/or dietary intake and host characteristics between responders (R) and non-responders (NR) in studies which have demonstrated differing gut microbiota responses to a dietary

Gut microbiota responsiveness							
Study information			**Responsiveness definition**	**Observed baseline differences**		**Observed differences in gut microbiota response**	**Reference**
Subjects	**Intervention**	**Analysis method**		**Gut microbiota composition and function**	**Dietary intake and host characteristics**		
10 healthy	100g of cracker containing 33g of RS for 3 weeks- either RS2 Hi-Maize or RS4 Fibersym	454 pyrosequencing, DGGE and qPCR	Magnitude of change in gut microbiota composition	Not investigated	Not investigated	The magnitude of change varied considerably between individuals. None of the community shifts were observed in all participants.	Martinez *et al.* (2010)[131]
18 healthy	4 weeks of each of the following- chews with 0g, 2.5g, 5g and 10g/d of GOS	454 pyrosequencing	Change in major bacterial groups analysed- *Bifidobacterium* and *Bacteroides*	No baseline differences in gut microbiota composition between R and NR	Not investigated	R- Significant change in at least 1 of the major bacterial groups analysed after the 5g and/or 10g/d GOS NR- Bacterial groups analysed were unaffected by 5g and 10g/d GOS	Davis *et al.* (2011)[23]
14 over-weight men*	1 week M diet, 3 week high RS and 3 week high NSP and 3 week weight loss diet (high protein, low CHO)	Pyrosequencing, DGGE and qPCR	Distance between baseline and post-intervention samples on a PCoA	Differing baseline gut microbiota composition between R and NR- did not elaborate	Not investigated	R- post-intervention samples a distance away from the baseline sample on a PCoA NR- post-intervention and baseline samples cluster on a PCoA	Walker *et al.* (2011)[194]
31 healthy	Biscuits containing 6.6g/d FOS and 3.4g/d guar gum for 21 days	FISH	Change in bifidobacteria	Lower baseline bifidobacteria levels in R compared to NR. NR had a baseline bifidobacteria ≥9.3 $\log_{10}$ cells/g	Not investigated	R- Increase in bifidobacteria NR- No change in bifidobacteria	Tuohy *et al.* (2001)[24]
9 healthy	8g/d of IN for 2 weeks	FISH	Change in bifidobacteria	Lower baseline bifidobacteria levels in R compared to NR	Not investigated	R- Higher response in bifidobacteria NR- Lower response in bifidobacteria	Tuohy *et al.* (2001)[195]
8 healthy individuals per group	Consumed either 2.5g, 5g, 7.5g or 10g/day of SC-FOS, SBOS, GOS or RS for 7 days (16 groups)	Culture based	Change in bifidobacteria	Lower baseline bifidobacteria levels in R compared to NR	Not investigated	R- Higher response in bifidobacteria NR- Lower response in bifidobacteria	Bouhnik *et al.* (2004)[196]
30 healthy	2 weeks 5g/day of IN, 1 week washout and 2 weeks of 8g/day of IN	FISH	Change in bifidobacteria	Significantly lower baseline bifidobacteria levels in R compared to NR for 5 and 8g/day of IN	Not investigated	R- Increase in bifidobacteria NR- No change in bifidobacteria	Kolida *et al.* (2007)[197]
39 healthy	5g/day IN for 4 weeks	Culture based	Change in bifidobacteria	Lower baseline bifidobacteria levels in R compared to NR	Not investigated	R- Higher response in bifidobacteria NR- Lower response in bifidobacteria	Bouhnik *et al.* (2007)[162]
19 healthy	For 4 weeks of Lactulose 10g twice a day OR Synergy 1 (FOS:IN) 10g twice a day	qPCR and DGGE	Change in bifidobacteria	Lower baseline bifidobacteria levels in R compared to NR	Not investigated	R- Higher response in bifidobacteria NR- Lower response in bifidobacteria	de Preter *et al.* (2008)[198]
12 healthy	5g Synergy 1 (FOS:IN) twice a day for 3 weeks	qPCR	Change in bifidobacteria	No significant difference in baseline bifidobacteria between R and NR	Not investigated	R- Higher response in bifidobacteria NR- Lower response in bifidobacteria	Fuller *et al.* (2007)[199]
19 healthy	10 g/day or 40 g/day of dietary fibre (cross-over design) with a 2 week washout phase	454 pyrosequencing and qPCR	Microbial stability	R- Low microbial diversity (OTU numbers) NR- High microbial diversity (OTU numbers)	NR had a higher diversity of vegetable intake compared to R	R- Higher microbial change NR- Higher microbial stability	Tap *et al.* (2015)[20]

45 over-weight and obese^	6 weeks of calorie-restriction, high protein, low glycaemic index diet followed by a 6 week M diet	SOLiD sequencing	Microbial gene richness stability	R- LGR NR- HGR	R less fruit, vegetables and fish products and there was a trend towards lower dietary fibre intakes compared NR.	R- Significant increase in gene richness however gene richness was still significantly lower than in NR NR- No change in gene richness	Cotillard *et al.* (2013)[200]
14 over-weight men*	1 week M diet, 3 week high RS and 3 week high NSP and 3 week weight loss diet (high protein, low CHO)	HITChip and qPCR	Distance between baseline and post-intervention samples on PCoA	R had a significantly lower microbial diversity (inverse Simpson index) than NR	No difference in habitual diets between R and NR. NSP intake correlated with microbial diversity in R but not NR	R- post-intervention samples a distance away from the baseline sample on a PCoA NR- post-intervention and baseline samples cluster on a PCoA	Salonen *et al.* (2014)[21]
45 over-weight and obese^	6 weeks of calorie-restriction, high protein, low glycaemic index diet followed by a 6 week M diet	SOLiD sequencing	Change in gut microbiota composition	Baseline differences in *Bifidobacterium adolescentis, Faecalibacterium prausnitzii* and *Eubacterium rectale* between R and NR groups	Not investigated	R- Increase in *Bacteroides thetaitaomicron* and a decrease in *Lactobacillus reuteri* and *F. prausnitzii.* NR- Decrease in *L. reuteri*	Shoaie *et al.* (2015)[193]
46 healthy	4 weeks 25g/d NSP, 2 week washout and 4 weeks 25g/d NSP + 22g/d RS diet	SCFA only- Gas chromatography	Increase in butyrate conc.	Baseline butyrate conc. differences- grouped into quartiles	NR (highest quartile) had higher BMI, energy and protein intakes compared to R (lowest quartile). Fibre intake did not differ	R- Increase in butyrate conc. NR- Decrease or no change in butyrate conc.	McOrist *et al.* (2011)[22]
14 healthy females	7 weeks of fermented milk product (FMP; 2 x 4 oz. per day)	16S rRNA bacterial gene sequencing	Persistence of *Lactococcus lactis* strains after FMP stopped	R- *Lactococcus* carrier. Higher abundance of *Barnesiellaceae, Odoribacteraceae* and *Clostridiaceae.* NR- *Lactococcus* non-carrier. Lower abundance of *Barnesiellaceae, Odoribacteraceae* and *Clostridiaceae.*	Not investigated	R- Persistence of *L. lactis* (probiotic within FMP) after FMP stopped (n=5). NR- No *L. lactis* persistence after FMP stopped (n=9). Significantly higher weighted UniFrac distance after 1 week of FMP in R compared to NR	Zhang *et al.* (2016)[25]
29 healthy	3x3 week interventions with a 1 week washout phase between each intervention phase. Chocolate chews with 0, 5 or 7.5g of agave IN	16S rRNA Illumina sequencing	Change in *Bifidobacterium* relative abundance	Not investigated	Higher dietary fibre intakes were associated with increased butyrate production and a trend towards higher *Bifidobacterium*	R- Significant increase in *Bifidobacterium* relative abundance NR- No significant increase in *Bifidobacterium* relative abundance	Holscher *et al.* (2015)[166]
10 healthy donors + 30 gnotobiotic mice	Faecal samples from 5 CRON individuals and 5 AMER individuals. Inoculated gnotobiotic mice were fed a representative CRON or AMER diet	16S rRNA bacterial gene sequencing	Shift in dietary pattern associated bacteria	R- Higher abundance of *Bacteroides* species (*cellulosilyticus, thetaiotaomicron*), *Parabacteroides goldsteinii* and *Allstipes putredinis.* NR- Higher abundance of *Dorea longicatena, Blautia, Coporoccus comes* and *Clostridium clostridioforme.*	CRON donors consumed 42.1% less calories, 48.6% less fat, 33.5% less carbohydrates, 37.6% less total protein and 60.8% less protein from animal sources compared to AMER donors	R- CRON inoculated mice had a shift towards the CRON or AMER dietary pattern associated bacterial profile to match the diet (CRON or AMER) consumed. NR- AMER inoculated mice weaker shift towards a CRON associated bacterial profile on the CRON diet	Griffin *et al.* (2017)[26]
22 healthy	50g/day palm date for 3 weeks and 3.9g maltodextrin and 33.19g dextrose per day for 3 weeks with a 2 week washout period between interventions	FISH	Change in gut microbiota composition	R- Lower baseline *Bacteroides* conc. NR- Higher baseline *Bacteroides* conc.	R had a higher dietary fibre intake (18.5g/d) then NR (6g/d).	R- Significant increase in total bacteria, *Bacteroides*, *Lactobacillus/Enterococcus*, *C.coccoides-E. rectale*, *Ruminococcus bromii + flavefaciens* and *Roseburia + E. rectale* groups NR- No change in gut microbiota	Eid *et al.* (2015)[27]

34 healthy	16 g/d of inulin-type fructan prebiotic for 3 weeks	16S rRNA Illumina sequencing	Change in gut microbiota composition	R- Lower abundance of unknown genus of *Lachnospiraceae* NR- Higher abundance of unknown genus of *Lachnospiraceae*	R had higher dietary fibre, energy, fat, PUFA, MUFA, CHO, fibre per 1000kJ, vegetable, fruit, nut and seed intakes than NR	R- Increase in *Bifidobacterium* and *Faecalibacterium*, decrease in *Coprococcus*, *Dorea*, and *Ruminococcus* NR- Increase in *Bifidobacterium* only	Under review

Abbreviations: AMER- American diet with no calorie-restriction, CHO- carbohydrate, CRON- calorie-restricted adequate nutrition diet, DGGE- denaturing gradient gel electrophoresis, FOS- fructo-oligosaccharide, FISH- fluorescence *in situ* hybridisation, FMP- fermented milk product, GOS- galacto-oligosaccharide, HITChip- human intestinal tract chip, IN- inulin, M-maintenance, MUFA- mono-unsaturated fatty acids, NSP- non-starch polysaccharide, PCoA- principal co-ordinate analysis, PUFA- poly-unsaturated fatty acids, qPCR- quantitative polymerase chain reaction, RS-resistant starch, rRNA- ribosomal RNA, SCFA- short chain fatty acid, SC-FOS- short chain fructo-oligosaccharide, SBOS-soybean oligosaccharide. *Same study cohort, ^Same study cohort

2.9.2 Host response to dietary change

Dietary interventions have been shown to elicit differential gut microbiota responses between individuals. A given dietary intervention also leads to variable host responses. While dietary interventions affect host metabolic outcomes in an individualised way, host parameters such as inter-individual variability in gut microbiota composition and habitual dietary intakes may prove useful in predicting host responses.

2.9.2.1 Baseline gut microbiota composition

As gut microbiota structure and function are implicated in disease risk, it is imperative to investigate whether gut microbiota composition is linked to inter-individual variability in host responses. Emerging research suggests that an association between baseline gut microbiota composition and host responses does exist (Table 2-2). A study conducted in 800 healthy individuals demonstrated that glycaemic response to a given food is highly variable between individuals. Researchers were able to use baseline gut microbiota composition, blood parameters, anthropometric measurements and self-reported lifestyle behaviour (i.e. dietary habits and physical activity levels) data to identify factors associated with individual variability in glycaemic response[202]. A machine-learning algorithm was devised, based on the factors associated with individual variability, to accurately predict glycaemic responses. The algorithm was used in a human intervention study (n = 26) to generate personalised dietary interventions. The algorithm-derived personalised dietary interventions were shown to significantly lower glycaemic responses and alter gut microbiota composition[202]. This study highlights the potential in utilising host outcome parameters and baseline gut microbiota data to better predict dietary intervention success. A number of other studies have also demonstrated a link between glycaemic response and baseline gut microbiota composition. Individuals with higher baseline *Prevotella* abundance experienced an incremental blood glucose area and

insulin area under the curve decrease after 3 days of consuming barley kernel-based bread[203]. The improvement in glycaemic response was also associated with an increase in *Prevotella:Bacteroides* ratio. Individuals with lower baseline *Prevotella* abundance did not experience a host or gut microbiota response after the dietary intervention suggesting a more resilient host phenotype and gut microbiota profile[203]. Recent research suggests that artificial sweeteners may not be detrimental to health. Suez and co-authors[204] demonstrated that saccharin led to glucose intolerance in a subset of their participant cohort. Baseline principal co-ordinate analysis (PCoA) data demonstrated that individuals who had no saccharin related change in glycaemic response (non-responders) had gut microbiota profiles that clustered together but that clustered separately from individuals that experienced a glycaemic response (responders) demonstrating that responders and non-responders had differing gut microbiota compositions at baseline. Saccharin responders also experienced a more pronounced change in gut microbiota composition due to the saccharin intervention compared to non-responders suggesting that dysbiosis may have led to the observed saccharin-related glucose intolerance.

Other host metabolic outcomes, such as cholesterol and weight reduction, have been associated with baseline gut microbiota composition differences[205–207]. Individuals with low baseline *A. muciniphila* abundance were shown to be less metabolically healthy but more metabolically resilient to a 6-week calorie-restriction diet than individuals with high baseline *A. muciniphila* abundance[206]. In individuals with high baseline *A. muciniphila* abundance, the calorie-restriction intervention led to a higher Disse index (assesses insulin sensitivity), greater improvements in LDL cholesterol, a continued reduction in waist circumference and a reduction in *A. muciniphila* abundance, even after the calorie-restriction diet was discontinued and participants were commenced on a

weight maintenance diet[206]. Using the same participant cohort, Kong and co-authors[205] demonstrated that weight loss success after the calorie-restriction diet was dependent on baseline concentrations of the *Lactobacillus/Leuconostoc/Pediococcus* group. Individuals with lower concentrations of *Lactobacillus/Leuconostoc/Pediococcus* experienced a 4.4% reduction in total body weight during the calorie-restriction diet phase but regained 2.9% of their total body weight during the 6-week weight maintenance diet phase. Conversely, individuals with higher concentrations of *Lactobacillus/Leuconostoc/Pediococcus* at baseline had a 7.6% reduction in total body weight during the calorie-restriction diet phase and by the end of the weight maintenance diet phase had lost an additional 2.4% of their total body weight. Korpela and co-authors[207] used three previously published dietary intervention cohorts to determine whether a baseline gut microbiota signature was associated with more pronounced host-specific metabolic changes. A very high or very low abundance of *Eubacterium ruminantium* and *Clostridium felsineum* were shown to be associated with a more responsive gut microbiota. Interestingly, individuals with more responsive gut microbiota also had greater changes in cholesterol demonstrating the prognostic value of baseline gut microbiota. A number of studies have revealed that inflammatory responses to dietary change[200,208] and low FODMAP diet related reductions in IBS associated pain[157,209] are also linked to baseline gut microbiota composition providing further evidence of the role baseline gut microbiota composition has in host response.

At present, the efficacy of a dietary intervention in changing gut microbiota composition and host health outcomes is highly individualised and unpredictable. Mounting evidence does suggest that microbial-specific biomarkers could be used to predict the individualised success a given dietary intervention may have on host responses.

2.9.2.2 Habitual dietary intake

Recent research has suggested that habitual dietary intake may also be associated with variability in host responses (Table 2-2). One study, in 45 overweight and obese individuals, demonstrated that three distinctive dietary pattern clusters were linked with distinctions in metabolic and inflammatory variables, and microbial gene richness. Cluster 1 was associated with the least healthy dietary pattern, cluster 3 had the healthiest dietary pattern and cluster 2 had a dietary pattern between cluster 1 and 3. Individuals with the healthiest dietary pattern cluster had lower levels of the inflammatory marker sCD14, higher levels of anti-inflammatory adipose tissue macrophage CD163 and the highest microbial gene richness compared to cluster 1 and 2[210]. In the same participant cohort, Cotillard and co-authors[200] demonstrated that a calorie-restriction diet led to more pronounced improvements in inflammatory markers in participants with the healthiest dietary pattern (i.e. cluster 3 from the Kong and co-author study). Previous research has shown that individuals with healthier dietary patterns also have higher microbial gene richness[17]. Therefore, individuals with higher microbial gene richness may harbour gut microbiota with a larger repertoire of bacterial genes that are metabolically capable of coping with extreme changes in macronutrient intake leading to a greater potential to influence host outcomes.

A number of studies have been unable to demonstrate dietary intake differences between individuals that have or have not experienced changes in host outcomes after a given dietary intervention[157,206,209]. In these studies, participants had not been actively recruited based on distinctions in dietary intakes and dietary assessment methods that only assessed current rather than habitual dietary intakes were employed. As previously discussed we recruited individuals with distinctive habitual dietary fibre intakes and demonstrated that habitual dietary fibre intakes influence gut microbiota response to an

inulin-type prebiotic intervention (under review). In the same participant cohort we also demonstrated an association between habitual dietary fibre intake and host appetite ratings. In the HDF group, the inulin-type fructan prebiotic led to a significant reduction in satisfaction before lunch and in hunger after dinner, and a significant increase in fullness and satisfaction after lunch. Changes in appetite ratings were not associated with a change in weight or a reduction in caloric intake but as the length of the prebiotic intervention was only 3 weeks this may not have been long enough to initiate changes in these host outcomes. Conversely, the LDF group did not experience any significant changes in appetite ratings, weight or caloric intake after the prebiotic intervention. The HDF group also had gut microbiota that were more responsive to the inulin-type fructan prebiotic than the LDF group. This is the only study in humans that has been conducted with the primary aim of determining whether differing habitual dietary intakes influence gut microbiota and host response to a dietary intervention. Therefore, it is essential that additional studies are conducted in this area.

Table 2-2. The observed differences in baseline gut microbiota composition and function and/or dietary intake and host characteristics between responders (R) and non-responders (NR) in studies which have demonstrated differing host responses to a dietary intervention

Host responsiveness								
Study information			**Responsiveness definition**	**Observed baseline differences**		**Observed differences in response**		**Reference**
Subjects	**Intervention**	**Analysis method**		**Gut microbiota composition and function**	**Dietary intake and host characteristics**	**Host clinical outcome**	**Gut microbiota**	
12 healthy	Algorithm derived personalised diet	16S rRNA bacterial gene sequencing	PPGR	Used as a factor to help predict glycaemic response- Enterobacteriaceae, Proteobacteria and Actinobacteria	Dietary patterns used as a factor to help predict glycaemic response	R- Significantly higher PPGR during the "bad" diet phase compared to the "good" diet phase NR- No difference in PPGR between the "good" and "bad" diet phases	Inter-individual variability in gut microbiota responses- did not analyse R and NR separately	Zeevi *et al.* (2015)[202]
20 healthy	3 days BKB followed by 3 days of WWB	454 pyro-sequencing	Improved glucose metabolism	R- Higher *Prevotella* abundance NR- Lower *Prevotella* abundance	Not investigated	R- Incremental blood glucose area decreased by at least 25%, total AUC decreased and insulin AUC decreased by at least 15% NR- No difference in glucose and/or insulin response	R- Increase in the *Prevotella/Bacteroidetes* ratio NR- No change in *Prevotella/Bacteroidetes* ratio	Kovatcheva-Datchary *et al.* (2015)[203]
7 healthy	1 week saccharin intervention- 15mg/kg body weight	16S rRNA bacterial gene sequencing	Glycaemic response	PCoA differences between R and NR	Not investigated	R- Poor glycaemic response to saccharin NR- No glycaemic response to saccharin	R- Pronounced change in gut microbiota composition NR- No change. PCoA differences after the intervention between R and NR	Suez *et al.* (2014)[204]
49 over-weight and obese*	6 week calorie-restriction diet followed by a 6 week M diet	SOLiD high-throughput sequencing and qPCR	Metabolic improvements	R- High *Akkermansia muciniphilia* NR- Low *A. muciniphilia*	R- More metabolically healthy NR- Less metabolically healthy No difference in diet quality between R and NR	R- Higher Disse index, greater improvement in LDL cholesterol and a continued decrease in waist circumference NR- Less benefit from intervention	R- Reduction in *A. muciniphilia* (abundance still higher than NR group) NR- Minimal change in *A. muciniphilia*	Dao *et al.* (2015)[206]
78 over-weight and obese	Cohort 1 (n=52)- high fibre rye and WG diet and a low fibre refined grain diet. Cohort 2 (n=13)- 8g of IN and 8g of FOS. Cohort 3- (n=13)- 3 week WL diet (high protein, low CHO)	HITChip	Change in cholesterol	R- High or low abundance of *Eubacterium ruminantium* and *Clostridium felsineum*. Lower bifidobacteria NR- Average abundance of *E. ruminantium* and *C. felsineum*. Higher bifidobacteria	Not investigated	R- Decrease (39%) or no change (62%) in cholesterol NR- Decrease (24%) or an increase (23%) in cholesterol	R- Microbial stability < 0.87. Higher response in bifidobacteria NR- Microbial stability > 0.92. Lower response in bifidobacteria	Korpela *et al.* (2014)[207]
49 over-weight and obese*	6 weeks of calorie-restriction diet followed by a 6 week M diet	qPCR	Intervention driven WL and M	R- Higher conc. of *Lactobacillus/ Leuconostoc/Pediococcus* group NR- Lower conc. of *Lactobacillus/ Leuconostoc/Pediococcus* group	NR had a higher starch and oil intake and a lower intake of protein compared to R	R- 7.6% WL at 6 weeks and 10% at 12 weeks NR- 4.4% WL at 6 weeks and 2.9% weight regain at 12 weeks (only a 1.5% WL over the 12 weeks)	Not investigated	Kong *et al.* (2013)[205]

28 healthy	Three 4 week wholegrain flake interventions- WGB, BR+WGB and BR	454 pyro-sequencing	Immunological response IL-6	Higher abundance of *Dialister* and a lower abundance of *Coriobacteriaceae* in R compared to NR	Not investigated	R- Highest tertile change in IL-6 due to the BR+WGB intervention NR- Lowest tertile change in IL-6 due to the BR+WGB intervention	Not investigated	Martinez *et al.* (2013)[208]
45 over-weight and obese*	6 weeks of calorie-restriction diet followed by a 6 week M diet	SOLiD sequencing	Change in inflammatory markers	R- HGR NR- LGR	NR less fruit, vegetables, fish products and trend towards lower dietary fibre compared to R. NR had higher insulin resistance and fasting serum TAGs compared to R	R- More marked improvement in inflammation markers NR- Less pronounced improvements in inflammatory markers	R- No change in gene richness NR- Significant increase in gene richness however gene richness was still significantly lower than in R	Cotillard *et al.* (2013)[200]
8 children with IBS	1 week of LFSD	454 pyro-sequencing	Reduction in pain frequency	R- Higher abundance of an OTU from *Ruminococcaceae* (specifically *Sporobacter* and *Subdoligranulum*). NR- Higher abundance of *Bacteroidales* and an OTU from *Ruminococcaceae*	No difference in dietary intake between R and NR	R- ≥50% reduction in abdominal pain frequency and lower hydrogen production NR- <50% reduction in abdominal pain frequency and higher hydrogen production	R- Increased abundance of *Prevotellaceae* and unclassified *Coriobacteriaceae* and a reduction in *Dialister* NR- Increased abundance of *Actetivibrio cellulolyticus*, *Bacteroides* and *Dialister*	Chumpitazi *et al.* (2014)[209]
33 children with paediatric Rome III-defined IBS	48 hour low FODMAP or typical American childhood diet (cross-over design) with a 5 day washout phase	454 pyro-sequencing	Reduction in pain frequency	R- Higher abundance of *Bacteroides, Ruminococcaceae, Dorea, Faecalibacterium prausnitzii* and cc_115 (family *Erysipilotrichaceae*). 3 enriched CHO metabolism specific KEGG orthologues at baseline NR- Higher abundance of *Turicibacter* from the family *Turicibacteraceae*. No enriched KEGG orthologues	No differences in baseline dietary intake (1x 3-day diet record) between R and NR	R- Significant improvement in pain episodes during low FODMAP diet phase NR- No improvement in pain episodes during low FODMAP diet phase	Not investigated	Chumpitazi *et al.* (2015)[157]
34 healthy	16 g/d of inulin-type fructan prebiotic for 3 weeks	16S rRNA Illumina sequencing	Improvement in appetite ratings	R- Lower abundance of unknown genus of *Lachnospiraceae* NR- Higher abundance of unknown genus of *Lachnospiraceae*	R had higher dietary fibre, energy, fat, PUFA, MUFA, CHO, fibre per 1000kJ, vegetable, fruit, nut and seed intakes than NR	R- Significant reduction in satisfaction before lunch and hunger after dinner, and a significant increase in fullness and satisfaction after lunch. NR- no significant change in appetite ratings	R- Increase in *Bifidobacterium* and *Faecalibacterium*, decrease in *Coprococcus*, *Dorea*, and *Ruminococcus* NR- Increase in *Bifidobacterium* only	Under review

Abbreviations: AUC- area under curve, BR- brown rice, BR+WGB- brown rice and wholegrain barley, BKB- barley kernel-based bread, CDI- *Clostridium difficile* infection, CHO- carbohydrate, FODMAP- fermentable oligosaccharides disaccharides monosaccharides and polyols, HITChip- human intestinal tract chip, HGR- high bacterial gene richness, IL-6- interleukin 6, IBS- irritable bowel syndrome, KEGG- Kyoto encyclopaedia of genes and genomes, LDL- low density lipoprotein, LFSD- low fermentable substrate diet, LGR- low bacterial gene richness, M-maintenance, OTU- operational taxonomic unit, PCoA- principal co-ordinate analysis, PPGR- post-prandial glycaemic response, qPCR- quantitative polymerase chain reaction, rRNA- ribosomal RNA, SOLiD- sequencing of oligonucleotides by ligation, WL- weight loss, WG- wholegrain, WWB- white wheat flour bread, WGB- wholegrain barley. *Same study cohort

2.10 Conclusion

Modulation of the gut microbiota to improve human health is an attractive strategy for reducing the increasing prevalence of metabolic and inflammatory-related disease. The multifaceted interactions that exist within a gut microbiota community make it difficult to predict how a specific dietary intervention may influence gut microbiota composition and host outcomes. In addition, the complexity and individuality of gut microbiota and host responsiveness make any progress in this area challenging. Gaining a better understanding of the factors implicated in inter-individual variability in gut microbiota and host responsiveness may help improve dietary intervention success and subsequently enhance human health outcomes.

The majority of studies reviewed suggest that baseline gut microbiota composition and habitual dietary intakes are implicated in gut microbiota and host responsiveness. A limited number of studies have found no link between responsiveness and baseline gut microbiota composition[23,199] or dietary intake[157,206,209]. The discrepancies in results between studies may be related to heterogeneity in participant characteristics (i.e. age, gender, ethnicity, BMI) and the differing dietary assessment methods, dietary interventions and gut microbial analysis methods used. Larger, less heterogeneous cohorts and analytical methods should be used in the future to provide further insight into the influence baseline gut microbiota composition and habitual dietary intake have on gut microbiota and host response to dietary interventions.

At present, only a small number of studies have been conducted with the primary aim of determining whether differences in baseline gut microbiota composition[202,203,206,207,209] and habitual dietary intake[26,202] can be used to predict likely responses to a dietary intervention. The other studies reviewed relied on post hoc analysis to determine what factors influenced gut microbiota and host response. Therefore,

additional studies that primarily aim to determine what factors are implicated in gut microbiota and host responsiveness are urgently required.

Preliminary evidence suggests that future studies aiming to modulate the gut microbiota to improve host outcomes should take baseline gut microbiota composition and habitual dietary intakes into consideration either when recruiting participants or when analysing study data. This will help provide a deeper understanding of the influence gut microbiota composition and habitual dietary intake have on gut microbiota and host responsiveness so that these factors can be controlled for more effectively. This will help determine the true efficacy of a given dietary intervention and provide better consistency of results between studies.

Future developments of predictive mathematical models that integrate various gut microbiota, habitual dietary intake, and host physiological and behavioural parameters, as introduced by Zeevi and co-authors[202], have promise in ensuring dietary interventions are tailored to the individual to improve their success. Continuing advancements in -omics technologies and a deeper understanding of gut microbiota and host physiology resilience may help facilitate the advancement of more robust personalised microbiota-targeted dietary approaches in the future.

2.11 References

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CHAPTER THREE

SECONDARY DATA ANALYSIS

DIFFERING HABITUAL DIETARY INTAKES INFLUENCE GUT MICROBIOTA RESPONSIVENESS TO ACTAZIN™; A GREEN KIWIFRUIT POWDER

I would like to take the opportunity to thank Anagenix for allowing me to conduct secondary analysis using the data generated for the human intervention study they provided products (i.e. Actazin™ and Livaux™) and partial funding for.

Chapter 3 Secondary data analysis

3.1 Abstract

Large inter-individual variability in gut microbiota response to dietary interventions has been shown. Preliminary evidence indicates that baseline gut microbiota composition and diversity influence gut microbiota response to a dietary intervention. As distinctions in habitual dietary intakes led to differing gut microbiota compositions it is plausible that habitual dietary intake also influence gut microbiota responsiveness to a dietary intervention. However, to date no studies have demonstrated whether there is any validity to this hypothesis. Secondary data analysis was undertaken using data from a previous randomised, double-blind, placebo-controlled, cross-over study designed to determine whether baseline gut microbiota composition and diversity, and gut microbiota response to a 4 week high-dose (2400 mg/d) Actazin™ (green kiwifruit powder) differed due to distinctions in habitual dietary intake. Nutrient and food group intake information, ascertained from two 3-day diet records, was used to group individuals into two comparative intake groups (low *versus* high or low *versus* moderate *versus* high) for 13 different dietary groups. For each dietary group, differences in baseline gut microbiota composition and diversity, and gut microbiota response (16S rRNA bacterial gene sequencing) to high-dose Actazin™ between low *versus* high or low *versus* moderate *versus* high consumers were determined. In nine of the 13 dietary groups analysed significant baseline differences in gut microbiota composition were observed. The dietary groups that had the largest number of bacterial taxa that significantly differed between low *versus* high or low *versus* moderate *versus* high consumers were Vegetable, Fruit and Carbohydrate:Protein ratio. Significantly different gut microbiota responses to high-dose Actazin™ were demonstrated in nine of the 13 dietary groups with the Dietary fibre, Wholegrain, Vegetable, Plant protein and Carbohydrate:Protein ratio dietary

groups appearing to be particularly influential. The secondary data analysis results suggest that habitual dietary intake does influence gut microbiota response to a dietary intervention. It appears that dietary groups rich in dietary fibre were particularly influential. Therefore, an *in vitro* experiment and a human intervention study will be conducted to determine whether habitual dietary fibre intakes influence gut microbiota response to a dietary intervention.

3.2 Introduction

Diet has a major influence on the composition and functional capacity of the bacteria that reside within the gastrointestinal tract[1]. Research relating to the influence fermentable dietary substrates have on the ecology and function of the gut microbiota has increased recently; however, much remains unknown. It appears that differing habitual dietary intakes are in part responsible for the distinctive inter-individual variations in gut microbiota composition[1–4]. Acquiring an enhanced understanding of the role that certain dietary components and patterns have on the composition and function of the colonic microbiota will help enhance diet-specific therapeutic strategies designed to enhance human health via gut microbiota manipulation.

Interestingly, emerging research suggests that gut microbiota respond to a given dietary intervention in a distinctive manner among individuals, secondary to distinction in baseline gut microbiota profiles. A study, in healthy participants, conducted by Tuohy and co-authors[5] demonstrated that lower baseline concentrations of bifidobacteria led to a larger increase in bifidobacteria after a 2 week inulin prebiotic intervention. A number of other studies have demonstrated similar results[6–10]. Baseline microbial diversity also appears to influence gut microbiota response to a dietary intervention. It has been shown that individuals with lower alpha diversity at baseline harbour gut microbiota that are more responsive to dietary interventions than individuals with higher baseline alpha diversity[11]. These studies provide preliminary evidence to suggest that baseline gut microbiota composition and diversity influence gut microbiota response to a dietary intervention.

As diet significantly influences gut microbiota composition, it is plausible that the differing responses to a dietary intervention may also be related to variability in habitual dietary intakes. Presently, no studies have been conducted to determine whether

habitual dietary intake influences gut microbiota response. Therefore, research is required to determine which dietary components may significantly influence inter-individual variability in gut microbiota responsiveness to a dietary intervention.

The aim of this study was to use the data from a previous randomised, double-blind, placebo-controlled, cross-over study[12] to determine whether (1) baseline differences in bacterial taxa and alpha diversity exist between individuals with differing habitual dietary intakes and (2) differences in bacterial taxa response to high-dose Actazin™ exist between individuals with differing habitual dietary intakes. We hypothesised that baseline differences in gut microbiota composition and diversity, and gut microbiota response to high-dose Actazin™ will be evident among individuals with distinctive habitual dietary intakes.

3.3 Methods

Secondary data analysis was conducted using data from a previous randomised, double-blind, placebo-controlled, cross-over study[12]. Ethical approval for the original study was granted by the New Zealand Human Disability and Ethics Committee (12/STH/72/AM01). The original study was also registered with the Australia New Zealand Clinical Trials Registry (ACTRN: 12612001270808). Secondary data analysis was undertaken in late 2014/early 2015.

3.3.1 Intervention

In the original study participants consumed four different interventions; each for 4 weeks with a 2 week washout period between interventions. The four intervention were: placebo (isomalt; 2400 mg/d), low-dose Actazin™ (600 mg/d of green kiwifruit powder), high-dose Actazin™ (2400 mg/d of green kiwifruit powder) and high-dose Livaux™ (2400 mg/d of gold kiwifruit powder). Each participant took 4 x 600 mg capsules per day with all interventions being identical in appearance. During the low-dose Actazin™ intervention phase participants consumed 1 x 600 mg Actazin™ capsule and 3 x 600 mg placebo capsules. Subjects were asked not to take high-fibre supplements (i.e. Metamucil, Benefiber or Phloe) and to continue their usual habitual dietary intakes and physical activity levels throughout the duration of the study. For the purposes of secondary data analysis we used the data collected at baseline and after the high-dose Actazin™ intervention phase.

3.3.2 Participants

A total of 28 participants completed the original study. Prior to conducting secondary data analysis three of the original participants were excluded as they experienced a significant increase or decrease in weight (> 5% change in their total body weight) during the study (n = 2) or appeared to have under- or over-reported on their 3-day diet records

(n = 1). A Goldberg cut-off of < 1.0 or > 2.4 was used to classify participants as under- or over-reporters; respectively[13]. Therefore, the data from 25 participants (21 females and 4 males) were used for secondary data analysis. Seventeen participants were healthy and eight were classified as having functional constipation based on the Rome III criteria[14]. The data from the two groups were analysed together as there were no differences in baseline bacterial taxa or gut microbiota response to high-dose Actazin™ between the two groups (Table 3-1).

Table 3-1. Comparison of baseline bacterial taxa (% relative abundance) and change in bacterial taxa after the dietary intervention between constipated and healthy participants[1]

	Constipated		Healthy		
Bacterial taxa	**Mean**	**SD**	**Mean**	**SD**	**P value**
Baseline					
Bifidobacterium	2.81	2.99	3.09	5.25	0.669
Collinsella	1.01	0.61	1.51	2.41	0.344
Bacteroides	14.50	8.10	16.40	13.00	0.977
Parabacteroides	1.91	1.49	1.17	1.07	0.238
Prevotella	0.75	0.96	1.57	3.24	0.262
Rikenellaceae (family)	2.92	1.98	2.72	2.62	0.549
Clostridiales (order)	7.12	5.42	5.78	4.29	0.475
Lachnospiraceae (family)	11.93	7.34	12.38	8.27	0.842
Blautia	4.41	2.80	4.80	2.98	0.798
Coprococcus	5.44	4.11	5.89	3.84	0.549
Dorea	0.97	0.63	1.01	1.08	0.669
Lachnospira	0.76	0.90	1.59	1.83	0.157
Roseburia	0.27	0.28	0.86	1.30	0.374
Ruminococcaceae (family)	20.73	10.45	19.04	12.64	0.711
Faecalibacterium	5.20	4.40	6.75	6.46	0.406
Oscillospira	1.27	0.48	1.08	0.77	0.406
Ruminococcus	4.58	2.37	4.08	3.72	0.238
Akkermansia	3.90	5.99	1.78	2.90	0.215
Change after high-dose Actazin™					
Bifidobacterium	0.55	1.98	1.26	3.88	0.754
Collinsella	-0.41	0.72	0.41	1.60	0.175
Bacteroides	-5.44	10.86	-6.36	18.39	0.754
Parabacteroides	-0.22	0.88	0.05	0.67	0.798
Prevotella	-0.14	0.74	-0.70	4.57	0.842
Rikenellaceae (family)	-0.72	1.55	-0.33	3.57	0.628
Clostridiales (order)	1.28	3.70	-1.12	5.09	0.066
Lachnospiraceae (family)	5.01	8.52	3.05	8.13	0.669
Blautia	-0.46	4.37	0.09	4.51	0.798
Coprococcus	-0.53	4.06	0.92	3.75	0.374
Dorea	-0.03	0.75	-0.39	1.05	0.669
Lachnospira	-1.64	2.34	0.06	1.17	0.066
Roseburia	0.17	0.34	0.41	1.04	0.798
Ruminococcaceae (family)	1.96	11.10	1.17	11.28	0.887
Faecalibacterium	-0.12	5.11	1.18	4.68	0.157
Oscillospira	-0.48	0.75	-0.47	1.09	0.754
Ruminococcus	-0.26	2.10	0.39	4.47	0.628
Akkermansia	0.69	5.72	0.28	2.53	0.511

[1]Mann-Whitney test. P value < 0.05 is considered significant. Bacterial taxa are genera unless otherwise specified. SD: standard deviation.

3.3.3 Dietary intake analysis

Each participant completed two 3-day diet records; one at the beginning of the study and one 22 weeks later; at the end of the study. Nutrient analysis was conducted using FoodWorks version 7.0.3016 (FOODfiles 2010). Food group serves were determined from each participants' 3-day diet records using the suggested food group serving sizes outlined in the Ministry of Health Food and Nutrition guidelines[15].

3.3.4 Dietary groupings

Thirteen dietary groups were chosen to help determine whether differing habitual dietary intakes had an influence on baseline bacterial composition and diversity, and gut microbiota response to high-dose Actazin™. The following dietary groups were used: Sugar (g/d), Dietary fibre (g/d), Energy from fat (%), Energy from protein (%), Energy from carbohydrates (CHO) (%), Fruits (serves/d), Vegetables (serves/d), Wholegrains (serves/d), Lean meats etc. (serves/d), Plant protein (serves/d), Animal Protein (serves/d), Carbohydrate:Fat ratio and Carbohydrate:Protein ratio. The 13 dietary groups were chosen as previous research has demonstrated that these nutrients and food groups have an influence gut microbiota composition[1–4,16,17].

Each dietary group was split into one of two comparative intake groups; low *versus* high or low *versus* moderate *versus* high consumers. The specific comparative intake group split for each dietary group was chosen based on how the split compared to the Ministry of Health Food and Nutrition guidelines[15] (Table 3-2). Generally, for each dietary group, low consumers would not have met the Ministry of Health guidelines, whereas high consumers would have met or exceeded the dietary recommendations. There were some exceptions; for example, all participants exceeded the recommended intake for sugar, therefore participants were split into low, moderate and high sugar consumers. Additionally, some dietary groups, i.e. Plant protein, Carbohydrate:Fat ratio,

do not have specific dietary recommendations. In these cases a dietary group classification was chosen to ensure the greatest distinction in habitual dietary intakes between dietary groupings would occur (Table 3-2).

Table 3-2. Mean nutrient and food group intakes of the entire cohort, and for low, moderate and high consumers compared to mean intakes in New Zealand and the Ministry of Health Food and Nutrition guidelines

Dietary group	Mean	SD	Mean intakes in NZ	MoH guidelines	Mean intakes by dietary grouping		
					Low	Moderate	High
Nutrient							
Sugar (g/d)	97.6	32.4	96	< 10% TE equal to <48-54 g/d	64	103	138
Dietary fibre (g/d)	25.7	6.8	17.5	> 25-30 g/d	19		31
Energy from protein (%)	18.3	3.3	16.4	15-25% TE	14	18	21
Energy from fat (%)	31.4	5.4	33.8	20-35% TE	25	32	37
Energy from CHO (%)	45.6	6.4	47.1	45-65% TE	39	47	53
CHO:Protein ratio	3.2	1.0	NS	NS	2.3	2.7	4.3
CHO:Fat ratio	3.9	1.1	NS	NS	2.7	3.8	5.1
Food group							
Fruit (serves/d)	1.7	1.2	NS	> 2 serves/d	0.5	1.7	3.2
Vegetable (serves/d)	3.5	1.5	NS	> 3 serves/d	2.2		4.6
Wholegrain (serves/d)	1.8	1.2	NS	> 2 serves/d	0.8		2.9
Plant protein (serves/d)	0.5	0.6	NS	NS	0.1		0.9
Lean meat etc. (serves/d)	2.3	0.8	NS	> 1 serve/d	1.4	2.3	3.3
Animal protein (serves/d)	1.4	1.0	NS	NS	0.3	1.4	2.5

SD: standard deviation, NZ: New Zealand, MoH: Ministry of Health, CHO: carbohydrate, NS: not specified, TE: total energy.

3.3.5 Bacterial DNA extraction

Faecal samples, collected at baseline and after the high-dose Actazin™ intervention phase, were stored at -20 ^{o}C prior to analysis. DNA was extracted from a 250 mg portion of each homogenised faecal sample using the MO-BIO PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions

with minor alterations. Each sample was added to a bead beating tube (0.1 mm zirconia beads) and a Disruptor Genie (Scientific Industries, Bohemia, NY, USA) was used to mechanically disrupt the bacterial cells (2 850 rpm for 3 x 30 secs with 1 min on ice between each cycle). To help isolate gram-positive bacteria, the lysis temperature was increased to 95 ºC.

3.3.6 16S rRNA bacterial gene sequencing

An initial polymerase chain reaction (PCR) was run to amplify the V3-V4 variable regions of the 16S rRNA bacterial gene (position 341-805 in the *Escherichia coli* rRNA gene) using the following forward and reverse primers[18]:

Forward: Bakt_341F-

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CCTACGGGNGGCWGCAG**

Reverse: Bakt_805R-

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**GACTACHVGGGTATCTAATCC**

The bold section of the sequence binds to the amplicon region of interest. The remaining section of the primer sequence binds to the Illumina index adaptors. Each PCR run consists of 25 µL of HotStartTaq master mix (QIAGEN, Melbourne, Australia), 12 µL of each of the forward and reverse primers made to a concentration of 0.1 µM and 1 µL of template DNA or sterile water (negative control); equalling 50 µL total volume. The following PCR conditions were used: initial denaturation of 95 ºC for 15 mins followed by 30 cycles of 30 secs denaturation at 95 ºC, 30 secs annealing at 55 ºC and 30 secs extension at 72 ºC, then lastly a 5 min extension at 72 ºC. Column purification of the PCR products was undertaken using a QIAquick PCR purification kit (QIAGEN,

Melbourne, Australia). The Qubit 2.0 fluorometer (Life Technologies) was used to quantify the PCR products before they were sent to the New Zealand Genomics Ltd (NZGL) Massey Genome Service (MGS) for the second PCR step using an Illumina MiSeq sequencer. The amplicons were library quality control checked, diluted and pooled prior to being loaded onto the Illumina MiSeq sequencer (three 2x250bp PE runs).

3.3.7 Bioinformatics

The Illumina MiSeq sequencing data were interpreted using Quantitative Insights Into Microbial Ecology (QIIME) software version 1.8.0[19]. PANDASeq was used to assemble the forward and reverse reads into a continuous sequence ensuring at least a 40 bp overlap with a minimum of 350 bp and a maximum of 500 bp length[20]. Chimera filtered sequences and reads were clustered into operational taxonomic units (OTUs) based on an identity threshold value of 97% using USEARCH and UCLUST[21]. Sequence alignment with the Greengenes core reference database (version 13_8) was carried out using PyNAST[22]. The RDP Naïve Bayesian classifier was used to provide taxonomic assignment[23].

3.3.8 Statistics

Baseline gut microbiota and changes in bacterial taxa after high-dose Actazin™ data were analysed using non-parametric Mann-Whitney (low *versus* high consumers) or Kruskal-Wallis (low *versus* moderate *versus* high consumers) tests (GenStat 14th edition). Only bacterial taxa with a mean baseline relative abundance > 1% were included in the analysis unless the bacterial taxa has been shown in the literature to be influenced by dietary change (i.e. *Roseburia*)[24,25]. Baseline alpha diversity (PD_whole tree) data were analysed using a non-parametric two sample t-test (QIIME version 1.8.0).

3.4 Results

3.4.1 Differences in baseline gut microbiota

Significant differences in baseline gut microbiota composition were observed for comparatively different intake groups in nine of the 13 dietary groups analysed. The dietary groups with the greatest number of significant baseline bacterial taxa differences between low and high, or low, moderate and high consumers were Vegetable, Fruit and Carbohydrate:Protein ratio (Table 3-3). Baseline alpha diversity was also significantly different between low, moderate and high dietary fibre consumers for the Animal protein, CHO:Protein ratio, Fruit and Lean meat etc. dietary groups (Table 3-4).

Table 3-3. Dietary groups that had significantly different baseline bacterial taxa (% relative abundance) between low *versus* high, or low *versus* moderate *versus* high consumers[1]

		Dietary grouping			
Dietary group	**Bacterial taxa**	**Low**	**Moderate**	**High**	**P value**
Dietary fibre	*Bifidobacterium*	3.5		2.7	0.017
	Ruminococcaceae (family)	12.3		23.7	0.020
Vegetable	*Parabacteroides*	0.9		2.0	0.005
	Rikenellaceae (family)	2.0		3.6	0.040
	Lachnospiraceae (family)	15.7		8.5	0.008
	Blautia	6.1		3.2	0.010
	Coprococcus	7.7		3.6	0.005
	Dorea	1.4		0.5	0.035
	Roseburia	1.1		0.2	0.002
	Ruminococcaceae (family)	13.9		25.7	0.010
	Oscillospira	0.8		1.5	0.022
	Verrucomicrobia (phylum)	0.8		4.4	0.002
	Akkermansia	0.8		4.3	0.002
Plant protein	*Bacteroides*	10.4		20.8	0.016
	Prevotella	2.5		0.2	0.036
	Coprococcus	7.3		4.3	0.035
Energy from protein (%)	*Parabacteroides*	0.7^{a}	1.7^{b}	2.0^{b}	0.036
Lean meat etc.	*Rikenellaceae* (family)	1.3^{a}	4.6^{b}	2.7^{a}	0.021
Animal protein	*Rikenellaceae* (family)	1.2^{a}	4.4^{b}	2.5^{ab}	0.028
	Ruminococcaceae (family)	19.7^{a}	27.3^{b}	10.8^{ab}	0.006
Fruit	*Collinsella*	0.8^{ab}	2.8^{a}	0.5^{b}	0.049
	Bacteroides	18.3^{a}	7.3^{b}	21.1^{a}	0.008
	Prevotella	0.8^{a}	2.1^{b}	1.1^{a}	0.046
	Dorea	1.2^{a}	1.3^{a}	0.5^{b}	0.038
Sugar	*Parabacteroides*	2.2^{a}	1.1^{b}	0.9^{b}	0.039
CHO:Protein ratio	Actinobacteria (phylum)	10.7^{a}	2.12^{b}	5.34^{c}	0.027
	Bifidobacterium	7.0^{a}	0.7^{b}	2.5^{b}	0.037
	Parabacteroides	1.6^{a}	2.0^{a}	0.7^{b}	0.038
	Clostridiales (order)	2.8^{a}	6.9^{b}	7.8^{b}	0.039
	Blautia	7.3^{a}	2.8^{b}	5.0^{c}	0.005
	Coprococcus	8.9^{a}	3.3^{b}	6.4^{a}	0.015
	Dorea	1.7^{a}	0.4^{b}	1.2^{a}	0.011
	Ruminococcaceae (family)	10.2^{a}	27.4^{b}	17.1^{a}	0.023

[1]Mann-Whitney (low *versus* high) or Kruskal-Wallis (low *versus* moderate *versus* high) tests. P value of < 0.05 is considered significant. Values with differing superscripts are significantly different from each other. Bacterial taxa are genera unless otherwise specified. CHO: carbohydrate.

Table 3-4. Dietary groups with significantly different baseline alpha diversity (PD_whole tree index) between low *versus* moderate *versus* high consumers[1]

	Dietary grouping			
Dietary group	**Low**	**Moderate**	**High**	**P value**
Animal protein	49.0[a]	69.2[b]	65.7[ab]	0.011
CHO:Protein ratio	64.5[ab]	67.8[a]	52.8[b]	0.039
Fruit	73.0[a]	52.8[b]	59.4[ab]	0.010
Lean meat etc.	49.4[a]	73.3[b]	63.7[ab]	0.002

[1]Non-parametric two-sample t-test. P value of < 0.05 is considered significant. Values with differing superscripts are significantly different from each other. CHO: carbohydrate.

3.4.2 Differences in gut microbiota response to high-dose Actazin™

Significant differences in gut microbiota response to the high-dose Actazin™ were observed for comparatively different intake groups in nine of the 13 dietary groups (Table 3-5). The dietary groups with the greatest number of bacterial taxa that responded in a distinctive manner between low, moderate and high consumers were Dietary fibre, Wholegrain, Vegetable, Plant protein and Carbohydrate:Protein ratio (Table 3-5).

Table 3-5. Dietary groups that had bacterial taxa (% relative abundance) that changed in response to the high-dose Actazin™ in a significantly different way between low *versus* high, or low *versus* moderate *versus* high consumers[1]

		Dietary grouping			
Dietary group	**Bacterial taxa**	**Low**	**Moderate**	**High**	**P value**
Dietary fibre	*Lachnospiraceae* (family)	- 8.4		- 1.0	0.020
	Coprococcus	- 2.5		0.7	0.043
	Roseburia	- 0.9		0.0	0.008
Wholegrain	*Bifidobacterium*	0.1		- 2.2	0.035
	Bacteroides	12.2		0.4	0.040
	Blautia	- 2.0		2.0	0.022
	Coprococcus	- 2.6		1.5	0.014
	Oscillospira	0.9		0.1	0.040
Vegetable	*Lachnospiraceae* (family)	- 7.2		0.1	0.019
	Parabacteroides	0.4		- 0.4	0.005
	Roseburia	- 0.7		0.0	0.010
	Faecalibacterium	- 3.5		2.2	< 0.001
Plant protein	Firmicutes (phylum)	- 13.0		1.2	0.019
	Bacteroidetes (phylum)	16.0		- 1.0	0.014
	Bacteroides	15.6		- 2.7	0.005
Lean meat etc.	*Rikenellaceae* (family)	1.2[a]	0.8[ab]	- 0.7[b]	0.042
Energy from CHO (%)	*Ruminococcus*	0.54[a]	- 0.18[b]	0.03[b]	0.021
Sugar	*Ruminococcus*	0.58[a]	0.0[ab]	0.12[b]	0.034
CHO:Protein ratio	Firmicutes (phylum)	- 16.0[a]	3.0[b]	8.2[b]	0.034
	Bacteroidetes (phylum)	21.0[a]	- 2.1[b]	- 8.5[b]	0.034
	Bacteroides	20.1[a]	- 2.8[b]	6.6[b]	0.017
	Blautia	- 4.1[a]	1.5[b]	1.3[b]	0.016
	Faecalibacterium	- 3.8[a]	1.8[b]	- 1.6[a]	0.038
CHO:Fat ratio	*Ruminococcus*	0.53[a]	- 0.29[a]	0.11[b]	0.038

[1]Mann-Whitney (low *versus* high) or Kruskal-Wallis (low *versus* moderate *versus* high) tests. P value of < 0.05 is considered significant. Values with differing superscripts are significantly different from each other. Bacterial taxa are genera unless otherwise specified. CHO: carbohydrate.

For the Dietary fibre dietary group, high-dose Actazin™ led to significantly different responses between low and high consumers for *Lachnospiraceae*, *Coprococcus* and *Roseburia* (Figure 3-1). A more pronounced reduction in *Lachnospiraceae* (family) relative abundance occurred in low- compared to high-dietary fibre consumers (-8.4% *versus* -1.0%; respectively [p = 0.020]). *Coprococcus* relative abundance decreased in low-dietary fibre consumers (-2.6%) but increased in high-dietary fibre consumers (0.7%) (p = 0.043). Lastly, *Roseburia* relative abundance did not change in high-dietary fibre consumers but reduced in low-dietary fibre consumers (-0.9%) (p = 0.008).

For the Wholegrain dietary group, high-dose Actazin™ led to significantly different responses between low and high consumers for *Bifidobacterium, Bacteroides, Blautia, Coprococcus* and *Oscillospira* (Figure 3-2). Minimal change (0.1%) in *Bifidobacterium* occurred in low wholegrain consumers, however, in high-wholegrain consumers *Bifidobacterium* decreased in relative abundance by 2.2% (p = 0.035). A large increase in *Bacteroides* relative abundance (12.2%) was evident for low-wholegrain consumers, whereas, *Bacteroides* only increased marginally (0.4%) in high-wholegrain consumers (p = 0.040). *Blautia* (p = 0.022) and *Coprococcus* (p = 0.014) relative abundance increased in high-wholegrain consumers (2.0 and 1.5%; respectively) but decreased in low-wholegrain consumers (-2.0 and -2.6%; respectively). Lastly, *Oscillospira* relative abundance increased in both low- and high-wholegrain consumers (0.9 and 0.1%; respectively), however, the increase was more pronounced in low-wholegrain consumers (p = 0.040) (Table 3-5).

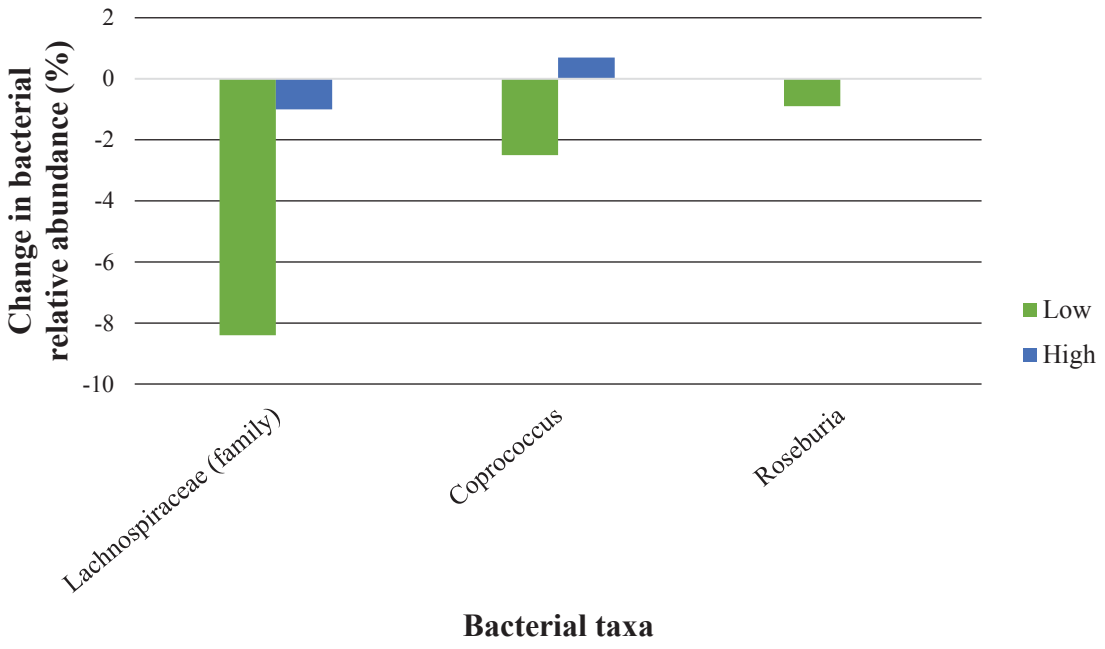

Figure 3-1. Bacterial taxa that responded in a significantly different way after the high-dose Actazin™ for the low- *versus* high-dietary fibre consumers

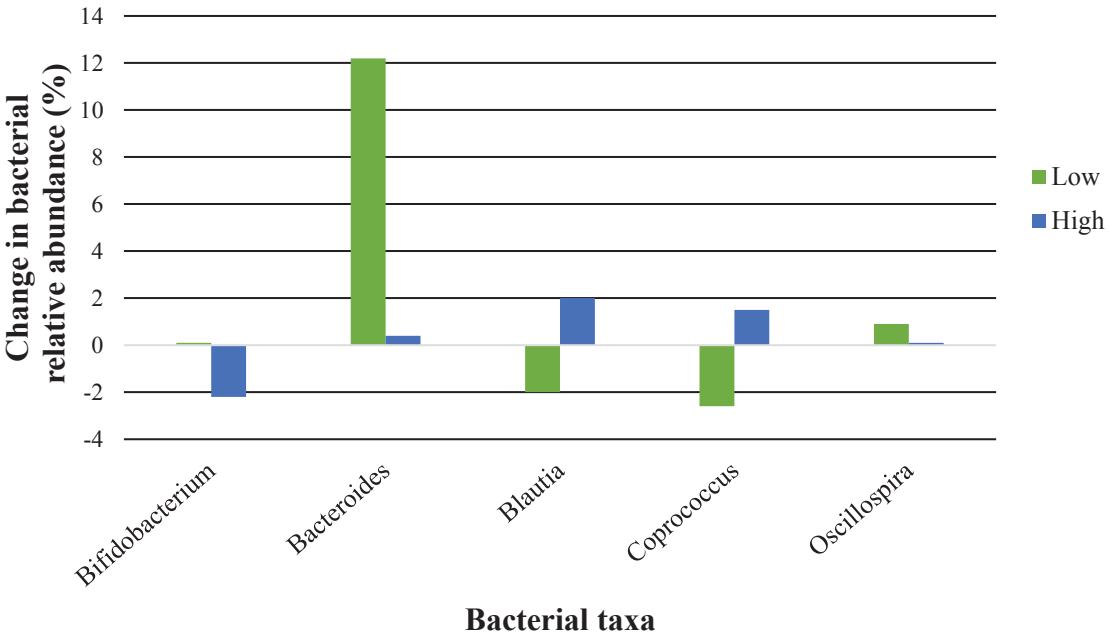

Figure 3-2. Bacterial taxa that responded in a significantly different way after the high-dose Actazin™ for the low- *versus* high-wholegrain consumers

For the Vegetable dietary group, high-dose Actazin™ led to significantly different responses between low and high consumers for *Parabacteroides,*

Lachnospiraceae, Roseburia and *Faecalibacterium* (Figure 3-3). *Parabacteroides* relative abundance increased in low-vegetable consumers (0.4%) and decreased in high-vegetable consumers (-0.4%) ($p = 0.005$). Large decreases in *Lachnospiraceae* ($p = 0.019$) and *Faecalibacterium* ($p < 0.001$) relative abundance were observed in low-vegetable consumers (-7.2 and -3.5%; respectively), whereas, increases in these bacterial taxa were observed in high-vegetable consumers (0.1 and 2.2%; respectively). Lastly, no change in *Roseburia* relative abundance was observed in high-vegetable consumers, however, a decrease in *Roseburia* occurred in low-vegetable consumers (-0.7%) ($p = 0.010$).

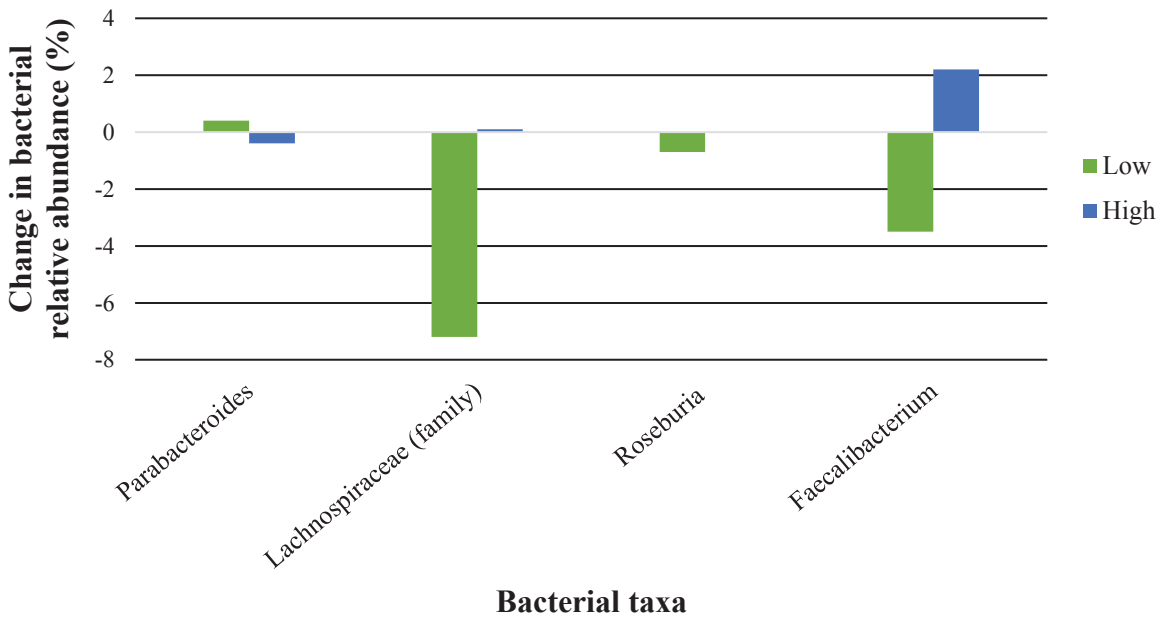

Figure 3-3. Bacterial taxa that responded in a significantly different way after the high-dose Actazin™ for the low- *versus* high-vegetable consumers

For the Plant protein dietary group, high-dose Actazin™ led to significantly different responses between low and high consumers for Firmicutes, Bacteroidetes and *Bacteroides* (Figure 3-4). A reduction in Firmicutes relative abundance occurred in low-plant protein consumers (-13.0%) but an increase occurred in high- plant protein consumers (1.2%) ($p = 0.019$). Bacteroidetes and *Bacteroides* relative abundance

increased in low-plant protein consumers (16.0 and 15.6%; respectively) but decreased in high-plant protein consumers (-1.0 and -2.7%; respectively) ($p = 0.014$ and $p = 0.005$; respectively).

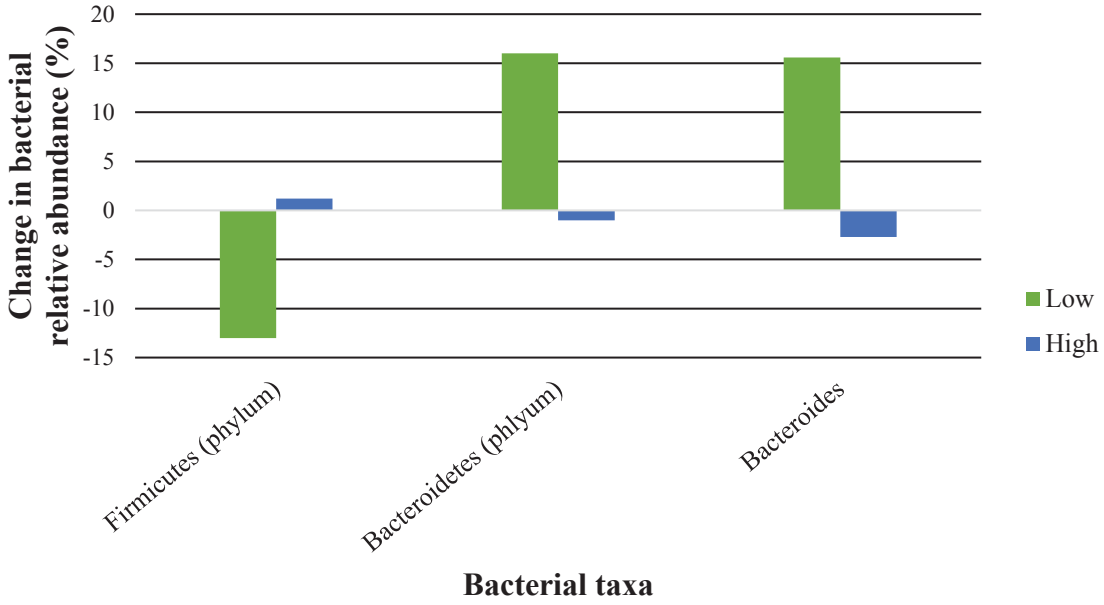

Figure 3-4. Bacterial taxa that responded in a significantly different way after the high-dose Actazin™ for the low- *versus* high-plant protein consumers

For the Carbohydrate:Protein ratio dietary group, high-dose Actazin™ led to significantly different responses between low, moderate and high consumers for Firmicutes, Bacteroidetes, *Bacteroides*, *Blautia* and *Faecalibacterium* (Figure 3-5). Low Carbohydrate:Protein ratio consumers experienced a large decrease in Firmicutes ($p = 0.034$) and *Blautia* ($p = 0.016$) relative abundance (-16.0 and -4.1%; respectively), whereas, an increase in these bacterial taxa were observed in moderate (3.0 and 1.5%; respectively) and high Carbohydrate:Protein ratio consumers (8.2 and 1.3%; respectively). Bacteroidetes relative abundance decreased in moderate and high Carbohydrate:Protein ratio consumers (-2.1 and -8.5%; respectively) but increased in low Carbohydrate:Protein ratio consumers (21.0%) ($p = 0.034$). The increase in *Bacteroides*

relative abundance observed in high Carbohydrate:Protein ratio consumers (20.1%) was significantly different from the changes that occurred in moderate (-2.8%) and high Carbohydrate:Protein ratio consumers (6.6%) ($p = 0.017$). Lastly, *Faecalibacterium* relative abundance increased in moderate Carbohydrate:Protein ratio consumers (1.8%), however, in low and high Carbohydrate:Protein consumers there was a decrease in *Faecalibacterium* relative abundance (-3.8 and -1.6%; respectively) ($p = 0.038$).

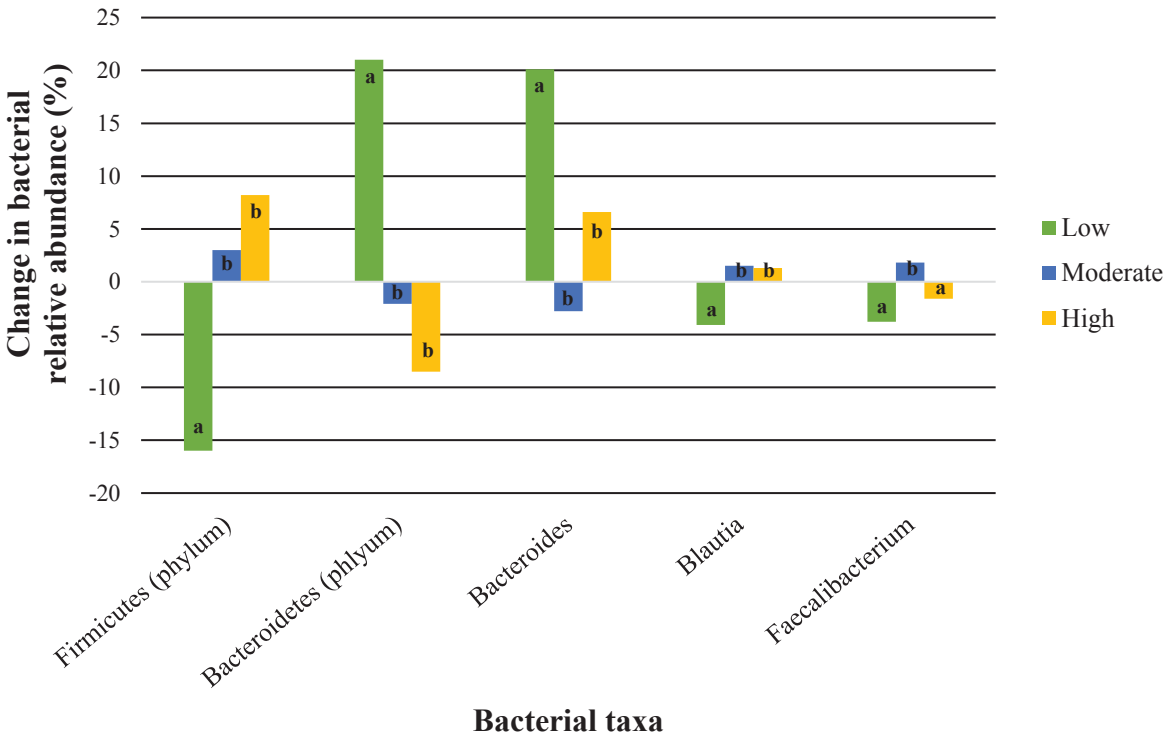

Figure 3-5. Bacterial taxa that responded in a significantly different way after the high-dose Actazin™ between low- *versus* moderate- *versus* high-Carbohydrate:Protein ratio consumers. Bars with differing superscripts are significantly different from each other (within each bacterial taxa)

3.5 Discussion

Secondary data analysis provided preliminary evidence to support our hypothesis that distinctive habitual dietary intakes influence baseline gut microbiota composition and diversity, and gut microbiota response to high-dose Actazin™. We established that baseline differences in gut microbiota composition existed in nine of the 13 dietary groups analysed. Vegetable, Fruit and Carbohydrate:Protein ratio had the greatest number of taxonomic differences between comparatively different intake groups (low *versus* high or low *versus* moderate *versus* high consumers) within each dietary group. We also demonstrated that comparatively different intakes in nine of the 13 dietary groups analysed led to significant differences in gut microbiota response to high-dose Actazin™. The dietary groups that appeared to have the largest impact on gut microbiota responsiveness were Dietary fibre, Wholegrain, Vegetable, Plant protein and Carbohydrate:Protein ratio.

A number of studies have demonstrated that dietary interventions can elicit significant changes in gut microbiota composition and function[24,26,27]. It does, however, appear that inter-individual variability in gut microbiota response to dietary interventions exists. Baseline gut microbiota composition[7,10,28] and diversity[11] have been suggested to influence gut microbiota response to a dietary intervention. As diet has a major impact on the structure and function of the gut microbiota, it is plausible that habitual dietary intakes may also influence gut microbiota responsiveness. To date, no studies have been conducted with the primary aim of determining what influence habitual dietary intake has on gut microbiota response. Therefore, the results of this study are the first to suggest that habitual dietary intake may influence gut microbiota response to a dietary intervention.

There are some limitations to this study that require discussion including the retrospective analysis of data obtained from a previous randomised, double-blind, placebo-controlled, cross-over study with relatively small participant numbers. For the purposes of this study the retrospective analysis of the previously collected data helped determine whether there was any validity to our hypothesis before progressing to more time- and resource-intensive studies. Another limitation of this study is that the dietary intake information collected, using two 3-day diet records, may not have provided dietary intake information that was representative of habitual dietary intakes. The two diet records were completed 22 weeks apart so it is possible that the dietary intake information collected may represent habitual dietary intakes. However, in future studies it will be important to collect habitual dietary intake information using multiple diet records over a number of months or through food frequency questionnaires which require participants to indicate the number of serves of various foods consumed over the past year.

The majority of dietary groups that led to distinctive gut microbiota responses to the high-dose Actazin™ were rich in dietary fibre, i.e. Dietary fibre, Wholegrain, Vegetable, Plant Protein and Energy from carbohydrates (%). We, therefore, believe that focusing on the influence differing habitual dietary fibre intakes have on gut microbiota response to a dietary intervention should be our primary research objective in the future. To test our hypothesis that habitual dietary fibre intake will influence gut microbiota response to a dietary intervention, an *in vitro* three-stage continuous colonic model system experiment was designed and conducted.

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CHAPTER FOUR

IN VITRO THREE-STAGE COLONIC MODEL SYSTEM

VARIABILITY IN GUT MICROBIOTA RESPONSE TO AN INULIN-TYPE FRUCTAN PREBIOTIC WITHIN AN *IN VITRO* THREE-STAGE CONTINUOUS COLONIC MODEL SYSTEM

This chapter is published as:

Healey G, Murphy R, Butts C, Brough L, Rosendale D, Blatchford P, Stoklosinski H & Coad J. Variability in gut microbiota response to an inulin-type fructan prebiotic within an *in vitro* three-stage continuous colonic model system. *Bioactive Carbohydrates and Dietary Fibre*. 2017;11:26-37.

The *in vitro* chapter presented in this thesis has been altered to include a document summarising the development of the two differing fermentable carbohydrate media (Appendix 4-1).

The published *in vitro* study is available as Appendix 4-2. A scanned copy of the statement of contribution to doctoral thesis containing publications is available as Appendix 4-3.

Chapter 4 *In vitro* three-stage colonic model system

4.1 Abstract

Gut microbiota have a major influence on human health and disease. Dietary interventions have been shown to beneficially modulate the gut microbiota and improve health; however, it appears there is large inter-individual variability in gut microbiota responsiveness to dietary interventions. We aimed to determine whether different fermentable carbohydrate content media, mimicking Western and Prudent style dietary patterns, influence the response of the gut microbiota to an inulin-type fructan prebiotic using an *in vitro* three-stage continuous colonic model system (i.e. gut model). We demonstrated that the addition of an inulin-type fructan prebiotic led to shifts in the organic acid concentrations and bacterial taxa in both the LFC and high HFC gut models. The shifts that occurred after the addition of the prebiotic significantly differed between the LFC and HFC gut models. Acetate increased in the HFC gut models but decreased in the LFC gut models ($p = 0.021$). The increases that occurred in Firmicutes ($p = 0.026$), *Lactobacillus* ($p = 0.045$) and *Mitsuokella* ($p = 0.012$) were significantly greater in the HF gut models. *Megasphaera* ($p = 0.033$) and an unknown genus of *Enterobacteriaceae* ($p = 0.011$) decreased in the LFC gut models but increased in the HFC gut models. The reduction in an unknown genus of *Lachnospiraceae* (other) was significantly greater in LFC gut models ($p = 0.040$). Additionally, large inter- and intra-donor variability in gut microbiota responsiveness to the prebiotic were demonstrated. This study demonstrates that media with different fermentable carbohydrate contents caused variability in gut microbiota responsiveness to an inulin-type fructan prebiotic; however, these results will need to be replicated in an i*n vivo* study.

4.2 Introduction

Numerous studies have suggested that diet plays a major role in the aetiology of certain diseases, such as cancer, obesity, type II diabetes mellitus (T2DM) and cardiovascular disease. Healthy dietary patterns rich in fruit, vegetables, legumes, wholegrains and fish, such as Prudent-style (PS) or Mediterranean dietary patterns, are thought to protect against certain diseases while unhealthy dietary patterns, such as a Western-style (WS) dietary pattern, which is high in sugar, animal protein and saturated fat, appear to increase the risk of developing disease[1–3]. Links between the microbiota that reside in the human gastrointestinal (GI) tract (gut) and disease have also been postulated. Dysbiotic gut microbiota have been associated with obesity[4], inflammatory bowel disease (IBD)[5], T2DM[6] and colon cancers[7]. It is, however, difficult to determine whether a dysbiotic gut microbiota are causally linked to these diseases or are the result of these diseases or the dietary patterns associated with disease. There are a number of other factors which are known to alter the composition or balance of the gut microbiota including genetics[8], age[9], gender[10], antibiotic use[11] and diet[12]. Diet appears to be particularly influential with 57% of the variation in gut microbiota composition being attributed to dietary differences[13]. It has been shown that individuals who have distinctive dietary patterns also have correspondingly distinctive colonic microbial consortia. Vegans have a distinctive gut microbiota composition (i.e. lower counts of *Bacteroides* spp., *Bifidobacterium* spp., *Escherichia coli* and *Enterobacteriaceae*) when compared with individuals consuming an omnivorous diet[14]. Individuals consuming a diet rich in plant-based foods have a lower Firmicutes:Bacteroidetes ratio and higher organic acid production, microbial diversity and richness when compared with individuals consuming a high-fat, high-sugar containing diet[12]. Short and longer term dietary interventions have also been shown to alter the gut microbiota composition. One study, which investigated the influence of

animal- and plant-based diets on microbial community structure, showed that the gut microbiota responded rapidly to the short-term shifts in macronutrient composition[15]. Studies have been conducted to establish the influence of prebiotics on the gut microbiota. Prebiotics are defined as "selectively fermented ingredients that result in specific changes in composition and/or activity in the GI microbiota, thus conferring benefit(s) upon human health"[16]. Inulin-type fructan prebiotics, such as inulin, fructo-oligosaccharides and oligo-fructose, have been widely studied and are known to stimulate the growth of bifidobacteria and increase butyrate production (a marker of microbial activity and substrate utilisation) which are often the main targets of prebiotic intervention studies[17–19]. The majority of studies to date have used targeted microbial enumeration techniques, such as fluorescence *in situ* hybridisation (FISH) and quantitative real-time polymerase chain reaction (qPCR), to investigate the influence of inulin-type fructan prebiotics on certain bacterial taxa[17,20]. Very few studies have used high-throughput sequencing technology to demonstrate what influence inulin-type fructan prebiotics have on the entire microbial community[21].

Even though the composition and functional capacity of the gut microbiota can be altered with dietary change, considerable variability in gut microbiota responsiveness to dietary interventions has been demonstrated. A recent *in vitro* batch fermentation study[22] demonstrated that donors (n = 18) with differing dietary patterns had gut microbiota that responded in a distinctive manner when fermenting corn, oat, rye and wheat flour or bran. Donors with a healthy dietary pattern had less of a reduction in diversity, a higher butyrate production and a gut microbiota consortia that were better equipped at utilizing carbohydrates found in grains compared to the donors with an unhealthy dietary pattern[22]. Several *in vivo* studies have grouped participants as responders and non-responders based on whether the gut microbiota changed as a result

of a dietary intervention. Individuals with higher microbial diversity[23,24] or bifidobacteria concentrations[18,25] at baseline were shown to be less responsive to dietary interventions. Preliminary research suggests that habitual dietary fibre intake may also influence how a microbial community responds to a dietary intervention[26]. It has been proposed that the development of novel therapeutic strategies designed to modulate the gut microbiota may prove helpful in the prevention, management and treatment of disease. However, given the variability in gut microbiota response to particular dietary interventions it is imperative we gain a better understanding of the factors that influence gut microbiota responsiveness in order to develop personalised dietary interventions that are tailored to modulate an individual's gut microbiota and thereby improve host health outcomes.

In this *in vitro* study, two media were designed to represent a WS and a PS dietary pattern (low fermentable carbohydrate medium [LFC] and high fermentable carbohydrate medium [HFC]; respectively) to mimic the background fermentable carbohydrate amounts and types commonly consumed by humans. The primary aim of this study was to determine what influence different fermentable carbohydrate content media had on the responsiveness of the gut microbiota to an inulin-type fructan prebiotic using an *in vitro* three-stage continuous colonic model system ("gut model"). Donor-specific differences in responsiveness were also considered, as inter-individual variability in responsiveness has been reported in human studies. Variability in gut microbiota responsiveness to dietary change has, to the best of our knowledge, not previously been studied within an *in vitro* gut model. The *in vitro* three-stage continuous colonic model system is useful as it has been validated against the colonic contents of sudden death victims to confirm that it simulates the physiologically relevant conditions found in the human colon[27]. The *in vitro* gut model can, therefore, be used to test research hypotheses before progressing to animal or human studies. A number of studies have

utilised the *in vitro* three-stage continuous colonic model system to analyse the impact dietary interventions have on the compositional and functional capacity of colonic microbial communities[28–30].

4.3 Methods

4.3.1 *In vitro* three-stage continuous colonic model system

The validated *in vitro* three-stage continuous colonic model system simulates the physiologically relevant conditions found in the human colon. The *in vitro* gut model consists of three vessels connected in series, which mimic the proximal (V1), transverse (V2) and distal (V3) colon[27]. The temperature of the vessels were maintained at 37ºC, with a pH of 5.4–5.6 (V1), 6.1–6.3 (V2) and 6.7–6.9 (V3) and were kept anaerobic by sparging with oxygen free nitrogen gas. The contents within each vessel were stirred to keep them homogenous. Media flowed from a 5 L media vessel into V1 via peristaltic pump with V2 and V3 being fed from the overflow of V1 and V2, respectively. Media which overflowed from V3 passed into a waste bottle. The transit time for the gut model was 36 hours (flow rate of 25 mL/hr).

Two different media were used; LFC and HFC. The basal medium originally developed by Macfarlane and co-authors[27] was modified to produce the LFC and HFC. The non-carbohydrate media ingredients (i.e. peptone water, casein, magnesium sulfate) were not altered; however, the fermentable carbohydrate media ingredients (i.e. pectin, resistant starch, xylan) were altered to allow the LFC to represent a WS dietary pattern and the HFC to represent a PS dietary pattern, which are low and high in fermentable carbohydrates; respectively. Dietary pattern information[31–33] and food composition tables, which provide information on the inulin-type fructan[34], resistant starch[35] and non-starch polysaccharide[36] content of the commonly consumed foods, were used to define the types, amounts and proportions of each fermentable carbohydrate ingredient added to the LFC and HFC (Appendix 4-1 & 4-4).

In total, six gut models (three LFC gut models and three HFC gut models) were used; however, at any one time only three *in vitro* gut models (each containing three

vessels per gut model) were run simultaneously (Figure 4-1). The first three gut models contained the HFC and the second three gut models contained the LFC. All three vessels within each *in vitro* gut model were inoculated with 100 mL of fresh faecal slurry. The fresh faecal slurry was prepared by diluting (1 in 5) fresh faeces with phosphate buffered saline and 0.05% L-cysteine hydrogen chloride and filtering it through a sterile BagPage® filter. A baseline sample was taken once the gut models were inoculated. After inoculation the gut models were run as a batch culture for 24 hours to acclimatise the bacteria prior to initiating media flow. The gut models were then run for 8 full turnovers (one turnover is 900 mL- the total volume of each gut model) to allow for steady state one (SS1) to be reached[29]. Steady state was deemed to be reached when daily measured acetate, butyrate and propionate were assessed as being stable (percentage coefficient of variation <10%) for at least 2 consecutive days. Once SS1 had been reached, a pre-intervention sample was taken and the prebiotic was added to the media. The prebiotic (Beneo Orafti® Synergy 1; 50:50 inulin:fructo-oligosaccharide mix) was added to the media in a proportional amount to that commonly used in human prebiotic intervention studies. The gut model was run for an additional 8 turnovers to allow for steady state two (SS2) to be reached. Once SS2 was reached a post-intervention sample was taken (Figure 4-1). The baseline, pre-intervention and post-intervention samples were immediately stored as 1 mL aliquots at -20 °C.

4.3.2 Faecal donors

Three faecal donors were recruited for this study (Health and Disability Ethics Committee: 13/CEN/144) as this number of faecal donors have been used in past *in vitro* gut model experiments[28,29]. A questionnaire was used to collect information from faecal donors relating to age, gender, ethnicity, health status, weight and height. A validated food frequency questionnaire was used to determine whether faecal donors had low,

moderate or high habitual dietary fibre intakes[37]. Healthy faecal donors of the same gender and ethnicity, and similar BMI and dietary fibre intakes were used. The 3 faecal donors had not experienced any recent weight loss or change in dietary intake over the past year and had not taken laxatives, gastric motility medications, prebiotic or probiotic containing foods, drinks or supplements in the past month or antibiotics in the past 6 months.

Each donor provided a fresh faecal donation on two separate occasions; one for the HFC gut model run and one for the LFC gut model run (Figure 4-1). Faecal donations were collected in a sterile container and were immediately stored in an anaerobic bag which contained an anaerobic sachet until the donation was processed. Processing of the faecal donation occurred within 30 min of the sample being voided.

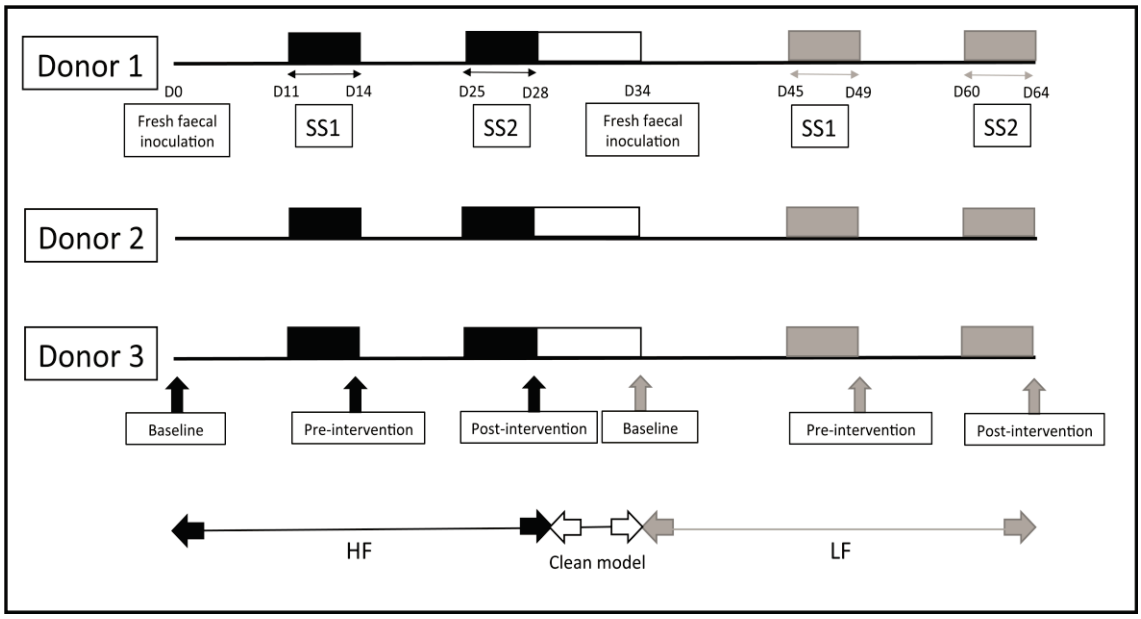

Figure 4-1. Diagram of the study design of the in vitro three-stage continuous colonic model system ("gut model"). Fresh faeces from each donor was used to inoculate each gut model twice through-out the experiment. The gut models were cleaned and autoclaved between each media type run. Samples were taken at baseline (after inoculation), pre-intervention (after steady state 1 [SS1] was reached) and post-intervention (after steady state 2 [SS2] was reached) for the high fermentable carbohydrate medium (HF) gut models and low fermentable carbohydrate medium (LF) gut models. D: day.

4.3.3 Dietary intake analysis

Each donor completed a 3-day diet record during the 3 days leading up to each faecal donation. Therefore, six days' worth of dietary intake information was collected from each donor. Nutrient and food group analysis was conducting using FoodWorks version 8.0 software (Xyris Software Pty Ltd). The Australian database in FoodWorks was used (AusBrands and AusFoods 2015 data sources) so nutrient intake and food group analysis could be conducted.

4.3.4 Media vessel sampling

The media vessels for each gut model had to be changed every 8 days to ensure a constant supply of media was provided. When a new media vessel was connected the sterility of the previous media vessel was assessed to ensure the media vessel had not become contaminated during the running of the experiment. The samples taken from the used media vessels were analysed for total bacteria concentrations using qPCR (method outlined below). Samples were sent for 16S rRNA bacterial gene sequencing (method outlined below) if contamination was suspected based on the qPCR results.

4.3.5 Bacterial DNA extraction

Samples were thawed at 4 °C and 1 mL aliquots were centrifuged at 14,000 x g for 10 min. The supernatant was removed, the pellet was resuspended in 750 µL of bead solution and the sample was transferred to a PowerLyzer® glass bead tube. Bacterial DNA was extracted from the samples using the MoBio PowerLyzer® Powersoil DNA® isolation kit according to the manufacturer's instructions (from step 4 of the protocol) with minor alterations. A FastPrep-24™ 5G (MP Biomedicals) was used to homogenise the samples at a speed of 5.5 m/sec for four 90 sec cycles with a 60 sec break between each cycle. The DNA was eluted in 10 mM Tris. NanoDrop 1000 spectrophotometry was used to quantify the DNA concentration.

4.3.6 16S rRNA bacterial gene sequencing and bioinformatics

The extracted DNA was used as a template for initial PCR amplification of the V3-V4 hyper-variable region of the 16S rRNA bacterial gene using the barcoded fusion primers:

16SR_V4- (5′-CAAGCAGAAGACGGCATACGAGAT-barcode-AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT-3′)

16SF_V3- (5′-AATGATACGGCGACCACCGAGATCTACAC-barcode-TATGGTAATTGGCCTACGGGAGGCAGCAG -3′)

These primers also contain adaptors for downstream Illumina MiSeq sequencing. Each sample was amplified with a pair of unique (8 base) barcoded primers. The PCR reagents used were Invitrogen AccuPrime™ Pfx SuperMix (part number 12344-040) (17 µL), 10 µM 16SR_V4 Primer (1 µL), 10 µM 16SF_V3 Primer (1 µL) and Ambion nuclease-free water (catalog number: AM9932) to normalise to 5 ng/µL (1 µL). The following PCR conditions were used; a hold at 95 °C for 2 min followed by 30 cycles of 95 °C for 20 sec (denaturation), 55 °C for 15 sec (annealing), 72 °C for 5 min (extension) finishing with a hold at 72 °C for 10 min. Library clean-up utilised an Invitrogen SequalPrep Normalisation Plate Kit (Thermo Fisher). Eighteen microlitres of the PCR product was used in the library clean-up with an elution volume of 12 µL. A Qubit DNA HS assay was used to measure the library concentration and a Bioanalyzer DNA HS assay was used for library sizing. The libraries were pooled by equal volume. Sequencing was undertaken on an Illumina MiSeq machine, using 2 x 250 base pair (bp) read length, at the Massey Genome Service (Massey University, Palmerston North, New Zealand). The data obtained from Illumina MiSeq sequencing were analysed using Quantitative Insights Into Microbial Ecology (QIIME)[38]. Paired-end assembler for DNA sequencing (PANDASeq) was used to assemble the forward and reverse reads into continuous sequences ensuring at least a 50 bp overlap with a minimum of 350 bp and a maximum

of 500 bp length[39]. Chimera filtered sequences and reads were clustered into operational taxonomic units (OTUs) based on an identity threshold value of 97% using USEARCH 6.1 and UCLUST[40]. Sequence alignment with the Greengenes core reference database (version 13_5) was carried out using PyNAST[41]. The RDP Naïve Bayesian classifier was used to provide taxonomic assignment[42].

4.3.7 Quantitative PCR

Total bacteria and bifidobacteria concentrations were determined using the LightCycler® 480 system (Roche Life Science). Total bacteria standard template DNA was prepared using *Escherichia coli* (Nissle) and bifidobacteria standard template DNA was prepared using *Bifidobacterium bifidium* (DSM20082). Bacterial DNA was extracted using the MoBio PowerLyzer® Powersoil DNA® isolation kit as described above. The following primers were used: Total bacteria- forward (TCCTACGGGAGGCAGCAGT) and reverse (GGACTACCAGGGTATCTAATCCTGTT) primers[43]; bifidobacteria- forward (GGGTGGTAATGCCGGATG) and reverse (CCACCGTTACACCGGGAA) primers[44]. Quantitative PCR was performed in triplicate with 10 µL of SyBr Green Master (Roche Life Science), 1 µL of each of the forward and reverse primers (5 µM), 7 µL of PCR grade water and 1 µL of template DNA. The conditions used for total bacteria PCR amplification were initial denaturation at 95 °C for 10 min followed by 40 cycles of denaturation (95 °C for 15 sec), annealing (60 °C for 1 min), extension (72 °C for 20 sec) and finished with a melt curve (95 °C for 30 sec, 65 °C for 1 min and 95 °C continuous- 5 per °C acquisitions). The conditions used for bifidobacteria PCR amplification were initial denaturation at 95 °C for 5 min followed by 40 cycles of denaturation (95 °C for 1 min), annealing (66 °C for 45 sec), extension (72 °C for 1 min) and finished with a melt curve (95 °C for 30 sec, 65 °C for 1 min and 95 °C continuous- 5 per °C acquisitions).

4.3.8 Organic acid analysis

Organic acids for steady state and at pre- and post-intervention were measured by gas chromatography (GC) using a modified GC method[45]. One millilitre of fermenta was transferred into a 1.5 mL Eppendorf tube and centrifuged at 14,000 x g for 10 min at room temp. Then 100 µL of supernatant was transferred into a 2 mL Eppendorf for immediate steady state analysis or frozen at -80 °C until required for pre- and post-intervention quantification. The 100 µL of supernatant in 2 mL Eppendorf tubes were diluted with 400 µL of 0.01 M phosphate buffered saline containing 2-ethylbutyric acid (6.25 mM) as an internal standard, with a final internal standard concentration of 5 mM. The sample was then acidified with 250 µL concentrated hydrochloric acid and 1000 µL diethyl ether added. After mixing, to allow acids to transfer to the diethyl ether phase, the sample was centrifuged at 10,000 x g for 5 min (4 °C). The diethyl ether phase was derivatised immediately for steady state or stored at -80 °C for pre- and post-intervention quantification. In a capped GC vial 100 µL of the diethyl ether phase was derivatised with 20 µL N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide with 1% tert-butyldimethylchlorosilane (MTBSTFA + TBDMSCI, 99:1; Sigma-Aldrich) by heating to 80 °C in a water bath for 20 min. Samples were then transferred to a 200 µL glass insert, placed back into the GC vial and re-capped. Steady state samples, measuring acetate, butyrate and propionate, were injected immediately onto the GC, whilst pre- and post-intervention samples were left for 48 hours at room temperature before analysis to allow complete derivatisation of all acids quantified (i.e. lactate). Analysis was performed on a Shimadzu capillary gas chromatograph system (GC-2010 Plus, Tokyo, Japan) equipped with a flame ionization detector (FID) and fitted with a Restek column (SH-Rtx-1, 30 m × 0.25 mm ID × 0.25 µm) (Shimadzu, USA). The carrier gas was helium with a total flow rate of 21.2 mL/min and pressure of 131.2 kPa. Make up gas was

nitrogen. The temperature program began at 70 °C increasing to 115 °C at 6 °C/min, with a final increase to 300 °C at 60 °C/min, holding for 3 min. Flow control mode was set to linear velocity; 37.5 cm/sec. Injector temperature was 260 °C and detector temperature was 310 °C. Samples were injected (1 µL) with a split injection (split ratio 1:10). The GC instrument was controlled and data processed using Shimadzu GC Work Station LabSolutions Version 5.3. Steady state analysis was determined by peak area to internal standard peak area ratio of the acids acetate, propionate and butyrate. Pre- and post-intervention sample analysis was determined by quantifying peak area to internal standard peak area ratio against organic acid standard curves (acetate, propionate, butyrate and lactate) containing 2-ethylbutyric acid (5 mM) as an internal standard. The standards were prepared for derivatisation alongside the pre- and post-intervention diluted fermenter samples. Data acquired provided a final sample result of µmol organic acid/mL fermenta.

4.3.9 Statistical analysis

Gut microbiota and organic acid data were analysed using paired t-tests. A non-parametric test was not used to analyse the data due to the small sample size. A p value of < 0.05 was considered significant. Statistical analysis was carried out using R version 3.3.2[46]. PCoA graphs were generated using unweighted UniFrac distances within QIIME[38].

4.4 Results

4.4.1 Donor characteristics and dietary intake

Donor characteristics may influence how the gut microbiota respond to a dietary intervention, therefore, differences and similarities in characteristics between donors were determined. All donors (n = 3) were healthy, the same gender and ethnicity, and of a normal BMI (Table 4-1). Food group and nutrient analysis helps provide insight into the differences and similarities in habitual dietary intake between donors. Differences in habitual dietary intake may also influence gut microbiota responsiveness and interestingly nutrient intakes and food group serves did differ between donors (Table 4-1). The New Zealand Ministry of Health has implemented guidelines for recommended food groups serves to help encourage good health[47]. Donor 1 (D1) and donor 3 (D3) meet the recommended serves per day for fruits and vegetables. None of the donors meet the recommended serves per day for grains and donor 2 (D2) and D3 did not meet the recommendation to consume more wholegrain than refined grain serves. All donors were very close to or exceeded the recommended dietary fibre intake of 25–30 g/day. D3 met the dairy and protein (legumes, nuts, seeds, meat, etc.) serve recommendations, however, D1 and D2 had lower than recommended intakes of these food groups.

4.4.2 Baseline differences in gut microbiota

Each donor provided two fresh faecal donations: one to inoculate the LFC gut model and one to inoculate the HFC gut model (Figure 4-1). Baseline samples were analysed, after inoculation, to determine whether there were differences in gut microbiota composition between the first and second faecal void for each donor. The principal co-ordinate analysis (PCoA) graph demonstrates that the baseline samples for each donor tend to cluster together suggesting that the bacterial community used to inoculate the LFC and HFC gut models were similar for each donor (Figure 4-2A).

Table 4-1. Donor characteristics, nutrient intakes and food group serves

	Donor 1	Donor 2	Donor 3
Donor characteristics			
Gender	Female	Female	Female
Age (years)	33	51	29
Ethnicity	NZ European/ Maori	NZ European/ Maori	NZ European/ Maori
BMI (kg/m^2)	21.1	21.2	21.9
Smoking status	Non-smoker	Non-smoker	Non-smoker
Health status	Healthy; no chronic diseases	Healthy; no chronic diseases	Healthy; no chronic diseases
Medications	No regular	No regular	No regular
Nutrient intake			
Energy (kJ/day)	9630.0	8257.3	9383.1
Protein (g/day)	52.6	70.4	109.2
Total fat (g/day)	108.4	86.4	97.7
Saturated fat (g/day)	46.0	32.9	29.4
Polyunsaturated fat (g/day)	16.6	13.6	15.9
Monounsaturated fat (g/day)	35.6	32.7	43.6
Carbohydrate (g/day)	258.0	193.3	196.6
Sugars (g/day)	141.0	93.7	88.5
Dietary fibre (g/day)	35.0	24.4	41.2
Alcohol (g/day)	0.9	13.7	6.9
Energy from protein (%)	9.3	14.5	19.7
Energy from fat (%)	41.3	38.7	38.8
Energy from carbohydrate (%)	44.4	38.7	34.6
Energy from fibre (%)	2.9	2.4	3.6
Food group serves per day			
Total grains	5.5	5.4	5.8
Refined grains	2.7	2.9	4.4
Wholegrains	2.9	2.5	1.4
Fruit	2.8	1.1	2.0
Vegetables	3.6	2.8	7.3
Meat	0.0	0.6	1.4
Nuts & seeds	0.7	0.5	0.6
Legumes	0.2	0.3	0.5
Dairy	0.9	2.0	2.2

4.4.3 Media vessel contamination

Samples were taken, for subsequent analysis, from the used 5 L media vessels whenever new 5 L media vessels were connected, to monitor the sterility of the media throughout the running of the experiment. Total bacteria qPCR analysis of the used media samples revealed that one sample from the donor 2 LFC gut model media vessel, taken just prior to when the post-intervention sample was collected, had a high total bacteria concentration (3.6×10^8 gene copies/mL) when compared with the other media vessel samples, which were all below the limit of detection. This result was consistent with bacterial contamination and was confirmed with 16S rRNA bacterial gene sequencing which demonstrated that the high total bacteria concentration media vessel sample had 99.5% relative abundance of an unknown genus of *Enterobacteriaceae*. Based on the

sequencing results it was difficult to ascertain whether the bacteria caused a perturbation of the entire bacterial community within the contaminated gut model. However, as medium contamination was confirmed, the post-intervention data from donor 2 LFC gut model for all three vessels, were not included in the analysis (Appendix 4-5).

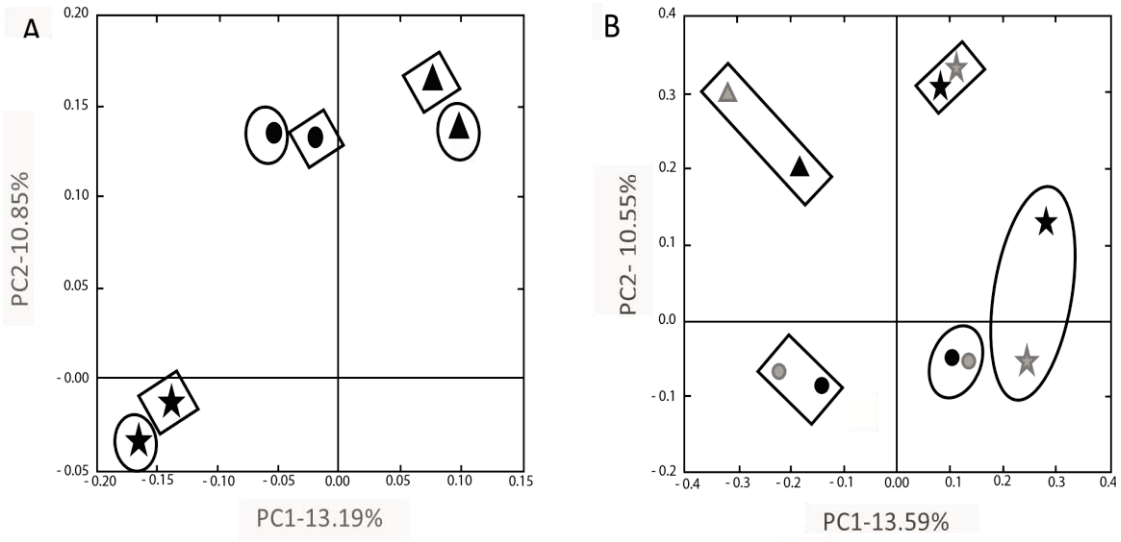

Figure 4-2. Principal co-ordinate analysis graphs (unweighted UniFrac distances) illustrating the differences and similarities in the bacterial communities between donors and media types at baseline (post inoculation) (A) and within vessel 1 comparing the pre- and post-intervention samples (B). LFC data from donor 2 was not included in Figure 4-2B. Samples that cluster together are more similar in bacterial composition and samples that are further apart have a more distinctive bacterial composition. Key: Donor 1 ★, Donor 2 ▲ and Donor 3 ●. High fermentable carbohydrate medium ◯ and low fermentable carbohydrate medium □. Black shapes- pre-intervention samples (or baseline samples in Figure 4-2A), Grey shapes- post-intervention samples. PC: principal co-ordinate.

4.4.4 Prebiotic driven changes in gut microbiota and organic acid concentrations

Differential prebiotic driven changes in gut microbiota composition and organic acid concentrations were observed between the LFC and HFC gut models. Changes in organic acid concentrations, in response to the prebiotic, differed significantly between the LFC and HFC gut models for acetate only. Acetate increased in V1 of the HFC gut models but decreased in V1 of the LFC gut models (+16.8 *versus* -0.9 µmol/mL; respectively [$p = 0.021$]) (Table 4-2A & B). There were significant differences in how certain bacterial taxa responded to the prebiotic between the LFC and HFC gut models. In V1, the increase

that occurred in Firmicutes as a result of the prebiotic was significantly greater in the HFC than the LFC gut models (+9.3% *versus* +2.2%; respectively [p = 0.026]). Also in V1, an unknown genus of *Enterobacteriaceae* decreased as a result of the prebiotic in the LFC gut models but increased in the HFC gut models (-0.6% *versus* +0.5%; respectively [p = 0.011]). In V2, *Lactobacillus* increased in the HFC gut models secondary to the prebiotic but did not change in the LFC (+0.4% *versus* 0.0%; respectively [p = 0.045]). Also in V2, *Megasphaera* increased in the HFC gut models but decreased in the LFC gut models (+3.0% *versus* -1.0%; respectively [p = 0.033]). Lastly, in V3 the decrease that occurred in an unknown genus of *Lachnospiraceae* (other) in the LFC gut models, as a result of the addition of the prebiotic, was significantly greater than the decrease that occurred in the HFC gut models (-2.9% *versus* -0.3%; respectively [p = 0.040]). Also, the increase that occurred in *Mitsuokella* was significantly greater in the HFC gut models than the LFC gut models (+2.5% *versus* +0.1%; respectively [p = 0.012]) (Table 4-2A & B).

Despite the prebiotic driven changes that occurred in some of the other bacterial taxa and organic acids, the large variability in donor responses rendered a lot of the changes as statistically non-significant. For example, lactate concentrations in V1 of the HFC gut models increased from 0.3 to 23.1 μmol/mL but did not change in V1 of the LFC gut models. The large variability in responses between donors for the HFC gut models meant the difference was not significant. *Bifidobacterium* relative abundance increased more than 2 fold in V2 of the HFC gut models and did not change in V2 of the LFC gut models; however, again the difference was not significant (Table 4-2A & B). Therefore, we examined the pre-intervention differences between the two media types and change in gut microbiota in response to the prebiotic on an individual donor basis.

Table 4-2. Pre- and post-intervention organic acid concentrations, phylum and genus level relative abundance for vessels 1, 2 and 3 of the high fermentable carbohydrate medium (LFC) gut models (**A**) and low fermentable carbohydrate medium (HFC) gut models (**B**)[1]

(A)	**High fermentable carbohydrate**											
	Vessel 1				**Vessel 2**				**Vessel 3**			
	Pre		**Post**		**Pre**		**Post**		**Pre**		**Post**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Organic acid (µmol/mL)												
Acetate	23.0*	18.2	39.8*	21.3	46.2	20.4	54.2	13.7	57.2	24.4	68.5	9.5
Propionate	21.5	18.4	17.5	5.7	36.8	15.9	34.7	17.2	38.8	15.0	36.5	18.7
Butyrate	36.9	7.4	45.2	36.1	55.1	6.4	74.8	24.8	57.2	10.6	73.9	0.9
Lactate	0.3	0.0	23.1	25.9	0.3	0.0	0.3	0.0	0.3	0.0	0.3	0.0
Phylum (% relative abundance)												
Actinobacteria	16.3	22.8	19.4	27.3	11.1	14.9	14.9	20.2	12.7	15.6	15.6	21.0
Bacteroidetes	17.3	0.5	3.7	2.7	25.9	26.9	26.9	28.6	20.3	10.6	20.9	24.7
Firmicutes	64.6*	22.9	73.9*	28.4	61.4	56.8	56.8	8.5	63.8	5.9	61.0	2.0
Proteobacteria	1.4	0.6	2.3	1.8	1.0	0.7	0.7	0.5	1.3	0.8	0.7	0.2
Genus (% relative abundance)												
Bifidobacterium	7.0	9.7	12.0	16.8	4.2	5.7	9.0	12.5	3.5	4.8	8.4	11.6
Collinsella	6.9	9.7	6.2	8.7	4.6	6.5	4.0	5.7	5.7	8.1	4.3	6.0
Bacteroides	8.6	11.7	0.7	0.6	17.3	15.8	17.0	19.7	14.9	15.9	13.9	17.8
Prevotella	8.6	12.2	3.0	3.3	3.3	4.7	8.5	7.4	1.9	2.7	5.9	5.8
Lactobacillus	0.1	0.1	1.7	1.4	0.1*	0.0	0.5*	0.0	0.1	0.0	0.5	0.1
Lachnospiraceae, unknown genus (other)	2.7	2.9	0.9	0.5	2.6	0.2	2.1	0.3	3.6*	2.8	3.3*	0.0
Lachnospiraceae, unknown genus	33.1	17.2	22.5	15.3	17.0	3.2	10.9	2.6	15.2	0.2	9.8	2.7
Coprococcus	1.0	1.3	0.2	0.2	0.9	0.9	0.6	0.4	1.1	1.1	0.7	0.5
Dorea	0.2	0.1	0.3	0.4	0.6	0.5	1.0	0.7	0.8	0.2	1.3	1.0
Ruminococcaceae, unknown genus	2.0	0.6	6.7	9.4	2.8	0.5	6.2	5.8	5.5	1.2	5.7	2.1
Ruminococcus	0.1	0.0	0.0	0.0	9.6	4.7	3.9	0.7	6.9	0.6	5.3	1.1
Dialister	3.8	4.2	9.3	11.2	6.6	0.2	7.9	5.3	7.4	0.6	7.9	0.7
Megasphaera	7.4	3.2	8.0	5.4	7.7*	5.9	10.7*	6.5	7.2	3.6	7.7	0.5
Mitsuokella	2.8	3.9	13.3	16.0	0.3	0.2	1.9	1.1	0.3*	0.0	2.8*	0.0
Enterobacteriaceae, unknown genus	0.5*	0.5	1.0*	0.0	0.1	0.1	0.3	0.1	0.2	0.1	0.3	0.2
(B)	**Low fermentable carbohydrate**											
	Vessel 1				**Vessel 2**				**Vessel 3**			
	Pre		**Post**		**Pre**		**Post**		**Pre**		**Post**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Organic acid (µmol/mL)												
Acetate	25.3*	3.9	24.4*	1.5	28.0	3.2	35.5	3.3	36.5	1.3	45.6	5.6
Propionate	24.4	1.0	42.3	4.6	29.5	0.4	48.4	5.3	30.4	0.2	50.9	8.6
Butyrate	42.9	9.5	62.6	7.8	54.5	11.8	74.2	8.0	55.0	5.4	78.2	11.9
Lactate	0.3	0.0	0.3	0.0	0.3	0.0	0.3	0.0	0.3	0.0	0.3	0.0
Phylum (% relative abundance)												
Actinobacteria	7.0	9.1	1.6	1.2	7.4	9.3	3.1	3.2	9.2	11.4	4.6	5.4
Bacteroidetes	22.8	0.2	27.4	13.2	11.4	3.2	27.9	14.0	9.0	1.0	20.1	3.6
Firmicutes	67.4*	7.7	69.4*	13.6	76.8	10.2	67.1	16.0	76.9	10.0	73.0	7.8
Proteobacteria	2.4	1.2	1.3	0.7	4.0	2.1	1.6	1.1	4.4	2.4	1.8	1.2
Genus (% relative abundance)												
Bifidobacterium	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Collinsella	2.2	3.2	0.5	0.7	1.8	2.6	0.9	1.3	2.4	3.3	1.7	2.4
Bacteroides	1.4	1.2	0.2	0.1	2.1	1.4	2.1	1.7	1.9	0.8	12.3	0.9
Prevotella	18.5	2.3	27.2	13.3	7.3	2.6	25.4	16.1	4.3	0.3	6.3	2.1
Lactobacillus	0.0	0.0	0.0	0.0	0.0*	0.0	0.0*	0.0	0.0	0.0	0.0	0.0
Lachnospiraceae, unknown genus (other)	2.7	0.7	1.6	1.5	3.4	2.1	1.1	0.7	4.0*	2.8	1.1*	0.2
Lachnospiraceae, unknown genus	10.7	6.8	9.1	1.6	9.3	6.6	5.1	2.6	8.6	4.0	4.6	3.6
Coprococcus	0.3	0.0	6.3	8.7	0.5	0.1	8.5	11.8	0.7	0.4	13.3	18.5
Dorea	5.3	1.7	1.9	0.1	7.6	4.7	2.3	0.2	8.2	5.5	3.7	0.0
Ruminococcaceae, unknown genus	11.6	0.3	15.8	4.3	15.2	0.6	14.4	5.9	11.5	1.6	11.4	3.2
Ruminococcus	7.1	3.9	6.2	3.7	5.3	2.7	4.4	3.2	3.0	1.3	2.7	0.9
Dialister	13.4	6.5	11.4	6.4	13.2	5.4	10.2	5.3	11.4	4.2	9.9	2.7
Megasphaera	2.2	2.6	1.6	1.8	5.0*	1.6	4.0*	2.6	7.2	0.7	3.9	2.3
Mitsuokella	0.0	0.0	0.1	0.1	0.0	0.0	0.2	0.0	0.0*	0.0	0.1*	0.0
Enterobacteriaceae, unknown genus	1.5*	0.0	0.9*	0.5	0.9	0.1	0.8	0.0	0.8	0.2	0.7	0.4

[1] Paired t-test of 16S rRNA bacterial gene sequencing data. The change in mean values from pre- to post-intervention are significantly different between the LFC and HFC gut models; *$p < 0.05$. Only data from donor 1 and 3 are included in the analysis. SD: standard deviation, Pre: pre-intervention, Post: post-intervention.

Table 4-3. Pre-intervention differences in organic acid concentrations, phylum and genus level relative abundance between donors in vessel 1 (V1), 2 (V2) and 3 (V3) for the low and high fermentable carbohydrate medium

		High fermentable carbohydrate			Low fermentable carbohydrate		
	Donor	**V1**	**V2**	**V3**	**V1**	**V2**	**V3**
Organic acids (µmol/mL)							
Acetate	**1**	35.9	60.6	74.4	22.5	25.7	37.4
	2	25.2	53.0	55.1	25.9	27.8	29.1
	3	10.2	31.8	39.9	28.0	30.2	35.5
Propionate	**1**	34.6	48.1	49.4	23.7	29.8	30.3
	2	11.5	23.6	25.5	23.4	25.8	27.3
	3	8.5	25.6	28.2	25.1	29.2	30.6
Butyrate	**1**	31.7	50.6	49.7	49.6	62.8	58.8
	2	43.1	65.2	64.7	46.8	57.7	59.3
	3	42.1	59.6	64.7	36.2	46.1	51.2
Lactate	**1**	0.3	0.3	0.3	0.3	0.3	0.3
	2	3.2	0.3	0.3	0.3	0.3	0.3
	3	0.3	0.3	0.3	0.3	0.3	0.3
Phylum (% relative abundance)							
Actinobacteria	**1**	32.4	21.3	23.7	13.4	14.0	17.3
	2	18.1	9.7	9.4	0.9	1.2	1.0
	3	0.2	1.0	1.6	0.5	0.9	1.1
Bacteroidetes	**1**	17.6	22.4	12.8	22.7	13.7	9.6
	2	0.9	21.6	22.1	38.8	31.6	29.2
	3	16.9	29.4	27.8	23.0	9.1	8.3
Firmicutes	**1**	48.4	55.0	59.6	62.0	69.6	69.9
	2	75.5	66.5	67.1	57.3	64.7	68.3
	3	80.7	67.7	67.9	72.8	84.1	84.0
Proteobacteria	**1**	0.9	0.6	0.7	1.6	2.4	2.7
	2	4.8	1.4	0.7	2.5	1.9	1.2
	3	1.8	1.4	1.9	3.2	5.5	6.2
Genus (% relative abundance)							
Bifidobacterium	**1**	13.8	8.2	6.9	0.1	0.1	0.1
	2	17.8	9.5	9.1	0.0	0.0	0.0
	3	0.1	0.1	0.1	0.0	0.0	0.0
Collinsella	**1**	13.7	9.2	11.4	4.5	3.7	4.7
	2	0.1	0.1	0.1	0.0	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0	0.0
Bacteroides	**1**	0.3	6.1	3.6	0.5	1.1	1.3
	2	0.6	21.4	21.7	38.7	31.5	28.8
	3	16.9	28.5	26.1	2.3	3.1	2.5
Prevotella	**1**	17.2	6.6	3.8	16.9	9.1	4.5
	2	0.2	0.0	0.0	0.0	0.0	0.1
	3	0.0	0.0	0.0	20.1	5.4	4.2
Lactobacillus	**1**	0.1	0.1	0.1	0.0	0.0	0.0
	2	13.5	7.8	8.1	0.0	0.0	0.0
	3	0.2	0.1	0.1	0.0	0.0	0.0
Lachnospiraceae, unknown genus (other)	**1**	4.8	2.4	1.7	2.2	1.9	2.0
	2	8.7	5.9	3.9	2.2	2.5	1.9
	3	0.7	2.7	5.6	3.2	4.8	5.9
Lachnospiraceae, unknown genus	**1**	21.0	14.7	15.3	15.5	13.9	11.4
	2	16.1	9.3	10.4	6.5	4.4	5.3
	3	45.3	19.3	15.1	5.9	4.6	5.8
Coprococcus	**1**	1.9	1.5	1.9	0.3	0.4	0.4
	2	5.2	4.4	5.0	1.2	1.1	0.8
	3	0.1	0.2	0.4	0.3	0.6	0.9
Dorea	**1**	0.2	1.0	1.0	4.1	4.2	4.3
	2	0.1	0.3	0.5	0.4	0.5	1.0
	3	0.1	0.3	0.7	6.4	10.9	12.1
Ruminococcaceae, unknown genus	**1**	2.4	2.5	4.7	11.4	15.6	12.7
	2	0.4	2.6	3.4	12.5	12.7	10.3
	3	1.5	3.2	6.4	11.9	14.8	10.4
Ruminococcus	**1**	0.1	6.2	6.5	4.3	3.5	2.1
	2	1.4	5.2	6.1	12.0	12.2	10.1
	3	0.1	12.9	7.3	9.9	7.2	3.9
Dialister	**1**	0.8	6.7	7.8	8.8	9.4	8.5
	2	8.4	4.6	2.9	5.4	5.4	6.8
	3	6.8	6.5	6.9	18.0	17.0	14.4
Megasphaera	**1**	9.7	11.8	9.8	4.1	6.2	6.8
	2	6.0	10.4	8.3	3.9	12.9	12.9
	3	5.1	3.5	4.7	0.4	3.8	7.7
Mitsuokella	**1**	0.1	0.2	0.3	0.0	0.0	0.0
	2	5.5	2.1	1.4	0.6	0.6	0.6
	3	5.6	0.5	0.2	0.0	0.0	0.0
Enterobacteriaceae, unknown genus	**1**	0.8	0.2	0.2	1.5	1.0	1.0
	2	4.8	1.3	0.6	2.3	1.6	0.7
	3	0.1	0.1	0.1	1.4	0.9	0.7

4.4.5 Individual donor pre-intervention differences in gut microbiota and organic acid concentrations between media types

Examining the results on an individual donor basis revealed that there were considerable inter-donor differences in pre-intervention organic acid concentrations for the LFC and HFC gut models (Figure 4-3A). For example, D3-HFC gut model produced less acetate in all vessels (V1- 10.2, V2- 31.8 and V3- 39.9 µmol/mL) when compared with D1-HFC (V1- 35.9, V2- 60.6 and V3- 74.4 µmol/mL) and D2-HFC (V1- 25.2, V2- 53.0 and V3- 55.1 µmol/mL) gut models (Table 4-3). Less variability in organic acid production between donors were observed in the LFC gut models (Table 4-3). There were also noticeable intra-donor differences in organic acid concentrations when comparing the HFC with the LFC gut models. For example, acetate, propionate and butyrate production in V1 of the D1-HFC gut model were 35.9, 34.6 and 31.7 µmol/mL respectively, but were 22.5, 23.7, 49.6 µmol/mL; respectively, in V1 of the D1-LFC gut model (Table 4-3).

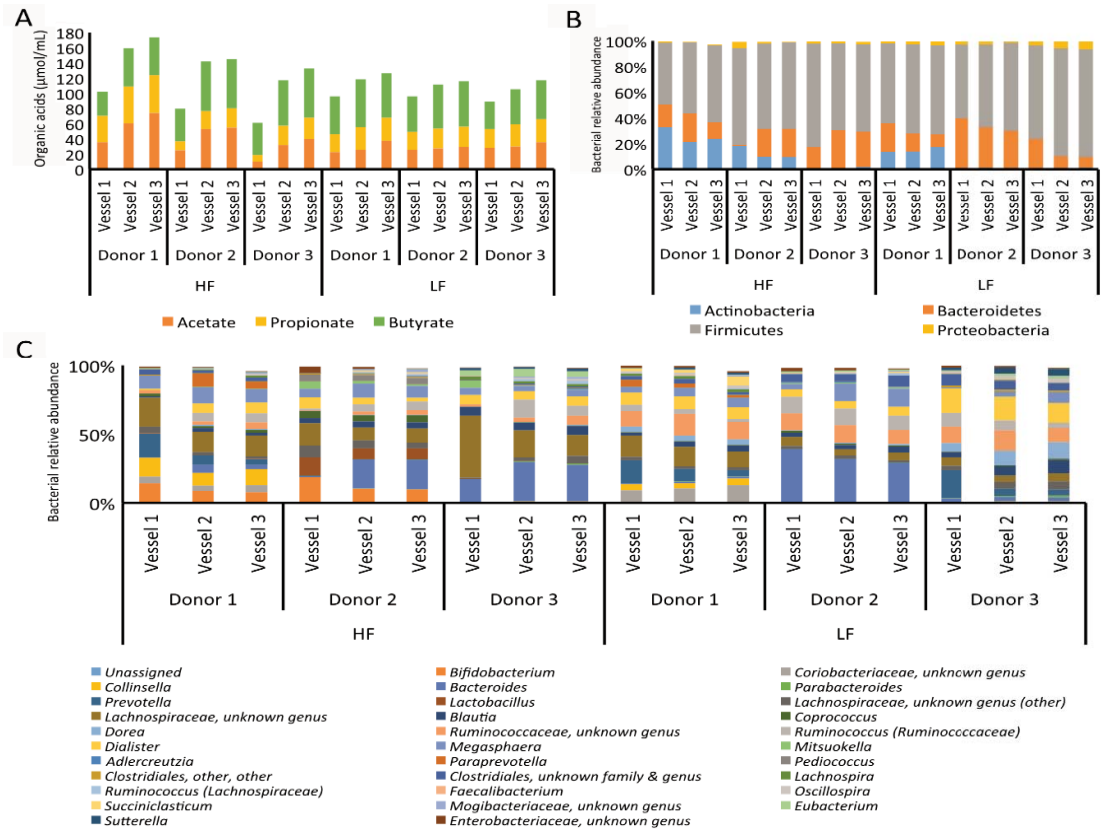

Figure 4-3. Stacked column graphs illustrating the inter- and intra-donor differences in pre-intervention organic acid concentrations (A), phylum level relative abundance (B) and genus level relative abundance (C) (16S rRNA bacterial gene sequencing) for each vessel (1, 2 and 3) and media type (high fermentable carbohydrate medium [HF] and low fermentable carbohydrate medium [LF]).

Pre-intervention inter-donor differences were also observed in gut microbiota composition at a phylum and genus level. Marked differences in phylum level relative abundance between donors for the LFC and HFC gut models occurred (Figure 4-3B). For example, in V1 of the D1-HFC and D2-HFC gut models there was a much higher relative abundance of Actinobacteria (32.4% and 18.1%; respectively) than in the D3-HFC gut model (0.2%) (Table 4-3). There were also noticeable intra-donor differences in phylum level relative abundance between the LFC and HFC gut models. For example, the relative abundance of Actinobacteria and Firmicutes clearly differed in V1 between the D1-LFC (13.4% and 62.0%; respectively) and D1-HFC (32.4% and 48.4%; respectively) gut models (Table 4-3). At a genus level there were also inter- and intra-donor distinctions

between the LFC and HFC gut models (Figure 4-3C). The D1-HFC gut model was characterised by a predominance of *Bifidobacterium* (V1- 13.8%, V2- 8.2% and V3- 6.9%), *Collinsella* (V1- 13.7%, V2- 9.2% and V3- 11.4%), *Prevotella* (V1-17.2%, V2- 6.6% and V3- 3.8%) and an unknown genus of *Lachnospiraceae* (V1- 21.0%, V2- 14.7% and V3- 15.3%); D2-HFC gut model by a predominance of *Bifidobacterium* (V1- 17.8%, V2- 9.5% and V3- 9.1%), *Lactobacillus* (V1- 13.5%, V2- 7.8% and V3- 10.4%) and an unknown genus of *Lachnospiraceae* (V1- 16.1%, V2- 9.3% and V3- 10.4%) and D3-HFC gut model by a predominance of *Bacteroides* (V1- 16.9%, V2- 28.5% and V3- 26.1%) and an unknown genus of *Lachnospiraceae* (V1- 45.3%, V2- 19.3% and V3- 15.1%) (Table 4-3). The predominant bacteria in each LFC gut model also differed between donors (Table 4-3).

4.4.6 Individual donor prebiotic driven changes in gut microbiota and organic acid concentrations

The PCoA graph shows each donor's entire bacterial community response to the prebiotic in the LFC and HFC gut models (only V1 data presented) (Figure 4-2B). The bacterial community within the D2-HFC gut model appears to respond to the prebiotic as the pre- and post-intervention samples are further apart whereas the pre- and post-intervention samples from the D1-HFC and D3-HFC gut models are more clustered, suggesting their bacterial community as a whole were less responsive. The bacterial community within the D1-LFC gut model appeared to be more responsive than in the D3-LFC gut model (Figure 4-2B).

Inter- and intra-donor differences in gut microbiota responsiveness and organic acid concentration changes, after the prebiotic was introduced, were evident between the LFC and HFC gut models (Figure 4-4). Using the results from V1 as an example, the D1-HFC and D2-HFC gut models had an increase in butyrate concentrations and

Bifidobacterium relative abundance after the prebiotic was introduced whereas the D3-HFC gut model had a reduction in butyrate and no change in *Bifidobacterium*. Concentrations of acetate, propionate and lactate and the relative abundance of *Lactobacillus*, *Dialister* and *Mitsuokella* increased in response to the prebiotic in the D3-HFC gut model. D1-HFC gut model experienced an increase in an unknown genus of *Ruminococcaceae*, *Megasphaera* and *Mitsuokella* and D2-HFC gut model experienced an increase in *Lactobacillus*, *Coprococcus* and *Mitsuokella* relative abundance.

In the D1-LFC and D3-LFC gut models there was an increase in butyrate and propionate concentrations; however, the relative abundance of *Bifidobacterium* did not change. *Prevotella* relative abundance increased in the D1-LFC gut model and there was an increase in the relative abundance of an unknown genus of *Lachnospiraceae, Coprococcus* and an unknown genus of *Ruminococcaceae* in the D3-LFC gut model as a result of the prebiotic. Gut microbiota responses also varied between donors in V2 and V3 (Figure 4-4).

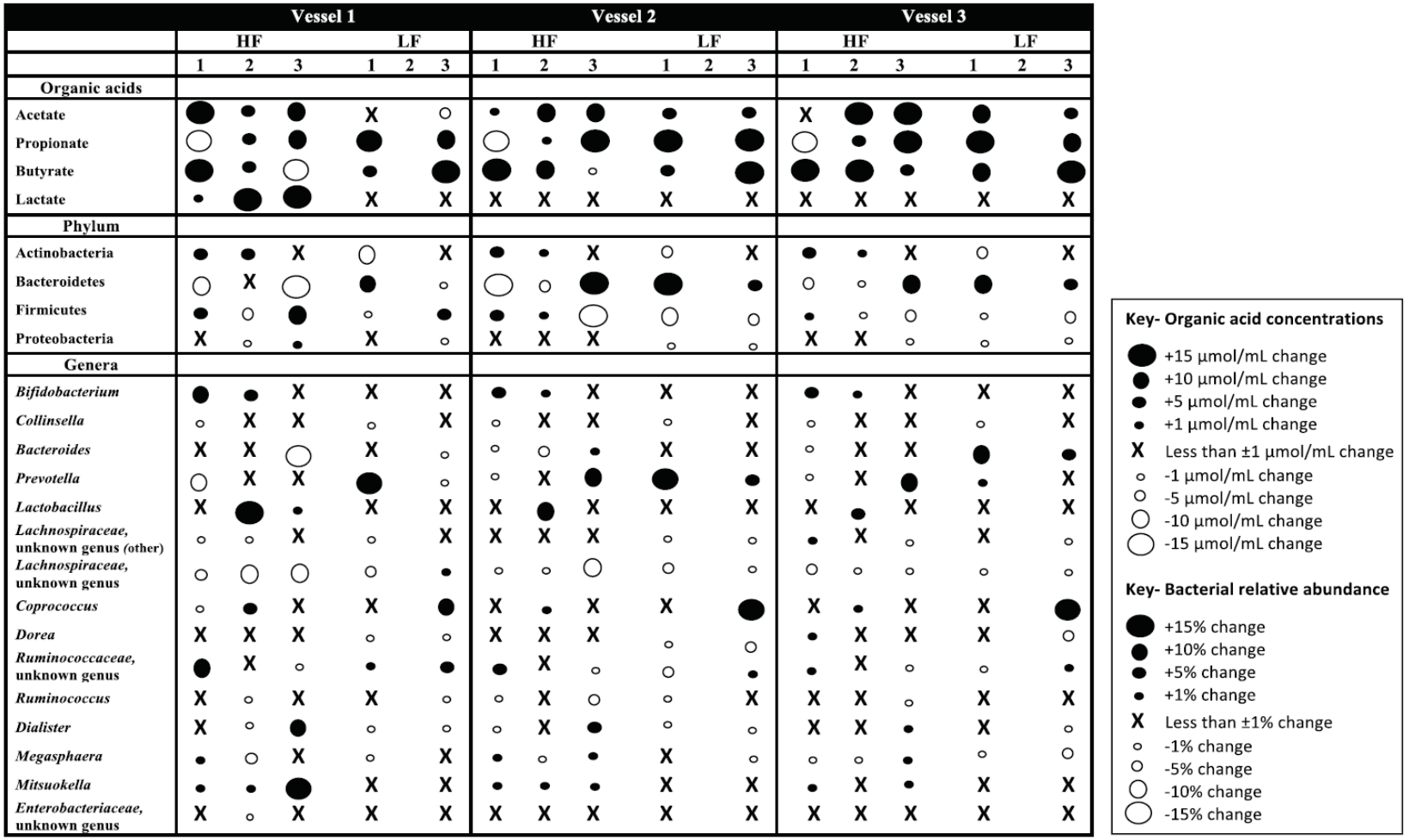

Figure 4-4. Bubble plot illustrating the distinctive changes in organic acid concentrations and bacterial relative abundance that occurred between donors (1, 2 and 3) and media types (high fermentable carbohydrate medium [HF] and low fermentable carbohydrate medium [LF]) in vessel 1, vessel 2 and vessel 3 in response to the prebiotic. The black circles represent increases in organic acid concentrations and bacterial relative abundance. The white circles represent decreases in organic acid concentrations and bacterial relative abundance. The X represents a less than ±1 µmol/mL change in organic acid concentrations or a less than ±1% change in bacterial relative abundance. LF data from donor 2 was not included.

Previous research has shown that baseline bifidobacteria concentrations influence how the gut microbiota respond to a dietary intervention[18,19,25]. Therefore, the baseline bifidobacteria concentrations of the three donors were analysed in relation to prebiotic driven changes in bifidobacteria concentrations (Figure 4-5). Baseline bifidobacteria concentrations were low in the D2 and D3 gut models (HFC-1.3 x 10^5 and 1.3 x 10^4 gene copies/mL; respectively, LFC- D2 gut model data not included, 9.5 x 10^3 gene copies/mL) and high in the D1 gut models (HFC- 2.3 x 10^7 gene copies/mL, LFC- 1.9 x 10^8 gene copies/mL). Bifidobacteria concentrations increased post-intervention in all vessels for D1-HFC and D2-HFC gut models but decreased for D1-LFC gut model (D2-LFC gut model data not included). The D3 gut models had a more varied bifidobacteria response to the prebiotic, with a reduction in V1 but a slight increase in bifidobacteria concentrations in V2 and V3 of the HFC gut model and in all vessels in the LFC gut model (Figure 4-5).

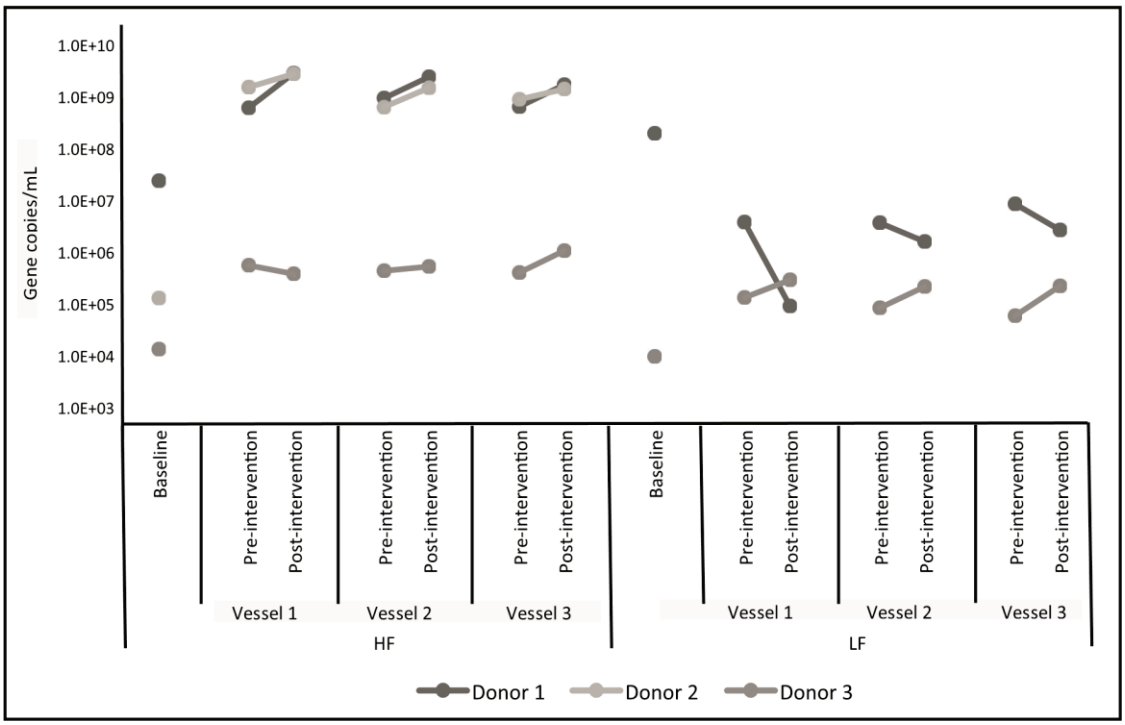

Figure 4-5. Baseline, pre- and post-intervention bifidobacteria concentrations (gene copies/mL) for each vessel (1, 2 and 3), media type (high fermentable carbohydrate medium [HF] and low fermentable carbohydrate medium [LF]) and donor (1, 2 and 3). Bifidobacteria concentrations analysed using qPCR. LF data from donor 2 was not included.

4.5 Discussion

In this study, two fermentable carbohydrate content media were developed to mimic WS and PS dietary patterns. The two media were used to determine what influence different fermentable carbohydrate contents had on gut microbiota responsiveness to an inulin-type fructan prebiotic using an *in vitro* gut model. We demonstrated that the addition of an inulin-type fructan prebiotic led to a shift in the gut microbiota in both the LFC and HFC gut models with the shift that occurred differing between the LFC and HFC gut models. There were significant differences in the amount of acetate produced and the changes that occurred in the relative abundance of Firmicutes, *Lactobacillus*, an unknown genus of *Lachnospiraceae* (other), *Megasphaera*, *Mitsuokella* and an unknown genus of *Enterobacteriaceae* between the LFC and HFC gut models. These results suggest that the fermentable carbohydrate content of the media has an influence on how the gut microbiota respond to an inulin-type fructan prebiotic. The results were also considered on an individual donor basis, which identified large inter- and intra-donor differences in gut microbiota responses to the prebiotic.

Factors which influence gut microbiota responsiveness from previous *in vivo* research include baseline gut microbiota composition and habitual dietary intakes. Previous studies have shown that individuals who have low baseline bifidobacteria concentrations experience a greater increase in bifidobacteria as a result of a dietary intervention than individuals with higher baseline bifidobacteria concentrations[18,19,25]. In the present study, there were inconsistencies in the influence baseline bifidobacteria concentrations had on changes in bifidobacteria. In the HFC gut models, the D1-HFC and D2-HFC gut models had an increase in bifidobacteria and the D3-HFC gut model had minimal change in bifidobacteria concentrations as a result of the prebiotic intervention, even though the D1-HFC gut model had a high baseline bifidobacteria

concentration and the D2-HFC and D3-HFC gut models had lower baseline bifidobacteria concentrations. In the LFC gut models, the results were more consistent with the literature as the D1-LFC gut model experienced a decrease and the D3-LFC gut model experienced an increase in bifidobacteria concentrations.

Habitual dietary fibre intakes have also been suggested to influence gut microbiota responsiveness[26]. All donors, in the present study, had dietary fibre intakes that would be considered high based on current recommendations as they were very close to or exceeded the recommended dietary fibre intake of 25–30 g/day[47]; however, dietary fibre intakes still ranged from 24.4 to 41.2 g/day. There were other distinctions in nutrient and food group intakes (e.g. protein, vegetable, carbohydrate intakes) between donors that may have influenced the responsiveness of the gut microbiota; however, it is difficult to draw any valid conclusions from the present study due to the small number of donors used. A sample size of three donors, used in the present study, is not uncommon for an *in vitro* three-stage continuous colonic model system study. Similar *in vitro* three-stage continuous colonic model system studies have also used three faecal donors[28,29]. Additionally, our results are consistent with previous *in vivo* studies, which have also observed inter-individual variability in gut microbiota responsiveness to dietary interventions[23,48,49]. Nevertheless, the influence habitual dietary intake has on gut microbiota responsiveness should be confirmed in *in vivo* studies with larger participant numbers.

Pre-intervention differences between the LFC and HFC gut models were expected as the gut microbiota consortia were exposed to different amounts and proportions of fermentable carbohydrates. The only significant pre-intervention gut microbiota difference observed between the LFC and HFC gut models was in an unknown genus of *Ruminococcaceae* (unpublished data). However, large inter-donor

differences in pre-intervention gut microbiota composition and organic acid production meant that a large proportion of the gut microbiota differences observed (*Bifidobacterium*, *Lactobacillus*, unknown genus of *Lachnospiraceae* etc.) between the LFC and HFC gut models were not significant. It was, therefore, important to discern the differences in pre-intervention gut microbiota and organic acid concentrations between the two media types on an individual donor level. By observing the results on an individual donor basis we demonstrated that each donor had a distinctive gut microbiota profile and organic acid production in the presence of the two different fermentable carbohydrate content media types.

This research is novel as media that were developed to mimic two diverse dietary patterns commonly consumed by humans (WS and PS dietary patterns) were used. The media were developed using dietary pattern food group information[31–33] and fermentable carbohydrate specific food composition tables[34–36], which has not been undertaken before. A further strength of this research is the way in which specific factors, such as media substrates, could be manipulated in an *in vitro* three-stage continuous colonic model system without the complication of confounding factors, such as immune parameters, dietary non-compliance and medication use, which are often present in human studies. The *in vitro* gut model also provides a validated platform for testing a hypothesis before progressing to *in vivo* studies. Another strength of this study is that the bacterial analysis was conducted using 16S rRNA bacterial gene sequencing. Very few *in vitro* three-stage continuous colonic model system or *in vivo* inulin-type fructan prebiotic studies have utilised Next-generation sequencing technology. Therefore, this research provides additional insight into whole bacterial community changes that occurred within an *in vitro* gut model after an inulin-type fructan prebiotic intervention. This is also the first time inter- and intra-donor variability has been discussed in detail in

an *in vitro* gut model, which demonstrates that this model could be used in the future to study the effects of individual variability in gut microbiota responsiveness. There are, however, some limitations to this study including the exclusion of the data collected from the D2-LFC gut model secondary to contamination. Fortunately the contamination occurred near the end of the experiment so all the D2-HFC gut model data, and the baseline and pre-intervention D2-LFC gut model data could still be used. As mentioned above, dietary pattern information and food composition data were used to help ensure the media types contained the types and amounts of fermentable carbohydrates present in WS and PS dietary patterns; however, food composition data was not available for all fermentable carbohydrate substrates present in the human diet (i.e. β-glucans). The two media recipes developed are representative of the best dietary fermentable carbohydrate information currently available. If additional fermentable carbohydrate food composition data becomes available in the future the media recipes can be refined before use in future *in vitro* studies.

In conclusion, this study demonstrated that media with different fermentable carbohydrate contents, which mimic WS and PS dietary patterns, influenced gut microbiota response to an inulin-type fructan prebiotic within an *in vitro* three-stage continuous colonic model system. Large inter- and intra-donor variability in gut microbiota responsiveness also appeared to have a profound influence on the prebiotic driven changes that occurred. The results of this study are novel and promising; however, they will need to be replicated in an *in vivo* study using a larger study cohort. Future research which aims to determine what factors (including habitual dietary intake) influence gut microbiota responsiveness to a dietary intervention may help enhance the success of gut microbiota modulation strategies.

4.6 References

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CHAPTER FIVE

VALIDATION STUDY

VALIDITY AND REPRODUCIBILITY OF A HABITUAL DIETARY FIBRE INTAKE SHORT FOOD FREQUENCY QUESTIONNAIRE

This chapter is published as:

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The validation study chapter presented in this thesis has been altered from the one published to include the associated study protocol (Appendix 5-1), study advertisements (Appendix 5-4), participant information sheet (Appendix 5-5), consent form (Appendix 5-6) and screening questionnaire (Appendix 5-7). The published validation study is available as Appendix 5-2.

A scanned copy of the statement of contribution to doctoral thesis containing publications is available as Appendix 5-3

Chapter 5 Validation study

5.1 Abstract

Low dietary fibre intake has been associated with poorer health outcomes, therefore, having the ability to be able to quickly assess an individual's dietary fibre intake would prove useful in clinical practice and for research purposes. Current dietary assessment methods such as food records and food frequency questionnaires are time-consuming and burdensome, and there are presently no published short dietary fibre intake questionnaires that can quantify an individual's total habitual dietary fibre intake and classify individuals as low, moderate or high habitual dietary fibre consumers. Therefore, we aimed to develop and validate a DFI-FFQ which can quickly and accurately classify individuals based on their habitual dietary fibre intake. In this study the DFI-FFQ was validated against the Monash University comprehensive nutrition assessment questionnaire (CNAQ). Fifty-two healthy, normal weight male (n = 17) and female (n = 35) participants, aged between 21 and 61 years, completed the DFI-FFQ twice and the CNAQ once. All eligible participants completed the study, however the data from 46% of the participants were excluded from analysis secondary to misreporting. The DFI-FFQ cannot accurately quantify total habitual dietary fibre intakes, however, it is a quick, valid and reproducible tool in classifying individuals based on their habitual dietary fibre intakes.

5.2 Introduction

Dietary fibre are non-digestible plant polysaccharides found in high amounts in fruits, vegetables, breads and cereals, legumes and nuts and seeds. Dietary fibre has been shown to have important implications for human health, including preventing and alleviating constipation, reducing gastrointestinal cancer incidence and blood glucose levels, lowering blood cholesterol levels and blood pressure, and beneficially modulating gut microbiota[1]. It is also possible that the efficacy of a dietary intervention is altered as a result of the influence habitual dietary fibre intake has on gut microbiota responsiveness and host outcomes. Therefore, being able to quickly assess an individual's habitual dietary fibre intake and classify individuals based on their dietary fibre intakes will prove useful in clinical practice and in nutrition and health research.

Dietary assessment methods such as diet records and food frequency questionnaires have inherent limitations such as being difficult to complete accurately, time-consuming and may not accurately assess a person's habitual diet[2]. A small number of dietary fibre assessment questionnaires have been developed, however these questionnaires assess general dietary behaviours, do not estimate total dietary fibre amounts, and/or do not classify individuals based on habitual dietary fibre intakes[3–6].

Therefore, the primary aim of this study was to determine whether a newly developed dietary fibre intake short food frequency questionnaire (DFI-FFQ) can accurately classify individuals based on their habitual dietary fibre intake and the secondary aim of the study was to determine whether the DFI-FFQ can accurately quantify total habitual dietary fibre intakes.

5.3 Methods

5.3.1 Subjects

Participants were recruited via email and poster advertisement (Appendix 5-4) in multiple locations around Palmerston North, New Zealand. A diverse cross-section of the population was targeted to help ensure a good representation of the New Zealand population was recruited. Sixty-eight individuals provided informed consent (Appendix 5-5 + Appendix 5-6) to participate in this study, of which fifty-two healthy participants met the inclusion criteria (aged >19 and <65 years, healthy, BMI >18.5 and <30 kg/m2, no significant weight loss or weight gain within the past year, no significant dietary change within the past year, not pregnant or breastfeeding, no food intolerances which cause gastrointestinal symptoms (i.e. lactose intolerance, gluten sensitivity), no adverse gastrointestinal symptoms, non-smoker and not high alcohol consumers) (Appendix 5-7). Participants completed the DFI-FFQ twice, at least 2 weeks apart, and the comprehensive nutrition assessment questionnaire (CNAQ) once. The DFI-FFQ was completed initially, followed by the CNAQ, and lastly the repeated DFI-FFQ was completed. The CNAQ and DFI-FFQ were both completed online. An energy intake: basal metabolic rate (EI:BMR) of <1.1 and >2.19 was used to exclude participants who appeared to have over- or under-reported using the CNAQ[7]. Ethical approval was obtained from the Massey University Human Ethics Committee (Southern A, Application 15/34).

5.3.2 Development of the DFI-FFQ

The DFI-FFQ (Appendix 5-8) was designed to quickly and accurately classify individuals as low, moderate or high habitual dietary fibre consumers and quantify an individual's habitual dietary fibre intake (g/day). The DFI-FFQ consists of five high dietary fibre containing food groups (vegetables, fruits, breads and cereals, nuts and

seeds and legumes) which account for 73.5% of the dietary fibre in a typical New Zealand diet[8]. Examples of what one serve is equivalent to, for each food group, is detailed within the DFI-FFQ. The frequency of consumption for the average number of serves consumed over the past year, was given as follows: Never, <1/month, 1–3/month, 1/week, 2–4/week, 5–6/week, 1/day, 2/day, 3/day, 4/day, 5/day and 6+/day.

5.3.3 DFI-FFQ Scoring Sheet

A scoring sheet (Appendix 5-9) was developed to quantify the amount of dietary fibre consumed and to classify individuals as low, moderate and high dietary fibre consumers. FoodWorks version 7.0.3016 (FOODfiles 2010; Xyris Software Pty Ltd., Brisbane, Queensland, Australia) was used to quantify the average amount of dietary fibre provided by the five food groups for each frequency of consumption. An individual's total dietary fibre intake was calculated by adding together the average amount of dietary fibre consumed from each food group in relation to the number of serves consumed.

5.3.4 Dietary Fibre Classification

The cut-offs used to classify individuals based on their dietary fibre intakes are outlined in Table 5-1. The high dietary fibre intake cut-offs were selected to reflect the New Zealand Ministry of Health recommended dietary fibre intake guidelines; >25 g/day for females and >30 g/day for males[9]. The low dietary fibre intake cut-offs were selected as the median dietary fibre intake in New Zealand was 17.5 g/day for females and 22.1 g/day for males, which are below recommended amounts[8]. Similar cut-offs have been used previously, however, the specific cut-offs used in this study were modified to be applicable to a New Zealand population[3].

Table 5-1. The dietary fibre intake cut-offs used to classify individuals as low, moderate and high dietary fibre consumers

	Females	**Males**
Low	< 18 g/day	< 22 g/day
Moderate	18–24.9 g/day	22–29.9 g/day
High	≥ 25 g/day	≥ 30 g/day

5.3.5 Dietary Assessment Method Used for Comparison

The Monash University online CNAQ was used for comparison with the DFI-FFQ. The 297-item food frequency questionnaire has been shown to be valid in assessing habitual dietary intakes when compared to four 7-day food records, each completed three months apart[10].

5.3.6 Statistical analysis

We aimed to recruit enough participants to ensure that correlations over 0.7 would be statistically significant and that the assumptions of chi-squared tests would not be over stretched. The relationship between results of the DFI-FFQ when compared to the CNAQ was determined using Spearman correlation, Pearson correlation, Bland-Altman plot, chi-squared test and linear weighted kappa score. Test-retest repeatability was assessed using Pearson correlation, Bland-Altman plot and Cronbach's alpha. T-tests were used to determine whether there were any differences in dietary fibre intakes between the DFI-FFQ and CNAQ and the repeated DFI-FFQ. A p value of < 0.05 is considered significant. Statistical analysis was carried out using GenStat 17th edition (VSNi Ltd., Hemel Hempstead, UK), Minitab 16th edition (Cronbach's alpha) (Minitab Inc., State College, PA, USA) and the calculator at http://vassarstats.net/kappa.html (kappa score).

5.4 Results

All eligible participants (n = 52) completed the study. The data from 28 participants (54%) were used as the data from 24 participants (46%) were excluded from the analysis secondary to likely misreporting on the CNAQ; with 18 participants (34.5%) having over-reported and six participants (11.5%) having under-reported their energy intakes. The group mean EI:BMR was 2.8 (SD 4.7) prior to exclusion and reduced to 1.6 (SD 0.3) after exclusion. Participant characteristics, total dietary fibre intakes and classifications determined by the DFI-FFQ and CNAQ are summarised in Table 5-2. The median dietary fibre intake in New Zealand (20.3 g/day)[8] is similar to the average dietary fibre intake of the study cohort, with dietary fibre intakes from both groups being below the New Zealand recommended dietary fibre intake guidelines[9]. The DFI-FFQ took on average 3.5 min to complete in comparison to the estimated completion time of 20–40 min for the CNAQ.

Table 5-2. Characteristics, dietary fibre intakes and classifications for the study participants

Mean (SD)	Male (n = 8)	Female (n = 20)	Total (n = 28)
Participant characteristics			
Age (years)	40 (11.02)	38 (9.37)	39 (9.91)
BMI (kg/m^2)	24 (1.9)	23 (3.1)	24 (2.82)
Ethnicity (No.)			
New Zealand European	4	14	18
Asian	3	0	3
Maori	0	2	2
Other	1	4	5
Dietary fibre intakes and classifications			
DFI-FFQ			
Dietary fibre intake (g/day)	27 (11.77)	23 (10.33)	24 (10.85)
Dietary fibre classification (No.)			
Low	2	5	7
Moderate	2	4	6
High	4	11	15
Monash CNAQ			
Dietary fibre intake (g/day)	31 (11.35)	29 (9.43)	29 (10.09)
Dietary fibre classification (No.)			
Low	1	4	5
Moderate	3	1	4
High	4	15	19

DFI-FFQ: dietary fibre intake short food frequency questionnaire, CNAQ: comprehensive nutrition assessment questionnaire, SD: standard deviation.

When comparing the DFI-FFQ to the CNAQ for dietary fibre classification, exact agreement occurred 79% of the time and gross misclassification occurred 7% of the time (Table 5-3). There was a significant difference in dietary fibre intakes between the DFI-FFQ and CNAQ (CNAQ was on average 5 g/day higher than the DFI-FFQ). The two dietary assessment methods were however correlated (Pearson correlation 0.65, Spearman correlation 0.53). A chi-squared test indicated an association between the classifications based on the DFI-FFQ and CNAQ (p = 0.002) and the linear weighted kappa score showed good agreement[11] (Table 5-4). The Bland-Altman plot is available within the Supplementary information (Figure S2A). Pearson correlation (0.94) and Cronbach's alpha (0.97) showed that the repeated DFI-FFQ correlated. The estimated dietary fibre intake from the second DFI-FFQ was significantly lower than the first DFI-FFQ by 1.8 g/day (Table 5-4). The Bland-Altman plot is available as Appendix 5-10.

Table 5-3. Comparison in dietary fibre classification (low, moderate and high) between the comprehensive nutrition assessment questionnaire (CNAQ) and the dietary fibre intake food frequency questionnaire (DFI-FFQ)

		CNAQ			
		Low	**Moderate**	**High**	**Total**
DFI-FFQ	Low	5 (18%)	0 (0%)	2 (7%)	7 (25%)
	Moderate	0 (0%)	3 (11%)	3 (11%)	6 (21%)
	High	0 (0%)	1 (3%)	14 (50%)	15 (54%)
Total		5 (18%)	4 (14%)	19 (68%)	28 (100%)

Table 5-4. Correlation and test–retest repeatability statistical analysis

Correlation between DFI-FFQ and CNAQ		**P value**
Pearson correlation	0.65	< 0.001
Spearman correlation	0.53	0.001
Chi-square test	9.6	0.002
Linear weighted kappa *	0.68	
Standard error	0.14	
Magnitude of agreement	Good	
Bland-Altman plot		
Limits of agreement (g/day)	−12.5–22.6	
Standard error	1.7	
Mean difference (g/day)	5	0.007
Test–Retest Repeatability		**P value**
Pearson correlation	0.94	< 0.001
Cronbach's alpha	0.97	
Bland-Altman plot		
Limits of agreement (g/day)	−6.0–9.6	
Standard error	0.72	
Mean difference (g/day)	1.8	0.019

CNAQ: comprehensive nutrition assessment questionnaire, DFI-FFQ: dietary fibre intake short food frequency questionnaire. * One category disagreement had a weight of ¾.

5.5 Discussion

Presently, there are no known short dietary fibre intake questionnaires that are able to classify individuals based on their habitual dietary fibre intake. Having the ability to be able to quickly and accurately classify an individual based on their dietary fibre intake will prove useful as low dietary fibre intakes have been associated with poorer health outcomes[12]. This study has shown that the DFI-FFQ can accurately classifying individuals based on their habitual dietary fibre intakes.

There was however, a significant difference in habitual dietary fibre intakes between the repeated DFI-FFQs and the DFI-FFQ and CNAQ, which suggests the DFI-FFQ might not accurately quantify total habitual dietary fibre intakes. Research has shown that large food item FFQs overestimate fruit and vegetable consumption, which may help explain the higher dietary fibre intakes determined from the CNAQ[13]. The addition of other dietary fibre contributing food groups, such as cakes and muffins, pies and pastries and biscuits, to the DFI-FFQ may have helped to improve the questionnaire's accuracy in quantifying total habitual dietary fibre intakes as these food groups collectively contribute 6.3% of the dietary fibre in a typical New Zealand diet[8]. Another reason why the DFI-FFQ may not have been able to accurately quantify total habitual dietary fibre intakes may be related to the serving size examples provided. The examples provided did not include all possible foods within a particular food group and relied on participants to use their own judgement regarding the number of serves consumed for foods that were not specifically listed.

There are a handful of short questionnaires that have been developed to assess dietary fibre intakes however these questionnaires assess general dietary behaviours[4–6], do not estimate total dietary fibre amounts[4–6], and/or do not classify individuals based on habitual dietary fibre intakes[3–6]. The DFI-FFQ is novel as it can accurately classify

individuals based on habitual dietary fibre intake. Unlike previously developed questionnaires, the DFI-FFQ was validated against an FFQ which assesses dietary intake over the past year, providing a more accurate account of long term rather than current dietary fibre intakes. Additionally, some of the questionnaires were validated using fairly homogenous populations, such as factory workers[3] and patients[5], making these questionnaires less useful in more diverse populations, such as in this study.

When comparing the study cohorts average dietary fibre intake to the Adult Nutrition Survey data[8] it appeared the study cohort has a similar dietary fibre intake to the New Zealand population. Therefore, the DFI-FFQ is a valid tool for classifying individuals based on their habitual dietary fibre intakes in New Zealand. In countries where dietary fibre intakes are distinctly different from New Zealand, the DFI-FFQ may need to be re-validated in these populations.

Forty-six percent of participants were excluded from the study secondary to misreporting on the CNAQ, which reduced the data available for analysis. A known limitation of FFQs is the high rate of misreporting, however the rate of misreporting in this study was much higher than previously reported[14]. It may therefore be useful to compare the DFI-FFQ to another dietary assessment method (i.e., 3- or 7-day diet records, or shorter validated FFQ) to confirm these results. The sample size for this study was small however a sufficient number of participants were recruited based on the sample size calculations, even after exclusion for misreporting. Additionally, other dietary questionnaire validation studies have similarly small participant numbers[15,16]. Despite the limitations discussed, we believe the DFI-FFQ will be a valuable tool in research and clinical practice as it is quick to complete (3.5 min on average), has low respondent burden and is a valid and reproducible method of classifying individuals based on their habitual dietary fibre intakes.

In conclusion, the DFI-FFQ has been shown to be a quick, valid and reproducible tool in classifying individuals based on their habitual dietary fibre intakes. The DFI-FFQ cannot however, accurately estimate total habitual dietary fibre intakes.

5.6 References

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CHAPTER SIX

STUDY PROTOCOL

INFLUENCE OF HABITUAL DIETARY FIBRE INTAKE ON THE RESPONSIVENESS OF THE GUT MICROBIOTA TO A PREBIOTIC: PROTOCOL FOR A RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED, CROSS-OVER, SINGLE-CENTRE STUDY

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The published study protocol is available as Appendix 6-1.

A scanned copy of the statement of contribution to doctoral thesis containing publications is available as Appendix 6-2.

Chapter 6 Study Protocol

6.1 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 29 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.

Trial registration number: ACTRN12615000922572; Pre-results.

6.2 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 entero-pathogenic organisms[1,2], extract nutrients from undigested dietary components[2,3], modulate the immune system[2] and synthesise essential vitamins[4]. It has been hypothesised that an aberrant host–microbe relationship is associated with a number of disease states including obesity, type II diabetes mellitus and inflammatory bowel disease[5–8]. There are a number of factors which can influence the composition and therefore balance of the gut microbiota including diet[9], genetics[10], life stage[11], gender[12], antibiotic use[9,13] and disease[5], with diet being particularly important.

Researchers are 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 (e.g. inulin, oligofructose and fructo-oligosaccharides), have been shown to be effective in eliciting beneficial alterations in gut microbiota composition and function (i.e. 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, Ruminococcus 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 lead to a significant increase in total bacteria, *Lactobacillus/Enterococcus* group, *Bacteroides, C. coccoides–E. rectale* group, *R. bromii + Ruminoccous 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.

6.3 Methods and Analysis

6.3.1 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.

6.3.2 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.

6.3.3 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.

6.3.4 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.

6.3.5 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.

6.3.6 Study setting

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

6.3.7 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/m^2;
- Significant weight loss or weight gain (> 5% of total body weight) within the past year;
- Significant dietary change within the past year (i.e. has become vegetarian, removed gluten from their diet, actively trying to lose weight);
- Pregnant, breast feeding or planning a pregnancy in the next 3 months;
- Food intolerance causing gastrointestinal symptoms (i.e. 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.

6.3.8 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 6-1.

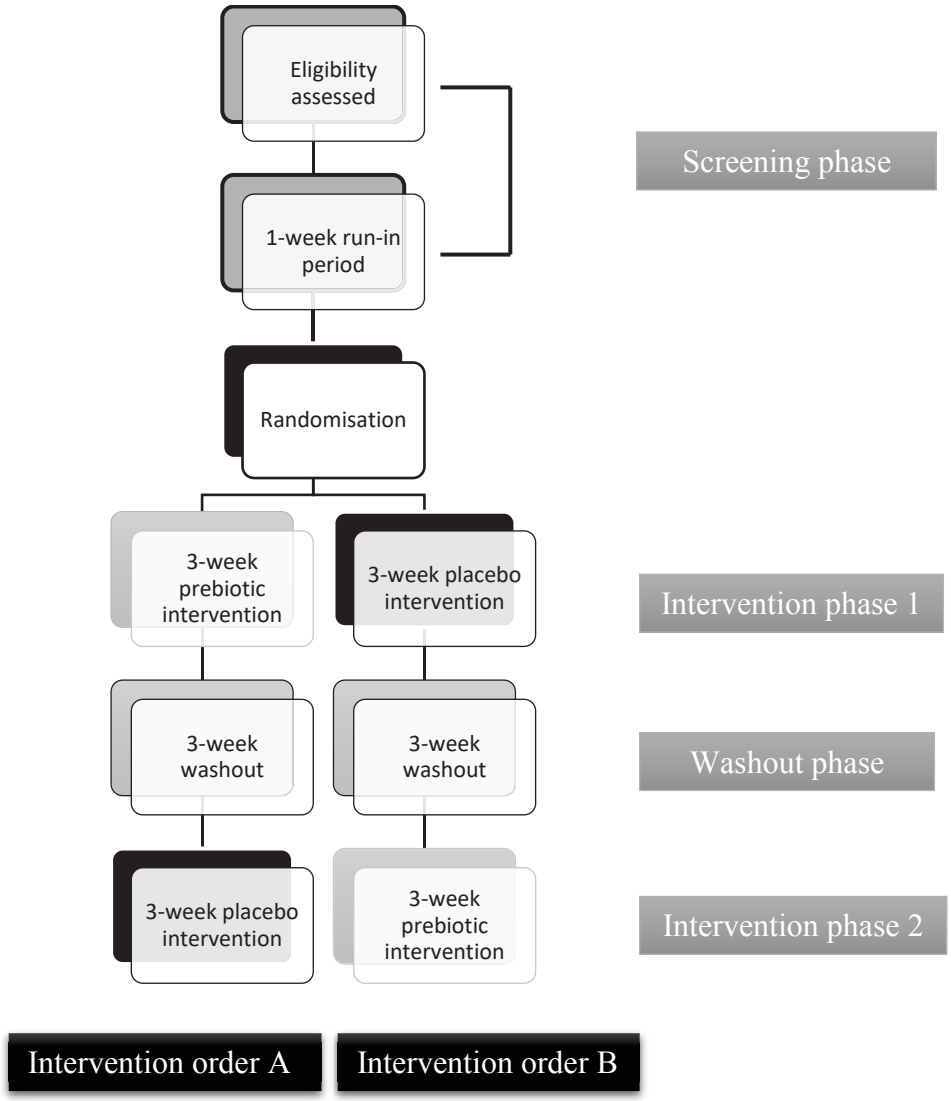

Figure 6-1. Flow diagram summarising the four separate study phases including the two possible intervention orders. The intervention orders may not be as described within the figure as they are blinded to the lead researcher, analysts and participants

6.3.9 Sample size calculations

In order to detect a significant difference in responsiveness of the key phylum and genera (i.e., Actinobacteria, *Ruminococcus*, *Faecalibacterium*, *Bifidobacterium*) 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.

6.3.10 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.

6.3.11 Participant screening

6.3.11.1 Screening questionnaire

Eligibility will initially be assessed using a screening questionnaire. Once participants have provided written 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, on the basis of the screening questionnaire, they will be invited to attend the screening phase visit (first research unit visit).

6.3.11.2 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[37]. 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[38]. The low dietary fibre intake cut-offs were 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[39].

6.3.11.3 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 complete a 3-day diet record and appetite questionnaire, and a fructan intake FFQ (FI-FFQ)[40] by a registered dietitian, as well as materials and 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 (i.e. liver disease, kidney disease, diabetes) will be excluded from the study. The screening visit will take place around 1 week prior to the initiation of IP1 and will provide a short run-in period.

6.3.12 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 prebiotic[15] and that a 3-week washout provides sufficient time for the gut microbiota to revert back to a baseline composition[41]. Participants will be asked to continue their usual food intake and physical activities throughout the duration of the study.

6.3.12.1 Randomisation and intervention concealment

Participants will be randomly allocated one of two intervention orders: intervention order A or B (Figure 6-1). The intervention order will be randomised using a computer-based pre-generated 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. Un-blinding 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.

6.3.12.2 Start of 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.

6.3.12.3 End of 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.

6.3.12.4 Start of 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.

6.3.12.5 End of 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 6-2 provides an illustration of the participant flow through the study including the measurements, questionnaires and samples that will be obtained.

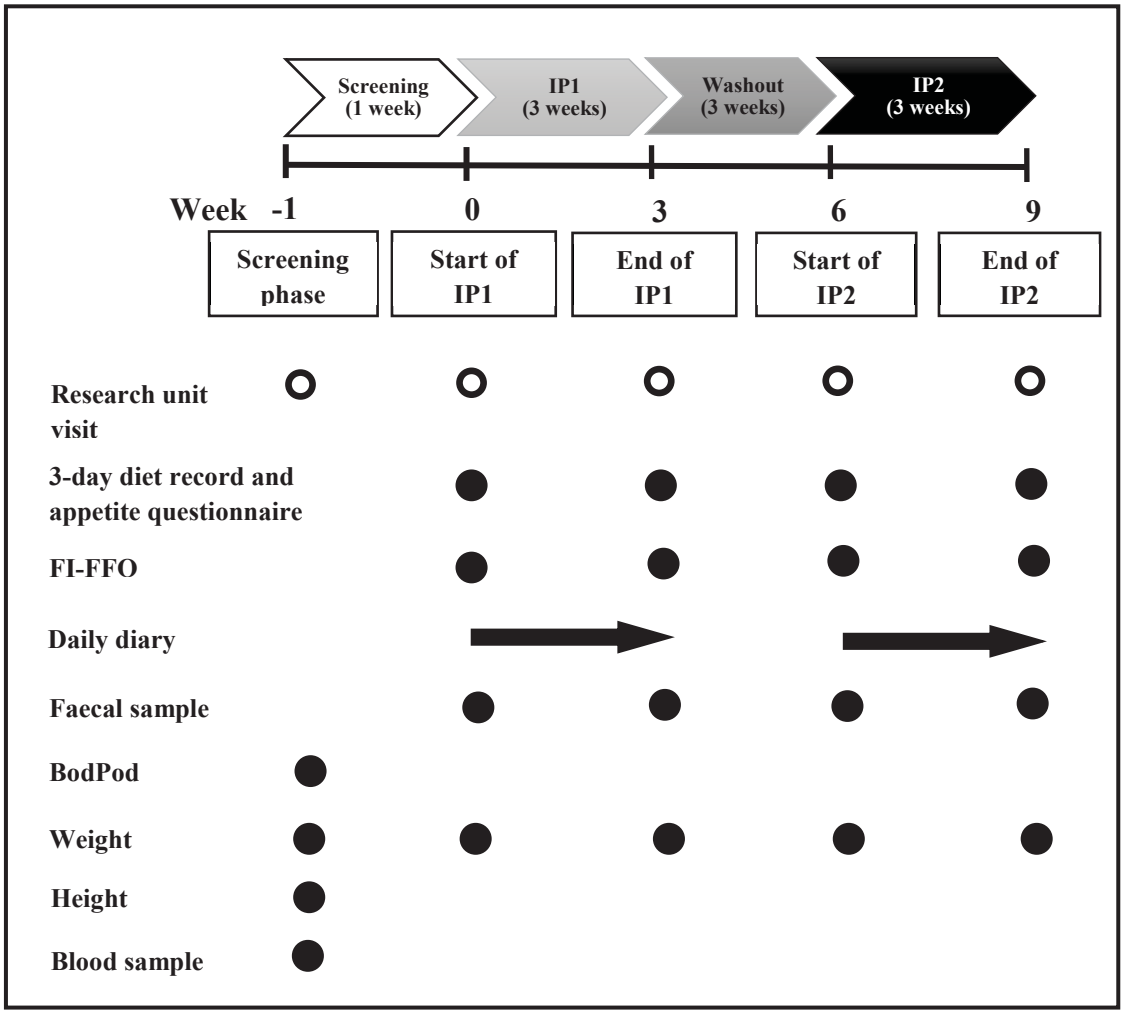

Figure 6-2. Participant flow through the study including measurements, questionnaires and samples taken at each Human Nutrition Research Unit visit. BodPod: air displacement plethysmography, FI-FFQ: fructan intake food frequency questionnaire, IP: intervention phase

6.3.12.6 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.

6.3.12.7 Adverse symptom monitoring

High intakes of inulin and fructo-oligosaccharides have been associated with mild gastrointestinal symptoms including flatulence, diarrhoea, borborygmi and bloating[23,42]. 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).

6.3.13 Outcome measures

6.3.13.1 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[43].

6.3.13.2 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 IP1 (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[44] will be used in conjunction with weight and dietary intake (assessed using the 3-day diet records) information.

6.3.14 Statistical analysis

Bacterial relative abundance and relative abundance of bacterial gene function differences (at baseline and between groups post prebiotic 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.

6.4 Ethics and Dissemination

6.4.1 Research ethics approval

A human ethics application was submitted. Ethics approval was granted by Massey University Human Ethics Committee in July 2015 (Massey University HEC: Southern A, Application 15/34).

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.

6.4.2 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.

6.5 Discussion

Habitual dietary intake plays a significant role in shaping the community of microbes which reside in the gastrointestinal tract[45]; 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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CHAPTER SEVEN

HUMAN INTERVENTION STUDY

HABITUAL DIETARY FIBRE INTAKE INFLUENCES GUT MICROBIOTA RESPONSE TO AN INULIN-TYPE FRUCTAN PREBIOTIC: A RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED, CROSS-OVER, HUMAN INTERVENTION STUDY

This chapter, in part, has been submitted for publication and is under review with the British Journal of Nutrition.

The human intervention study chapter presented in this thesis has been altered from the manuscript under review to include the paperwork provided to participants (Appendix 7-1 to 7-9) and to add additional results relating to PICRUSt analysis (within section 7.4.6) and bifidogenic response data analysis (section 7.4.8).

A copy of the poster, relating to the key results of this study, presented at the Gut Microbiota for Health World Summit conference in Paris, France in March 2017 is available as Appendix 7-20.

Chapter 7 Human intervention study

7.1 Abstract

Dysbiotic gut microbiota have been implicated in human disease. Diet-based therapeutic strategies have been utilised to manipulate the gut microbiota towards a more favourable profile. However, it has been demonstrated that large inter-individual variability exists in gut microbiota response to a dietary intervention. The primary objective of this study was to investigate whether habitual low (LDF) *versus* high dietary fibre (HDF) intakes influence gut microbiota response to an inulin-type fructan prebiotic. In this randomised, double-blind, placebo-controlled, cross-over study, 34 healthy participants were classified as LDF or HDF consumers. Gut microbiota composition (16S rRNA bacterial gene sequencing) and short-chain fatty acid concentrations (gas chromatography) were assessed following 3 weeks of daily prebiotic supplementation (Beneo Orafti® Synergy 1; 16 g/d) or placebo (Roquette Glucidex® 29 Premium; 16 g/d) as well as after 3 weeks of the alternative intervention, following a 3-week washout period. In the LDF group, the prebiotic intervention only led to an increase in *Bifidobacterium* ($p = 0.001$). In the HDF group, the prebiotic intervention led to an increase in *Bifidobacterium* ($p < 0.001$) and *Faecalibacterium* ($p = 0.010$) and decreases in *Coprococcus* ($p = 0.010$), *Dorea* ($p = 0.043$) and *Ruminococcus* (*Lachnospiraceae* family) ($p = 0.032$). This study demonstrates that those with HDF intakes have a greater gut microbiota response and are therefore more likely to benefit from an inulin-type fructan prebiotic than those with LDF intakes. Future studies aiming to modulate the gut microbiota using an inulin-type fructan prebiotic should take habitual dietary fibre intake into account.

7.2 Introduction

The commensal microbes that reside within the gastrointestinal tract are implicated in human health and disease. Host genetics[1], life stage[2], geographical location[2], gender[3] and antibiotic use[4] influence gut microbiota composition; however, diet plays a major role in modulating the community of microbes that reside within the gut[5]. Dietary interventions provide an opportunity to manipulate the commensal bacteria towards a more favourable profile to help enhance human health.

Numerous studies have demonstrated that dietary interventions can elicit significant changes in gut microbiota composition and short-chain fatty acid (SCFA) production. In a recent study, a short-term plant-based diet high in grains, legumes, fruits and vegetables or an animal-based diet composed of meat, eggs and cheese led to distinct shifts in bacterial relative abundance. Interestingly, the animal-based diet had a larger effect on the gut microbiota than the plant-based diet[6]. Another study demonstrated that a three-week intervention containing high levels of wholegrains or red meat altered numerous bacterial taxa, including *Collinsella aerofaciens* and certain *Clostridium sp.*, and led to an increase in microbial diversity during the high wholegrain dietary phase[7]. Dietary intake in economically-developed countries is characterised by intakes of dietary fibre well below recommendations, thus depriving the gut microbiota of valuable fermentable substrates[8,9]. One method of enriching the diet to positively modulate the gut microbiota is to supplement it with prebiotics. A prebiotics is "a substrate that is selectively utilized by host microorganisms conferring a health benefit"[10].

It is becoming increasingly evident that there is profound inter-individual variability in gut microbiota response to dietary interventions. Preliminary research has suggested that factors such as microbial diversity, baseline bifidobacteria concentrations and habitual diet are implicated in gut microbiota responsiveness. A study undertaken by

Tap and co-authors[11] demonstrated that a short-term alteration in dietary fibre intake in 19 healthy adults led to differing microbial responses among participants. Participants with higher baseline microbial richness had gut microbiota that were more resilient to change and, therefore, less responsive to the change in dietary fibre intake. Several studies have also established a link between baseline bifidobacteria concentrations and change in bifidobacteria in response to a dietary intervention[12–16]. Increases in bifidobacteria concentrations are more pronounced in individuals with lower baseline bifidobacteria compared to individuals with higher baseline bifidobacteria concentrations. Preliminary research has shown that habitual diet may also influence gut microbiota responsiveness[17,18]. A 21-day palm date intervention did not influence the numbers of select bacterial taxa, however, secondary analysis demonstrated that those with HDF intakes hosted microbiota that were more stable in response to the palm date intervention than those with LDF intakes[17].

To date, no human studies have been conducted with the primary aim of determining whether habitual dietary intake influences gut microbiota responsiveness to a dietary intervention. Therefore, we aimed to investigate the influence of differing habitual dietary fibre intakes on the responsiveness of the gut microbiota to an inulin-type fructan prebiotic.

7.3 Methods

This randomised, double-blind, placebo-controlled, cross-over, human intervention study was conducted at Massey University, Palmerston North, New Zealand between March and August 2016. The Massey University Human Ethics Committee approved the study (Massey University HEC: Southern A application- 15/34). The study is registered in the Australian New Zealand Clinical trials registry (ACTRN12615000922572). The study protocol has previously been published[19].

7.3.1 Participants

Participants were recruited through email and poster advertisement around Palmerston North, New Zealand (Appendix 7-1). A total of forty-four eligible participants provided written informed consent (Appendix 7-2 & 7-3) to participate in this human intervention study (Figure 7-1). Participants completed a screening questionnaire (Appendix 7-4) to ensure they met the following inclusion criteria: aged between 19 and 65 years; BMI between 18.5 and 30 kg/m^2; healthy (self-reported and confirmed by a health screening blood test [liver and kidney function, blood glucose levels, electrolytes, complete blood count, calcium and C reactive protein] using standard clinical cut-offs). Exclusion criteria included: a significant change in weight (± 5% of total body weight) or dietary intake over the past year; taken antibiotics within the past 6 months; consumption of supplementary prebiotics or probiotic containing foods, drinks and supplements within the past 1 month; pregnancy or breastfeeding (or plans for a pregnancy within the following 3 months); food intolerances associated with gastrointestinal upset; current smoker and high alcohol consumer (> 15 standards drinks per week for males or > 10 standard drinks per week for females, and less than 2 days per week alcohol free).

Participants were also selected based on their habitual dietary fibre intakes. All eligible participants completed a validated habitual dietary fibre intake food frequency

questionnaire (DF-FFQ)[20] during the screening phase of the study to determine whether they were low, moderate or high dietary fibre consumers (Appendix 7-4). Only participants categorised with low (<18 g/d for females and <22 g/d for males) or high (≥25 g/d for females and ≥30 g/d for males) dietary fibre intakes were invited to participate in the study. The HDF categories were chosen to reflect the New Zealand recommended dietary fibre intake which is >25 g/d for females and >30 g/d for males[21]. The LDF categories were chosen as the average dietary fibre intake in New Zealand is 17.5 g/d for females and 22.1 g/d for males which is well below the recommended dietary fibre intakes[22]. To ensure that categorisation into low and high dietary fibre groups was as accurate as possible, once recruited, participants completed four 3-day diet records. If the average dietary fibre intake from these records was outside of the pre-defined categories described above, the participants' data were excluded from the analysis.

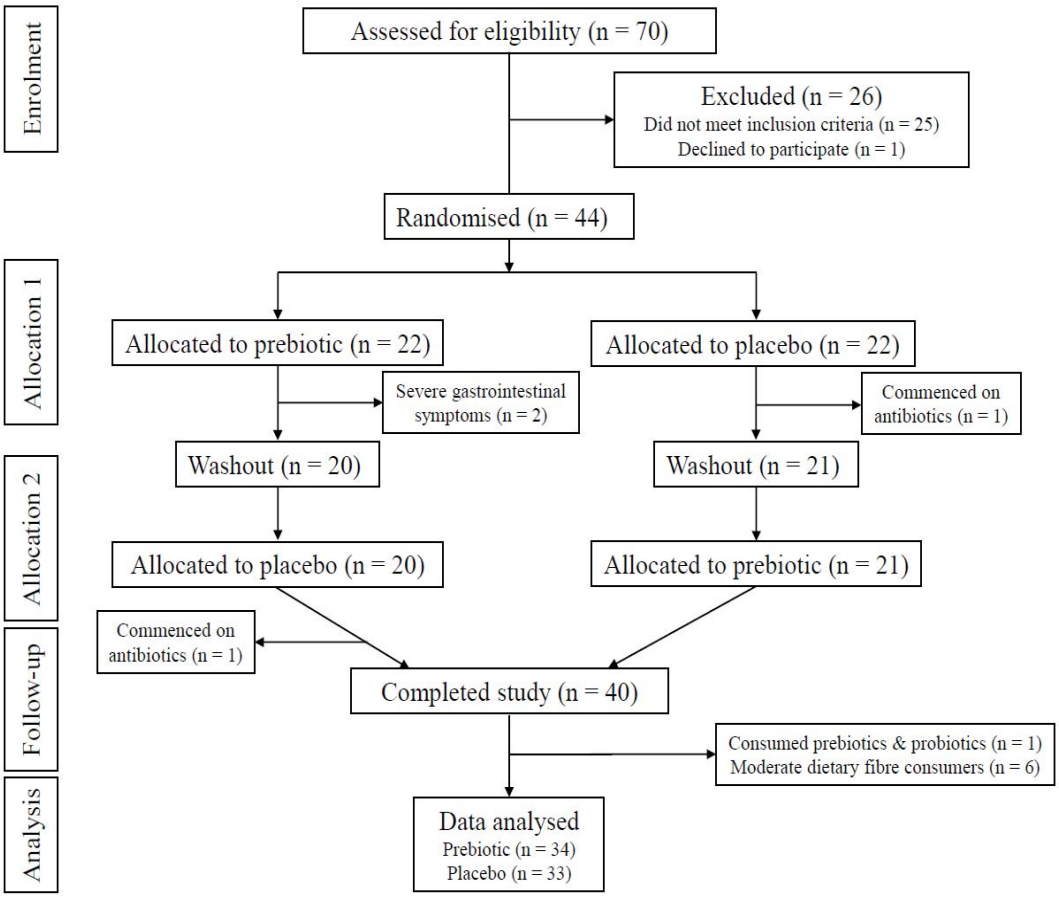

Figure 7-1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram

7.3.2 Interventions

The two interventions were either 16 g/d of powdered inulin-type fructan prebiotic (Beneo Orafti® Synergy1- 50:50 inulin to fructo-oligosaccharide mix) as two 8 g/d doses for three weeks or 16 g/d of powdered placebo (Roquette Glucidex® 29 Premium-digestible maltodextrin) as two 8 g/d doses for three weeks. The two doses were consumed 30 min before breakfast and 30 min before dinner mixed into hot or cold beverages that the participants regularly consumed. A washout period of three weeks was undertaken between the two intervention phases (Figure 7-1). Both interventions were presented in identical packaging and the powders were similar in taste and appearance and were both low in calories (prebiotic 34 kcal/d; placebo 62 kcal/d). Participants were advised not to change their habitual dietary intake or physical activity levels, or take supplementary prebiotics or probiotic containing foods, drinks or supplements for the duration of the study.

7.3.3 Study design

Participants attended an initial screening visit to the research unit where a fasted health screening blood sample was taken. Body composition was assessed using air displacement plethysmography (BodPod®; participants were fasted and wore skin tight clothing) and weight and height measurements were taken (Figure 7-2). Eligible participants were then randomised to one of two intervention orders (i.e. prebiotic then placebo or placebo then prebiotic) (Figure 7-1). The intervention order was randomised using a computer-based pre-generated intervention order. The researcher involved in participant recruitment and data collection, and the participants were blinded to the intervention order. Participants completed a participant questionnaire (Appendix 7-5) at the beginning of the study. They also completed a 3-day diet record & appetite

questionnaire (Appendix 7-6), fructan food frequency questionnaire (Fructan-FFQ) (Appendix 7-7), and had a weight measurement taken at the beginning and end of each intervention phase. A daily diary was completed by each participant during both intervention phases to assess compliance to the intervention, stool frequency and gastrointestinal symptoms (Appendix 7-8). A fresh faecal sample was voided at the beginning and end of each intervention phase into a sterile container, immediately sealed in an anaerobic bag containing an anaerobic sachet and stored at -20ºC until processing (Appendix 7-9).

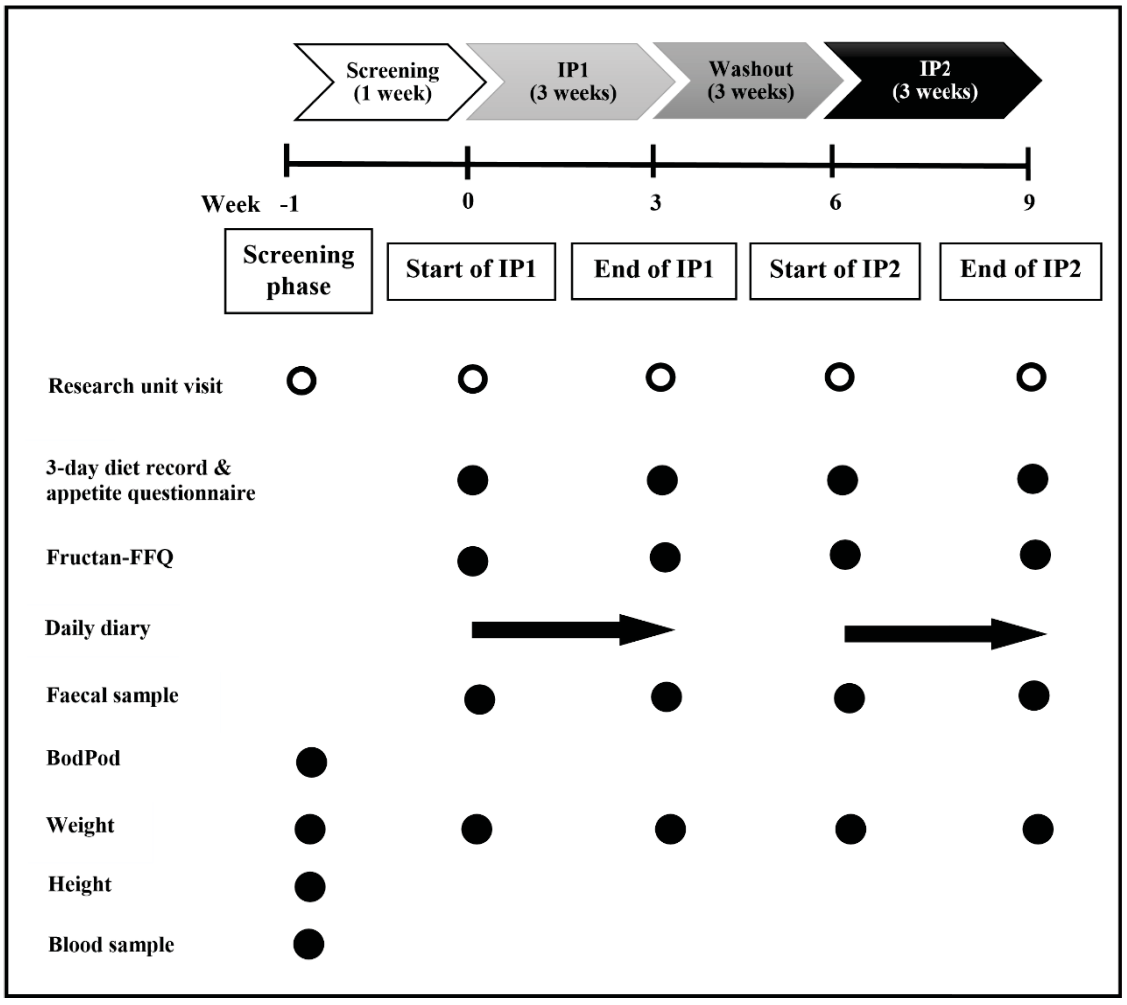

Figure 7-2. Participant flow through the study including measurements, questionnaires and samples taken at each research unit visit. IP: intervention phase, Fructan-FFQ: fructan food frequency questionnaire, BodPod: air displacement plethysmography

7.3.4 Dietary intake analysis

Nutrient intake and food group serves were evaluated using four 3-day diet records. The 3-day diet records were completed on the three days leading up to the start of each intervention phase and the last three days of each intervention phase. The information collected in the 3-day diet records was entered into FoodWorks version 8.0 (Xyris Software Pty Ltd) by a registered Dietitian. The Australian database in FoodWorks was used (AusBrands and AusFoods 2015 data sources) so nutrient intake and food group analysis could be conducted.

Due to the absence of data regarding inulin-type fructan composition in dietary analysis software, fructan intake from diet was evaluated using a validated Fructan-FFQ[23]. Four Fructan-FFQs were completed during the study, one at the beginning and one at the end of each intervention phase. The Fructan-FFQ comprised twenty-three food and drink items contributing to inulin and oligofructose intake. For each food or drink item, participants indicated the usual portion size (i.e. small, medium or large) and the number of portions consumed in the previous 7 days. Inulin and oligofructose consumed from food commodities (i.e. onion or garlic), were determined using published food composition data[24], whereas for composite foods (i.e. noodles and biscuits) the inulin and oligofructose intakes were determined first by calculating the food commodity content (i.e. wheat) of the composite food using the Food Commodity Intake Database (US Department of Agriculture and the US Environmental Protection Agency, USA) and then calculating the inulin and oligofructose amounts of each food commodity present in each composite food item, as previously performed[25]. Portion sizes were estimated using standard portion size information[26].

7.3.5 Appetite rating analysis

Appetite rating was evaluated using an anchored 100 mm visual analogue scale[27]. Hunger, fullness, satisfaction and how much can be consumed were assessed. Participants were instructed to mark with a cross at the point on the scale where they felt the cross best represented their appetite at the time the questionnaire was completed. Appetite ratings were assessed 30 min before and 30 min after main meals (breakfast, lunch and dinner) on the three days leading up to the start of each intervention phase and the last three days of each intervention phase.

7.3.6 Bacterial DNA extraction

Faecal microbiota were measured using 16S rRNA bacterial gene sequencing and bifidobacteria concentrations were analysed using quantitative polymerase chain reaction (PCR). Bacterial DNA was extracted from the faecal samples using the MoBio PowerLyzer® Powersoil DNA® isolation kit according to the manufacturer's instructions with minor alterations. Faecal subsamples were taken from the outer region of the sample only (to reduce variability) and weighed (0.25 ± 0.025 g) into PowerLyzer® glass bead tubes. A FastPrep-24™ 5G (MP Biomedicals) was used to homogenise the samples at a speed of 5.5 m/sec for four 90 sec cycles with a 60 sec break between each cycle. The DNA was eluted in 10 mM Tris. NanoDrop 1000 spectrophotometry was used to quantify the DNA concentration.

7.3.7 16S rRNA bacterial gene sequencing

The extracted bacterial DNA was used as a template for initial PCR amplification of the V3-V4 hyper-variable region of the 16S rRNA bacterial gene using the barcoded fusion primers:

16SR_V4- (5′-CAAGCAGAAGACGGCATACGAGAT-barcode-AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT-3′)

16SF_V3- (5′-AATGATACGGCGACCACCGAGATCTACAC-barcode-TATGGTAATTGGCCTACGGGAGGCAGCAG -3′).

These primers also contain adaptors for downstream Illumina MiSeq sequencing. Each sample was amplified with a pair of unique (8 base) barcoded primers. The PCR reagents used were Invitrogen AccuPrime™ Pfx SuperMix (part number 12344-040) (17 μL), 10 μM 16SR_V4 Primer (1 μL), 10 μM 16SF_V3 Primer (1 μL) and Ambion nuclease-free water (catalog number: AM9932) to normalise to 5 ng/μL (1 μL). The following PCR conditions were used; a hold at 95 °C for 2 min followed by 30 cycles of 95 °C for 20 sec (denaturation), 55 °C for 15 sec (annealing), 72 °C for 5 min (extension) finishing with a hold at 72 °C for 10 min. Library clean-up utilised an Invitrogen SequalPrep Normalisation Plate Kit (Thermo Fisher). Eighteen μL of the PCR product was used in the library clean-up with an elution volume of 12 μL. A Qubit DNA high sensitivity assay was used to measure the library concentration and a Bioanalyzer DNA HS assay was used for library sizing. The libraries were pooled by equal volume. Sequencing was undertaken on an Illumina MiSeq machine, using 2 x 250 base pair (bp) read length, at the Massey Genome Service (Massey University, Palmerston North, New Zealand).

The data obtained from Illumina MiSeq sequencing were analysed using Quantitative Insights Into Microbial Ecology (QIIME)[28]. Paired-end assembler for DNA sequencing (PANDAseq) was used to assemble the forward and reverse reads into continuous sequences ensuring at least a 50 bp overlap with a minimum of 350 bp and a maximum of 500 bp length[29]. Chimera filtered sequences and reads were clustered into operational taxonomic units (OTUs) based on an identity threshold value of 97% using USEARCH 6.1 and UCLUST[30]. Sequence alignment with the Greengenes core reference

database (version 13_5) was carried out using PyNAST[31]. The RDP Naïve Bayesian classifier was used to provide taxonomic assignment[32].

Phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt)[33] was performed using the 16S rRNA bacterial gene data to predict the functional capacity of the gut microbiota. PICRUSt analysis was undertaken within the Galaxy web application (https://huttenhower.sph.harvard.edu/galaxy/). The OTU table generated in QIIME was normalised by copy number and metagenomes were predicted using the KEGG catalogue (at level 3)[34].

7.3.8 Quantitative PCR

Previous research has demonstrated that baseline bifidobacteria concentrations influence bifidobacteria response to certain dietary interventions[12–16]. Therefore, bifidobacteria concentrations were determined using the LightCycler® 480 system (Roche Life Science). Standard template DNA was prepared using *Bifidobacterium bifidium* (DSM20082). *Bifidobacterium bifidum* was grown in MRS (De Man, Rogosa, Sharpe) broth (Oxoid, Adelaide, Australia) + 0.05% cysteine at 37 °C for 2 days under anaerobic conditions. The culture was counted using a haemocytometer and adjusted to a final concentration of 1×10^9 cells/mL. Bacterial DNA was extracted using the MoBio PowerLyzer® Powersoil DNA® isolation kit as described above. The following primers were used: forward (GGGTGGTAATGCCGGATG) and reverse (CCACCGTTACACCGGGAA) primers[35]. Quantitative PCR was performed in triplicate with 10 µL of SyBr Green Master (Roche Life Science), 1 µL of each of the forward and reverse primers (5 µM), 7 µL of PCR grade water and 1 µL of template DNA. The conditions used for PCR amplification were initial denaturation at 95 °C for 5 min followed by 40 cycles of denaturation (95 °C for 1 min), annealing (66 °C for 45

sec), extension (72 °C for 1 min) and finished with a melt curve (95 °C for 30 sec, 65 °C for 1 min and 95 °C continuous– 5 per °C acquisitions).

7.3.9 Faecal short-chain fatty acid analysis

Short-chain fatty acids were measured by gas chromatography (GC) using a modified method[36]. While still frozen, 0.5 g to 1.0 g of faecal sample was weighed into a 15 mL Eppendorf tube; 0.01 M phosphate buffered saline containing 2-ethylbutyric acid (5.56 mM) as an internal standard was added to the faecal sample to make an aqueous faecal solution (dilution factor of 10) containing 5 mM 2-ethylbutyric acid. The samples were kept on ice and mixed to disperse faecal matter. Aqueous faecal solutions were centrifuged at 3000 x g for 10 min (4 °C), 500 µL of the supernatant was transferred to a 2 mL Eppendorf tube and was acidified with 250 µL concentrated hydrochloric acid and 1000 µL diethyl ether added. Following a 10 sec vortex, to allow acids to transfer to the diethyl ether phase, the samples were centrifuged at 10,000 x g for 5 min (4 °C). In a capped GC vial 100 µL of the diethyl ether phase was derivatised with 20 µL N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide with 1% tert-butyldimethylchlorosilane (MTBSTFA + TBDMSCI, 99:1; Sigma-Aldrich) in a water bath at 80 °C for 20 min. Once cooled, the derivatised sample was transferred to a 200 µL vial insert and recapped. To ensure complete derivatisation, the samples were left for 48 h at room temperature before analysis using GC. Standards containing 2-ethylbutyric acid (5 mM) as an internal standard were prepared for derivatisation alongside the samples.

Analysis was performed on a Shimadzu capillary gas chromatograph system (GC-2010 Plus, Tokyo, Japan) equipped with a flame ionisation detector and fitted with a Restek column (SH-Rtx-1, 30 m × 0.25 mm ID × 0.25 µm) (Shimadzu, USA). The carrier gas was helium with a total flow rate of 21.2 mL/min and pressure of 131.2 kPa. Make-up gas was nitrogen. The temperature program began at 70 °C increasing to

115 °C at 6 °C/min, with a final increase to 300 °C at 60 °C/min, holding for 3 min. Flow control mode was set to linear velocity; 37.5 cm/sec. Injector temperature was 260 °C and detector temperature was 310 °C. Samples were injected (1 µL) with a split injection (split ratio 10:1). The GC instrument was controlled and data processed using Shimadzu GC Work Station LabSolutions Version 5.3. Data acquired provided a final sample result of µmol SCFA/g wet faeces.

7.3.10 Sample size calculations

In order to detect a significant difference in responsiveness of the key phylum and genera (i.e. *Actinobacteria, Lactobacillus, Faecalibacterium, Bifidobacterium)* to the prebiotic intervention (difference of 3% in bacterial composition with a variance of 9% between and within individuals) between the LDF and HDF groups (with a power of 80% and significance of 5%) thirty-four participants were required[37]. To allow for participant withdrawal we aimed to recruit approximately forty participants.

7.3.11 Statistical analysis

Mann-Whitney tests were used to determine whether there were significant differences in baseline (start of intervention phase 1) bacterial taxa, SCFA concentrations and dietary intakes (fructan intakes, nutrient intakes and food group serves) between the LDF and HDF groups, and the bifidogenic responders and non-responders. One-way repeated measures analysis of variance (ANOVA) was used to determine whether nutrient intakes changed throughout the duration of the study in the whole cohort. Differences in participant characteristics between the LDF and HDF groups, and bifidogenic responders and non-responders were assessed using t-tests and Chi-squared tests. McNemar's tests were used to determine whether there were any differences in gastrointestinal symptom frequency during the placebo and prebiotic intervention phases in the whole cohort and the LDF and HDF groups. Differences in bacterial taxa and SCFA concentrations

between the start of intervention phase 1 (baseline) and start of intervention phase 2 (after the washout period) where determine using a Mann-Whitney test. Mann-Whitney test was also used to determine whether the bacterial taxa or SCFA concentrations changed during the placebo intervention phase. Two-way repeated measures ANOVA, blocked by participant, were used to determine whether there were differences in appetite ratings, SCFA concentrations and bacterial taxa during the prebiotic and placebo intervention phases in the whole cohort and the LDF and HDF groups. Two-way repeated measures ANOVA, blocked by participant, was also used to determine whether there were differences in prebiotic driven gut microbiota response between the LDF and HDF groups. Bacterial taxa with skewed data were log transformed to help normalise the data. Spearman's rank correlation test was used to analyse the correlation between baseline (start of intervention phase 1) bifidobacteria concentrations and change in bifidobacteria concentrations after the prebiotic intervention for the LDF and HDF groups, and the bifidogenic responders and non-responders. Two-way repeated measures ANOVA, blocked by participant, was used to determine whether there were differences in predicted functional capacity, using PICRUSt, between the low and high dietary fibre groups during the prebiotic intervention phase. Statistical analysis was carried out using Genstat version 17.1.0.14713. QIIME[28] was used to conducted the statistical analysis (non-parametric two-sample t-test) to compare baseline (start of intervention phase 1) alpha diversity between the dietary fibre groups and the change in alpha diversity in the LDF and HDF groups after the prebiotic intervention.

7.4 Results

7.4.1 Participants

Of the forty-four eligible participants who provided informed consent to participate in the study, four did not complete the study as they either experienced severe gastrointestinal symptoms (i.e. disabling abdominal pain, cramps and bloating) due to the prebiotic (n = 2) or were commenced on antibiotics at the beginning of the study (n = 1). One participant was also commenced on antibiotics at the end of the study (during the placebo intervention phase), however, the data collected during the prebiotic intervention phase was still able to be used. Forty participants completed the study, however, the data from seven participants were excluded as the participants were either assessed as being moderate dietary fibre consumers, based on the data collected from the four 3-day diet records (n = 6), or were found to have consumed supplementary prebiotics and probiotic containing foods and drinks during the study (n = 1). The data collected from thirty-four participants were used for the prebiotic intervention analysis and the data collected from thirty-three participants were used for the placebo intervention analysis (Figure 7-1).

7.4.2 Baseline dietary intake and participant characteristic differences

Categorisation into different dietary fibre intake groups was successful as dietary fibre intakes were significantly different ($p < 0.001$) between the LDF (n = 14; 18.0 g/d) and HDF (n = 20; 38.6 g/d) groups. There were several additional significant differences ($p < 0.05$) in baseline nutrient intakes between the LDF and HDF groups. HDF consumers had higher energy, total fat, polyunsaturated fat, monounsaturated fat, carbohydrate and dietary fibre per 1000 kJ compared to the LDF group. There were, however, no differences in fructan intake between the two groups (Table 7-1). Energy from fat (%) and energy from protein (%) were not significantly different, however, energy from fibre

(%) was significantly different between dietary fibre groups ($p < 0.001$). Therefore, the only macronutrient that continued to be significantly different between dietary fibre groups after energy intakes were controlled for was dietary fibre (Table 1). The LDF group had a lower intake of fruits ($p = 0.009$), vegetables ($p < 0.001$) (dark green [$p = 0.007$] and red orange vegetables [$p = 0.039$]), protein foods ($p = 0.036$) and nuts and seeds ($p = 0.001$) compared to the HDF group (Figure 7-3). Dietary intakes did not change throughout the duration of the study (Appendix 7-10). Despite similar age, sex and BMI, there were significant differences in body composition between the two dietary fibre groups. The LDF group had a significantly lower fat free mass ($p = 0.021$) and significantly higher fat mass ($p = 0.021$) compared to the HDF group (Table 7-2).

Table 7-1. Baseline dietary intake differences between the low and high dietary fibre groups[1]

	Low dietary fibre (n=14)		High dietary fibre (n=20)		
Dietary intake	**Mean**	**SD**	**Mean**	**SD**	**P value**
Energy (kJ/d)	7161.1	2285.2	10013.9	2769.8	**0.002**
Protein (g/d)	83.1	28.5	112.7	45.5	0.066
Total fat (g/d)	67.8	26.0	95.9	29.7	**0.012**
Saturated fat (g/d)	26.5	13.3	33.3	14.6	0.259
Polyunsaturated fat (g/d)	10.8	4.2	16.3	5.9	**0.005**
Monounsaturated fat (g/d)	24.6	8.9	38.0	10.8	**0.001**
Carbohydrate (g/d)	178.0	83.0	241.3	84.0	**0.015**
Sugars (g/d)	77.8	47.0	106.2	40.4	0.051
Starch (g/d)	99.2	39.5	132.0	59.9	0.104
Dietary fibre (g/d)	18.0	3.4	38.6	13.0	**<0.001**
Dietary fibre (g/d) per 1000kJ	2.7	0.8	3.9	1.0	**<0.001**
Total inulin (g/d)	3.1	1.3	2.9	1.1	0.796
Total oligofructose (g/d)	3.0	1.2	2.8	1.0	0.769
Water (g/d)	2048.2	746.3	2781.4	1428.1	0.104
Alcohol (g/d)	3.1	6.8	4.2	12.3	0.565
Energy from protein (%)	20.6	8.0	19.1	4.5	0.877
Energy from fat (%)	34.4	5.2	35.9	8.5	0.986
Energy from saturated fat (%)	13.1	3.9	12.3	4.5	0.457
Energy from carbohydrate (%)	40.6	10.4	39.3	7.4	0.823
Energy from alcohol (%)	1.2	2.6	1.0	2.7	0.601
Energy from fibre (%)	2.2	0.7	3.1	0.8	**<0.001**

[1] Mann-Whitney test. Significant results ($p < 0.05$) are in **bold**. SD: standard deviation

Table 7-2. Participant characteristic comparison between the low and high dietary fibre groups[1]

	Low dietary fibre (n=14)	High dietary fibre (n=20)	P value
Age (years)	37.7 ± 10.6	37.2 ± 14.4	0.902
BMI (kg/m^2)	24.3 ± 2.7	22.5 ± 2.8	0.061
Male : Female	6 : 8	7 : 13	0.643
Fat mass (%)	27.5 ± 7.5	20.3 ± 9.0	**0.021**
Fat free mass (%)	72.6 ± 7.5	79.7 ± 9.0	**0.021**
Ethnicity (no.)			0.938
NZ European	6	9	
Maori	1	2	
Other	7	9	
Skip meals (Yes : No)	6 : 8	5 : 15	0.273
Snack consumed per day (no.)	2.0 ± 0.9	2.4 ± 0.6	0.091
Activity level^	5.1 ± 0.9	5.6 ± 1.2	0.183
Stools passed per week (no.)	6.7 ± 3.6	8.6 ± 3.8	0.155

[1] Chi-squared test and unpaired t-test. Significant results ($p < 0.05$) are in **bold**. Values are means ± standard deviations. ^ Activity level of 5 = Seated work with some moving around and strenuous leisure activity

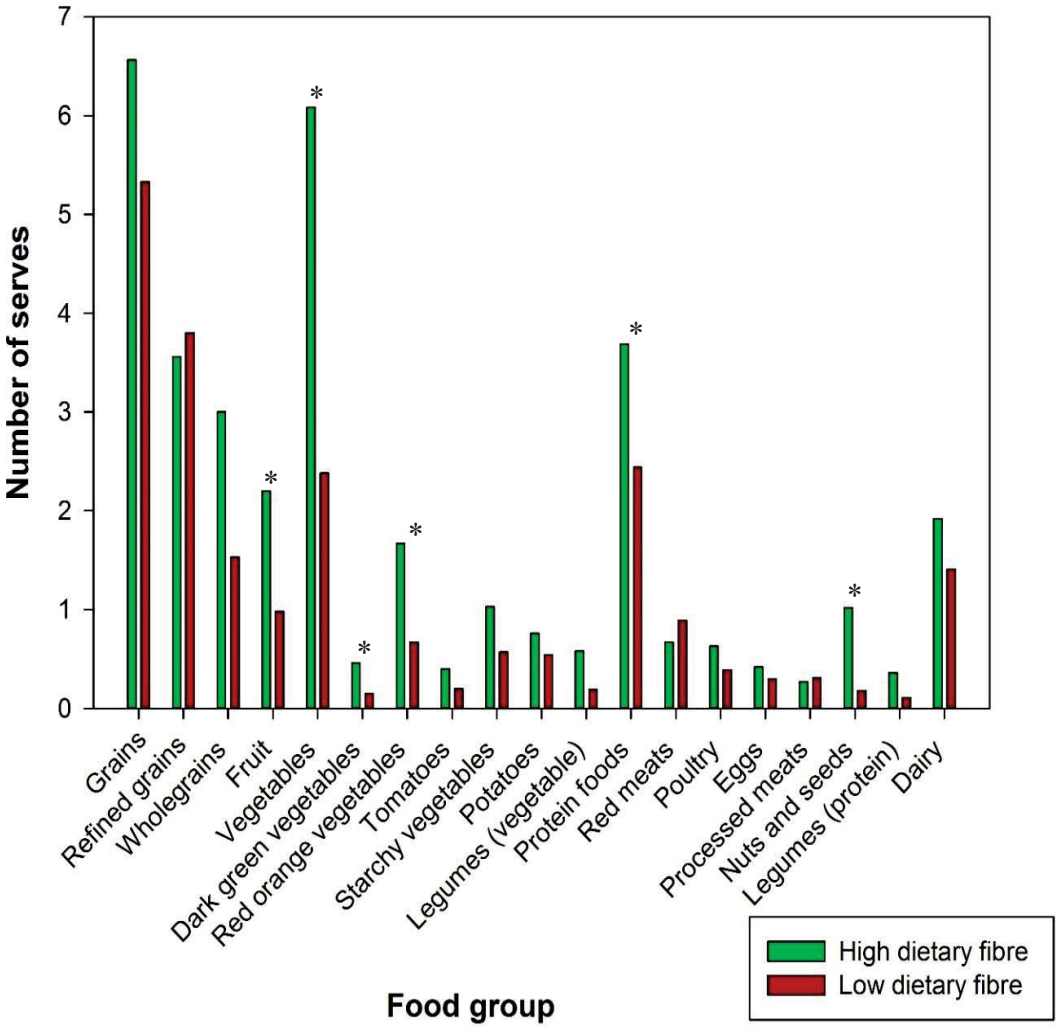

Figure 7-3. Baseline differences in the average number of food group serves consumed per day (as assessed using four 3-day diet records) between the low and high dietary fibre groups. Changes that are significantly different ($p < 0.05$) between dietary fibre groups are indicated with an asterisks (*) as analysed by a Mann-Whitney test.

7.4.3 Baseline SCFA concentration and gut microbiota differences

From the 138 faecal samples analysed, a total of 12,420,607 high quality 16S rRNA bacterial gene sequence reads were generated. The average number of sequence reads generated per faecal sample was 90,004 (33,906 to 196,843 reads per sample).

There were no significant baseline differences in SCFA concentrations (Table 7-3) or any of the alpha diversity indices measured (Observed species, Shannon, Chao and PD_whole tree) (Appendix 7-11) between the LDF and HDF groups. At baseline the relative abundance of an unknown genus of *Lachnospiraceae* (other) was significantly higher in the LDF group compared to the HDF group (2.38% *versus* 1.50% [p = 0.043]). The LDF group also had a trend towards a higher relative abundance of *Bifidobacterium* compared to the HDF group (9.81% *versus* 4.51%); however, statistical significance was not reached (p = 0.066) (Table 7-3).

Table 7-3. Baseline differences in short-chain fatty acid concentrations and bacterial taxa between low and high dietary fibre groups[1]

	Low dietary fibre (n=14)		High dietary fibre (n=20)		
	Mean	**SD**	**Mean**	**SD**	**P value**
Short-chain fatty acids (µmol/g)					
Acetate	28.97	18.23	33.32	19.74	0.592
Butyrate	7.77	5.10	9.08	5.80	0.545
Propionate	9.99	6.31	10.05	8.90	0.666
Sum of short-chain fatty acids	51.02	29.00	56.57	33.12	0.877
Phylum (% relative abundance)					
Actinobacteria	13.98	9.42	8.87	5.97	0.104
Bacteroidetes	11.31	8.94	16.82	11.44	0.169
Firmicutes	72.82	8.79	72.12	12.15	0.931
Proteobacteria	0.65	0.93	0.47	0.47	0.823
Verrucomicrobia	0.33	0.58	0.26	0.33	0.304
Genus (% relative abundance)					
Bifidobacterium	9.81	7.78	4.51^	4.10	0.066
Collinsella	2.95	3.09	3.15	2.69	0.616
Bacteroides	6.77	5.01	6.81	3.66	0.931
Prevotella	2.94	4.89	6.79	11.03	0.666
Lactobacillus	0.59	1.29	0.03	0.06	0.609
Lachnospiraceae, other, unknown genus	2.38	1.46	1.50*	0.78	0.043
Lachnospiraceae, unknown genus	11.85	7.20	13.04	5.58	0.377
Blautia	10.42	5.57	9.53	4.36	0.569
Coprococcus	3.83	2.13	4.97	2.74	0.204
Dorea	2.01	1.22	1.57	0.71	0.341
Ruminococcus (Lachnospiraceae)	2.55	1.99	1.81	1.20	0.306
Ruminococcaceae, unknown genus	15.95	5.08	14.74	3.12	0.569
Faecalibacterium	0.39	0.29	0.42	0.18	0.500
Oscillospira	1.21	0.65	1.00	0.42	0.478
Ruminococcus (Ruminococcaceae)	5.80	4.26	5.27	3.47	0.849
Dialister	1.00	1.65	1.12	1.81	0.568

[1] Mann-Whitney test. Mean values are significantly different from the low dietary fibre group. *p < 0.05, ^trend towards significance (p < 0.1). SD: standard deviation

7.4.4 Gastrointestinal symptoms

In the whole cohort, the frequency of mild and severe gastrointestinal symptoms were statistically similar ($p > 0.05$) during the placebo and prebiotic intervention phases. There was, however, a significantly higher frequency of moderate symptoms (42% *versus* 12% [$p = 0.013$]), particularly moderate flatulence (30% *versus* 3% [$p = 0.012$]), experienced during the prebiotic compared to the placebo intervention phase (Appendix 7-12).

After categorisation into dietary fibre intake groups, the HDF group also experienced an increased frequency of moderate symptoms (58% *versus* 11% [$p = 0.004$]), particularly moderate flatulence (37% versus 0% [$p = 0.016$]), during the prebiotic compared to the placebo intervention phase. There were no significant differences in gastrointestinal symptom frequency between the placebo and prebiotic intervention phases in the LDF group (Appendix 7-12).

7.4.5 Prebiotic driven changes in appetite ratings

In the whole cohort, there were no significant differences in appetite ratings before or after breakfast, lunch or dinner during the prebiotic intervention phase (Appendix 7-13).

After categorising participants based on their dietary fibre intakes, appetite ratings did not significantly change before or after breakfast, lunch or dinner during the prebiotic intervention phase in the LDF group (Appendix 7-14). There were, however, a number of significant changes in appetite ratings during the prebiotic intervention phase in the HDF group. The prebiotic intervention led to a significant reduction in satisfaction before lunch and in hunger after dinner (36.3 to 28.8 [$p = 0.042$] and 20.3 to 15.8 [$p = 0.006$]; respectively), and a significant increase in fullness and satisfaction after lunch (70.4 to 73.9 [$p = 0.002$] and 69.3 to 74.7 [$p = 0.044$]) (Appendix 7-15).

7.4.6 Prebiotic driven changes in SCFA concentrations and gut microbiota

Gut microbiota composition and SCFA concentrations after the washout period were not significantly different from baseline (Appendix 7-16). There were also no significant changes in gut microbiota composition or SCFA concentrations during the placebo intervention (Appendix 7-17).

Table 7-4. Short-chain fatty acid concentration and bacterial taxa changes during the placebo and prebiotic intervention phases in the whole cohort[1]

	Placebo (n = 33)				Prebiotic (n = 34)			
	Before intervention		After intervention		Before intervention		After intervention	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Short-chain fatty acids (µmol/g)								
Acetate	31.78	17.80	33.80	18.97	31.53	18.97	39.50	20.96
Butyrate	9.75	6.12	9.44	5.62	8.54	5.48	10.16	5.62
Propionate	10.09	6.19	11.63	7.78	10.03	7.83	11.94	7.47
Sum of short-chain fatty acids	55.52	28.69	59.48	32.28	54.28	31.16	65.51	32.48
Phylum (% relative abundance)								
Actinobacteria	10.88	6.43	10.84	7.24	10.98	7.87	19.95**	10.20
Bacteroidetes	14.30	12.09	13.09	8.39	14.55	10.70	12.46	8.33
Firmicutes	72.90	11.31	73.85	11.02	72.41	10.75	65.71**	11.03
Proteobacteria	0.43	0.40	0.51	0.61	0.54	0.69	0.36^	0.42
Verrucomicrobia	0.33	0.66	0.38	0.85	0.29	0.45	0.17	0.33
Genus (% relative abundance)								
Bifidobacterium	6.56	5.21	6.50	5.88	6.69	6.37	15.07**	8.54
Collinsella	3.36	2.52	3.15	2.80	3.07	2.82	3.81	2.79
Bacteroides	6.49	3.81	6.45	4.31	6.80	4.19	5.86	3.40
Prevotella	5.36	12.16	3.66	5.98	5.20	9.12	4.85	7.98
Lactobacillus	0.24	0.92	0.44	1.96	0.26	0.86	1.26	3.83
Lachnospiraceae, other, unknown genus	2.07	1.11	1.91	1.24	1.86	1.18	1.55	0.62
Lachnospiraceae, unknown genus	13.27	5.79	13.43	5.62	12.55	6.22	14.74	6.30
Blautia	10.78	5.81	9.45	4.43	9.90	4.83	7.67	3.88
Coprococcus	3.80	1.83	4.16	2.20	4.50	2.54	3.55*	1.65
Dorea	1.65	0.86	1.61	0.86	1.75	0.96	1.20*	0.66
Ruminococcus (Lachnospiraceae)	1.85	1.66	1.95	1.64	2.11	1.59	1.15**	1.04
Ruminococcaceae, unknown genus	16.33	4.82	16.86	4.29	15.24	4.01	14.50	4.12
Faecalibacterium	0.47	0.32	0.53	0.30	0.41	0.22	0.61^	0.32
Oscillospira	1.10	0.67	1.11	0.70	1.08	0.53	0.78*	0.46
Ruminococcus (Ruminococcaceae)	5.60	3.73	5.52	4.00	5.49	3.76	4.40	3.32
Dialister	0.77	1.15	1.00^	1.56	1.07	1.72	0.94	1.59

[1] Two-way repeated-measures ANOVA (blocked by participant) and least significant difference test. Mean values are significantly different from those of the placebo intervention and prebiotic before intervention or in the case of *Dialister* different from those of the prebiotic intervention and placebo before intervention; *p < 0.05, **p < 0.01, ^trend towards significance (p < 0.1). SD: standard deviation

In the whole cohort, there were no significant changes in SCFA concentrations due to the prebiotic intervention (Table 7-4). There were, however, a number of prebiotic driven changes in bacterial taxa. At a phylum level, Actinobacteria relative abundance significantly increased (10.98% to 19.95% [p < 0.001]) and Firmicutes relative abundance significantly decreased (72.41% to 65.71% [p = 0.007]). There was also a

trend towards a reduction in Proteobacteria relative abundance (0.54% to 0.36% [p = 0.070]) during the prebiotic intervention phase (Table 7-4). At a genus level, there was a prebiotic driven increase in the relative abundance of *Bifidobacterium* (6.69% to 15.07% [p < 0.001]) and a reduction in *Coprococcus* (4.50% to 3.55% [p = 0.016]), *Dorea* (1.75% to 1.20% [p = 0.029]), *Ruminococcus* (*Lachnospiraceae* family) (2.11% to 1.15% [p = 0.007]) and *Oscillospira* relative abundance (1.08% to 0.78% [p = 0.031]). There was also a trend towards an increase in *Faecalibacterium* relative abundance (0.41% to 0.61% [p = 0.088]) during the prebiotic intervention phase (Table 7-4).

Table 7-5. Short-chain fatty acid concentration and bacterial taxa changes during the placebo and prebiotic intervention phases in the low dietary fibre group[1]

	Low dietary fibre							
	Placebo (n = 14)				Prebiotic (n = 14)			
	Before intervention		After intervention		Before intervention		After intervention	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Short-chain fatty acids (µmol/g)								
Acetate	31.46	18.07	33.64	17.90	28.97	18.23	34.26	22.33
Butyrate	10.34	7.16	9.38	5.42	7.77	5.10	8.27	5.28
Propionate	10.02	5.76	12.62	8.30	9.99	6.31	10.88	7.08
Sum of short-chain fatty acids	55.73	28.84	61.43	32.34	51.02	29.00	57.59	33.95
Phylum (% relative abundance)								
Actinobacteria	12.81	6.11	13.16	7.99	13.98	9.42	23.17**	9.59
Bacteroidetes	13.07	8.06	12.84	5.33	11.31	8.94	9.56	5.43
Firmicutes	72.30	7.36	71.59	9.73	72.82	8.79	65.96	9.74
Proteobacteria	0.62	0.53	0.78	0.85	0.65	0.93	0.30^	0.36
Verrucomicrobia	0.41	0.69	0.68	1.18	0.33	0.58	0.20	0.46
Genus (% relative abundance)								
Bifidobacterium	8.40	5.53	8.62	6.83	9.81	7.78	18.01**	7.85
Collinsella	3.51	2.64	3.30	3.13	2.95	3.09	3.94	3.22
Bacteroides	7.98	3.82	7.63	4.38	6.77	5.01	5.73	3.91
Prevotella	3.08	5.50	2.39	3.62	2.94	4.89	2.48	3.62
Lactobacillus	0.56	1.38	1.02	2.98	0.59	1.29	2.97	5.64
Lachnospiraceae, other, unknown genus	2.50	1.31	2.41	1.58	2.38	1.46	1.84	0.64
Lachnospiraceae, unknown genus	12.37	7.68	12.28	5.14	11.85	7.20	13.24	7.36
Blautia	11.72	7.02	9.25	4.33	10.42	5.57	8.10	4.08
Coprococcus	3.43	1.82	3.31	1.88	3.83	2.13	3.07	1.87
Dorea	1.93	1.01	1.60	0.85	2.01	1.22	1.19	0.54
Ruminococcus (Lachnospiraceae)	1.91	2.09	1.94	1.74	2.55	1.99	1.24^	1.31
Ruminococcaceae, unknown genus	15.63	5.19	15.78	4.38	15.95	5.08	13.10	4.84
Faecalibacterium	0.42	0.39	0.51	0.34	0.39	0.29	0.45	0.33
Oscillospira	1.10	0.62	1.25	0.84	1.21	0.65	0.91^	0.47
Ruminococcus (Ruminococcaceae)	5.35	3.28	5.67	4.39	5.80	4.26	5.04	4.01
Dialister	0.86	1.36	0.97	1.71	1.00	1.65	1.09	2.13

[1] Two-way repeated-measures ANOVA (blocked by participant) and least significant difference test. Mean values are significantly different from those of the placebo intervention and prebiotic before intervention; *p < 0.05, **p < 0.01, ^trend towards significance (p < 0.1). SD: standard deviation

After categorisation into dietary fibre intake groups, there were no significant prebiotic driven changes in SCFA concentrations in the LDF (Table 7-5) or HDF groups

(Table 7-6) which was consistent with the whole cohort analysis (Table 7-1). At a phylum level, both dietary fibre groups had a significant increase in Actinobacteria relative abundance (LDF 13.98% to 23.17% [p = 0.007] and HDF 8.87% to 17.70% [p = <0.001]); however, the reduction in Firmicutes relative abundance was only significant in the HDF group (LDF 72.82% to 65.96% [p = 0.127] and HDF 72.12% to 65.54% [p = 0.027]) (Tables 7-5 & 7-6). At a genus level, the only significant change that occurred during the prebiotic intervention in the LDF group was an increase in the relative abundance of *Bifidobacterium* (9.81% to 18.01 [p = 0.001]) (Table 7-5). The prebiotic intervention did, however, lead to a number of significant changes in the HDF group including a significant increase in *Bifidobacterium* (4.51% to 13.02% [p < 0.001]) and *Faecalibacterium* relative abundance (0.42% to 0.72% [p = 0.010]), and a significant reduction in *Coprococcus* (4.97% to 3.88% [p = 0.010]), *Dorea* (1.57% to 1.20% [p = 0.043]) and *Ruminococcus* (*Lachnospiraceae* family) relative abundance (1.81% to 1.10% [p = 0.032]) (Table 7-6). There was a trend towards a reduction in Shannon index (6.28 to 5.99 [p = 0.060]) in the HDF group and an increased in Chao index (5655 to 6977 [p = 0.060]) in the LDF group during the prebiotic intervention (Appendix 7-11).

Table 7-6. Short-chain fatty acid concentration and bacterial taxa changes during the placebo and prebiotic intervention phases in the high dietary fibre group[1]

	High dietary fibre							
	Placebo (n = 19)				Prebiotic (n = 20)			
	Before intervention		After intervention		Before intervention		After intervention	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Short-chain fatty acids (µmol/g)								
Acetate	32.02	18.08	33.92	20.21	33.32	19.74	43.17	19.69
Butyrate	9.32	5.39	9.49	5.92	9.08	5.80	11.48	5.60
Propionate	10.14	6.64	10.90	7.52	10.05	8.90	12.69	7.83
Sum of short-chain fatty acids	55.37	29.37	58.05	33.05	56.57	33.12	71.06	31.05
Phylum (% relative abundance)								
Actinobacteria	9.45	6.45	9.13	6.32	8.87	5.97	17.70**	10.23
Bacteroidetes	15.20	14.53	13.27	10.23	16.82	11.44	14.50	9.48
Firmicutes	73.34	13.71	75.52	11.85	72.12	12.15	65.54*	12.09
Proteobacteria	0.30	0.21	0.32	0.22	0.47	0.47	0.41	0.45
Verrucomicrobia	0.27	0.66	0.16	0.41	0.26	0.33	0.15	0.21
Genus (% relative abundance)								
Bifidobacterium	5.20	4.65	4.94	4.65	4.51	4.10	13.02**	8.58
Collinsella	3.25	2.50	3.05	2.62	3.15	2.69	3.72	2.53
Bacteroides	5.38	3.49	5.58	4.16	6.81	3.66	5.96	3.10
Prevotella	7.05	15.29	4.60	7.20	6.79	11.03	6.51	9.73
Lactobacillus	0.01	0.01	0.02	0.04	0.03	0.06	0.07	0.24
Lachnospiraceae, other, unknown genus	1.75	0.82	1.54	0.78	1.50	0.78	1.35	0.54
Lachnospiraceae, unknown genus	13.94	3.99	14.27	5.94	13.04	5.58	15.80	5.39
Blautia	10.09	4.82	9.59	4.61	9.53	4.36	7.36	3.82
Coprococcus	4.08	1.84	4.79	2.26	4.97	2.74	3.88*	1.42
Dorea	1.44	0.69	1.62	0.89	1.57	0.71	1.20*	0.75
Ruminococcus (Lachnospiraceae)	1.80	1.31	1.95	1.61	1.81	1.20	1.10*	0.83
Ruminococcaceae, unknown genus	16.84	4.60	17.65	4.15	14.74	3.12	15.49	3.31
Faecalibacterium	0.50	0.27	0.56	0.28	0.42	0.18	0.72*	0.28
Oscillospira	1.10	0.72	0.99	0.59	1.00	0.42	0.70	0.45
Ruminococcus (Ruminococcaceae)	5.78	4.11	5.41	3.80	5.27	3.47	3.94	2.77
Dialister	0.70	1.00	1.03*	1.48	1.12	1.81	0.84	1.14

[1] Two-way repeated-measures ANOVA (blocked by participant) and least significant difference test. Mean values are significantly different from those of the placebo intervention and prebiotic before intervention or in the case of *Dialister* significantly different from those of the prebiotic intervention and placebo before intervention; *p < 0.05, **p < 0.01. SD: standard deviation

The between dietary fibre group comparison demonstrated that there were no differences in phylum level gut microbiota response to the prebiotic between the LDF and HDF groups (Table 7-7). There were also no differences in prebiotic driven SCFA production between the LDF and HDF groups (Table 7-7). The gut microbiota did; however, respond differently between the LDF and HDF groups at a genus level for *Lactobacillus*, an unknown genus of *Ruminococcaceae* and *Faecalibacterium* (Figure 7-4). There was minimal change (0.1%) in *Lactobacillus* relative abundance due to the prebiotic in the HDF group; however, *Lactobacillus* increased from 0.6% to 3.0% in the LDF group (p = 0.025). The relative abundance of an unknown genus of *Ruminococcaceae* increased (0.8%) in the HDF group but decreased (-2.9%) in the LDF

group (p = 0.018). The relative abundance of *Faecalibacterium* increased more markedly in the HDF group (0.4 to 0.7%) then the LDF group (0.4 to 0.5%) (p = 0.009) (Table 7-7).

Table 7-7. Short-chain fatty acid concentrations and bacterial taxa before and after the prebiotic intervention in low and high dietary fibre groups[1]

	Low dietary fibre (n=14)					High dietary fibre (n=20)					
	Before intervention		After intervention		Change	Before intervention		After intervention		Change	
	Mean	SD	Mean	SD		Mean	SD	Mean	SD		P value
Short-chain fatty acids (µmol/g)											
Acetate	29	18.2	34.3	22.3	5.3	33.3	19.7	43.2	19.7	9.9	0.534
Butyrate	7.8	5.1	8.3	5.3	0.5	9.1	5.8	11.5	5.6	2.4	0.375
Propionate	10	6.3	10.9	7.1	0.9	10.1	8.9	12.7	7.8	2.6	0.424
Sum of short-chain fatty acids	51	29	57.6	34	6.6	56.6	33.1	71.1	31.1	14.5	0.475
Phylum (% relative abundance)											
Actinobacteria	14	9.4	23.2	9.6	9.2	8.9	6	17.7	10.2	8.8	0.907
Bacteroidetes	11.3	8.9	9.6	5.4	-1.7	16.8	11.4	14.5	9.5	-2.3	0.829
Firmicutes	72.8	8.8	66	9.7	-6.9	72.1	12.2	65.5	12.1	-6.6	0.933
Proteobacteria	0.7	0.9	0.3	0.4	-0.4	0.5	0.5	0.4	0.5	-0.1	0.188
Verrucomicrobia	0.3	0.6	0.2	0.5	-0.1	0.3	0.3	0.2	0.2	-0.1	0.947
Genus (% relative abundance)											
Bifidobacterium	9.8	7.8	18	7.9	8.2	4.5	4.1	13	8.6	8.5	0.9
Collinsella	3	3.1	3.9	3.2	1	3.2	2.7	3.7	2.5	0.6	0.681
Bacteroides	6.8	5	5.7	3.9	-1	6.8	3.7	6	3.1	-0.9	0.909
Prevotella	2.9	4.9	2.5	3.6	-0.5	6.8	11	6.5	9.7	-0.3	0.898
Lactobacillus	0.6	1.3	3	5.6	2.4	0	0.1	0.1	0.2	0.1	**0.025**
Lachnospiraceae, other, unknown genus	2.4	1.5	1.8	0.6	-0.5	1.5	0.8	1.4	0.5	-0.2	0.261
Lachnospiraceae, unknown genus	11.9	7.2	13.2	7.4	1.4	13	5.6	15.8	5.4	2.8	0.522
Blautia	10.4	5.6	8.1	4.1	-2.3	9.5	4.4	7.4	3.8	-2.2	0.917
Coprococcus	3.8	2.1	3.1	1.9	-0.8	5	2.7	3.9	1.4	-1.1	0.65
Dorea	2	1.2	1.2	0.5	-0.8	1.6	0.7	1.2	0.8	-0.4	0.253
Ruminococcus (Lachnospiraceae)	2.6	2	1.2	1.3	-1.3	1.8	1.2	1.1	0.8	-0.7	0.249
Ruminococcaceae, unknown genus	16	5.1	13.1	4.8	-2.9	14.7	3.1	15.5	3.3	0.8	**0.018**
Faecalibacterium	0.4	0.3	0.5	0.3	0.1	0.4	0.2	0.7	0.3	0.3	**0.009**
Oscillospira	1.2	0.7	0.9	0.5	-0.3	1	0.4	0.7	0.5	-0.3	0.986
Ruminococcus (Ruminococcaceae)	5.8	4.3	5	4	-0.8	5.3	3.5	3.9	2.8	-1.3	0.617
Dialister	1	1.7	1.1	2.1	0.1	1.1	1.8	0.8	1.1	-0.3	0.356

[1] Two-way repeated-measures ANOVA (blocked by participant) and least significant difference test. The changes in bacterial relative abundance that were significantly different between the low and high dietary fibre groups are in **bold** ($p < 0.05$). SD: standard deviation.

PICRUSt analysis was performed to determine whether there were any predicted functional capacity differences between the LDF and HDF groups during the prebiotic intervention phase. There were no significant differences between the two dietary fibre groups for any of the bacterial substrate metabolism KEGG orthology groups analysed (Appendix 7-18).

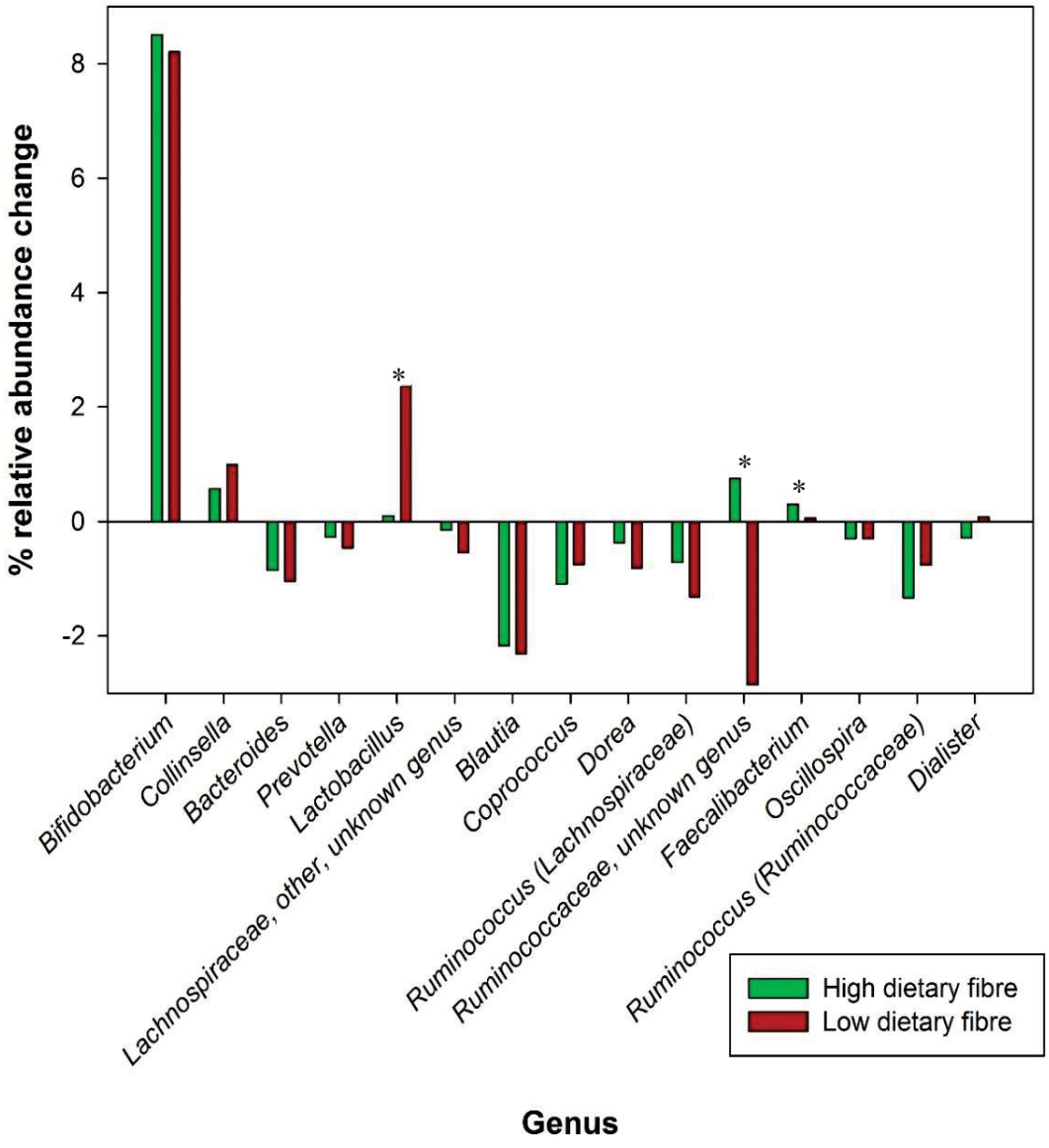

Figure 7-4. Genus level changes after the prebiotic intervention between the low and high dietary fibre groups. A significant change ($p < 0.05$) is indicated with an asterisk (*) as analysed by a two-way repeated-measures ANOVA (blocked by participant) and least significant difference test

7.4.7 Correlation between baseline bifidobacteria concentrations and change in bifidobacteria

The quantitative PCR data were used to determine whether there was a correlation between baseline bifidobacteria concentrations and change in bifidobacteria concentrations due to the prebiotic intervention. A significant correlation was

demonstrated for both the LDF (p = 0.017) and HDF (p = 0.004) groups. The strength of the correlation was similar between the dietary fibre groups (Figure 7-5).

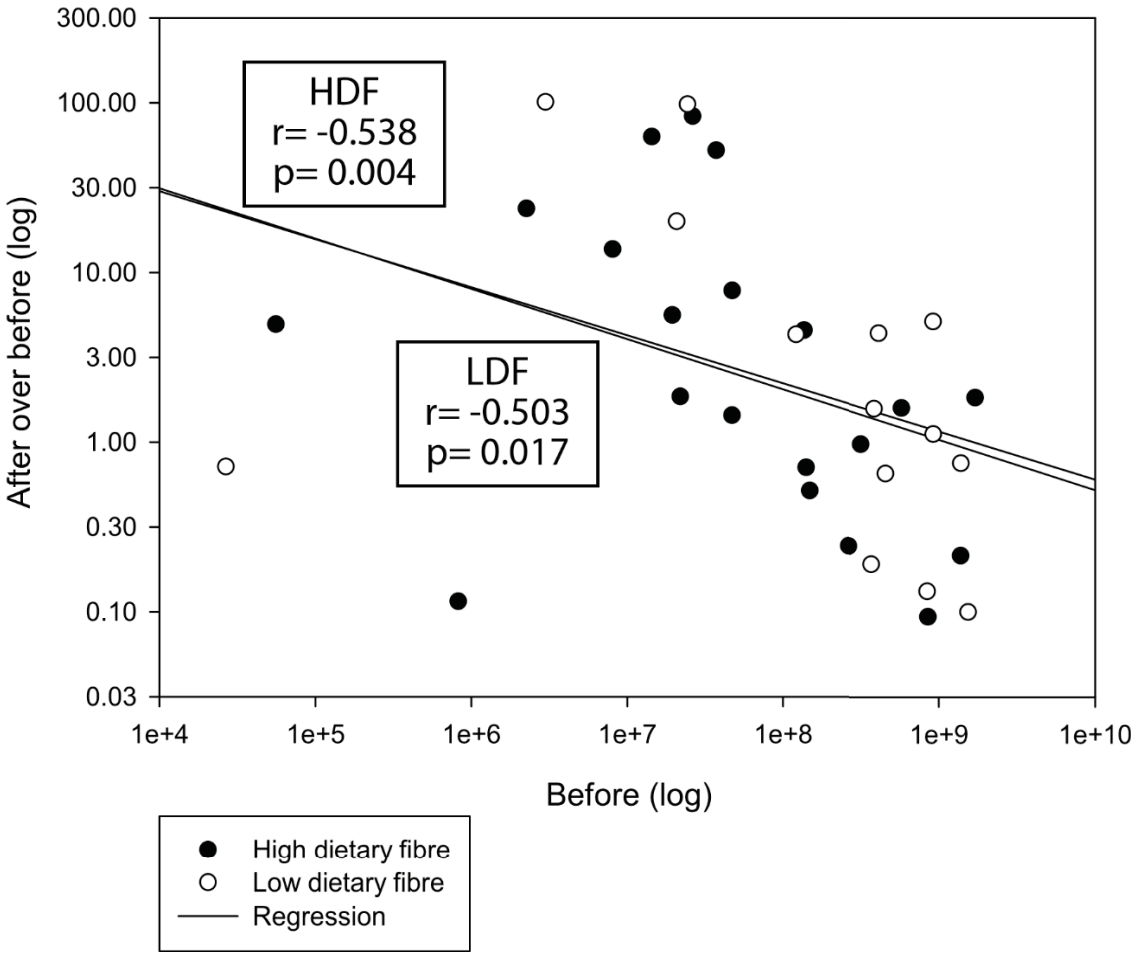

Figure 7-5. The correlation between baseline bifidobacteria concentrations (Before [log]) and change in bifidobacteria concentrations (After over before [log]) during the prebiotic intervention between the low and high dietary fibre groups. Bifidobacteria concentrations were determined using quantitative PCR. P values <0.05 are considered significant as analysed by a Pearson's rank correlation test

7.4.8 Bifidogenic response during the prebiotic intervention phase

7.4.8.1 Grouping participants as responders and non-responders

Participants were grouped as responders or non-responders based on whether they had a pronounced increase in *Bifidobacterium* relative abundance during the prebiotic intervention phase. The placebo intervention phase was used to demonstrate what a

"normal" fluctuation in *Bifidobacterium* relative abundance is. Approximately, 90% of participants had a change in *Bifidobacterium* relative abundance of ± 8% during the placebo phase (Appendix 7-19). Therefore, participants that experienced an increase in *Bifidobacterium* relative abundance of > 8% during the prebiotic intervention phase were classified as responders (n = 18) and those that experienced < 8% increase in *Bifidobacterium* relative abundance were classified as non-responders (n = 16).

7.4.8.2 Participant characteristic and dietary intake differences

There were no significant differences in participant characteristics between responders and non-responders (Table 7-8).

Table 7-8. Participant characteristic comparison between the bifidogenic responders and non-responders[1]

	Responders (n=18)	**Non-responders (n=16)**	**P value**
Age (years)	37.8 ± 11.2	36.9 ± 14.8	0.831
BMI (kg/m^2)	23.3 ± 3.1	23.2 ± 2.7	0.990
Male : Female	7 : 11	6 : 10	0.934
Fat mass (%)	22.5 ± 7.5	24.1 ± 10.7	0.631
Fat free mass (%)	77.5 ± 7.5	75.9 ± 10.7	0.631
Ethnicity (no.)			0.402
NZ European	6	9	
Maori	2	1	
Other	10	6	
Skip meals (Yes : No)	6 : 11	4 : 12	0.388
Snack consumed per day (no.)	2.1 ± 0.7	2.4 ± 0.9	0.234
Activity level^	5.4 ± 1.0	5.3 ± 1.3	0.739
Stools passed per week (no.)	7.1 ± 2.5	8.6 ± 4.8	0.270

[1] Chi-squared test and unpaired t-test. A p value of < 0.05 is considered significant. Values are means ± standard deviations. ^ Activity level of 5 = Seated work with some moving around and strenuous leisure activity

There were a number of baseline dietary intake differences between responders and non-responders. Non-responders had significantly higher intakes of starch (p = 0.027), starchy vegetables (p = 0.039), potatoes (p = 0.019), dairy (p = 0.036) and milk (p = 0.042) compared to responders (Table 7-9).

Table 7-9. Baseline dietary intake differences between bifidogenic responders and non-responders[1]

	Responders (n = 18)		Non-responders (n = 16)		
	Mean	**SD**	**Mean**	**SD**	**P value**
Nutrient intakes					
Energy (kJ/d)	8302.93	2707.22	9442.59	3109.96	0.297
Protein (g/d)	93.38	34.27	108.53	48.49	0.772
Total fat (g/d)	83.81	36.23	84.89	25.50	0.646
Saturated fat (g/d)	30.46	16.98	30.54	11.00	0.646
Polyunsaturated fat (g/d)	13.65	6.12	14.47	5.80	0.463
Monounsaturated fat (g/d)	32.42	13.39	32.46	10.61	0.772
Carbohydrate (g/d)	191.53	78.75	241.89	92.98	0.075
Sugars (g/d)	90.80	47.37	98.69	42.95	0.597
Starch (g/d)	99.10	45.93	140.25	56.04	**0.027**
Dietary fibre (g/d)	28.90	11.23	31.45	17.54	0.932
Water (g/d)	2556.79	1549.23	2392.54	791.16	0.721
Alcohol (g/d)	3.96	12.86	3.51	6.62	0.373
Energy from protein (%)	19.93	7.55	19.42	4.08	0.905
Energy from fat (%)	36.60	8.14	33.80	6.06	0.266
Energy from saturated fat (%)	12.84	4.54	12.44	4.00	0.851
Energy from carbohydrate (%)	38.09	10.36	41.75	5.99	0.347
Energy from alcohol (%)	1.06	3.00	1.08	2.18	0.389
Energy from fibre (%)	2.82	0.73	2.63	1.05	0.484
Food groups (serves/d)					
Grains	5.32	3.07	6.88	3.03	0.187
Refined grains	3.17	2.37	4.21	2.72	0.330
Wholegrains	2.15	1.57	2.66	2.88	0.980
Fruit	2.11	1.90	1.24	0.84	0.251
Vegetables	3.84	2.40	5.37	3.99	0.266
Dark green vegetables	0.37	0.33	0.28	0.44	0.161
Red orange vegetables	1.02	0.88	1.53	2.16	1.000
Tomatoes	0.28	0.29	0.36	0.36	0.531
Starchy vegetables	0.46	0.76	1.27	1.40	**0.039**
Potatoes	0.31	0.58	1.07	1.23	**0.019**
Legumes (vegetable)	0.22	0.38	0.64	0.82	0.089
Protein foods	3.23	1.62	3.11	1.92	0.670
Red meats	0.87	0.90	0.64	0.57	0.674
Poultry	0.54	0.64	0.52	0.64	0.841
Eggs	0.34	0.34	0.41	0.42	0.772
Processed meats	0.35	0.44	0.20	0.27	0.448
Nuts seeds	0.75	0.79	0.59	0.91	0.214
Legumes (protein)	0.13	0.23	0.40	0.52	0.092
Dairy	1.33	1.00	2.14	1.22	**0.036**
Milk	0.74	0.60	1.24	0.76	**0.042**

[1] Mann-Whitney test. Significant results ($p < 0.05$) are in **bold**. SD: standard deviation

Fructan intakes also significantly differed between responders and non-responders. Responders consumed significantly less inulin (2.65 ± 1.11 g/d *versus* 3.63 ± 1.42 g/d; respectively [p = 0.036]) and oligofructose (2.54 ± 1.05 g/d *versus* 3.50 ± 1.41 g/d; respectively [p = 0.046]) compared to non-responders (Figure 7-6).

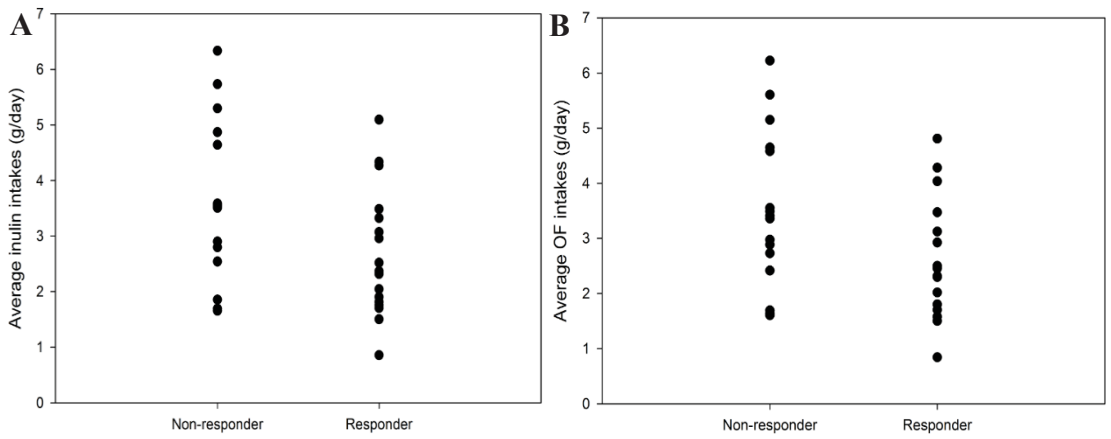

Figure 7-6. Average inulin (A) and oligofructose (OF; B) intake differences between bifidogenic responders and non-responders

7.4.8.3 Baseline differences in SCFA concentrations and gut microbiota

At baseline, there were no significant differences in SCFA concentrations or bacterial taxa at the phylum level between responders and non-responders (Table 7-10). There was, however, a significantly higher relative abundance of *Dialister* and a trend towards a significant lower relative abundance of *Collinsella* in the non-responder group compared to the responder group (Table 7-10).

7.4.8.4 Correlation between baseline bifidobacteria concentrations and change in bifidobacteria

A strong positive correlation between baseline bifidobacteria concentrations and prebiotic driven change in bifidobacteria was observed in bifidogenic responders ($r = 0.773$; $p < 0.001$) (Figure 7-7). A significant but weaker correlation was observed in non-responders ($r = 0.382$; $p = 0.035$) (Figure 7-7).

Table 7-10. Baseline differences in short-chain fatty acid concentrations and bacterial taxa between bifidogenic responders and non-responders[1]

	Responders (n =18)		Non-responders (n =16)	
	Mean	**SD**	**Mean**	**SD**
Short-chain fatty acids (µmol/g)				
Acetate	29.81	15.79	33.47	22.4
Butyrate	8.18	4.53	8.94	6.53
Propionate	9.18	5.31	10.98	10.05
Sum of organic acids	51.21	24.51	57.74	37.82
Phylum (% relative abundance)				
Actinobacteria	11.55	6.06	10.33	9.69
Bacteroidetes	14.02	9.85	15.15	11.88
Firmicutes	73.17	9.54	71.55	12.24
Proteobacteria	0.41	0.35	0.7	0.93
Verrucomicrobia	0.23	0.45	0.36	0.45
Genus (% relative abundance)				
Bifidobacterium	6.54	4.7	6.86	8.01
Collinsella	3.79	2.69	2.25^	2.81
Bacteroides	6.56	4.05	7.06	4.47
Prevotella	5.26	7.84	5.13	10.65
Lactobacillus	0.26	1.03	0.25	0.66
Lachnospiraceae, other, unknown genus	1.99	1.36	1.72	0.94
Lachnospiraceae, unknown genus	13.09	5.15	11.95	7.38
Blautia	10.59	4.35	9.12	5.36
Coprococcus	5.1	2.44	3.83	2.55
Dorea	1.77	0.83	1.73	1.12
Ruminococcus (Lachnospiraceae)	1.92	1.17	2.33	1.98
Ruminococcaceae, unknown genus	15.43	4.07	15.01	4.07
Faecalibacterium	0.44	0.22	0.37	0.23
Oscillospira	0.97	0.51	1.21	0.54
Ruminococcus (Ruminococcaceae)	5.65	3.52	5.31	4.12
Dialister	0.69	1.51	1.5*	1.9

[1] Mann-Whitney test. Mean values are significantly different from the responder group. *p < 0.05, ^trend towards significance (p < 0.1). SD: standard deviation

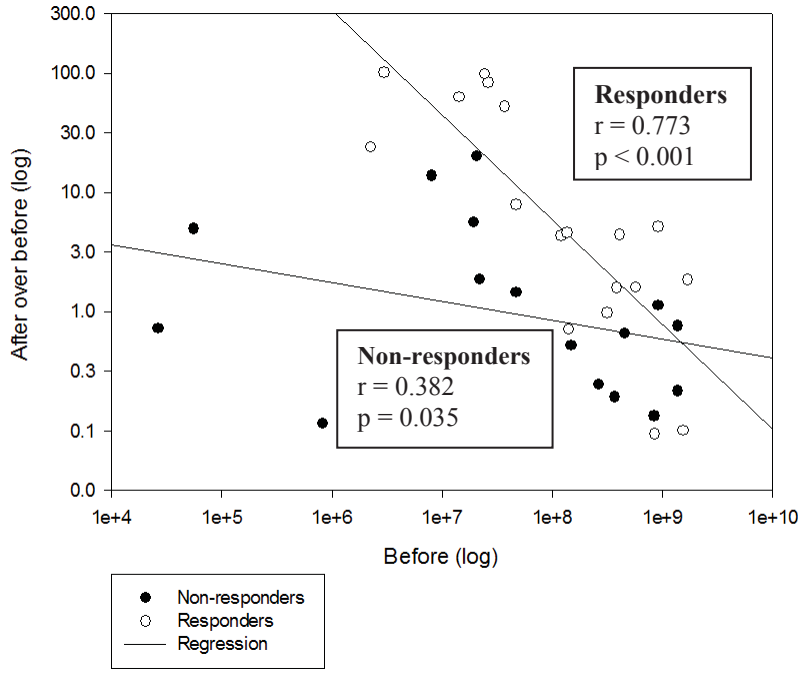

Figure 7-7. The correlation between baseline bifidobacteria concentrations (Before [log]) and change in bifidobacteria concentrations (After over before [log]) during the prebiotic intervention between bifidogenic responders and non-responders. Bifidobacteria concentrations were determined using quantitative PCR. P values <0.05 are considered significant as analysed by a Pearson's rank correlation test

7.5 Discussion

In the present study, the inulin-type fructan prebiotic led to several microbial changes in the whole cohort including an increase in *Bifidobacterium* and a decrease in *Coprococcus*, *Dorea*, *Ruminococcus* (*Lachnospiraceae* family) and *Oscillospira* relative abundances. There was also a trend towards an increase in *Faecalibacterium* relative abundance. Previous inulin-type fructan prebiotic intervention studies have demonstrated similar results with increases in *Bifidobacterium* and/or *Faecalibacterium* being reported in a number of studies[14,38–41]. Short-chain fatty acid concentrations did not differ after the prebiotic intervention. *In vitro* studies have shown that inulin-type fructan prebiotics led to an enhanced production of butyrate[42,43]; however, this result is often not replicated in human prebiotic intervention studies[37,40,41]. This is not overly surprising as over 95% of the SCFAs produced in the human colon are used by the microbiota that reside within the gut, are rapidly utilised by colonocytes and are absorbed into the host's systemic circulation[44,45]. Additionally, there were no significant changes in appetite ratings during the prebiotic intervention phase in the whole cohort.

Interestingly, categorisation into LDF and HDF groups led to a number of distinctions in gut microbiota response. In the LDF group, the only significant genus level microbiota change elicited by the inulin-type fructan prebiotic was an increase in *Bifidobacterium* relative abundance. In the HDF group, the inulin-type fructan prebiotic led to a significant increase in the relative abundance of *Bifidobacterium* and *Faecalibacterium* and a significant reduction in *Coprococcus, Dorea* and *Ruminococcus* (*Lachnospiraceae* family). The LDF group appeared to harbour a gut microbiota community that were more resilient to change and, therefore, less responsive to the inulin-type fructan prebiotic than the HDF group. A study conducted by Eid and co-authors[17] demonstrated that individuals with an average dietary fibre intake of 18 g/d,

hosted gut microbiota that were more resilient to a palm date intervention. In their study, individuals with an average dietary fibre intake of 18 g/d were classified as HDF, whereas, in the present study LDF consumers had an average dietary fibre intake of 18 g/d. A recent animal study, which used germ-free (gnotobiotic) mice colonised with human gut microbiota from donors with two varying dietary patterns (typical American style dietary pattern [AMER] or a plant-rich, calorie-restricted diet with optimal nutrient composition [CRON]), demonstrated that mice inoculated with AMER microbiota were less responsive to the CRON type diet when compared to mice inoculated with CRON microbiota[18]. A recent *in vitro* batch fermentation study demonstrated that donors with healthier dietary patterns harboured gut microbiota that were better equipped at utilising fermentable carbohydrates found in grains compared to donors with less healthy dietary patterns[46]. Therefore, the HDF group in the present study may have a gut microbiota consortia that is metabolically more capable of utilising high amounts of fermentable substrates as their habitual diet is already high in these substrates.

Host-specific responses, such as appetite rating changes and gastrointestinal symptoms, were significantly influenced by the inulin-type fructan prebiotic in the HDF group only. The HDF group reported a significantly higher frequency of moderate flatulence, an increase in fullness and satisfaction after lunch and a reduction in hunger after dinner due to the inulin-type fructan prebiotic. One of the key differences in gut microbiota response to the prebiotic intervention between dietary fibre groups was the significant increase in *Faecalibacterium* relative abundance observed only in the HDF group. Carbon dioxide and butyrate are primary metabolic by-products of indigestible substrate fermentation by *Faecalibacterium* in the colon[47]. A primary component of flatus is CO_2 gas[48] and butyrate has been shown to be involved in regulating appetite-associated gut hormones[49]. Therefore, it is plausible that the significant change in

appetite ratings and the increased frequency of moderate flatulence experienced by the HDF group after the prebiotic intervention may be associated with the increased abundance of *Faecalibacterium*. Nevertheless, further investigation is required to demonstrate whether a link between *Faecalibacterium* and host response in healthy individuals exists.

It is likely that the whole cohort bacterial taxa results may have changed if the number of LDF and HDF participants recruited were different. In the present study, the data from more HDF than LDF consumers (20 and 14 participants; respectively) were used in the analysis, therefore, the whole cohort results are more reflective of the results generated from the HDF group. If the proportion of recruited HDF to LDF participants differed from the present study, then this could have had implications on the whole cohort results. For example, if more LDF than HDF consumers were recruited, the prebiotic may have led to less pronounced changes in the gut microbiota community; outside of an increase in *Bifidobacterium* relative abundance.

Consideration towards inter-individual variability in gut microbiota responsiveness will be particularly important when researching the prebiotic potential of a given dietary intervention for the first time. If a greater proportion of participants with less responsive gut microbiota communities are recruited then it may appear that the dietary intervention does not have an influence on the gut microbiota which may not be representative of the true prebiotic efficacy of the dietary intervention for all participants. Gaining additional insight into the factors which influence gut microbiota responsiveness, so they can be controlled for more effectively, will help determine the true prebiotic efficacy of a dietary intervention and provide better consistency of results between studies.

Quantitative PCR data were utilised to investigate whether a correlation between baseline bifidobacteria concentrations and prebiotic driven change in bifidobacteria concentrations exists in our study cohort. We also aimed to determine whether the strength of the correlation differed between the LDF and HDF groups. A significant correlation existed between baseline bifidobacteria concentration and prebiotic driven change in bifidobacteria concentration with lower baseline bifidobacteria concentrations being correlated with a more pronounced bifidogenic response. The strength of the correlation did not differ between the LDF and HDF groups, suggesting that habitual dietary fibre intakes do not have an influence on the correlation. The majority of previous studies are in agreement with our results as they have also observed that lower baseline bifidobacteria concentrations lead to a more pronounced increase in bifidobacteria in response to a dietary intervention[12–16].

An increase in *Bifidobacterium* was the most consistent change observed between the LDF and HDF groups, however, not all participants experienced a change in *Bifidobacterium* relative abundance. Therefore, we decided to group participants based on whether or not they experienced a prebiotic driven bifidogenic response. By grouping participants based on bifidogenic responses we aimed to determine whether there were any baseline differences in habitual dietary intake or baseline gut microbiota composition between bifidogenic responders and non-responders. At baseline, only the relative abundance of *Dialister* significantly differed between responders and non-responders. Interestingly, a strong, significant correlation between baseline bifidobacteria concentrations and change in bifidobacteria was observed during the prebiotic intervention phase in bifidogenic responders. A significant but much weaker correlation was demonstrated in non-responders suggesting that the influence baseline bifidobacteria concentrations have on prebiotic driven change in bifidobacteria may be more

pronounced in responders compared to non-responders. However, it does appear that a few outliers in the non-responder group may have led to the weaker correlation observed making it difficult to draw to any strong conclusions. A significantly higher intake of inulin and oligofructose were demonstrated in non-responders compared to responders. This result is interesting as it appears that individuals with lower fructan intakes harbour bifidobacteria that are more responsive to an inulin-type fructan prebiotic than individuals with higher fructan intakes. It is possible that bifidogenic responders previously had a fructan intake deficit that was made up by the inulin-type fructan prebiotic supplementation. Whereas, bifidogenic non-responders may have already been consuming an adequate amount of fructans, thereby, rendering the inulin-type fructan prebiotic supplement as unrequired. To the best of our knowledge the link between fructan intakes and bifidogenic response to an inulin-type fructan has not previously been researched. This study was not specifically designed to look at bifidogenic responsiveness and fructan intakes, however, the post-hoc analysis conducted does suggest that future research in this area may be justified.

There are a number of strengths of this study including the robust study design: a randomised, double-blind, placebo-controlled, cross-over human intervention study. This is also the first study to recruit participants based on pre-defined habitual dietary fibre intake categories to demonstrate what influence habitual dietary fibre intake has on gut microbiota responsiveness. Another strength of this study is the utilisation of next-generation sequencing technology which allowed for the characterisation of the whole microbial community rather than focusing on changes that occur in only select bacterial taxa. A limitation of the present study is that the interpretation of our results is limited to inulin-type fructan prebiotic interventions, in particular, a mixed inulin:fructo-oligosaccharide prebiotic. The influence habitual dietary fibre has on gut microbiota

responsiveness will likely differ depending on the dietary intervention studied. The influence habitual dietary fibre intake has on the responsiveness of the gut microbiota to other dietary interventions, such as calorie restriction, increased resistant starch and high wholegrains diets, will need to be researched in the future.

In conclusion, it is difficult to predict how the gut microbiota will respond to a dietary intervention. Gaining a better understanding of the factors implicated in inter-individual variability in gut microbiota responsiveness may help improve dietary intervention success and subsequently enhance human health outcomes. In this study, we identified that individuals with HDF intakes have a greater gut microbiota response to an inulin-type fructan prebiotic. These individuals also experienced greater benefits in appetite but reported more GI symptoms. Future studies aiming to modulate the gut microbiota using an inulin-type fructan prebiotic should take habitual dietary fibre intake into account either when recruiting participants or during data analysis to help minimise the influence that inter-individual variability in gut microbiota responsiveness has on study outcomes. Lastly, post-hoc analysis demonstrated that a link between fructan intakes and bifidogenic response to an inulin-type fructan prebiotic may exist. Therefore, future studies should be designed to demonstrate whether habitual fructan intake influences bifidogenic response to an inulin-type fructan prebiotic.

7.6 References

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CHAPTER EIGHT

DISCUSSION, CONCLUSION AND AREAS OF FUTURE RESEARCH

Chapter 8 Discussion, conclusion and areas of future research

8.1 Discussion

Initially, secondary analysis (Chapter 3) was undertaken using data from a previous randomised, double-blind, placebo-controlled, cross-over study to determine whether baseline differences in bacterial taxa and alpha diversity exist between individuals with differing habitual dietary intakes and whether differences in bacterial taxa response to high-dose Actazin™ occurred between individuals with differing habitual dietary intakes. The hypothesis was that baseline differences in gut microbiota composition and diversity, and gut microbiota response to a given dietary intervention (i.e. high-dose Actazin™) would occur between individuals with distinctive habitual dietary intakes. It was, therefore, necessary to test the validity of this hypothesis by conducting secondary analysis of data from a previous human intervention study to help determine what nutrients and/or food groups had the greatest impact on gut microbiota responsiveness. It was established, that baseline differences in gut microbiota existed in nine of the 13 dietary groups analysed. The Vegetable, Fruit and Carbohydrate:Protein ratio dietary groups had the highest number of bacterial taxa differences between low and high, or low, moderate and high consumers. It was also demonstrated after secondary data analysis that nine of the 13 dietary groups analysed had significant distinctions in gut microbiota response to high-dose Actazin™. The Dietary fibre, Wholegrain, Vegetable, Plant protein and Carbohydrate:Protein ratio had the greatest impact on gut microbiota responsiveness. A large proportion of the dietary groups that led to differences in gut microbiota response to high-dose Actazin™ were dietary groups that were rich in dietary fibre (i.e. Dietary fibre, Wholegrains, Vegetables, Plant Protein and Energy from carbohydrates [%]). Therefore, focusing on the influence that habitual dietary fibre intake has on gut microbiota responses became the primary research aim.

An *in vitro* three-stage colonic model system study (Chapter 4) was the next study to be conducted. The validated *in vitro* three-stage colonic model system was an ideal model to use as it simulates the physiologically relevant conditions found in the human colon[1]. Additionally, the *in vitro* three-stage colonic model system is not affected by some of the confounding factors associated with *in vivo* studies making it a valid platform to test a hypothesis before progressing to a human intervention study. For this *in vitro* study, two media were developed which had distinctive fermentable carbohydrate contents to mimic WS and PS dietary patterns. The aim of this *in vitro* study was to demonstrate what influence differing fermentable carbohydrate content media had on gut microbiota responsiveness to an inulin-type fructan prebiotic using an *in vitro* three-stage colonic model system. The addition of the inulin-type fructan prebiotic led to significant changes in the gut microbiota in both the low (LFC) and high fermentable carbohydrate medium (HFC) gut models. Interestingly, the bacterial taxa and short-chain fatty acid (SCFA) concentration changes that occurred between the gut models differed. Significant differences in the amount of acetate that was produced and the changes in relative abundance of Firmicutes, *Lactobacillus*, an unknown genus of *Lachnospiraceae* (other), *Megasphaera*, *Mitsuokella* and an unknown genus of *Enterobacteriaceae* were evident between the LFC and HFC gut models. Previous *in vivo* research has demonstrated large inter-individual variability in gut microbiota response to dietary interventions. Therefore, the results from this study were also analysed on an individual donor basis which established that large inter-individual variability in gut microbiota response to the differing fermentable carbohydrate content media existed within the *in vitro* three-stage colonic model system. It is worth discussing one of the key limitations of this study which was the media vessel contamination resulting in the exclusion of data collected from the donor 2 (D2)-LFC gut model. It was fortunate that the contamination

occurred near the end of the experiment, however, it did mean that the data from only two donors could be used in the statistical analysis. Repeating the experiment for the D2-LFC gut model was not an option as a number of months had passed between when D2 provided the initial faecal sample and when the contamination was confirmed using next-generation sequencing. The results of the *in vitro* three-stage colonic model system provide preliminary evidence to suggest that the fermentable carbohydrate (i.e. dietary fibre) content of the media has an influence on gut microbiota response to an inulin-type fructan prebiotic.

The results of the secondary data analysis (Chapter 3) and *in vitro* three-stage colonic model system (Chapter 4) studies supported the hypothesis. Therefore, a human intervention study to determine what influence differing habitual dietary fibre intakes had on gut microbiota response to an inulin-type fructan prebiotic was designed and carried out. It was imperative that individuals with distinctive habitual dietary fibre intakes participated in the human intervention study, however, no short dietary fibre questionnaires were available that could accurately classify individuals based on their habitual dietary fibre intakes. Therefore, prior to conducting the human intervention study a dietary fibre intake short food frequency questionnaire (DFI-FFQ) (Chapter 5) was designed and validated for use in the screening phase of the human intervention study to ensure only LDF and HDF consumers were recruited. The results of the DFI-FFQ study showed that the DFI-FFQ was not able to accurately quantify total habitual dietary fibre intakes, however, the questionnaire was able to accurately and reproducibly classify individuals as low, moderate or high dietary fibre consumers. For the human intervention study, the intention was to recruit individuals with LDF and HDF intakes, therefore, for the purposes of screening individuals based on habitual dietary fibre intakes the DFI-FFQ was shown to be a valid questionnaire to use.

The main focus of this research was to design (Chapter 6) and conduct a randomised, double-blind, placebo-controlled, cross-over, human intervention study (Chapter 7) to investigate the influence differing habitual dietary fibre intakes have on gut microbiota response to an inulin-type fructan prebiotic. The results of this study demonstrated that the inulin-type fructan prebiotic led to a greater number of significant changes in bacterial taxa in the HDF compared to LDF group. This was contrary to the original primary hypothesis which suggested that individuals with LDF intakes will harbour bacterial taxa that change more significantly in response to an inulin-type fructan prebiotic than individuals with a HDF intake. It was also hypothesised that LDF individuals would experience a more pronounced change in appetite compared to HDF individuals in response to the inulin-type fructan prebiotic. A contrary result to the hypothesis was again demonstrated with the HDF group not the LDF group experiencing a significant change in appetite due to the prebiotic intervention. The rationale behind the hypothesis was that LDF individuals would harbour gut microbiota that were more responsive to the additional dietary fibre in their diet (i.e. inulin-type fructan prebiotic) as bacterial taxa that were previously deprived of fermentable substrates but are equipped to utilise them may have had the opportunity to increase in abundance due to their newly obtainable energy source. However, HDF individuals may harbour bacterial taxa that are more metabolically capable of utilising the additional dietary fibre leading to a more pronounced gut microbiota response in this group. Interestingly, PICRUSt analysis did not demonstrate any significant differences in functional capacity between the LDF and HDF groups but this is only a predictive measure of the bacterial functionality and may not represent the actual functional capacity of the microbial communities[2]. Nevertheless, the results of the human intervention study have demonstrated that habitual dietary fibre

intake does influence gut microbiota and host response to an inulin-type fructan prebiotic.

8.2 Conclusion

Gut microbiota modulation to enhance human health is an emerging concept which may help combat the rising prevalence of non-communicable disease. Presently, it is very difficult to predict how the gut microbiota or host will respond to a given dietary intervention. Previous research has demonstrated that large inter-individual variability in gut microbiota and host response may exist as a result of distinctions in baseline gut microbiota composition[3–6] and habitual dietary intakes[7,8]. However, the majority of these studies conducted post hoc analysis to demonstrate that baseline gut microbiota and habitual dietary intake help to predict gut microbiota and host responses. Therefore, gaining a deeper understanding of the factors implicated in gut microbiota and host response may enhance dietary intervention success and subsequently improve human health. Additionally, once the factors implicated in gut microbiota and host response are better understood they can be adequately controlled for to provide better consistency of results between studies to ensure the true efficacy of a given dietary intervention is demonstrated. Prior to conducting this research no studies had been undertaken to determine what influence habitual dietary intake has on gut microbiota and host response to a dietary intervention. This research demonstrates that habitual dietary fibre intake does influence gut microbiota and host response to an inulin-type fructan prebiotic. Future studies which aim to modulate the gut microbiota to enhance human health or demonstrate whether a novel fermentable substrate has prebiotic potential should take habitual dietary fibre intakes into consideration when recruiting participants and/or analysing the data.

8.3 Areas of future research

- Based on the secondary data analysis and human intervention study results it appears that *Coprococcus*, *Faecalibacterium* and *Oscillospira* were the bacterial taxa that were more likely to respond in a distinctive manner to the dietary interventions in individuals with differing habitual dietary intakes. In the future, it may be of interest to focus on these bacterial taxa as they appear to be particularly susceptible to the influence differing habitual dietary intakes have on gut microbiota response to a dietary intervention.
- It is plausible that the influence habitual dietary fibre has on gut microbiota and host response will differ depending on the dietary intervention studied. Therefore, the influence habitual dietary fibre intake has on gut microbiota and host response to other dietary interventions, such as calorie restriction and high wholegrain diets, should be investigated in the future.
- Preliminary evidence from the human intervention study suggests that a link between gut microbiota response and host response, particularly appetite and GI symptoms, exists. Future research in this area is required to determine the clinical relevance of the influence baseline gut microbiota composition and habitual diet has on gut microbiota and host response.
- Future studies which aim to determine whether baseline gut microbiota, habitual diet or a combination of the two factors appear to be more useful in predicting gut microbiota and host response are required.
- Preliminary evidence from the human intervention study suggests that bifidogenic responses to an inulin-type fructan prebiotic may be linked to fructan intakes suggesting that further investigations in this area are required.

- Future utilisation of metagenomic, metabolomic, metaproteomic and/or metatranscriptomic techniques should be considered to provide more accurate information relating to actual rather than predicted (PICRUSt) functional capacity differences between individuals with differing gut microbiota responses.
- Predictive mathematical models that integrate baseline gut microbiota, habitual dietary intake and host physiological parameters could be used in the future to personalise dietary advice and subsequently enhance dietary intervention success.

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APPENDICES

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Appendix 4-1. Media rationale

Development of the two differing fermentable carbohydrate media

There are a number of fermentable carbohydrate sources in the diet such as non-starch polysaccharides (NSP), lignin (not a carbohydrate but associated with fermentable carbohydrates), resistant starch (RS) and oligosaccharides. The amount and types of fermentable carbohydrates consumed vary considerably between individuals.

Two distinctive media have been developed to represent low and high fermentable carbohydrate (i.e. dietary fibre) intakes to help determine what influence differing background fermentable carbohydrate amounts have on the responsiveness of the gut microbiota to an inulin-type fructan prebiotic. The low fermentable carbohydrate medium (LFC) has been developed to represent a Western-style dietary pattern which is high in red meat, processed meat, butter, potatoes, refined grains, high fat dairy, sweets and desserts and eggs[1]. This dietary pattern is lower in NSP, lignin, RS and oligosaccharides and total fermentable carbohydrates. The high fermentable carbohydrate medium (HFC) has been developed to represent a Prudent-style dietary pattern which is high in vegetables, fruit, legumes, fish/seafood and wholegrain[1]. This dietary pattern is higher in NSP, lignin, RS and oligosaccharides and higher in total fermentable carbohydrates.

Determining the NSP and lignin content of the two media types

Data from the New Zealand Adult Nutrition Survey was used to determine the average NSP intake (20.3 g/day using the Englyst method which does not analyse lignin, RS or oligosaccharides) of New Zealanders and the major food group contributors of NSP in the diet[2] (Table 1).

The number of serves from each food group to be reflected in the LFC and HFC was determined based on the major food group contributors in the New Zealand diet (Table 1) and Western- *versus* Prudent-style dietary pattern information. For the HFC the majority of NSP will come from vegetable, fruit, legume and wholegrain based sources while for the LFC the majority of NSP will come from potato, refined grain, sweet and dessert based sources[1,3,4]. The NSP and lignin content of 228 commonly

consumed foods was used to determine the amount of each type of fermentable carbohydrate to be used in the HFC and LFC[5]. As the major food groups which contribute NSP and lignin differ between the two dietary patterns the amount of fermentable carbohydrate from each fermentable carbohydrate type (i.e. hemicellulose, cellulose, pectin or lignin) will also vary between the two media types (Table 2).

In New Zealand it is recommended that 25-30 g of fermentable carbohydrates (i.e. dietary fibre) are consumed each day[6]. Unfortunately a direct comparison between average New Zealand dietary fibre intakes (20.3 g/day of NSP) and the recommendations (25-30 g/day of total dietary fibre, i.e. NSP, RS, beta-glucan and oligosaccharides) cannot be made as the definition for dietary fibre differs between the recommendations and the average New Zealand dietary fibre intake. However, it is still important to ensure the NSP amounts within the media composites are representative of actual intakes. To ensure the LFC and HFC reflect realistically low and high intakes of NSP the aim was for the LFC to be equivalent to approximately 12.8 g/day of NSP and the HFC to be equivalent to approximately 28.5 g/day of NSP. These NSP amounts represent the 10th and 90th percentiles for NSP intake (excluding lignin) in New Zealand; respectively[2]. The LFC contains the equivalent of 13.7 g/day of NSP and lignin (11.6 g/day excluding lignin) and the HFC contains the equivalent of 30.7 g/day of NSP and lignin (26.1 g/day excluding lignin) (Table 2).

Table 1. Summary of the non-starch polysaccharide (NSP) data taken from "A Focus on Nutrition: Key Findings of the 2008/09 New Zealand Adult Nutrition Survey". The average amount of NSP (g) contributed by each food group was determined based on an average NSP intake of 20.3 g/day[2].

Food group	Serving size	Percentage (%) of NSP contributed by each food group	Average amount of NSP (g) contributed by each food group
Bread	1 slice	17.1	3.5
Vegetables (including legumes)	½ cup cooked vegetables, 1 cup lettuce or 1 tomato ¾ cup cooked legumes	16.4	3.3
Potatoes, Kumara and Taro	1 medium	11.7	2.3
Fruit	1 large piece (banana), 2 small pieces (mandarin) or ½ cup berries	11.5	2.3
Grains and pasta	1 cup cooked	8.3	1.7
Breakfast cereals	½ cup wholegrain cereal (Muesli) or 1 cup refined cereal (Cornflakes)	7.2	1.5
Bread based dishes	Equivalent to 1 slice bread	5.1	1
Other- cakes and muffins, pies and pastries, nuts, biscuits, soups etc.	Varies between food groups	22.7	4.6

Table 2. The non-starch polysaccharide (NSP) and lignin amounts estimated to be consumed per day and the percentage of total NSP and lignin from each food group for the Western-style dietary pattern/low fermentable carbohydrate (W/LFC) and Prudent-style dietary pattern/high fermentable carbohydrate (P/HFC) based diets. The NSP + lignin data was extrapolated from the Marlett and co-authors publication[5]. * This information carries over to Table 3. ^ This information carries over to Table 4.

	No. of serves consumed per day*		**Hemi-cellulose (soluble)**		**Pectin (soluble)**		**Cellulose (insoluble)**		**Hemi-cellulose (insoluble)**		**Pectin (insoluble)**		**Lignin (insoluble)**		**Total (g) per day**		**Percentage from each food group**	
			Amount per day (g)		Amount per day (g)		Amount per day (g)		Amount per day (g)		Amount per day (g)		Amount per day (g)					
Food group	**W/ LFC**	**P/ HFC**	**W/ LFC**	**P/ HFC**	**W/ LFC**	**P/ HFC**	**W/ LFC**	**P/ HFC**	**W/ LFC**	**P/ HFC**	**W/ LFC**	**P/ HFC**	**W/ LFC**	**P/ HFC**	**W/ LFC**	**P/ HFC**	**W/ LFC**	**P/ HFC**
Bread	4.0	2.0	0.80	0.60	0.00	0.00	0.80	1.20	0.80	2.40	0.00	0.00	0.80	0.80	3.20	5.00	23.2	16.3
Vegetable	1.0	4.0	0.20	0.79	0.17	0.67	0.62	2.48	0.42	1.69	0.28	1.11	0.11	0.44	1.80	7.18	13.0	23.5
Legumes	0.0	0.5	0.00	0.31	0.00	0.13	0.00	0.68	0.00	0.69	0.00	0.17	0.00	0.30	0.00	2.29	0.0	7.5
Fruit	1.0	3.0	0.12	0.35	0.23	0.70	0.46	1.37	0.39	1.17	0.25	0.76	0.40	1.19	1.85	5.54	13.0	17.9
Potato, Kumara, Taro	1.5	0.5	0.45	0.15	0.35	0.08	1.25	0.42	0.89	0.30	0.30	0.10	0.12	0.04	3.36	1.09	23.9	3.6
Grains and Pasta	1.0	1.0	0.14	0.40	0.00	0.00	0.18	0.95	0.25	1.15	0.00	0.50	0.12	1.30	0.70	4.30	5.1	14.0
Breakfast Cereals	1.0	1.0	0.10	0.35	0.0	0.00	0.25	0.83	0.20	1.95	0.00	0.10	0.15	0.40	0.70	3.63	5.1	11.7
Others	3.0	1.0	0.62	0.15	0.00	0.10	0.44	0.65	0.69	0.45	0.06	0.20	0.36	0.15	2.17	1.70	15.9	5.5
Total^			**2.4**	**3.1**	**0.8**	**1.7**	**4.0**	**8.6**	**3.6**	**9.8**	**0.9**	**2.9**	**2.1**	**4.6**	**13.8**	**30.7**		

Determining the resistant starch content of the two media types

Major dietary sources of RS include potatoes, bananas, bread, legumes, cereals and pasta[7,8]. The amount of RS in each media type was determined using food composition data relating to the RS content of commonly consumed foods[8] and the major food group contributors of NSP in the New Zealand diet[2]. The average RS intake is approximately 5 g/day but can vary considerably between individuals (1.37-10.2 g/day)[7–9]. The LFC media contains the equivalent of 4.4 g/day of RS and the HFC contains the equivalent of 5.7 g/day of RS. The main food group contributors of RS for the LFC are potato, kumara and taro, breads, grains and cereals and for the HFC are legumes, fruit (bananas) and breakfast cereals (Table 3).

Determining the oligosaccharide content of the two media types

The predominant oligosaccharides in the diet are fructans (fructo-oligosaccharide and inulin) and galactans (galacto-oligosaccharide). The average galacto-oligosaccharide (GOS) intake is approximately 0.62 g/day[10] with legumes providing the majority of GOS in the diet[11]. As GOS only provides a very small proportion of the total fermentable carbohydrates in the diet it was not added to either of the media composites.

Fructans are represented within the media composites as the average intake is considerably higher than GOS at approximately 4 g/day for inulin and 3.8 g/day for fructo-oligosaccharides[12,13]. Intakes of fructans can vary considerably between individuals from 3-11 g/day[14]. The major foods which contribute fructans in the diet include wheat, onions, bananas and garlic. Wheat from bread will represent a major source of fructans for the LFC, whereas, fructans from vegetable and fruit sources will be represented in the HFC. For the LFC a low fructan intake (< 2.78 g/day inulin and < 2.70 g/day fructo-oligosaccharides) was represented and for the HFC a high fructan intake (≥ 4.19 g/day inulin and ≥ 4.06 g/day fructo-oligosaccharides) was represented[13]. The LFC contains the equivalent of 2 g/day of inulin and 2 g/day of fructo-oligosaccharides and the HFC contains the equivalent of 4.3 g/day of inulin and 4.2 g/day of fructo-oligosaccharides (Table 4).

Table 3. The resistant starch (RS) amounts estimated to be consumed per serve (g) and per day (g) for each food group, as well as the percentage of RS from each food group for Western-style dietary pattern/low fermentable carbohydrate (W/LFC) and Prudent-style dietary pattern/high fermentable carbohydrate intake (P/HFC) based diets. The RS data was extrapolated from the Murphy and co-authors publication[8] * This information was carried over from Table 2. ^ This information carries over to Table 4.

	No. of serves consumed per day*		RS per serve (g)		RS per day (g)		Percentage (%) of RS from each food group	
Food group	**W/LFC**	**P/HFC**	**W/LFC**	**P/HFC**	**W/LFC**	**P/HFC**	**W/LFC**	**P/HFC**
Bread	4.0	2.0	0.27	0.27	1.1	0.6	24.6	9.6
Vegetable	1.0	4.0	0.00	0.00	0.0	0.0	0.0	0.0
Legumes	0.0	0.5	0.00	2.36	0.0	1.2	0.0	20.7
Fruit	1.0	3.0	0.63	0.63	0.6	1.8	14.5	33.4
Potato, Kumara, Taro	1.5	0.5	1.00	1.00	1.5	0.5	33.9	8.7
Grains and Pasta	1.0	1.0	0.13	0.50	0.1	0.5	3.1	8.8
Breakfast Cereals	1.0	1.0	0.36	1.10	0.4	1.1	8.3	18.9
Others	3.0	1.0	0.23	0.00	0.7	0.0	15.6	0.0
Total^					**4.4**	**5.7**		

Table 4. The estimated amount of each fermentable carbohydrate per day in Western-style dietary pattern/low fermentable carbohydrate (W/LFC) and Prudent-style dietary pattern/high fermentable carbohydrate (P/HFC) diets, the amount of each fermentable carbohydrate required in 600 ml of media to be equivalent to a W/LFC for the low fermentable carbohydrate medium (LFC) and a P/HFC for the high fermentable carbohydrate medium (HFC), and the amount of each fermentable carbohydrate required in 5 L of media for the LFC and HFC media which is the standard volume of media made up for the *in vitro* model at any one time. ^ This information was carried over from Table 2 + Table 3 and the "Determining the oligosaccharide content of the two media types" section. * This information carries over to Table 5.

	W/LFC	LFC		P/HFC	HFC	
Fermentable carbohydrate	**Estimated amount in the diet per day (g)^**	**Amount in 600 ml of media**	**g / 5 L of media***	**Estimated amount in the diet per day (g)^**	**Amount in 600 ml of media**	**g / 5 L of media***
Resistant starch	4.4	1.3	11.0	5.7	1.7	14.3
Inulin	2.0	0.6	5.0	4.3	1.3	10.8
Fructo-oligosaccharide	2.0	0.6	5.0	4.2	1.3	10.5
Soluble Hemicellulose	2.4	0.7	5.9	3.1	0.9	7.8
Soluble Pectin	0.8	0.2	1.9	1.7	0.5	4.3
Insoluble Cellulose	4.0	1.2	10	8.6	2.6	21.5
Insoluble Hemicellulose	3.6	1.1	9.1	9.8	2.9	24.6
Insoluble Pectin	0.9	0.3	2.2	2.9	0.9	7.3
Insoluble Lignin	2.1	0.6	5.1	4.6	1.4	11.6
Total fermentable carbohydrates	**22.2**	**6.7**	**55.2**	**44.9**	**13.6**	**112.6**

Extrapolating the fermentable carbohydrate content of the diet to the amount of fermentable carbohydrate added to the LFC and HFC media

The standard media originally used in the *in vitro* three-stage colonic model system at Reading University is assumed to provide an average amount of fermentable carbohydrate as the system was validated against sudden death victims who were likely to have a fairly average fermentable carbohydrate intake[15]. In the United Kingdom, where the system was developed and validated, the average fermentable carbohydrate intake (NSP, RS and oligosaccharides) is approximately 26 g/day[9,13,16]. Therefore, if the standard media represents an average fermentable carbohydrate intake then the amount of dietary fermentable carbohydrate consumed per day needs to be compared to the amount of fermentable carbohydrate which passes through the system in 24 hrs.

Intestinal transit times range from 20 to 120 hrs in humans[17]. A flow rate of 600 ml per 24 hrs will be used during the experiment as this fits within the "normal" intestinal transit time range and has been used in previous *in vitro* three-stage colonic model system experiments[18]. The standard media contains 65 g of fermentable carbohydrate per 5 L of media, therefore, at a flow rate of 600 ml per 24 hrs approximately 7.8 g of fermentable carbohydrate passes through the system every 24 hrs (5000 / 600 = 8.3; 65 / 8.3 = 7.8). It is assumed that 7.8 g of fermentable carbohydrate within the system is equivalent to 26 g/day of dietary fermentable carbohydrate. To extrapolate this to the LFC and HFC, which are based on low and high fermentable carbohydrate diets, 26 needs to be divided by 7.8 (26 / 7.8 = 3.3). The amount of fermentable carbohydrate in the diet is therefore 3.3 times higher then what passes through the system. A low fermentable carbohydrate diet is estimated to provide 22.2 g/day of fermentable carbohydrate which would be equivalent to 6.7 g per 24 hrs within the system (22.2 / 3.3 = 6.7). A high fermentable carbohydrate diet is estimated to provide 44.9 g/day of fermentable carbohydrate which would be equivalent to 13.6 g per 24 hrs within the system (44.9 / 3.3 = 13.6) (Table 4).

It is highly unlikely that the amount of fermentable carbohydrate running through the system every 24 hrs is equivalent to the amount of dietary fermentable carbohydrate consumed as the ingredients used within the media are likely to be more readily available to the bacteria as a source of energy as the fermentable carbohydrate ingredients used are not bound in a food matrix, are very pure and are highly fermentable forms of each fibre type.

To determine the amount of each type of fermentable carbohydrate needed in the LFC and HFC per 24 hrs (i.e. in 600 ml of media) the estimated amount of each fermentable carbohydrate from the two dietary patterns were divided by 3.3. For example, the estimated amount of RS in a low fermentable carbohydrate diet is 4.4 g/day, therefore, to extrapolate this to the amount needed in the LFC you need to divide 4.4 by 3.3 which equals 1.3 (Table 4).

A combination of fermentable carbohydrate ingredients (i.e. apple pectin, potato fibre) have been used to achieve the required amount of the differing fermentable carbohydrate types within the LFC and HFC. Pure ingredients were not always able to be sourced, such as insoluble cellulose. In this case fibre mixes such as potato and wheat fibre were used. Insoluble pectin was also not able to be sourced and an appropriate fibre mix was also not available so additional soluble pectin was used. A mix of apple and citrus pectin were used to provide the required soluble pectin (Table 5). Figure 1 illustrates the steps involved in developing the LFC and HFC media. The recipes for the LFC and HFC media are provided as Appendix 4-2 within this thesis.

Table 5. The fermentable carbohydrate ingredients used and the amount and types of fermentable carbohydrate each of the ingredients provides in the low fermentable carbohydrate medium (LFC) and high fermentable carbohydrate medium (HFC). ^ This information was carried over from Table 4. * This information carriers over to Appendix 4-2. # Insoluble pectin is not available, therefore, the insoluble and soluble pectin from Table 4 have been added together to give the pectin amount provided in this table. FOS: Fructo-oligosaccharide.

			The amount of fermentable carbohydrate added to the LFC and HFC media															
Fermentable carbohydrate ingredients	Amount added to 5L of media (g)*		Resistant starch (g)		Inulin (g)		FOS (g)		Hemi-cellulose (soluble) (g)		Pectin (g)#		Cellulose (insoluble) (g)		Hemi-cellulose (insoluble) (g)		Lignin (insoluble) (g)	
	LFC	**HFC**	**LFC**	**HFC**	**LFC**	**HFC**	**LFC**	**HFC**	**LFC**	**HFC**	**LFC**	**HFC**	**LFC**	**HFC**	**LFC**	**HFC**	**LFC**	**HFC**
Citrus Pectin	2.0	5.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	5.8	0.0	0.0	0.0	0.0	0.0	0.0
Apple Pectin	2.1	5.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.1	5.8	0.0	0.0	0.0	0.0	0.0	0.0
Lignin	5.1	11.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.1	11.6
Hi Maize Resistant starch	7.9	10.4	8.1	10.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Potato fibre	10.0	12.2	2.9	3.5	0.0	0.0	0.0	0.0	0.7	0.8	0.0	0.0	3.2	3.8	2.7	3.2	0.0	0.0
Wheat fibre	9.1	23.6	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.6	0.0	0.0	6.8	17.7	2.2	5.6	0.0	0.0
Arabinogalactan	5.0	6.4	0.0	0.0	0.0	0.0	0.0	0.0	5.0	6.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Xylan	4.2	15.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.2	15.8	0.0	0.0
Orafti HP Inulin	5.0	10.8	0.0	0.0	5.0	10.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Orafti P95 FOS	5.0	10.5	0.0	0.0	0.0	0.0	5.0	10.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total^	**55.4**	**112.8**	**11**	**14.3**	**5.0**	**10.8**	**5.0**	**10.5**	**5.9**	**7.8**	**4.1**	**11.6**	**10.0**	**21.5**	**9.1**	**24.6**	**5.1**	**11.6**

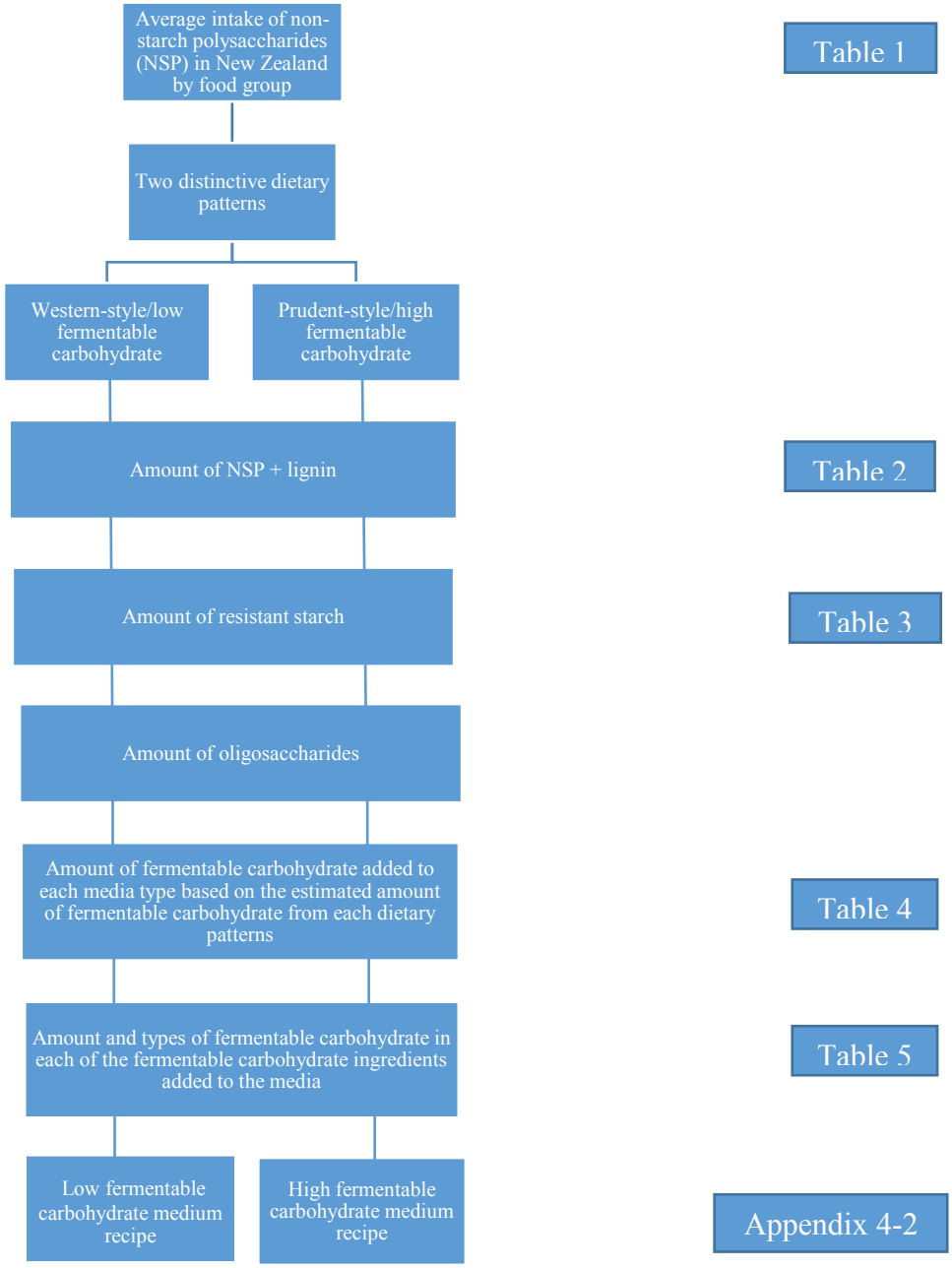

Figure 1. The steps involved in developing the LFC and HFC media including the tables which relate to each step.

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Appendix 4-2. *In vitro* three-stage colonic model system publication

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Variability in gut microbiota response to an inulin-type fructan prebiotic within an *in vitro* three-stage continuous colonic model system

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ABSTRACT

Gut microbiota have a major influence on human health and disease. Dietary interventions have been shown to beneficially modulate the gut microbiota and improve health; however, it appears there is large inter-individual variability in gut microbiota responsiveness to dietary interventions. We aimed to determine whether different fermentable carbohydrate content media, mimicking Western and Prudent style dietary patterns, influence gut microbiota response to an inulin-type fructan prebiotic using an *in vitro* three-stage continuous colonic model system. We demonstrated that the addition of an inulin-type fructan prebiotic led to shifts in the organic acid concentrations and bacterial taxa in both the low (LF) and high fermentable carbohydrate medium (HF) gut models. The shifts that occurred after the addition of the prebiotic significantly differed between the LF and HF gut models. Acetate increased in the HF gut models but decreased in the LF gut models ($p = 0.021$). The increases that occurred in Firmicutes ($p = 0.026$), *Lactobacillus* ($p = 0.045$) and *Mitsuokella* ($p = 0.012$) were significantly greater in the HF gut models. Megasphaera ($p = 0.033$) and an unknown genus of *Enterobacteriaceae* ($p = 0.011$) decreased in the LF gut models but increased in the HF gut models. The reduction in an unknown genus of *Lachnospiraceae* (other) was significantly greater in LF gut models ($p = 0.040$). Additionally, large inter- and intra-donor variability in gut microbiota responsiveness to the prebiotic were demonstrated. This study demonstrates that media with different fermentable carbohydrate contents caused variability in gut microbiota responsiveness to an inulin-type fructan prebiotic; however, these results will need to be replicated in an *in vivo* study.

1. Introduction

Numerous studies have suggested that diet plays a major role in the aetiology of certain diseases, such as cancer, obesity, type 2 diabetes and cardiovascular disease. Healthy dietary patterns rich in fruit, vegetables, legumes, wholegrains and fish, such as Prudent style (PS) or Mediterranean dietary patterns, are thought to protect against certain diseases while unhealthy dietary patterns, such as a Western style (WS) dietary pattern, which is high in sugar, animal protein and saturated fat, appear to increase the risk of developing disease (Fardet & Boirie, 2014; Hu et al., 2000; Satija et al., 2016). Links between the microbiota that reside in the human gastrointestinal tract (gut) and disease have also been postulated. Dysbiotic gut microbiota have been associated with obesity (Ley, Turnbaugh, Klein, & Gordon, 2006), inflammatory bowel disease (Walters, Xu, & Knight, 2014), type 2 diabetes (Wu et al., 2010) and colon cancers (Ou et al., 2013). It is, however, difficult to disentangle whether a dysbiotic gut microbiota are causally linked to these diseases or are simply the result of these diseases or the dietary patterns associated with disease. There are a number of other factors which are known to alter the composition or balance of the gut microbiota including genetics (Goodrich et al., 2016), age (Yatsunenko et al., 2012), gender (Mueller et al., 2005), antibiotic use (Jakobsson et al., 2010) and diet (De Filippo et al., 2010). Diet appears to be particularly influential with 57% of the variation in gut microbiota composition being attributed to dietary differences (Zhang et al., 2010). It has been shown that individuals who have distinctive dietary patterns also have correspondingly distinctive colonic microbial consortia. Vegans have a distinctive gut microbiota composition (*i.e.* lower counts of *Bacteroides* spp., *Bifidobacterium* spp., *Escherichia coli* and *Enterobacteriaceae*) when compared with individuals consuming an omnivorous diet (Zimmer et al., 2012). Individuals consuming a diet rich in plant-based foods have a lower Firmicutes:Bacteroidetes ratio and

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higher organic acid production, microbial diversity and richness when compared with individuals consuming a high-fat, high-sugar containing diet (De Filippo et al., 2010). Short and longer term dietary interventions have also been shown to alter the gut microbiota composition. One study, which investigated the influence of animal- and plant-based diets on microbial community structure, showed that the gut microbiota responded rapidly to the short-term shifts in macronutrient composition (David et al., 2013). Studies have been conducted to establish the influence of prebiotics on the gut microbiota. Prebiotics are defined as "selectively fermented ingredients that result in specific changes in composition and/or activity in the gastrointestinal microbiota, thus conferring benefit(s) upon human health" (Gibson et al., 2010). Inulin-type fructan prebiotics, such as inulin, fructo-oligosaccharides and oligo-fructose, have been widely studied and are known to stimulate the growth of bifidobacteria and increase butyrate production (a marker of microbial activity and substrate utilisation) which are often the main targets of prebiotic intervention studies (De Preter et al., 2008; Kolida, Meyer, & Gibson, 2007; Ramirez-Farias et al., 2009). The majority of studies to date have used targeted microbial enumeration techniques, such as fluorescence *in situ* hybridisation (FISH) and quantitative real-time polymerase chain reaction (qPCR), to investigate the influence of inulin-type fructan prebiotics on certain bacterial taxa (Ramirez-Farias et al., 2009; Ramnani et al., 2010). Very few studies have used high-throughput sequencing technology to demonstrate what influence inulin-type fructan prebiotics have on the entire microbial community (Holscher et al., 2015).

Even though the composition and functional capacity of the gut microbiota can be altered with dietary change, considerable variability in gut microbiota responsiveness to dietary interventions has been demonstrated. A recent *in vitro* batch fermentation study (Brahma et al., 2017) demonstrated that donors (n = 18) with differing dietary patterns had gut microbiota that responded in a distinctive manner when fermenting corn, oat, rye and wheat flour or bran. Donors with a healthy dietary pattern had less of a reduction in diversity, a higher butyrate production and a gut microbiota consortia that were better equipped at utilizing carbohydrates found in grains compared to the donors with an unhealthy dietary pattern (Brahma et al., 2017). Several *in vivo* studies have grouped participants as responders and non-responders based on whether the gut microbiota changed as a result of a dietary intervention. Individuals with higher microbial diversity (Salonen et al., 2014; Tap et al., 2015) or bifidobacteria concentrations (Bouhnik et al., 2007; de Preter et al., 2008) at baseline were shown to be less responsive to dietary interventions. Preliminary research suggests that habitual dietary fibre intake may also influence how a microbial community responds to a dietary intervention (Eid et al., 2015). It has been proposed that the development of novel therapeutic strategies designed to modulate the gut microbiota may prove helpful in the prevention, management and treatment of disease. However, given the variability in gut microbiota response to particular dietary interventions it is imperative we gain a better understanding of the factors that influence gut microbiota responsiveness in order to develop personalised dietary interventions that are tailored to modulate an individual's gut microbiota and thereby improve host health outcomes.

In this *in vitro* study, two media were designed to represent a WS and a PS dietary pattern (low fermentable carbohydrate medium [LF] and high fermentable carbohydrate medium [HF]; respectively) to mimic the background fermentable carbohydrate amounts and types commonly consumed by humans. The primary aim of this study was to determine what influence different fermentable carbohydrate content media had on the responsiveness of the gut microbiota to an inulin-type fructan prebiotic using an *in vitro* three-stage continuous colonic model system ("gut model"). Donor-specific differences in responsiveness were also considered, as inter-individual variability in responsiveness has been reported in human studies. Variability in gut microbiota responsiveness to dietary change has, to the best of our knowledge, not previously been studied within an *in vitro* gut model. The *in vitro* three-stage continuous colonic model system is useful as it has been validated against the colonic contents of sudden death victims to simulate the physiologically relevant conditions found in the human colon (Macfarlane, Macfarlane, & Gibson, 1998). The *in vitro* gut model can, therefore, be used to test research hypotheses before progressing to animal or human studies. A number of studies have utilised the *in vitro* three-stage continuous colonic model system to analyse the impact dietary interventions have on the compositional and functional capacity of colonic microbial communities (Costabile et al., 2015; Probert, Apajalahti, Rautonen, Stowell, & Gibson, 2004; Shen, Zhao, & Tuohy, 2012).

2. Methods

2.1. *In vitro* three-stage continuous colonic model system

The validated *in vitro* three-stage continuous colonic model system simulates the physiologically relevant conditions found in the human colon. The *in vitro* gut model consists of three vessels connected in series, which mimic the proximal (V1), transverse (V2) and distal (V3) colon (Macfarlane et al., 1998). The temperature of the vessels were maintained at 37 °C, with a pH of 5.4–5.6 (V1), 6.1–6.3 (V2) and 6.7–6.9 (V3) and were kept anaerobic by sparging with oxygen free nitrogen gas. The contents within each vessel were stirred to keep them homogenous. Media flowed from a 5 L media vessel into V1 *via* peristaltic pump with V2 and V3 being fed from the overflow of V1 and V2 a respectively. Media which overflowed from V3 passed into a waste bottle. The transit time for the gut model was 36 h (flow rate of 25 mL/h).

Two different media were used; low fermentable carbohydrate (LF) and high fermentable carbohydrate medium (HF). The basal medium originally developed by Macfarlane and co-authors (Macfarlane et al., 1998) was modified to produce the LF and HF. The non-carbohydrate media ingredients (*i.e.* peptone water, casein, magnesium sulfate) were not altered; however, the fermentable carbohydrate media ingredients (*i.e.* pectin, resistant starch, xylan) were altered to allow the LF to represent a WS dietary pattern and the HF to represent a PS dietary pattern, which are low and high in fermentable carbohydrates; respectively. Dietary pattern information (Hu et al., 1999; Hu, 2002; Schulze, Fung, Manson, Willett, & Hu, 2006) and food composition tables, which provide information on the inulin-type fructan (Anderson et al., 2015), resistant starch (Murphy, Douglass, & Birkett, 2008) and non-starch polysaccharide (Marlett & Cheung, 1997) content of commonly consumed foods, were used to define the types, amounts and proportions of each fermentable carbohydrate ingredient added to the LF and HF (Table S1).

In total, six gut models (three LF gut models and three HF gut models) were used; however, at any one time only three *in vitro* gut models (each containing three vessels per gut model) were run simultaneously (Fig. 1). The first three gut models contained the HF and the second three gut models contained the LF. All three vessels within each *in vitro* gut model were inoculated with 100 mL of fresh faecal slurry. The fresh faecal slurry was prepared by diluting (1 in 5) fresh faeces with phosphate buffered saline and 0.05% L-cysteine hydrogen chloride and filtering it through a sterile BagPage® filter. A baseline sample was taken once the gut models were inoculated. After inoculation the gut models were run as a batch culture for 24 h to acclimatise the bacteria prior to initiating media flow. The gut models were then run for 8 full turnovers (one turnover is 900 mL- the total volume of each gut model) to allow for steady state one (SS1) to be reached (Costabile et al., 2015). Steady state was deemed to be reached when daily measured acetate, butyrate and propionate were assessed as being stable (percentage coefficient of variation < 10%) for at least 2 consecutive days. Once SS1 had been reached, a pre-intervention sample was taken and the prebiotic was added to the media. The prebiotic (Beneo Orafti® Synergy 1; 50:50 inulin:fructo-oligosaccharide mix) was

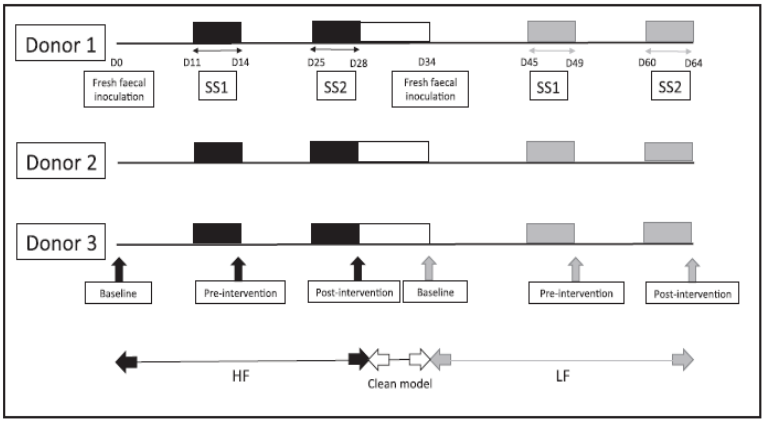

Fig. 1. Diagram of the study design of the *in vitro* three-stage continuous colonic model system ("gut model"). Fresh faeces from each donor was used to inoculate each gut model twice through-out the experiment. The gut models were cleaned and autoclaved between each media type run. Samples were taken at baseline (after inoculation), pre-intervention (after steady state 1 [SS1] was reached) and post-intervention (after steady state 2 [SS2] was reached) for the high fermentable carbohydrate medium (HF) gut models and low fermentable carbohydrate medium (LF) gut models. Abbreviation: D- day.

added to the media in a proportional amount to that commonly used in human prebiotic intervention studies. The gut model was run for an additional 8 turnovers to allow for steady state two (SS2) to be reached. Once SS2 was reached a post-intervention sample was taken (Fig. 1). The baseline, pre-intervention and post-intervention samples were immediately stored as 1 mL aliquots at −20 °C.

2.2. Faecal donors

Three faecal donors were recruited for this study (Health and Disability Ethics Committee: 13/CEN/144) as this number of faecal donors have been used in past *in vitro* gut model experiments (Costabile et al., 2015; Shen et al., 2012). A questionnaire was used to collect information from faecal donors relating to age, gender, ethnicity, health status, weight and height. A validated food frequency questionnaire was used to determine whether faecal donors had low, moderate or high habitual dietary fibre intakes (Healey et al., 2016). Healthy faecal donors of the same gender and ethnicity, and similar BMI and dietary fibre intakes were used. The 3 faecal donors had not experienced any recent weight loss or change in dietary intake over the past year and had not taken laxatives, gastric motility medications, prebiotic or probiotic containing foods, drinks or supplements in the past month or antibiotics in the past 6 months.

Each donor provided a fresh faecal donation on two separate occasions; one for the HF gut model run and one for the LF gut model run (Fig. 1). Faecal donations were collected in a sterile container and were immediately stored in an anaerobic bag which contained an anaerobic sachet until the donation was processed. Processing of the faecal donation occurred within 30 min of the sample being voided.

2.3. Dietary intake analysis

Each donor completed a 3-day diet record during the 3 days leading up to each faecal donation. Therefore, six days' worth of dietary intake information was collected from each donor. Nutrient and food group analysis was conducting using FoodWorks version 8.0 software (Xyris Software Pty Ltd). The Australian database in FoodWorks was used (AusBrands and AusFoods 2015 data sources) so nutrient intake and food group analysis could be conducted.

2.4. Media vessel sampling

The media vessels for each gut model had to be changed every 8 days to ensure a constant supply of media was provided. When a new media vessel was connected the sterility of the previous media vessel was assessed to ensure the media vessel had not become contaminated during the running of the experiment. The samples taken from the used media vessels were analysed for total bacteria concentrations using qPCR (method outlined below). Samples were sent for 16S rRNA bacterial gene sequencing (method outlined below) if contamination was suspected based on the qPCR results.

2.5. Bacterial DNA extraction

Samples were thawed at 4 °C and 1 mL aliquots were centrifuged at 14,000 × *g* for 10 min. The supernatant was removed, the pellet was resuspended in 750 µL of bead solution and the sample was transferred to a PowerLyzer® glass bead tube. Bacterial DNA was extracted from the samples using the MoBio PowerLyzer® Powersoil DNA® isolation kit according to the manufacturer's instructions (from step 4 of the protocol) with minor alterations. A FastPrep-24™ 5G (MP Biomedicals) was used to homogenise the samples at a speed of 5.5 m/s for four 90 s cycles with a 60 s break between each cycle. The DNA was eluted in 10 mM Tris. NanoDrop 1000 spectrophotometry was used to quantify the DNA concentration.

2.6. 16S rRNA bacterial gene sequencing and bioinformatics

The extracted DNA was used as a template for initial PCR amplification of the V3-V4 hyper-variable region of the 16S rRNA bacterial gene using the barcoded fusion primers 16SR_V4 (5′-CAAGCAGAAGACGGCATACGAGAT-barcode-AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT-3′) and 16SF_V3 (5′-AATGATACGGCGACCACCGAGATCTACAC-barcode-TATGGTAATTGGCCTACGGGAGGCAGCAG −3′), which also contain adaptors for downstream Illumina MiSeq sequencing. Each sample was amplified with a pair of unique (8 base) barcoded primers. The PCR reagents used were Invitrogen AccuPrime™ Pfx SuperMix (part number 12344-040) (17 µL), 10 µM 16SR_V4 Primer (1 µL), 10 µM 16SF_V3 Primer (1 µL) and Ambion nuclease-free water (catalog number: AM9932) to normalise to 5 ng/µL (1 µL). The following PCR conditions were used; a hold at 95 °C for 2 min followed by 30 cycles of 95 °C for 20 s (denaturation), 55 °C for 15 s (annealing), 72 °C for 5 min (extension) finishing with a hold at 72 °C for 10 min. Library clean-up utilised an Invitrogen SequalPrep Normalisation Plate Kit (Thermo Fisher). Eighteen microlitres of the PCR product was used in the library clean-up with an elution volume of 12 µL. A Qubit DNA HS assay was used to measure the library concentration and a Bioanalyzer DNA HS assay was used for library sizing. The libraries were pooled by equal volume. Sequencing was undertaken on an Illumina MiSeq machine, using 2 × 250 base pair (bp) read length, at the Massey Genome Service (Massey University, Palmerston North, New Zealand). The data obtained from

Illumina MiSeq sequencing were analysed using Quantitative Insights Into Microbial Ecology (QIIME) (Caporaso et al., 2010). Paired-end assembler for DNA sequencing (PANDASeq) was used to assemble the forward and reverse reads into continuous sequences ensuring at least a 50 bp overlap with a minimum of 350 bp and a maximum of 500 bp length (Masella, Bartram, Truszkowski, Brown, & Neufeld, 2012). Chimera filtered sequences and reads were clustered into operational taxonomic units (OTUs) based on an identity threshold value of 97% using USEARCH 6.1 and UCLUST (Edgar, 2010). Sequence alignment with the Greengenes core reference database (version 13_5) was carried out using PyNAST (DeSantis et al., 2006). The RDP Naïve Bayesian classifier was used to provide taxonomic assignment (Wang, Garrity, Tiedje, & Cole, 2007).

2.7. Quantitative PCR

Total bacteria and bifidobacteria concentrations were determined using the LightCycler® 480 system (Roche Life Science). Total bacteria standard template DNA was prepared using *Escherichia coli* (Nissle) and bifidobacteria standard template DNA was prepared using *Bifidobacterium bifidium* (DSM20082). Bacterial DNA was extracted using the MoBio PowerLyzer® Powersoil DNA® isolation kit as described above. The following primers were used: Total bacteria- forward (TCCTACGGGAGGCAGCAGT) and reverse (GGACTACCAGGGTATCTAATCCTGTT) primers (Nadkarni, Martin, Jacques, & Hunter, 2002); bifidobacteria- forward (GGGTGGTAATGCCGGATG) and reverse (CCACCGTTACACCGGGAA) primers (Kok et al., 1996). Quantitative PCR was performed in triplicate with 10 µL of SyBr Green Master (Roche Life Science), 1 µL of each of the forward and reverse primers (5 µM), 7 µL of PCR grade water and 1 µL of template DNA. The conditions used for total bacteria PCR amplification were initial denaturation at 95 °C for 10 min followed by 40 cycles of denaturation (95 °C for 15 s), annealing (60 °C for 1 min), extension (72 °C for 20 s) and finished with a melt curve (95 °C for 30 s, 65 °C for 1 min and 95 °C continuous- 5 per °C acquisitions). The conditions used for bifidobacteria PCR amplification were initial denaturation at 95 °C for 5 min followed by 40 cycles of denaturation (95 °C for 1 min), annealing (66 °C for 45 s), extension (72 °C for 1 min) and finished with a melt curve (95 °C for 30 s, 65 °C for 1 min and 95 °C continuous- 5 per °C acquisitions).

2.8. Organic acid analysis

Organic acids for steady state and at pre- and post-intervention were measured by gas chromatography (GC) using a modified GC method (Richardson, Calder, Stewart, & Smith, 1989). One millilitre of fermenta was transferred into a 1.5 mL Eppendorf tube and centrifuged at 14,000 × *g* for 10 min at room temp. Then 100 µL of supernatant was transferred into a 2 mL Eppendorf for immediate steady state analysis or frozen at −80 °C until required for pre- and post-intervention quantification. The 100 µL of supernatant in 2 mL Eppendorf tubes were diluted with 400 µL of 0.01 M phosphate buffered saline containing 2-ethylbutyric acid (6.25 mM) as an internal standard, with a final internal standard concentration of 5 mM. The sample was then acidified with 250 µL concentrated hydrochloric acid and 1000 µL diethyl ether added. After mixing, to allow acids to transfer to the diethyl ether phase, the sample was centrifuged at 10,000 × g for 5 min (4 °C). The diethyl ether phase was derivatised immediately for steady state or stored at −80 °C for pre- and post-intervention quantification. In a capped GC vial 100 µL of the diethyl ether phase was derivatised with 20 µL N-*tert*-butyldimethylsilyl-N-methyltrifluoroacetamide with 1% tert-butyldimethylchlorosilane (MTBSTFA + TBDMSCI, 99:1; Sigma-Aldrich) by heating to 80 °C in a water bath for 20 min. Samples were then transferred to a 200 µL glass insert, placed back into the GC vial and re-capped. Steady state samples, measuring acetate, butyrate and propionate, were injected immediately onto the GC, whilst pre- and post-intervention samples were left for 48 h at room temperature before analysis to allow complete derivatisation of all acids quantified (*i.e.* lactate). Analysis was performed on a Shimadzu capillary gas chromatograph system (GC-2010 Plus, Tokyo, Japan) equipped with a flame ionization detector (FID) and fitted with a Restek column (SH-Rtx-1, 30 m × 0.25 mm ID × 0.25 µm) (Shimadzu, USA). The carrier gas was helium with a total flow rate of 21.2 mL/min and pressure of 131.2 kPa. Make up gas was nitrogen. The temperature programme began at 70 °C increasing to 115 °C at 6 °C/min, with a final increase to 300 °C at 60 °C/min, holding for 3 min. Flow control mode was set to linear velocity; 37.5 cm/s. Injector temperature was 260 °C and detector temperature was 310 °C. Samples were injected (1 µL) with a split injection (split ratio 1:10). The GC instrument was controlled and data processed using Shimadzu GC Work Station LabSolutions Version 5.3. Steady state analysis was determined by peak area to internal standard peak area ratio of the acids acetate, propionate and butyrate. Pre- and post-intervention sample analysis was determined by quantifying peak area to internal standard peak area ratio against organic acid standard curves (acetate, propionate, butyrate and lactate) containing 2-ethylbutyric acid (5 mM) as an internal standard. The standards were prepared for derivatisation alongside the pre- and post-intervention diluted fermenter samples. Data acquired provided a final sample result of µmol organic acid/mL fermenta.

2.9. Statistical analysis

Gut microbiota and organic acid data were analysed using paired *t*-tests. A non-parametric test was not used to analyse the data due to the small sample size. A p value of < 0.05 was considered significant. Statistical analysis was carried out using R version 3.3.2 (R Core Team, 2016 Core Team, 2016). PCoA graphs were generated using unweighted UniFrac distances within QIIME (Caporaso et al., 2010).

3. Results

3.1. Donor characteristics and dietary intake

Donor characteristics may influence how the gut microbiota respond to a dietary intervention, therefore, differences and similarities in characteristics between donors whether determined. All donors (n = 3) were healthy, the same gender and ethnicity, and of a normal BMI (Table 1). Food group and nutrient analysis helps provide insight into the differences and similarities in habitual dietary intake between donors. Differences in habitual dietary intake may also influence gut microbiota responsiveness and interestingly nutrient intakes and food group serves did differ between donors (Table 1). The New Zealand Ministry of Health has implemented guidelines for recommended food groups serves to help encourage good health (Ministry of Health, 2015). Donor 1 (D1) and donor 3 (D3) meet the recommended serves per day for fruits and vegetables. None of the donors meet the recommended serves per day for grains and donor 2 (D2) and D3 did not meet the recommendation to consume more wholegrain than refined grain serves. All donors were very close to or exceeded the recommended dietary fibre intake of 25–30 g/day. D3 met the dairy and protein (legumes, nuts, seeds, meat, *etc.*) serve recommendations, however, D1 and D2 had lower than recommended intakes of these food groups.

3.2. Baseline differences in gut microbiota

Each donor provided two fresh faecal donations: one to inoculate the LF gut model and one to inoculate the HF gut model (Fig. 1). Baseline samples were analysed, after inoculation, to determine whether there were differences in gut microbiota composition between the first and second faecal void for each donor. The principal co-ordinate analysis (PCoA) graph demonstrates that the baseline samples for each donor tend to cluster together suggesting that the bacterial community used to inoculate the LF and HF gut models were similar for each donor (Fig. 2a).

Table 1
Donor characteristics, nutrient intakes and food group serves.

	Donor 1	Donor 2	Donor 3
Donor characteristics			
Gender	Female	Female	Female
Age (years)	33	51	29
Ethnicity	NZ European/ Maori	NZ European/ Maori	NZ European/ Maori
BMI (kg/m^2)	21.1	21.2	21.9
Smoking status	Non-smoker	Non-smoker	Non-smoker
Health status	Healthy; no chronic diseases	Healthy; no chronic diseases	Healthy; no chronic diseases
Medications	No regular	No regular	No regular
Nutrient intake			
Energy (kJ/day)	9630.0	8257.3	9383.1
Protein (g/day)	52.6	70.4	109.2
Total fat (g/day)	108.4	86.4	97.7
Saturated fat (g/day)	46.0	32.9	29.4
Polyunsaturated fat (g/day)	16.6	13.6	15.9
Monounsaturated fat (g/day)	35.6	32.7	43.6
Carbohydrate (g/day)	258.0	193.3	196.6
Sugars (g/day)	141.0	93.7	88.5
Dietary fibre (g/day)	35.0	24.4	41.2
Alcohol (g/day)	0.9	13.7	6.9
Energy from protein (%)	9.3	14.5	19.7
Energy from fat (%)	41.3	38.7	38.8
Energy from carbohydrate (%)	44.4	38.7	34.6
Energy from fibre (%)	2.9	2.4	3.6
Food group serves per day			
Total grains	5.5	5.4	5.8
Refined grains	2.7	2.9	4.4
Wholegrains	2.9	2.5	1.4
Fruit	2.8	1.1	2.0
Vegetables	3.6	2.8	7.3
Meat	0.0	0.6	1.4
Nuts & seeds	0.7	0.5	0.6
Legumes	0.2	0.3	0.5
Dairy	0.9	2.0	2.2

3.3. Media vessel contamination

Samples were taken, for subsequent analysis, from the used 5 L media vessels whenever new 5 L media vessels were connected, to monitor the sterility of the media throughout the running of the experiment. Total bacteria qPCR analysis of the used media samples revealed that one sample from the donor 2 LF gut model media vessel, taken just prior to when the post-intervention sample was collected, had a high total bacteria concentration (3.6×10^8 gene copies/mL) when compared with the other media vessel samples, which were all below the limit of detection. This result was consistent with bacterial contamination and was confirmed with 16S rRNA bacterial gene sequencing which demonstrated that the high total bacteria concentration media vessel sample had 99.5% relative abundance of an unknown genus of *Enterobacteriaceae*. Based on the sequencing results it was difficult to ascertain whether the bacteria caused a perturbation of the entire bacterial community within the contaminated gut model. However, as medium contamination was confirmed the post-intervention data from donor 2 LF gut model, for all three vessels, were not included in the analysis (Table S2).

3.4. Prebiotic driven changes in gut microbiota and organic acid concentrations

Differential prebiotic driven changes in gut microbiota composition and organic acid concentrations were observed between the LF and HF gut models. Changes in organic acid concentrations, in response to the prebiotic, differed significantly between the LF and HF gut models for acetate only. Acetate increased in V1 of the HF gut models but decreased in V1 of the LF gut models (+16.8 *versus* −0.9 μmol/mL; respectively [p = 0.021]) (Table 2a and b). There were significant differences in how certain bacterial taxa responded to the prebiotic between the LF and HF gut models. In V1, the increase that occurred in Firmicutes as a result of the prebiotic was significantly greater in the HF than the LF gut models (+9.3% *versus* +2.2%; respectively [p = 0.026]). Also in V1, an unknown genus of *Enterobacteriaceae* decreased as a result of the prebiotic in the LF gut models but increased in the HF gut models (−0.6% *versus* +0.5%; respectively [p = 0.011]). In V2, *Lactobacillus* increased in the HF gut models secondary to the prebiotic but did not change in the LF (+0.4% *versus* 0.0%; respectively [p = 0.045]). Also in V2, *Megasphaera* increased in the HF gut models but decreased in the LF gut models (+3.0% *versus* −1.0%; respectively [p = 0.033]). Lastly, in V3 the decrease that occurred in an unknown genus of *Lachnospiraceae* (other) in the LF gut models, as a result of the addition of the prebiotic, was significantly greater than the decrease that occurred in the HF gut models (−2.9% *versus* −0.3%; respectively

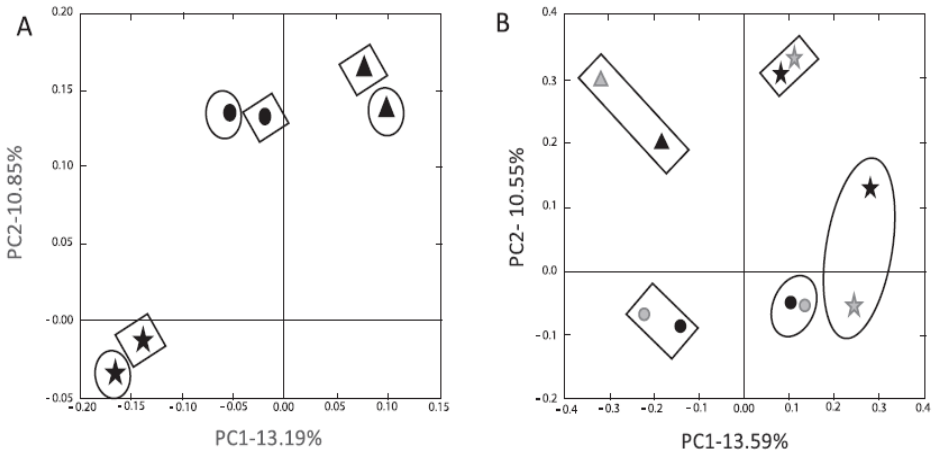

Fig. 2. Principal co-ordinate analysis graphs (unweighted UniFrac distances) illustrating the differences and similarities in the bacterial communities between donors and media types at baseline (post inoculation) (**a**) and within vessel 1 comparing the pre- and post-intervention samples (**b**). LF data from donor 2 was not included in Fig. 2b. Samples that cluster together are more similar in bacterial composition and samples that are further apart have a more distinctive bacterial composition. Key: Donor 1 ★, Donor 2 ▲ and Donor 3 ●. High fermentable carbohydrate medium □ and low fermentable carbohydrate medium ○. Black shapes- pre-intervention samples (or baseline samples in Fig. 2a), Grey shapes- post-intervention samples. Abbreviation: PC- principal co-ordinate.

Table 2
Pre- and post-intervention organic acid concentrations, phylum and genus level relative abundance for vessels 1, 2 and 3 of the high fermentable carbohydrate medium gut models **(a)** and low fermentable carbohydrate medium gut models **(b)**[a].

(a)	High fermentable carbohydrate											
	Vessel 1				Vessel 2				Vessel 3			
	Pre		Post		Pre		Post		Pre		Post	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Organic acid (μmol/mL)												
Acetate	23.0*	18.2	39.8*	21.3	46.2	20.4	54.2	13.7	57.2	24.4	68.5	9.5
Propionate	21.5	18.4	17.5	5.7	36.8	15.9	34.7	17.2	38.8	15.0	36.5	18.7
Butyrate	36.9	7.4	45.2	36.1	55.1	6.4	74.8	24.8	57.2	10.6	73.9	0.9
Lactate	0.3	0.0	23.1	25.9	0.3	0.0	0.3	0.0	0.3	0.0	0.3	0.0
Phylum (% relative abundance)												
Actinobacteria	16.3	22.8	19.4	27.3	11.1	14.9	14.9	20.2	12.7	15.6	15.6	21.0
Bacteroidetes	17.3	0.5	3.7	2.7	25.9	26.9	26.9	28.6	20.3	10.6	20.9	24.7
Firmicutes	64.6*	22.9	73.9*	28.4	61.4	56.8	56.8	8.5	63.8	5.9	61.0	2.0
Proteobacteria	1.4	0.6	2.3	1.8	1.0	0.7	0.7	0.5	1.3	0.8	0.7	0.2
Genus (% relative abundance)												
Bifidobacterium	7.0	9.7	12.0	16.8	4.2	5.7	9.0	12.5	3.5	4.8	8.4	11.6
Collinsella	6.9	9.7	6.2	8.7	4.6	6.5	4.0	5.7	5.7	8.1	4.3	6.0
Bacteroides	8.6	11.7	0.7	0.6	17.3	15.8	17.0	19.7	14.9	15.9	13.9	17.8
Prevotella	8.6	12.2	3.0	3.3	3.3	4.7	8.5	7.4	1.9	2.7	5.9	5.8
Lactobacillus	0.1	0.1	1.7	1.4	0.1*	0.0	0.5*	0.0	0.1	0.0	0.5	0.1
Lachnospiraceae, unknown genus (other)	2.7	2.9	0.9	0.5	2.6	0.2	2.1	0.3	3.6*	2.8	3.3*	0.0
Lachnospiraceae, unknown genus	33.1	17.2	22.5	15.3	17.0	3.2	10.9	2.6	15.2	0.2	9.8	2.7
Coprococcus	1.0	1.3	0.2	0.2	0.9	0.9	0.6	0.4	1.1	1.1	0.7	0.5
Dorea	0.2	0.1	0.3	0.4	0.6	0.5	1.0	0.7	0.8	0.2	1.3	1.0
Ruminococcaceae, unknown genus	2.0	0.6	6.7	9.4	2.8	0.5	6.2	5.8	5.5	1.2	5.7	2.1
Ruminococcus	0.1	0.0	0.0	0.0	9.6	4.7	3.9	0.7	6.9	0.6	5.3	1.1
Dialister	3.8	4.2	9.3	11.2	6.6	0.2	7.9	5.3	7.4	0.6	7.9	0.7
Megasphaera	7.4	3.2	8.0	5.4	7.7*	5.9	10.7*	6.5	7.2	3.6	7.7	0.5
Mitsuokella	2.8	3.9	13.3	16.0	0.3	0.2	1.9	1.1	0.3*	0.0	2.8*	0.0
Enterobacteriaceae, unknown genus	0.5*	0.5	1.0*	0.0	0.1	0.1	0.3	0.1	0.2	0.1	0.3	0.2
(b)	Low fermentable carbohydrate											
Organic acid (μmol/mL)												
Acetate	25.3*	3.9	24.4*	1.5	28.0	3.2	35.5	3.3	36.5	1.3	45.6	5.6
Propionate	24.4	1.0	42.3	4.6	29.5	0.4	48.4	5.3	30.4	0.2	50.9	8.6
Butyrate	42.9	9.5	62.6	7.8	54.5	11.8	74.2	8.0	55.0	5.4	78.2	11.9
Lactate	0.3	0.0	0.3	0.0	0.3	0.0	0.3	0.0	0.3	0.0	0.3	0.0
Phylum (% relative abundance)												
Actinobacteria	7.0	9.1	1.6	1.2	7.4	9.3	3.1	3.2	9.2	11.4	4.6	5.4
Bacteroidetes	22.8	0.2	27.4	13.2	11.4	3.2	27.9	14.0	9.0	1.0	20.1	3.6
Firmicutes	67.4*	7.7	69.4*	13.6	76.8	10.2	67.1	16.0	76.9	10.0	73.0	7.8
Proteobacteria	2.4	1.2	1.3	0.7	4.0	2.1	1.6	1.1	4.4	2.4	1.8	1.2
Genus (% relative abundance)												
Bifidobacterium	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Collinsella	2.2	3.2	0.5	0.7	1.8	2.6	0.9	1.3	2.4	3.3	1.7	2.4
Bacteroides	1.4	1.2	0.2	0.1	2.1	1.4	2.1	1.7	1.9	0.8	12.3	0.9
Prevotella	18.5	2.3	27.2	13.3	7.3	2.6	25.4	16.1	4.3	0.3	6.3	2.1
Lactobacillus	0.0	0.0	0.0	0.0	0.0*	0.0	0.0*	0.0	0.0	0.0	0.0	0.0
Lachnospiraceae, unknown genus (other)	2.7	0.7	1.6	1.5	3.4	2.1	1.1	0.7	4.0*	2.8	1.1*	0.2
Lachnospiraceae, unknown genus	10.7	6.8	9.1	1.6	9.3	6.6	5.1	2.6	8.6	4.0	4.6	3.6
Coprococcus	0.3	0.0	6.3	8.7	0.5	0.1	8.5	11.8	0.7	0.4	13.3	18.5
Dorea	5.3	1.7	1.9	0.1	7.6	4.7	2.3	0.2	8.2	5.5	3.7	0.0
Ruminococcaceae, unknown genus	11.6	0.3	15.8	4.3	15.2	0.6	14.4	5.9	11.5	1.6	11.4	3.2
Ruminococcus	7.1	3.9	6.2	3.7	5.3	2.7	4.4	3.2	3.0	1.3	2.7	0.9
Dialister	13.4	6.5	11.4	6.4	13.2	5.4	10.2	5.3	11.4	4.2	9.9	2.7
Megasphaera	2.2	2.6	1.6	1.8	5.0*	1.6	4.0*	2.6	7.2	0.7	3.9	2.3
Mitsuokella	0.0	0.0	0.1	0.1	0.0	0.0	0.2	0.0	0.0*	0.0	0.1*	0.0
Enterobacteriaceae, unknown genus	1.5*	0.0	0.9*	0.5	0.9	0.1	0.8	0.0	0.8	0.2	0.7	0.4

[a] Abbreviation: SD- standard deviation, Pre- pre-intervention, Post- post-intervention. Paired *t*-test of 16S rRNA bacterial gene sequencing data. The change in mean values from pre- to post-intervention are significantly different between the LF and HF gut models.
* $p < 0.05$. Only data from donor 1 and 3 are included in the analysis.

[p = 0.040]). Also, the increase that occurred in *Mitsuokella* was significantly greater in the HF gut models than the LF gut models (+2.5% *versus* +0.1%; respectively [p = 0.012]) (Table 2a and b).

Despite the prebiotic driven changes that occurred in some of the other bacterial taxa and organic acids, the large variability in donor responses rendered a lot of the changes as statistically non-significant. For example, lactate concentrations in V1 of the HF gut models increased from 0.3 to 23.1 μmol/mL but did not change in V1 of the LF gut models. The large variability in responses between donors for the HF gut models meant the difference was not significant. *Bifidobacterium* relative abundance increased more than 2 fold in V2 of the HF gut models and did not change in V2 of the LF gut models; however, again the difference was not significant (Table 2a and b). Therefore, we examined the pre-intervention differences between the two media types and change in gut microbiota response to the prebiotic on an individual donor basis.

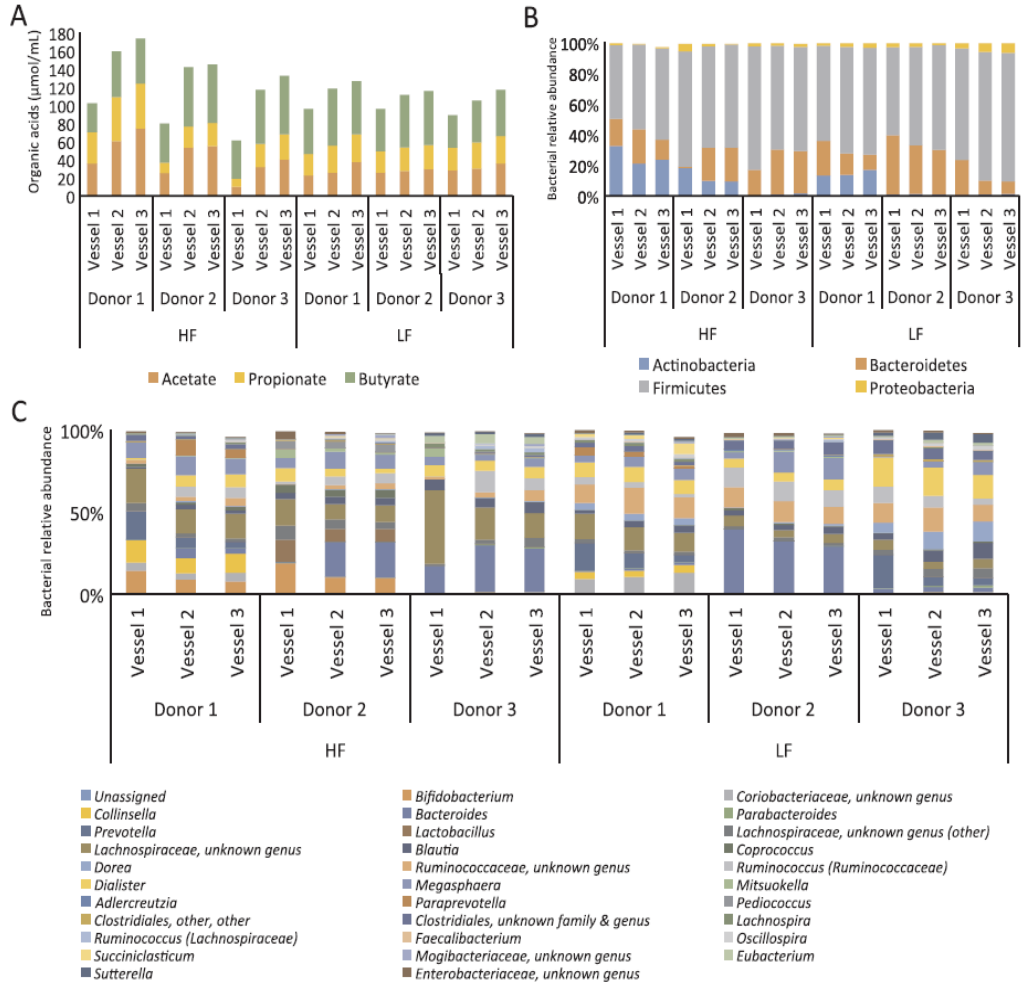

Fig. 3. - Stacked column graphs illustrating the inter- and intra-donor differences in pre-intervention organic acid concentrations (**a**), phylum level relative abundance (**b**) and genus level relative abundance (**c**) (16S rRNA bacterial gene sequencing) for each vessel (1, 2 and 3) and media type (high fermentable carbohydrate medium [HF] and low fermentable carbohydrate medium [LF]).

3.5. Individual donor pre-intervention differences in gut microbiota and organic acid concentrations between media types

Examining the results on an individual donor basis revealed that there were considerable inter-donor differences in pre-intervention organic acid concentrations for the LF and HF gut models (Fig. 3a). For example, D3-HF gut model produced less acetate in all vessels (V1- 10.2, V2- 31.8 and V3–39.9 µmol/mL) when compared with D1-HF (V1- 35.9, V2- 60.6 and V3–74.4 µmol/mL) and D2-HF (V1- 25.2, V2- 53.0 and V3–55.1 µmol/mL) gut models (Table 3). Less variability in organic acid production between donors were observed in the LF gut models (Table 3). There were also noticeable intra-donor differences in organic acid concentrations when comparing the HF with the LF gut models. For example, acetate, propionate and butyrate production in V1 of the D1-HF gut model were 35.9, 34.6 and 31.7 µmol/mL respectively, but were 22.5, 23.7, 49.6 µmol/mL; respectively, in V1 of the D1-LF gut model (Table 3).

Pre-intervention inter-donor differences were also observed in gut microbiota composition at a phylum and genus level. Marked differences in phylum level relative abundance between donors for the LF and HF gut models occurred (Fig. 3b). For example, in V1 of the D1-HF and D2-HF gut models there was a much higher relative abundance of Actinobacteria (32.4% and 18.1%; respectively) than in the D3-HF gut model (0.2%) (Table 3). There were also noticeable intra-donor differences in phylum level relative abundance between the LF and HF gut models. For example, the relative abundance of Actinobacteria and Firmicutes clearly differed in V1 between the D1-LF (13.4% and 62.0%; respectively) and D1-HF (32.4% and 48.4%; respectively) gut models (Table 3). At a genus level there were also inter- and intra-donor distinctions between the LF and HF gut models (Fig. 3c). The D1-HF gut model was characterised by a predominance of *Bifidobacterium* (V1- 13.8%, V2- 8.2% and V3- 6.9%), *Collinsella* (V1- 13.7%, V2- 9.2% and V3- 11.4%), *Prevotella* (V1-17.2%, V2- 6.6% and V3- 3.8%) and an unknown genus of *Lachnospiraceae* (V1- 21.0%, V2- 14.7% and V3- 15.3%); D2-HF gut model by a predominance of *Bifidobacterium* (V1- 17.8%, V2- 9.5% and V3- 9.1%), *Lactobacillus* (V1- 13.5%, V2- 7.8% and V3- 10.4%) and an unknown genus of *Lachnospiraceae* (V1- 16.1%, V2- 9.3% and V3- 10.4%) and D3-HF gut model by a predominance of *Bacteroides* (V1- 16.9%, V2- 28.5% and V3- 26.1%) and an unknown genus of *Lachnospiraceae* (V1- 45.3%, V2- 19.3% and V3- 15.1%) (Table 3). The predominant bacteria in each LF gut model also differed between donors (Table 3).

Table 3
Pre-intervention differences in organic acid concentrations, phylum and genus level relative abundance between donors in vessel 1 (V1), 2 (V2) and 3 (V3) for the low and high fermentable carbohydrate medium.

		High fermentable carbohydrate			Low fermentable carbohydrate		
	Donor	V1	V2	V3	V1	V2	V3
Organic acids (μmol/mL)							
Acetate	1	35.9	60.6	74.4	22.5	25.7	37.4
	2	25.2	53.0	55.1	25.9	27.8	29.1
	3	10.2	31.8	39.9	28.0	30.2	35.5
Propionate	1	34.6	48.1	49.4	23.7	29.8	30.3
	2	11.5	23.6	25.5	23.4	25.8	27.3
	3	8.5	25.6	28.2	25.1	29.2	30.6
Butyrate	1	31.7	50.6	49.7	49.6	62.8	58.8
	2	43.1	65.2	64.7	46.8	57.7	59.3
	3	42.1	59.6	64.7	36.2	46.1	51.2
Lactate	1	0.3	0.3	0.3	0.3	0.3	0.3
	2	3.2	0.3	0.3	0.3	0.3	0.3
	3	0.3	0.3	0.3	0.3	0.3	0.3
Phylum (% relative abundance)							
Actinobacteria	1	32.4	21.3	23.7	13.4	14.0	17.3
	2	18.1	9.7	9.4	0.9	1.2	1.0
	3	0.2	1.0	1.6	0.5	0.9	1.1
Bacteroidetes	1	17.6	22.4	12.8	22.7	13.7	9.6
	2	0.9	21.6	22.1	38.8	31.6	29.2
	3	16.9	29.4	27.8	23.0	9.1	8.3
Firmicutes	1	48.4	55.0	59.6	62.0	69.6	69.9
	2	75.5	66.5	67.1	57.3	64.7	68.3
	3	80.7	67.7	67.9	72.8	84.1	84.0
Proteobacteria	1	0.9	0.6	0.7	1.6	2.4	2.7
	2	4.8	1.4	0.7	2.5	1.9	1.2
	3	1.8	1.4	1.9	3.2	5.5	6.2
Genus (% relative abundance)							
Bifidobacterium	1	13.8	8.2	6.9	0.1	0.1	0.1
	2	17.8	9.5	9.1	0.0	0.0	0.0
	3	0.1	0.1	0.1	0.0	0.0	0.0
Collinsella	1	13.7	9.2	11.4	4.5	3.7	4.7
	2	0.1	0.1	0.1	0.0	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0	0.0
Bacteroides	1	0.3	6.1	3.6	0.5	1.1	1.3
	2	0.6	21.4	21.7	38.7	31.5	28.8
	3	16.9	28.5	26.1	2.3	3.1	2.5
Prevotella	1	17.2	6.6	3.8	16.9	9.1	4.5
	2	0.2	0.0	0.0	0.0	0.0	0.1
	3	0.0	0.0	0.0	20.1	5.4	4.2
Lactobacillus	1	0.1	0.1	0.1	0.0	0.0	0.0
	2	13.5	7.8	8.1	0.0	0.0	0.0
	3	0.2	0.1	0.1	0.0	0.0	0.0
Lachnospiraceae, unknown genus (other)	1	4.8	2.4	1.7	2.2	1.9	2.0
	2	8.7	5.9	3.9	2.2	2.5	1.9
	3	0.7	2.7	5.6	3.2	4.8	5.9
Lachnospiraceae, unknown genus	1	21.0	14.7	15.3	15.5	13.9	11.4
	2	16.1	9.3	10.4	6.5	4.4	5.3
	3	45.3	19.3	15.1	5.9	4.6	5.8
Coprococcus	1	1.9	1.5	1.9	0.3	0.4	0.4
	2	5.2	4.4	5.0	1.2	1.1	0.8
	3	0.1	0.2	0.4	0.3	0.6	0.9
Dorea	1	0.2	1.0	1.0	4.1	4.2	4.3
	2	0.1	0.3	0.5	0.4	0.5	1.0
	3	0.1	0.3	0.7	6.4	10.9	12.1
Ruminococcaceae, unknown genus	1	2.4	2.5	4.7	11.4	15.6	12.7
	2	0.4	2.6	3.4	12.5	12.7	10.3
	3	1.5	3.2	6.4	11.9	14.8	10.4
Ruminococcus	1	0.1	6.2	6.5	4.3	3.5	2.1
	2	1.4	5.2	6.1	12.0	12.2	10.1
	3	0.1	12.9	7.3	9.9	7.2	3.9
Dialister	1	0.8	6.7	7.8	8.8	9.4	8.5
	2	8.4	4.6	2.9	5.4	5.4	6.8
	3	6.8	6.5	6.9	18.0	17.0	14.4
Megasphaera	1	9.7	11.8	9.8	4.1	6.2	6.8
	2	6.0	10.4	8.3	3.9	12.9	12.9
	3	5.1	3.5	4.7	0.4	3.8	7.7
Mitsuokella	1	0.1	0.2	0.3	0.0	0.0	0.0
	2	5.5	2.1	1.4	0.6	0.6	0.6
	3	5.6	0.5	0.2	0.0	0.0	0.0
Enterobacteriaceae, unknown genus	1	0.8	0.2	0.2	1.5	1.0	1.0
	2	4.8	1.3	0.6	2.3	1.6	0.7
	3	0.1	0.1	0.1	1.4	0.9	0.7

3.6. Individual donor prebiotic driven changes in gut microbiota and organic acid concentrations

The PCoA graph shows each donor's entire bacterial community response to the prebiotic in the LF and HF gut models (only V1 data presented) (Fig. 2b). The bacterial community within the D2-HF gut model appears to respond to the prebiotic as the pre- and post-intervention samples are further apart whereas the pre- and post-intervention samples from the D1-HF and D3-HF gut models are more clustered, suggesting their bacterial community as a whole were less responsive. The bacterial community within the D1-LF gut model appeared to be more responsive than in the D3-LF gut model (Fig. 2b).

Inter- and intra-donor differences in gut microbiota responsiveness and organic acid concentration changes, after the prebiotic was introduced, were evident between the LF and HF gut models (Fig. 4). Using the results from V1 as an example, the D1-HF and D2-HF gut models had an increase in butyrate concentrations and *Bifidobacterium* relative abundance after the prebiotic was introduced whereas the D3-HF gut model had a reduction in butyrate and no change in *Bifidobacterium*. Concentrations of acetate, propionate and lactate and the relative abundance of *Lactobacillus*, *Dialister* and *Mitsuokella* increased in response to the prebiotic in the D3-HF gut model. D1-HF gut model experienced an increase in an unknown genus of *Ruminococcaceae*, *Megasphaera* and *Mitsuokella* and D2-HF gut model experienced an increase in *Lactobacillus*, *Coprococcus* and *Mitsuokella* relative abundance.

In the D1-LF and D3-LF gut models there was an increase in butyrate and propionate concentrations; however, the relative abundance of *Bifidobacterium* did not change. *Prevotella* relative abundance increased in the D1-LF gut model and there was an increase in the relative abundance of an unknown genus of *Lachnospiraceae*, *Coprococcus* and an unknown genus of *Ruminococcaceae* in the D3-LF gut model as a result of the prebiotic. Gut microbiota responses also varied between donors in V2 and V3 (Fig. 4).

Previous research has shown that baseline bifidobacteria concentrations influence how the gut microbiota respond to a dietary intervention (Bouhnik et al., 2007; de Preter et al., 2008; Kolida et al., 2007). Therefore, the baseline bifidobacteria concentrations of the three donors were analysed in relation to prebiotic driven changes in bifidobacteria concentrations (Fig. 5). Baseline bifidobacteria concentrations were low in the D2 and D3 gut models (HF-1.3×10^5 and 1.3×10^4 gene copies/mL; respectively, LF- D2 gut model data not included, 9.5×10^3 gene copies/mL) and high in the D1 gut models (HF-2.3×10^7 gene copies/mL, LF- 1.9×10^8 gene copies/mL). Bifidobacteria concentrations increased post-intervention in all vessels for D1-HF and D2-HF gut models but decreased for D1-LF gut model (D2-LF gut model data not included). The D3 gut models had a more varied bifidobacteria response to the prebiotic, with a reduction in V1 but a slight increase in bifidobacteria concentrations in V2 and V3 of the HF gut model and in all vessels in the LF gut model (Fig. 5).

4. Discussion

In this study, two fermentable carbohydrate content media were

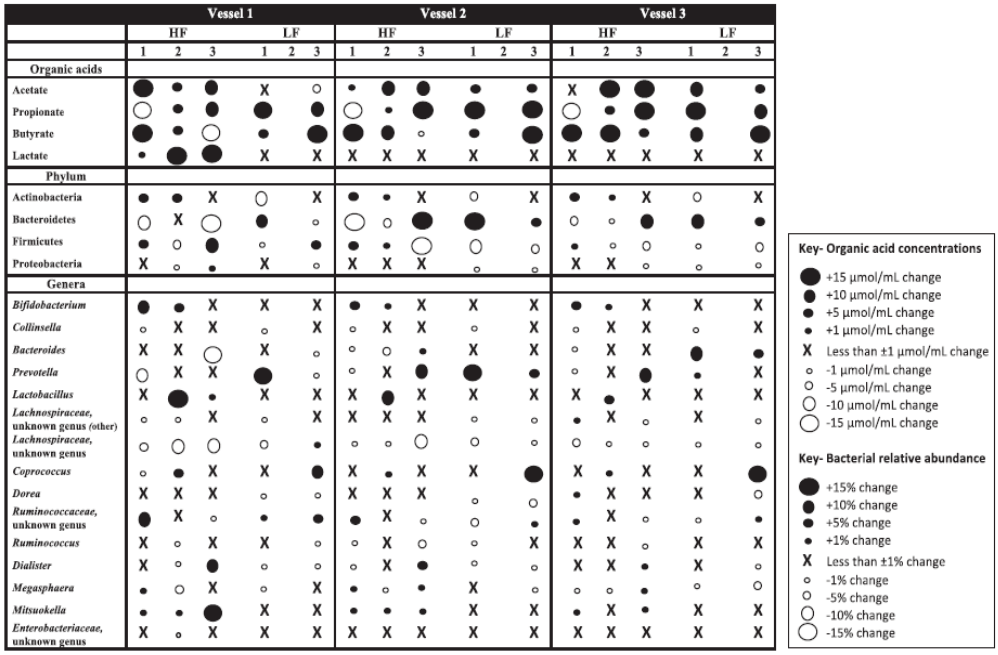

Fig. 4. - Bubble plot illustrating the distinctive changes in organic acid concentrations and bacterial relative abundance that occurred between donors (1, 2 and 3) and media types (high fermentable carbohydrate medium [HF] and low fermentable carbohydrate medium [LF]) in vessel 1, vessel 2 and vessel 3 in response to the prebiotic. The black circles represent increases in organic acid concentrations and bacterial relative abundance. The white circles represent decreases in organic acid concentrations and bacterial relative abundance. The **X** represents a less than ± 1 µmol/mL change in organic acid concentrations or a less than ± 1% change in bacterial relative abundance. LF data from donor 2 was not included.

developed to mimic WS and PS dietary patterns. The two media were used to determine what influence different fermentable carbohydrate contents had on gut microbiota responsiveness to an inulin-type fructan prebiotic using an *in vitro* gut model. We demonstrated that the addition of an inulin-type fructan prebiotic led to a shift in the gut microbiota in both the LF and HF gut models with the shift that occurred differing between the LF and HF gut models. There were significant differences in the amount of acetate produced and the changes that occurred in the relative abundance of Firmicutes, *Lactobacillus*, an unknown genus of *Lachnospiraceae* (other), *Megasphaera*, *Mitsuokella* and an unknown genus of *Enterobacteriaceae* between the LF and HF gut models. These results suggest that the fermentable carbohydrate content of the media has an influence on how the gut microbiota respond to an inulin-type fructan prebiotic. The results were also considered on an individual donor basis, which identified large inter- and intra-donor differences in gut microbiota responses to the prebiotic.

Factors which influence gut microbiota responsiveness from previous *in vivo* research include baseline gut microbiota composition and habitual dietary intakes. Previous studies have shown that individuals who have low baseline bifidobacteria concentrations experience a greater increase in bifidobacteria as a result of a dietary intervention than individuals with higher baseline bifidobacteria concentrations (Bouhnik et al., 2007; de Preter et al., 2008; Kolida et al., 2007). In the present study, there were inconsistencies in the influence baseline

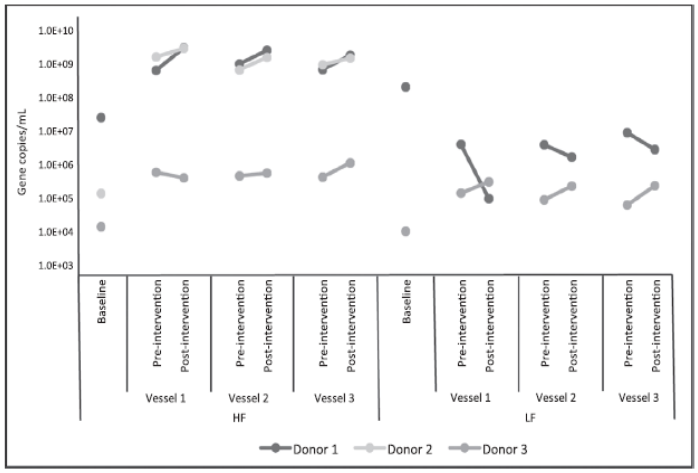

Fig. 5. - Baseline, pre- and post-intervention bifidobacteria concentrations (gene copies/mL) for each vessel (1, 2 and 3), media type (high fermentable carbohydrate medium [HF] and low fermentable carbohydrate medium [LF]) and donor (1, 2 and 3). Bifidobacteria concentrations analysed using qPCR. LF data from donor 2 was not included.

bifidobacteria concentrations had on changes in bifidobacteria. In the HF gut models, the D1-HF and D2-HF gut models had an increase in bifidobacteria and the D3-HF gut model had minimal change in bifidobacteria concentrations as a result of the prebiotic intervention, even though the D1-HF gut model had a high baseline bifidobacteria concentration and the D2-HF and D3-HF gut models had lower baseline bifidobacteria concentrations. In the LF gut models, the results were more consistent with the literature as the D1-LF gut model experienced a decrease and the D3-LF gut model experienced an increase in bifidobacteria concentrations.

Habitual dietary fibre intakes have also been suggested to influence gut microbiota responsiveness (Eid et al., 2015). All donors, in the present study, had dietary fibre intakes that would be considered high based on current recommendations as they were very close to or exceeded the recommended dietary fibre intake of 25–30 g/day (Ministry of Health, 2015); however, dietary fibre intakes still ranged from 24.4 to 41.2 g/day. There were other distinctions in nutrient and food group intakes (*e.g.* protein, vegetable, carbohydrate intakes) between donors that may have influenced the responsiveness of the gut microbiota; however, it is difficult to draw any valid conclusions from the present study due to the small number of donors used. A sample size of three donors, used in the present study, is not uncommon for an *in vitro* three-stage continuous colonic model system study. Similar *in vitro* three-stage continuous colonic model system studies have also used three faecal donors (Costabile et al., 2015; Shen et al., 2012). Additionally, our results are consistent with previous *in vivo* studies, which have also observed inter-individual variability in gut microbiota responsiveness to dietary interventions (Davis, Martínez, Walter, Goin, & Hutkins, 2011; Martínez, Kim, Duffy, Schlegel, & Walter, 2010; Salonen et al., 2014). Nevertheless, the influence habitual dietary intake has on gut microbiota responsiveness should be confirmed in *in vivo* studies with larger participant numbers.

Pre-intervention differences between the LF and HF gut models were expected as the gut microbiota consortia were exposed to different amounts and proportions of fermentable carbohydrates. The only significant pre-intervention gut microbiota difference observed between the LF and HF gut models was in an unknown genus of *Ruminococcaceae* (unpublished data). However, large inter-donor differences in pre-intervention gut microbiota composition and organic acid production meant that a large proportion of the gut microbiota differences observed (*Bifidobacterium*, *Lactobacillus*, unknown genus of *Lachnospiraceae etc.)* between the LF and HF gut models were not significant. It was, therefore, important to discern the differences in pre-intervention gut microbiota and organic acid concentrations between the two media types on an individual donor level. By observing the results on an individual donor basis we demonstrated that each donor had a distinctive gut microbiota profile and organic acid production in the presence of the two different fermentable carbohydrate content media types.

This research is novel as media that were developed to mimic two diverse dietary patterns commonly consumed by humans (WS and PS dietary patterns) were used. The media were developed using dietary pattern food group information (Hu et al., 1999; Hu, 2002; Schulze et al., 2006) and fermentable carbohydrate specific food composition tables (Anderson et al., 2015; Marlett & Cheung, 1997; Murphy et al., 2008), which has not been undertaken before. A further strength of this research is the way in which specific factors, such as media substrates, could be manipulated in an *in vitro* three-stage continuous colonic model system without the complication of confounding factors, such as immune parameters, dietary non-compliance and medication use, which are often present in human studies. The *in vitro* gut model also provides a validated platform for testing a hypothesis before progressing to *in vivo* studies. Another strength of this study is that the bacterial analysis was conducted using 16S rRNA bacterial gene sequencing. Very few *in vitro* three-stage continuous colonic model system or *in vivo* inulin-type fructan prebiotic studies have utilised Next-generation sequencing technology. Therefore, this research provides additional insight into whole bacterial community changes that occurred within an *in vitro* gut model after an inulin-type fructan prebiotic intervention. This is also the first time inter- and intra-donor variability has been discussed in detail in an *in vitro* gut model, which demonstrates that this model could be used in the future to study the effects of individual variability in gut microbiota responsiveness. There are, however, some limitations to this study including the exclusion of the data collected from the D2-LF gut model secondary to contamination. Fortunately the contamination occurred near the end of the experiment so all the D2-HF gut model data, and the baseline and pre-intervention D2-LF gut model data could still be used. As mentioned above, dietary pattern information and food composition data were used to help ensure the media types contained the types and amounts of fermentable carbohydrates present in WS and PS dietary patterns; however, food composition data was not available for all fermentable carbohydrate substrates present in the human diet (*i.e.* β-glucans). The two media recipes developed are representative of the best available dietary fermentable carbohydrate information currently available. If additional fermentable carbohydrate food composition data becomes available in the future the media recipes can be refined before use in future *in vitro* studies.

In conclusion, this study demonstrated that media with different fermentable carbohydrate contents, which mimic WS and PS dietary patterns, influenced gut microbiota response to an inulin-type fructan prebiotic within an *in vitro* three-stage continuous colonic model system. Large inter- and intra-donor variability in gut microbiota responsiveness also appeared to have a profound influence on the prebiotic driven changes that occurred. The results of this study are novel and promising; however, they will need to be replicated in an *in vivo* study using a larger study cohort. Future research which aims to determine what factors (including habitual dietary intake) influence gut microbiota responsiveness to a dietary intervention may help enhance the success of gut microbiota modulation strategies.

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Conflict of interest

The authors report no conflict of interest.

Authorship

GH was involved in the conception, study design, running of the experiment, sample processing, data collection, analysis and interpretation, and writing of the manuscript. LB, CB, RM and JC were involved in the conception, study design and data interpretation. DR, PB and HS were involved in the study design, running of the experiment and data interpretation. HS also carried out the organic acid sample processing and data analysis. All authors contributed to the development and approval of the manuscript.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bcdf.2017.07.001.

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Appendix 4-3. Statement of contribution to doctoral thesis containing publications

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Genelle Healey

Name/Title of Principal Supervisor: Professor Jane Coad

Name of Published Research Output and full reference:
Healey G, Murphy R, Butts C, Brough L, Rosendale D, Blatchford P, Stoklosinski H and Coad J. Variability in gut microbiota response to an inulin-type fructan prebiotic within an in vitro three-stage continuous colonic model system. Bioactive Carbohydrates and Dietary Fibre. 2017;11:26-37.

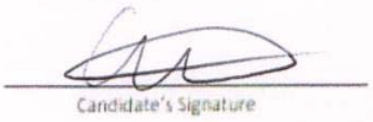
In which Chapter is the Published Work: Chapter 4

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:
  Involved in the conception, study design, donor recruitment, running of the experiment, sample collection and preparation, data analysis and interpretation, and writing the manuscript.



Candidate's Signature

26/09/2017.
Date

Jane Coad

Principal Supervisor's signature

29.9.17
Date

GRS Version 3– 16 September 2011

Appendix 4-4. Media recipes table

Medium recipe developed by Macfarlane and co-authors (standard) compared with the low fermentable carbohydrate medium (LFC) and high fermentable carbohydrate medium (HFC).

	Media ingredients	Standard	LFC	HFC
			g/5L media	
Non-carbohydrate	Peptone water	25.0	25.0	25.0
	Tryptone	25.0	25.0	25.0
	Yeast extract	22.5	22.5	22.5
	Sodium chloride	22.5	22.5	22.5
	Potassium chloride	22.5	22.5	22.5
	Mucin	20.0	20.0	20.0
	Casein	15	15	15
	Sodium bicarbonate	7.5	7.5	7.5
	Magnesium sulfate	6.25	6.25	6.25
	L-Cysteine HCl	4.0	4.0	4.0
	Potassium di-hydrogen phosphate	2.5	2.5	2.5
	Di-potassium hydrogen phosphate	2.5	2.5	2.5
	Bile salts	2	2	2
	Calcium chloride hexahydrate	0.75	0.75	0.75
	Hemin	0.25	0.25	0.25
	Ferrous sulfate heptahydrate	0.025	0.025	0.025
	Tween 80	5 mL	5 mL	5 mL
	Vitamin K1	50 µL	50 µL	50 µL
	Resazurin	-	4 mL	4 mL
Fermentable Carbohydrate	Soluble starch	25.0	-	-
	Guar gum	5.0	-	-
	Pectin- citrus peel	5.0	2.0	5.8
	Pectin- apple	5.0	2.1	5.8
	Lignin	-	5.1	11.6
	Resistant starch	-	7.9	10.4
	Potato fibre	-	10.0	12.2
	Wheat fibre	-	9.1	23.6
	Arabinogalactan	10.0	5.0	6.4
	Xylan	10	4.2	15.7
	Inulin	5.0	5.0	10.8
	Fructo-oligosaccharide	-	5.0	10.5
	Added to prebiotic containing media only			
	Beneo Orafti® Synergy 1	-	40.0	40.0

Appendix 4-5. Medium contamination table

Pre- and post-intervention genus level relative abundance for all donors (1, 2 and 3), vessels (1, 2 and 3) and media types (high and low fermentable carbohydrate medium)[1]

		High fermentable carbohydrate						Low fermentable carbohydrate					
		Vessel 1		Vessel 2		Vessel 3		Vessel 1		Vessel 2		Vessel 3	
Genus (% relative abundance)	**Donor**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Bifidobacterium	**1**	13.8	23.8	8.2	17.8	6.9	16.7	0.1	0.0	0.1	0.0	0.1	0.0
	2	17.8	27.4	9.5	12.0	9.1	11.7	0.0	0.0	0.0	0.0	0.0	0.0
	3	0.1	0.1	0.1	0.1	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Collinsella	**1**	13.7	12.3	9.2	8.1	11.4	8.5	4.5	1.0	3.7	1.8	4.7	3.4
	2	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bacteroides	**1**	0.3	0.3	6.1	3.1	3.6	1.4	0.5	0.1	1.1	1.0	1.3	12.9
	2	0.6	0.4	21.4	15.2	21.7	20.8	38.7	19.7	31.5	26.3	28.8	28.7
	3	16.9	1.1	28.5	30.9	26.1	26.5	2.3	0.3	3.1	3.3	2.5	11.7
Prevotella	**1**	17.2	5.3	6.6	3.3	3.8	1.8	16.9	36.6	9.1	36.8	4.5	7.8
	2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
	3	0.0	0.6	0.0	13.7	0.0	10.0	20.1	17.8	5.4	14.0	4.2	4.8
Lactobacillus	**1**	0.1	0.7	0.1	0.4	0.1	0.6	0.0	0.1	0.0	0.0	0.0	0.1
	2	13.5	29.6	7.8	18.5	8.1	16.9	0.0	0.0	0.0	0.0	0.0	0.0
	3	0.2	2.6	0.1	0.5	0.1	0.4	0.0	0.0	0.0	0.0	0.0	0.0
Lachnospiraceae, unknown genus (other)	**1**	4.8	1.2	2.4	1.9	1.7	3.3	2.2	0.6	1.9	0.6	2.0	1.2
	2	8.7	6.5	5.9	4.9	3.9	3.0	2.2	0.7	2.5	1.0	1.9	0.5
	3	0.7	0.6	2.7	2.3	5.6	3.3	3.2	2.6	4.8	1.6	5.9	1.0
Lachnospiraceae, unknown genus	**1**	21.0	11.7	14.7	12.8	15.3	7.9	15.5	10.3	13.9	6.9	11.4	7.2
	2	16.1	1.9	9.3	6.2	10.4	7.2	6.5	7.6	4.4	5.2	5.3	3.6
	3	45.3	33.3	19.3	9.1	15.1	11.7	5.9	8.0	4.6	3.3	5.8	2.0
Coprococcus	**1**	1.9	0.4	1.5	0.9	1.9	1.1	0.3	0.1	0.4	0.2	0.4	0.3
	2	5.2	12.5	4.4	7.7	5.0	7.9	1.2	18.9	1.1	19.0	0.8	19.5
	3	0.1	0.1	0.2	0.3	0.4	0.4	0.3	12.5	0.6	16.8	0.9	26.4
Dorea	**1**	0.2	0.6	1.0	1.5	1.0	2.0	4.1	1.8	4.2	2.2	4.3	3.7
	2	0.1	0.0	0.3	0.3	0.5	0.4	0.4	0.3	0.5	0.3	1.0	0.5
	3	0.1	0.0	0.3	0.5	0.7	0.6	6.4	2.0	10.9	2.4	12.1	3.7
Ruminococcaceae, unknown genus	**1**	2.4	13.3	2.5	10.3	4.7	7.2	11.4	12.8	15.6	10.2	12.7	9.1
	2	0.4	0.0	2.6	2.3	3.4	2.4	12.5	26.7	12.7	19.7	10.3	11.4
	3	1.5	0.0	3.2	2.1	6.4	4.3	11.9	18.8	14.8	18.6	10.4	13.7
Ruminococcus	**1**	0.1	0.0	6.2	4.4	6.5	6.0	4.3	3.6	3.5	2.1	2.1	2.0
	2	1.4	0.0	5.2	6.1	6.1	6.7	12.0	9.2	12.2	9.0	10.1	10.2
	3	0.1	0.0	12.9	3.4	7.3	4.5	9.9	8.9	7.2	6.6	3.9	3.3
Dialister	**1**	0.8	1.4	6.7	4.1	7.8	7.4	8.8	6.9	9.4	6.4	8.5	8.0
	2	8.4	5.4	4.6	4.0	2.9	2.3	5.4	3.5	5.4	3.8	6.8	4.6
	3	6.8	17.2	6.5	11.6	6.9	8.4	18.0	15.9	17.0	14.0	14.4	11.9
Megasphaera	**1**	9.7	11.8	11.8	15.3	9.8	7.3	4.1	2.9	6.2	5.8	6.8	5.6
	2	6.0	0.3	10.4	6.0	8.3	3.8	3.9	0.4	12.9	4.4	12.9	5.8
	3	5.1	4.2	3.5	6.0	4.7	8.1	0.4	0.4	3.8	2.2	7.7	2.3
Mitsuokella	**1**	0.1	2.0	0.2	1.2	0.3	2.8	0.0	0.0	0.0	0.1	0.0	0.1
	2	5.5	9.0	2.1	3.1	1.4	2.2	0.6	0.5	0.6	0.3	0.6	0.3
	3	5.6	24.6	0.5	2.7	0.2	2.9	0.0	0.1	0.0	0.2	0.0	0.1
Enterobacteriaceae, unknown genus*	**1**	0.8	1.0	0.2	0.3	0.2	0.5	1.5	0.5	1.0	0.8	1.0	1.0
	2	4.8	2.7	1.3	0.9	0.6	0.3	2.3	4.8	1.6	2.6	0.7	3.0
	3	0.1	1.1	0.1	0.2	0.1	0.1	1.4	1.2	0.9	0.7	0.7	0.4

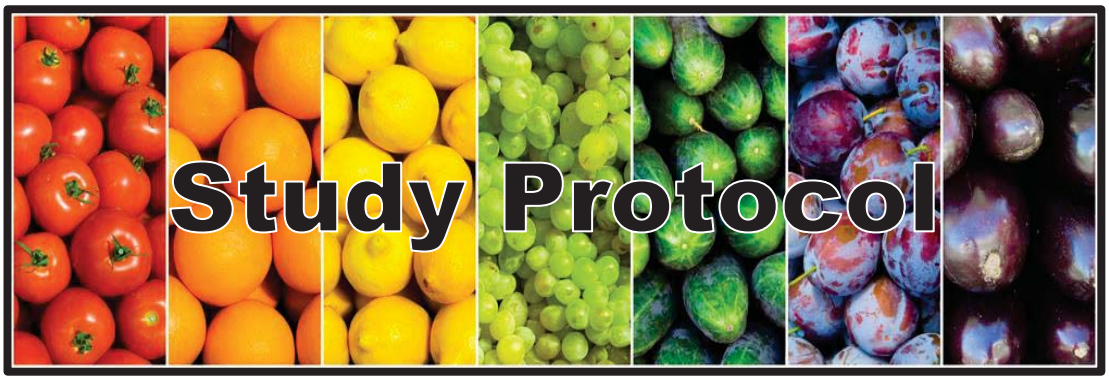
[1] Values determined using 16S rRNA bacterial gene sequencing. The relative abundances highlighted are from the contaminated gut model (donor 2 low fermentable carbohydrate medium). * indicates the contaminant bacteria. Pre: pre-intervention, Post: post-intervention.

Appendix 5-1. Validation study protocol

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Plant & Food RESEARCH
RANGAHAU AHUMĀRA KAI

Dietary fibre intake short food frequency questionnaire validation study



Background

As part of the screening process for the human clinical trial titled "The influence of habitual dietary fibre intake on responsiveness of the gut microbiota to a prebiotic" potential participants will be invited to complete a dietary fibre intake short food frequency questionnaire (DFI-FFQ), developed by the lead researcher. The DFI-FFQ is a brief food frequency questionnaire developed to assess dietary fibre intakes. Other methods utilised to collect dietary intake information such as food frequency questionnaires (FFQ) and food records can be resource and time consuming and require specialist skills to interpret which is why this quick dietary assessment questionnaire has been developed.

The aim of the DFI-FFQ is to accurately group individuals as low, medium or high dietary fibre consumers. Gaining accurate validated information on potential participants' dietary fibre intake is integral as only individuals with low or high dietary fibre intakes will be eligible for inclusion in the human clinical trial.

Study design

Aim

To validate the DFI-FFQ as a "quick method" to assess potential participants' dietary fibre so individuals can be accurately grouped as either low or high dietary fibre consumers for inclusion in the human clinical trial titled "The influence of habitual dietary fibre intake on responsiveness of the gut microbiota to a prebiotic".

Objectives

Primary objective

To validate the DFI-FFQ using an established food frequency questionnaire validated to assess habitual dietary fibre intake.

Hypotheses

The DFI-FFQ will be shown to be a valid and quick method to use in assessing dietary fibre intakes in healthy individuals.

Proposed study

The study is a validation study to determine the validity of a DFI-FFQ to assess potential participant's dietary fibre intakes so they can be classified as low, moderate or high dietary fibre consumers.

Individuals with a dietary fibre intake of <18g/d (females) or <22g/day (males) are considered as low dietary fibre consumers, 18-24.9g/day (females) or 22-29.9g/day (males) are considered as moderate dietary fibre consumers and ≥25g/day (females) and ≥30g/day (males) are considered high dietary fibre consumers. The dietary fibre intake value obtained from the dietary fibre intake screening tool will be compared to the dietary fibre intake value obtained from the CNAQ for each participant to determine whether the dietary fibre intake screening tool is valid tool in assessing habitual dietary fibre intakes.

30 participants will be recruited in total. Participants will be asked to complete an online version of the DFI-FFQ (twice) and an online validated food frequency questionnaire.

Participant recruitment

Potential study participants will be recruited via a number of avenues including word of mouth, radio and newspaper advertisement and flyer displays at Massey University, Plant and Food Research and on community notice boards in the Palmerston North area.

Participant criteria

Inclusion criteria

The target population for recruitment are healthy male and female individuals between the age of 19 and 65 years with a BMI of between 18.5 and 30kg/m^2.

Exclusion criteria

- Aged <19 and >65 years
- Unhealthy
- BMI <18.5 and >30 kg/m^2
- Significant weight loss or weight gain within the past year
- Significant dietary change within the past year
- Pregnant or breastfeeding
- Food intolerances which cause gastrointestinal symptoms (i.e. lactose intolerance, gluten sensitivity)
- Adverse gastrointestinal symptoms
- Smokers
- High alcohol consumers

Participant questionnaire

Participant eligibility will be determined using a participant questionnaire which will be emailed or posted to potential participants once they have expressed an interest in participating in the study. Information relating to the potential participant's gender, health status, medication and supplement use, age, BMI, recent weight and dietary changes, drinking patterns, smoking status and food intolerances will be collected.

Power calculations

Recruiting 30 people to test the validity of the DFI-FFQ will help ensure interesting levels of correlation (0.7 and above) are unlikely to occur by chance (with normally distributed random data and no association, 95% of correlation will have a magnitude less than 0.36). It also allows for reasonable numbers in each tertile for calculating agreement between the scales.

Methods

Validated food frequency questionnaire

A validated comprehensive nutrition assessment questionnaire (CNAQ), developed by researchers at Monash University, will be used to assess habitual dietary fibre intake (over the past year) (Barrett & Gibson, 2010). The food frequency questionnaire will be completed online by participants. A link to the FFQ will be emailed to participants which means they do not need to visit a research unit to complete it.

Dietary fibre intake short food frequency questionnaire

The DFI-FFQ was developed by the lead research as a quick method to assess potential participant's dietary fibre intake so they can be grouped as low, moderate or high dietary fibre consumers. The short food frequency questionnaire asks participants to record the frequency of consumption of the more predominant dietary fibre containing foods groups- fruit, vegetables, breads and cereals, nuts and seed and legumes (Ministry of Health, 2011). The DFI-FFQ will assess habitual intake of dietary fibre over the past year.

A scoring sheet has been developed using the average dietary fibre amount for each consumption frequency (i.e. 1/week, 5-6/week etc.) for each food group using dietary analysis information from FoodWorks version 7.0.3016 (Xyris software). Once participants have completed the DFI-FFQ a dietary fibre intake average for each food group can be determined. By adding the average dietary fibre intake from each food group together a total dietary fibre intake amount can be determined.

The dietary fibre intake data obtained from the CNAQ will be compared to the dietary fibre intake data obtained from the DFI-FFQ for validation purposes.

To demonstrate reproducibility the DFI-FFQ will be completed by participants twice; two weeks apart.

Statistical Analysis

To check whether the estimates of fermentable CHO consumption from the questionnaire agree with those from the food frequency questionnaire, correlation (either Pearson's

correlation coefficient or Spearman's rank-correlation coefficient) can be utilised. If the estimates are on the same scale (grams per day), using a Bland-Altman plot to estimate limits of agreement may also be worthwhile. Another option would be to use each set of estimates to split respondents into tertiles (three groups of equal numbers of respondent, consisting of the lowest, the highest or the middle responses according to one scale) and looking at how the allocation based on the two questionnaires agreed (possibly using a weighed kappa measure, c.f. Barrett et al, 2010).

To understand the behaviour of the questionnaire better, it might also be worth estimating the internal reliability (using Cronbach's alpha).

Test-retest reliability analysis (Barrett et al, 2010) of the DFI-FFQ could be conduct using individuals who complete the DFI-FFQ for the validation study and the intervention (ADAPT) study. There correlation (Spearman's and Pearson's) and calculating limits of agreement would be useful.

Participant Information

Participation and confidentiality

Participation within the study is voluntary and participants can withdraw from the study at any time with no explanation required. All information collected will be confidential and participants will only be identifiable by a participant identification number. All participants will be required to sign a consent form before participating in the study.

Approval from the Massey University Ethics Committee has been gained.

Participant reimbursement

A $25 grocery or petrol voucher will be provided to each participant to contribute towards travel costs and time taken to participate in the study.

Budget

Item	Description	Estimated cost	Financial year
Participant reimbursement	1x$25 (n=30)	750	2015
Food Frequency questionnaire	Monash University (n=30) ($15 each AUD= $17 NZD each)	510	2015
	TOTAL:	**1260**	

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Ministry of Health. (2011). *A Focus on Nutrition Key Findings of the 2008/09 New Zealand Adult Nutrition Survey* (pp. 1–332).

Appendix 5-2. Validation study publication

nutrients

MDPI

Communication

Validity and Reproducibility of a Habitual Dietary Fibre Intake Short Food Frequency Questionnaire

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Abstract: Low dietary fibre intake has been associated with poorer health outcomes, therefore having the ability to be able to quickly assess an individual's dietary fibre intake would prove useful in clinical practice and for research purposes. Current dietary assessment methods such as food records and food frequency questionnaires are time-consuming and burdensome, and there are presently no published short dietary fibre intake questionnaires that can quantify an individual's total habitual dietary fibre intake and classify individuals as low, moderate or high habitual dietary fibre consumers. Therefore, we aimed to develop and validate a habitual dietary fibre intake short food frequency questionnaire (DFI-FFQ) which can quickly and accurately classify individuals based on their habitual dietary fibre intake. In this study the DFI-FFQ was validated against the Monash University comprehensive nutrition assessment questionnaire (CNAQ). Fifty-two healthy, normal weight male (n = 17) and female (n = 35) participants, aged between 21 and 61 years, completed the DFI-FFQ twice and the CNAQ once. All eligible participants completed the study, however the data from 46% of the participants were excluded from analysis secondary to misreporting. The DFI-FFQ cannot accurately quantify total habitual dietary fibre intakes, however, it is a quick, valid and reproducible tool in classifying individuals based on their habitual dietary fibre intakes.

Keywords: dietary fibre; short food frequency questionnaire; validation

1. Introduction

Dietary fibre are non-digestible plant polysaccharides found in high amounts in fruits, vegetables, breads and cereals, legumes and nuts and seeds. Dietary fibre has been shown to have important implications on human health, including preventing and alleviating constipation, reducing gastrointestinal cancer incidence and blood glucose levels, lowering blood cholesterol levels and blood pressure, and beneficially modulating gut microbiota [1]. It is also possible that the efficacy of a dietary intervention is altered as a result of the influence habitual dietary fibre intake has on gut microbiota responsiveness and host outcomes. Therefore, being able to quickly assess an individual's habitual dietary fibre intake and classify individuals based on their dietary fibre intakes will prove useful in clinical practice and in nutrition and health research. Dietary assessment methods such as diet records and food frequency questionnaires have inherent limitations such as being difficult to complete accurately, time-consuming and may not accurately assess a person's habitual diet [2]. A small number of dietary fibre assessment questionnaires have been developed, however these

questionnaires assess general dietary behaviours, do not estimate total dietary fibre amounts, and/or do not classify individuals based on habitual dietary fibre intakes [3–6]. Therefore, the primary aim of this study was to determine whether a newly developed dietary fibre intake short food frequency questionnaire (DFI-FFQ) can accurately classify individuals based on their habitual dietary fibre intake and the secondary aim of the study was to determine whether the DFI-FFQ can accurately quantify total habitual dietary fibre intakes.

2. Methods

2.1. Subjects

Participants were recruited via email and poster advertisement in multiple locations around Palmerston North, New Zealand. A diverse cross-section of the population was targeted to help ensure a good representation of the New Zealand population was recruited. Sixty-eight individuals provided informed consent to participate in this study, of which fifty-two healthy participants met the inclusion criteria (aged >19 and <65 years, healthy, BMI >18.5 and <30 kg/m^2, no significant weight loss or weight gain within the past year, no significant dietary change within the past year, not pregnant or breastfeeding, no food intolerances which cause gastrointestinal symptoms (i.e., lactose intolerance, gluten sensitivity), no adverse gastrointestinal symptoms, non-smoker and not high alcohol consumers). Participants completed the DFI-FFQ twice, at least 2 weeks apart, and the comprehensive nutrition assessment questionnaire (CNAQ) once. The DFI-FFQ was completed initially, followed by the CNAQ, and lastly the repeated DFI-FFQ was completed. The CNAQ and DFI-FFQ were both completed online. An energy intake: basal metabolic rate (EI:BMR) of <1.1 and >2.19 was used to exclude participants who appeared to have over- or under-reported using the CNAQ [7]. Ethical approval was obtained from the Massey University Human Ethics Committee (Southern A, Application 15/34).

2.2. Development of the DFI-FFQ

The DFI-FFQ (Figure S1) was designed to quickly and accurately classify individuals as low, moderate or high habitual dietary fibre consumers and quantify an individual's habitual dietary fibre intake (g/day). The DFI-FFQ consists of five high dietary fibre containing food groups (vegetables, fruits, breads and cereals, nuts and seeds and legumes) which account for 73.5% of the dietary fibre in a typical New Zealand diet [8]. Examples of what one serve is equivalent to, for each food group, is detailed within the DFI-FFQ. The frequency of consumption for the average number of serves consumed over the past year, was given as follows: Never, <1/month, 1–3/month, 1/week, 2–4/week, 5–6/week, 1/day, 2/day, 3/day, 4/day, 5/day and 6+/day.

2.3. DFI-FFQ Scoring Sheet

A scoring sheet was developed to quantify the amount of dietary fibre consumed and to classify individuals as low, moderate and high dietary fibre consumers. FoodWorks version 7.0.3016 (Xyris Software Pty Ltd., Brisbane, Queensland, Australia) was used to quantify the average amount of dietary fibre provided by the five food groups for each frequency of consumption. An individual's total dietary fibre intake was calculated by adding together the average amount of dietary fibre consumed from each food group in relation to the number of serves consumed.

2.4. Dietary Fibre Classification

The cut-offs used to classify individuals based on their dietary fibre intakes are outlined in Table 1. The high dietary fibre intake cut-offs were selected to reflect the New Zealand Ministry of Health recommended dietary fibre intake guidelines; >25 g/day for females and >30 g/day for males [9]. The low dietary fibre intake cut-offs were selected as the median dietary fibre intake in New Zealand was 17.5 g/day for females and 22.1 g/day for males, which are below recommended amounts [8].

Similar cut-offs have been used previously however the specific cut-offs used in this study were modified to be applicable to a New Zealand population [3].

Table 1. The dietary fibre intake cut-offs used to classify individuals as low, moderate and high dietary fibre consumers.

	Females	Males
Low	<18 g/day	<22 g/day
Moderate	18–24.9 g/day	22–29.9 g/day
High	≥25 g/day	≥30 g/day

2.5. Dietary Assessment Method Used for Comparison

The Monash University online CNAQ was used for comparison with the DFI-FFQ. The 297-item food frequency questionnaire has been shown to be valid in assessing habitual dietary intakes when compared to four 7-day food records, each completed three months apart [10].

2.6. Statistical Analysis

We aimed to recruit enough participants to ensure that correlations over 0.7 would be statistically significant and that the assumptions of *chi-squared* tests would not be over stretched. The relationship between results of the DFI-FFQ when compared to the CNAQ was determined using Spearman correlation, Pearson correlation, Bland-Altman plot, *chi-squared* test and linear weighted kappa score. Test-retest repeatability was assessed using Pearson correlation, Bland-Altman plot and Cronbach's alpha. *T*-tests were used to determine whether there were any differences in dietary fibre intakes between the DFI-FFQ and CNAQ and the repeated DFI-FFQ. A p value of < 0.05 is considered significant. Statistical analysis was carried out using GenStat 17th edition (VSNi Ltd., Hemel Hempstead, UK), Minitab 16th edition (Cronbach's alpha) (Minitab Inc., State College, PA, USA) and the calculator at http://vassarstats.net/kappa.html (kappa score) [11].

3. Results

All eligible participants ($n = 52$) completed the study. The data from 28 participants (54%) were used as the data from 24 participants (46%) were excluded from the analysis secondary to likely misreporting on the CNAQ; with 18 participants (34.5%) having over-reported and six participants (11.5%) having under-reported their energy intakes. The group mean EI:BMR was 2.8 (SD 4.7) prior to exclusion and reduced to 1.6 (SD 0.3) after exclusion. Participant characteristics, total dietary fibre intakes and classifications determined by the DFI-FFQ and CNAQ are summarised in Table 2. The median dietary fibre intake in New Zealand (20.3 g/day) [8] is similar to the average dietary fibre intake of the study cohort, with dietary fibre intakes from both groups being below the New Zealand recommended dietary fibre intake guidelines [9]. The DFI-FFQ took on average 3.5 min to complete in comparison to the estimated completion time of 20–40 min for the CNAQ.

When comparing the DFI-FFQ to the CNAQ for dietary fibre classification, exact agreement occurred 79% of the time and gross misclassification occurred 7% of the time (Table 3). There was a significant difference in dietary fibre intakes between the DFI-FFQ and CNAQ (CNAQ was on average 5 g/day higher than the DFI-FFQ). The two dietary assessment methods were however correlated (Pearson correlation 0.65, Spearman correlation 0.53). A *chi-squared* test indicated an association between the classifications based on the DFI-FFQ and CNAQ ($p = 0.002$) and the linear weighted kappa score showed good agreement [12] (Table 4). The Bland-Altman plot is available within the Supplementary information (Figure S2A).

Table 2. Characteristics, dietary fibre intakes and classifications for the study participants.

Mean (SD)	Male (n = 8)	Female (n = 20)	Total (n = 28)
Participant characteristics			
Age (years)	40 (11.02)	38 (9.37)	39 (9.91)
BMI (kg/m^2)	24 (1.9)	23 (3.1)	24 (2.82)
Ethnicity (No.)			
New Zealand European	4	14	18
Asian	3	0	3
Maori	0	2	2
Other	1	4	5
Dietary fibre intakes and classifications			
DFI-FFQ			
Dietary fibre intake (g/day)	27 (11.77)	23 (10.33)	24 (10.85)
Dietary fibre classification (No.)			
Low	2	5	7
Moderate	2	4	6
High	4	11	15
Monash CNAQ			
Dietary fibre intake (g/day)	31 (11.35)	29 (9.43)	29 (10.09)
Dietary fibre classification (No.)			
Low	1	4	5
Moderate	3	1	4
High	4	15	19

DFI-FFQ: dietary fibre intake short food frequency questionnaire; CNAQ: comprehensive nutrition assessment questionnaire; SD: standard deviation.

Table 3. Comparison in dietary fibre classification between the comprehensive nutrition assessment questionnaire (CNAQ) and the dietary fibre intake food frequency questionnaire (DFI-FFQ).

		CNAQ			Total
		Low	Moderate	High	
DFI-FFQ	Low	5 (18%)	0 (0%)	2 (7%)	7 (25%)
	Moderate	0 (0%)	3 (11%)	3 (11%)	6 (21%)
	High	0 (0%)	1 (3%)	14 (50%)	15 (54%)
Total		5 (18%)	4 (14%)	19 (68%)	28 (100%)

Table 4. Correlation and test-retest repeatability statistical analysis.

Correlation between DFI-FFQ and CNAQ		p Value
Pearson correlation	0.65	<0.001
Spearman correlation	0.53	0.001
Chi-square test	9.6	0.002
Linear weighted kappa *	0.68	
Standard error	0.14	
Magnitude of agreement	Good	
Bland-Altman plot		
Limits of agreement (g/day)	−12.5–22.6	
Standard error	1.7	
Mean difference (g/day)	5	0.007
Test-Retest Repeatability		**p value**
Pearson correlation	0.94	<0.001
Cronbach's alpha	0.97	
Bland-Altman plot		
Limits of agreement (g/day)	−6.0–9.6	
Standard error	0.72	
Mean difference (g/day)	1.8	0.019

CNAQ: comprehensive nutrition assessment questionnaire; DFI-FFQ: dietary fibre intake short food frequency questionnaire; * One category disagreement had a weight of 3/4 .

Pearson correlation (0.94) and Cronbach's alpha (0.97) showed that the repeated DFI-FFQ correlated. The estimated dietary fibre intake from the second DFI-FFQ was significantly lower than the first DFI-FFQ by 1.8 g/day (Table 4). The Bland-Altman plot is available within the Supplementary information (Figure S2B).

4. Discussion

Presently, there are no known short dietary fibre intake questionnaires that are able to classify individuals based on their habitual dietary fibre intake. Having the ability to be able to quickly and accurately classify an individual based on their dietary fibre intake will prove useful as low dietary fibre intakes have been associated with poorer health outcomes [13]. This study has shown that the DFI-FFQ can accurately classifying individuals based on their habitual dietary fibre intakes.

There was however, a significant difference in habitual dietary fibre intakes between the repeated DFI-FFQs and the DFI-FFQ and CNAQ, which suggests the DFI-FFQ might not accurately quantify total habitual dietary fibre intakes. Research has shown that large food item FFQs overestimate fruit and vegetable consumption, which may help explain the higher dietary fibre intakes determined from the CNAQ [14]. The addition of other dietary fibre contributing food groups, such as cakes and muffins, pies and pastries and biscuits, to the DFI-FFQ may have helped to improve the questionnaire's accuracy in quantifying total habitual dietary fibre intakes as these food groups collectively contribute 6.3% of the dietary fibre in a typical New Zealand diet [8]. Another reason why the DFI-FFQ may not have been able to accurately quantify total habitual dietary fibre intakes may be related to the serving size examples provided. The examples provided did not include all possible foods within a particular food group and relied on participants to use their own judgement regarding the number of serves consumed for foods that were not specifically listed.

There are a handful of short questionnaires that have been developed to assess dietary fibre intakes however these questionnaires assess general dietary behaviours [4–6], do not estimate total dietary fibre amounts [4–6], and/or do not classify individuals based on habitual dietary fibre intakes [3–6]. The DFI-FFQ is novel as it can accurately classify individuals based on habitual dietary fibre intake. Unlike previously developed questionnaires, the DFI-FFQ was validated against an FFQ which assesses dietary intake over the past year, providing a more accurate account of long term rather than current dietary fibre intakes. Additionally, some of the questionnaires were validated using fairly homogenous populations, such as factory workers [3] and patients [5], making these questionnaires less useful in more diverse populations, such as in this study.

When comparing the study cohorts average dietary fibre intake to the Adult Nutrition Survey data [8] it appeared the study cohort has a similar dietary fibre intake to the New Zealand population. Therefore, the DFI-FFQ is a valid tool for classifying individuals based on their habitual dietary fibre intakes in New Zealand. In countries where dietary fibre intakes are distinctly different from New Zealand, the DFI-FFQ may need to be re-validated in these populations.

Forty-six percent of participants were excluded from the study secondary to misreporting on the CNAQ, which reduced the data available for analysis. A known limitation of FFQs is the high rate of misreporting, however the rate of misreporting in this study was much higher than previously reported [15]. It may therefore be useful to compare the DFI-FFQ to another dietary assessment method (i.e., 3- or 7-day diet records, or shorter validated FFQ) to confirm these results. The sample size for this study was small however a sufficient number of participants were recruited based on the sample size calculations, even after exclusion for misreporting. Additionally, other dietary questionnaire validation studies have similarly small participant numbers [16,17]. Despite the limitations discussed, we believe the DFI-FFQ will be a valuable tool in research and clinical practice as it is quick to complete (3.5 min on average), has low respondent burden and is a valid and reproducible method of classifying individuals based on their habitual dietary fibre intakes.

5. Conclusions

The DFI-FFQ has been shown to be a quick, valid and reproducible tool in classifying individuals based on their habitual dietary fibre intakes. The DFI-FFQ cannot however, accurately estimate total habitual dietary fibre intakes.

Author Contributions: G.H. was involved in the conception, study design, recruitment, conducting the study, and writing and editing the manuscript. J.C., C.B., R.M. and L.B. were involved in the conception, study design and editing of the manuscript. D.H. conducted the statistical analysis and was involved in editing the manuscript. All authors read and approved the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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Appendix 5-3. Statement of contribution to doctoral thesis containing publications

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Genelle Healey

Name/Title of Principal Supervisor: Professor Jane Coad

Name of Published Research Output and full reference:

Healey G, Brough L, Murphy R, Hedderley D, Butts C, Coad J. Validity and reproducibility of a habitual dietary fibre intake short food frequency questionnaire. Nutrients. 2016;8 (9):3–9. doi:10.3390/nu8090558

In which Chapter is the Published Work: Chapter 5

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Involved in the conception, study design, participant recruitment, running of the study and data collection, analysis and interpretation, as well as writing the manuscript.

Candidate's Signature — 27/4/17 Date

Jane Coad
Principal Supervisor's signature — 27.4.17 Date

GRS Version 3– 16 September 2011

Appendix 5-4. Validation study advertising

Dear.....

Thank you for showing an interest in participating in this study. We would like to invite you to participate in an exciting study. The aim of the study is to validate a screening questionnaire which will be used in a larger study to determine the fermentable carbohydrate intakes of potential participants.

The study Information Sheet, which explains the study in detail, has been attached to this email.

If you are interested in taking part in the study and/or know of anyone who would want to participate, please send an email to **genelle.healey@plantandfood.co.nz** or phone during work hours on **06 355 6108.**

Thank you very much for your time,

Genelle Healey
Lead Researcher

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 15/34. If you have any concerns about the conduct of this research, please contact Mr Jeremy Hubbard, Acting Chair, Massey University Human Ethics Committee: Southern A, telephone 04 801 5799 ext 63487, email humanethicsouthb@massey.ac.nz

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Plant & Food RESEARCH
RANGAHAU AHUMĀRA KAI

Screening questionnaire validation study

Participants needed

To help validate a fermentable carbohydrate intake screening questionnaire

Are you a healthy male or female aged between 19-65 years and interested in participating in this study?

Please contact Genelle Healey via email on **genelle.healey@plantandfood.co.nz** or phone **06 355 6108** during work hours

Appendix 5-5. Validation study participant information

Study title: Validation of a fermentable carbohydrate intake screening questionnaire

Lead researcher's details

Name: Genelle Healey **Contact phone number:** 06 355 6108

Email address: genelle.healey@plantandfood.co.nz

Supervisor's details

Name: Associate Professor Jane Coad **Contact phone number:** 06 951 6321

Email: j.coad@massey.ac.nz

We would like to invite you to participant in our study which aims to validate a fermentable carbohydrate screening questionnaire.

Read through the information sheet carefully before making a decision on whether or not to participate. The information sheet provides information on what the purpose of the study is, whether you meet the study inclusion criteria, what is involved in the study, the risks and benefits of participating in the study and participant rights.

If you agree to participate in the study you will need to complete a Consent Form.

WHAT IS THE PURPOSE OF THE STUDY?

The purpose of the study is to validate a fermentable carbohydrate intake screening questionnaire which will be used as part of a larger study as a quick method of assessing potential participants dietary fibre intakes.

It is important that the screening questionnaire is validated to ensure it can accurately determine a person's dietary fibre intake as the information gained from the screening questionnaire will help decide whether potential participants are eligible for inclusion in the larger study.

STUDY INCLUSION CRITERIA

To be able to participate in this study you need to meet the following criteria:

- Aged between 19 and 65 years
- Healthy
- BMI between 18.5 and 35 kg/m^2
- No significant weight loss or weight gain within the past year
- No significant dietary change within the past year
- Not pregnant or breastfeeding
- No food intolerances which cause gastrointestinal symptoms (i.e. lactose intolerance, gluten sensitivity)
- No adverse gastrointestinal symptoms
- Non-smoker
- Do not have a high alcohol intake

WHAT IS INVOLVED IN THE STUDY?

You will be asked to complete a questionnaire to ensure you meet the above inclusion criteria.

If you are eligible for inclusion in the validation study you will be asked to complete the fermentable carbohydrate intake screening questionnaire. You will then be invited to complete an online food frequency questionnaire which will be used to analyse your nutrient intake over the past year.

It will take approximately 30-50 minutes of your time to complete both the fermentable carbohydrate screening questionnaire and the food frequency questionnaire.

Two weeks after you have completed the fermentable carbohydrate intake screening questionnaire and the food frequency questionnaire you will be asked to complete the fermentable carbohydrate intake screening questionnaire again. It will take approximately 10 minutes of your time to complete the questionnaire.

All questionnaires can be completed online which means you do not have to visit a Research Unit.

WHAT ARE THE BENEFITS AND RISKS OF TAKING PART IN THIS STUDY?

Benefits

A potential benefit of being involved in this study is that you can request a copy of the nutrient intake information generated from the food frequency questionnaire.

If you are interested we can also send you a copy of the main findings of the study.

Risks

There are no perceived risks associated with participating in this study.

PARTICIPATING IN THE STUDY

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- Decline to answer any particular question
- Withdraw from the study at any time
- Ask any questions about the study at any time during participation
- Provide information on the understanding that your name will not be used unless you give permission to the researcher
- Be given access to a summary of the findings of the study when it is concluded

You will receive a $25 grocery or petrol voucher to contribute towards the time you have committed to participating in the study.

ETHICS COMMITTEE APPROVAL

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 15/34. If you have any concerns about the conduct of this research, please contact Mr Jeremy Hubbard, Acting Chair, Massey University

Human Ethics Committee: Southern A, telephone 04 801 5799 ext 63487, email humanethicsoutha@massey.ac.nz

PROJECT PROCEDURES

The data will be used only for the purposes of this study and no individual will be identified. Only the researchers and administrators of the study will have access to personal information and this will be kept secure and strictly confidential. Participants will be identified only by a participant identification number. Results of this study may be published or presented at conferences or seminars. No individual will be able to be identified.

At the end of this study the list of participants and their study identification number will be disposed of. Any raw data on which the results of the study depend will be retained in secure storage for 10 years, after which it will be destroyed.

Thank you very much for considering participating in this study

Appendix 5-6. Validation study consent form

MASSEY UNIVERSITY
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Plant & Food RESEARCH
RANGAHAU AHUMĀRA KAI

Screening questionnaire validation study

This consent form will be held for a period of five (5) years

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I consent for data to be stored for future research (please tick): Yes ☐ No ☐

I agree to participate in this study under the conditions set out in the Information Sheet.

Signature: _____ **Date:** _____

Printed full name: _____

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 15/34. If you have any concerns about the conduct of this research, please contact Mr Jeremy Hubbard, Acting Chair, Massey University Human Ethics Committee: Southern A, telephone 04 801 5799 ext 63487, email humanethicsoutha@massey.ac.nz

Appendix 5-7. Validation study screening questionnaire

Screening questionnaire validation study

Thank you for expressing an interest in participating in our research project. To ensure you are eligible to participate in the research project we would appreciate it if you can answer the following questions.

If you have any comments or questions relating to the research project or the questionnaire please feel free to contact **Genelle Healey** during working hours on **06 355 6108** or email **genelle.healey@plantandfood.co.nz**

Or

Associated Professor Jane Coad during work hours on **06 951 6321** or email **j.coad@massey.ac.nz**

Name: _____

Email address: _____

Postal address: _____

Day time phone number: _____

General practitioner: _____

Date of birth: _____

Current weight: _____kg **Height**: _____cm

Gender (please tick): Male ☐ Female ☐

Ethnicity (please tick all that apply):

NZ European ☐ Maori ☐ Samoan ☐ Cook Island Maori ☐

Tongan ☐ Indian ☐ Chinese ☐ Other ☐ please specify: _____

Do you smoke cigarettes (please tick)? Yes ☐ No ☐

Do you drink alcohol (please tick)? Yes ☐ No ☐

If yes, how many standard drinks do you consume per week? _____

(1 standard drink is 1 can/bottle of standard beer (330ml), 100ml wine or 30ml of spirits)

If yes, on how many occasions would you drink alcohol per week? _____

Have you been diagnosed with or experienced any of the following (tick for yes)?

Heart disease	
Stroke	
High cholesterol	
High blood pressure	
Kidney disease	
Cancer	
Diabetes (type 1 or 2, or prediabetes)	
Inflammatory bowel disease	
Irritable bowel syndrome	
Bowel or gastrointestinal surgery	
Food intolerance or allergies causing diarrhoea, bloating, cramping or constipation	
Long term diarrhoea or constipation	
Autoimmune disease (e.g. Coeliac disease, Rheumatoid arthritis, Multiple sclerosis)	
Liver disease	
Other (please specify):	

Are you taking any medications (traditional or homeopathic) **or nutritional supplements?**

Type of medication/supplement	**Taking?** (please tick)	**If you have answered <u>YES</u> for any of the medication or supplement options please provide the below information**	
		Medication/supplement name	***Dose and frequency***
Antibiotics	Yes ☐ No ☐		
Blood pressure lowering	Yes ☐ No ☐		
Cholesterol lowering	Yes ☐ No ☐		
Vitamins or minerals	Yes ☐ No ☐		
Laxatives	Yes ☐ No ☐		
Metamucil or Benefibre	Yes ☐ No ☐		
Phloe or Kiwicrush	Yes ☐ No ☐		
Probiotics	Yes ☐ No ☐		
Prebiotics	Yes ☐ No ☐		
Antacids or anti-reflux	Yes ☐ No ☐		
Anti-inflammatory	Yes ☐ No ☐		
Other	Yes ☐ No ☐		
Other	Yes ☐ No ☐		

Are you pregnant or breastfeeding (please tick)?

Yes ☐ No ☐

Has your weight been stable over the <u>past year</u> (please tick)? Yes ☐ No ☐

If no, please record the amount of weight you have lost or gained? _____kg

Have you made any significant changes to your food intake (i.e. become vegetarian/vegan, stopped consuming gluten, dairy or sugar, increased your fruit and vegetable intake or increased/decreased the amount of food you are eating) **over the <u>past year</u>** (please tick)? Yes ☐ No ☐

If yes, what changes to your food intake have you made?

_____

Do you regularly experience any of the following (please tick all that apply)?

Abdominal pain ☐ Abdominal bloating ☐

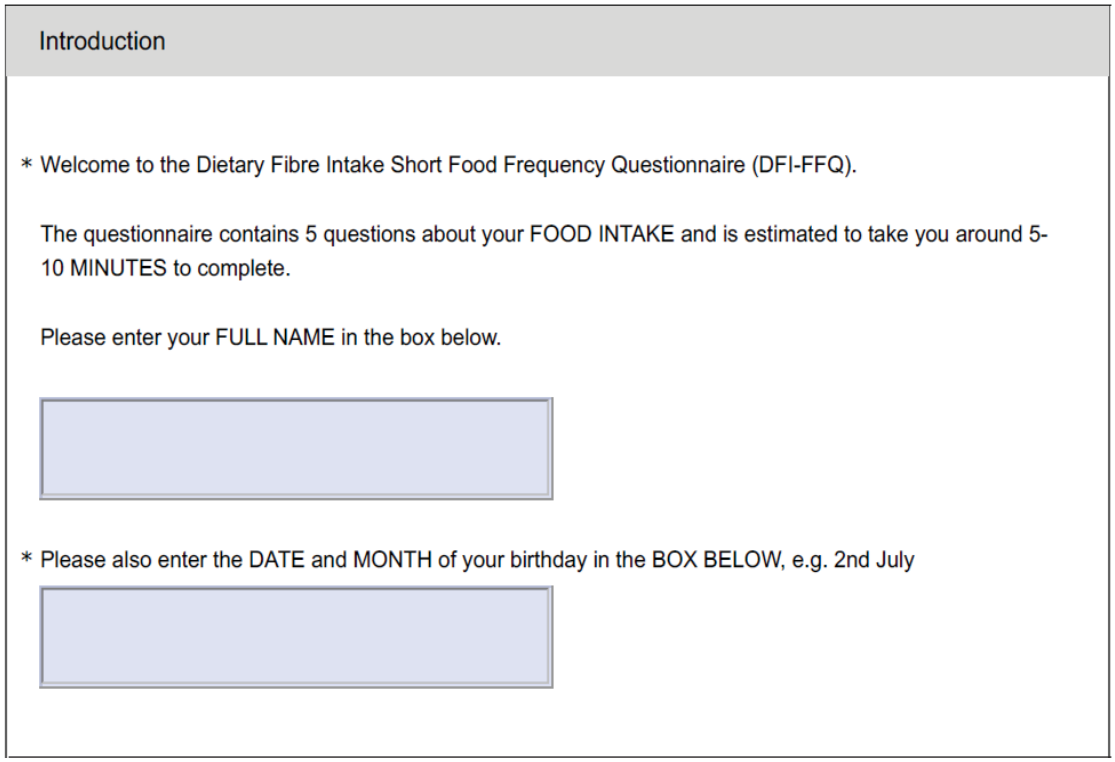
Flatulence/wind ☐

If you experience abdominal pain, bloating or flatulence/wind is it mild (nagging/annoying)**, moderate** (strong negative influence on your daily living) **or severe** (disabling) (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Abdominal pain				
Abdominal bloating				
Flatulence/wind				

Thank you very much for taking the time to complete the screening questionnaire. We will be in contact with you shortly.

Appendix 5-8. Validation study SurveyMonkey DFI-FFQ



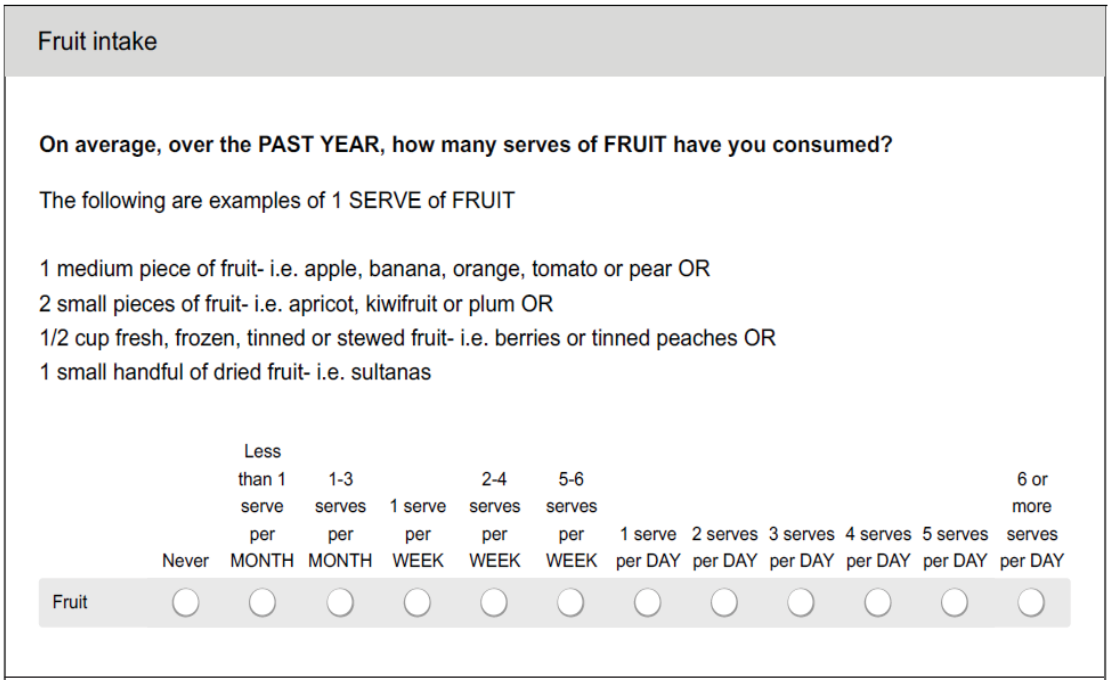
Introduction

* Welcome to the Dietary Fibre Intake Short Food Frequency Questionnaire (DFI-FFQ).

The questionnaire contains 5 questions about your FOOD INTAKE and is estimated to take you around 5-10 MINUTES to complete.

Please enter your FULL NAME in the box below.

* Please also enter the DATE and MONTH of your birthday in the BOX BELOW, e.g. 2nd July



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	○	○	○	○	○	○	○	○	○	○	○	○

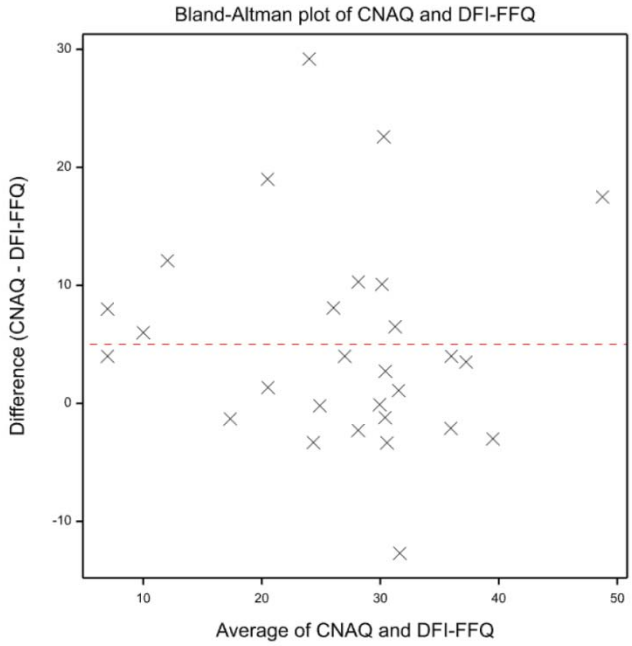
Appendix 5-9. Validation study DFI-FFQ scoring sheet

Food group	**Average dietary fibre content (g) per frequency of consumption for each food group**											
	Never	**<1/ month**	**1-3/ month**	**1/ week**	**2-4/ week**	**5-6/ week**	**1/ day**	**2/ day**	**3/ day**	**4/ day**	**5/ day**	**6+/ day**
Fruit	0.0	0.0	0.0	0.3	0.9	1.7	2.1	4.28	6.4	8.6	10.7	12.8
Vegetables	0.0	0.0	0.0	0.4	1.3	2.3	2.9	5.8	8.7	11.6	14.6	17.5
Breads and cereals	0.0	0.0	0.0	0.4	1.2	2.2	2.8	5.6	8.4	11.2	14.0	16.7
Nuts and seeds	0.0	0.0	0.0	0.5	1.6	3.0	3.8	7.6	11.4	15.2	19.0	22.8
Legumes- beans, peas and lentils	0.0	0.0	0.0	1.2	3.6	6.6	8.4	16.9	25.3	33.7	42.2	50.6

Appendix 5-10. Validation study Bland-Altman plots

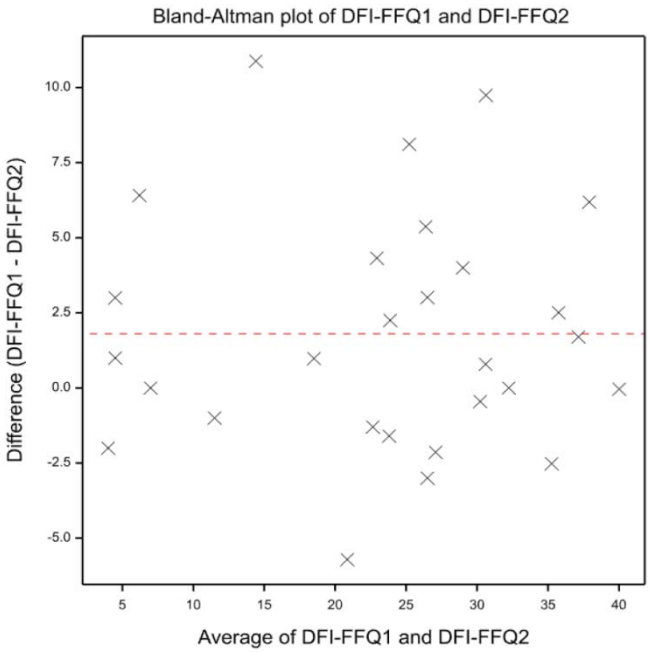
Bland-Altman plots for comparison between the CNAQ and DFI-FFQ **(A)**

(A)



Bland-Altman plots for comparison between the repeated DFI-FFQs **(B)**

(B)



Appendix 6-1. Study protocol publication

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Open Access Protocol

BMJ Open

Influence of habitual dietary fibre intake on the responsiveness of the gut microbiota to a prebiotic: protocol for a randomised, double-blind, placebo-controlled, cross-over, single-centre study

Genelle Healey,[1,2] Louise Brough,[1] Chrissie Butts,[2] Rinki Murphy,[3] Kevin Whelan,[4] Jane Coad[1]

To cite: Healey G, Brough L, Butts C, *et al*. Influence of habitual dietary fibre intake on the responsiveness of the gut microbiota to a prebiotic: protocol for a randomised, double-blind, placebo-controlled, cross-over, single-centre study. *BMJ Open* 2016;**6**:e012504. doi:10.1136/bmjopen-2016-012504

► Prepublication history for this paper is available online. To view these files please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2016-012504).

Received 3 May 2016
Revised 28 June 2016
Accepted 5 August 2016

For numbered affiliations see end of article.

Correspondence to
Genelle Healey; genelle.healey@plantandfood.co.nz

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 29 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,[1 2] extract nutrients from undigested dietary components,[2 3] modulate the immune system[2] and synthesise essential vitamins.[4] 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.[5–8] There are a number of factors which can influence the composition and therefore balance of the gut microbiota including diet,[9] genetics,[10] life stage,[11]

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gender,[12] antibiotic use[9 13] and disease,[5] with diet being particularly important.

Researchers are 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 well-being 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*, *Ruminococcus 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 lead 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

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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/m^2;
- 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

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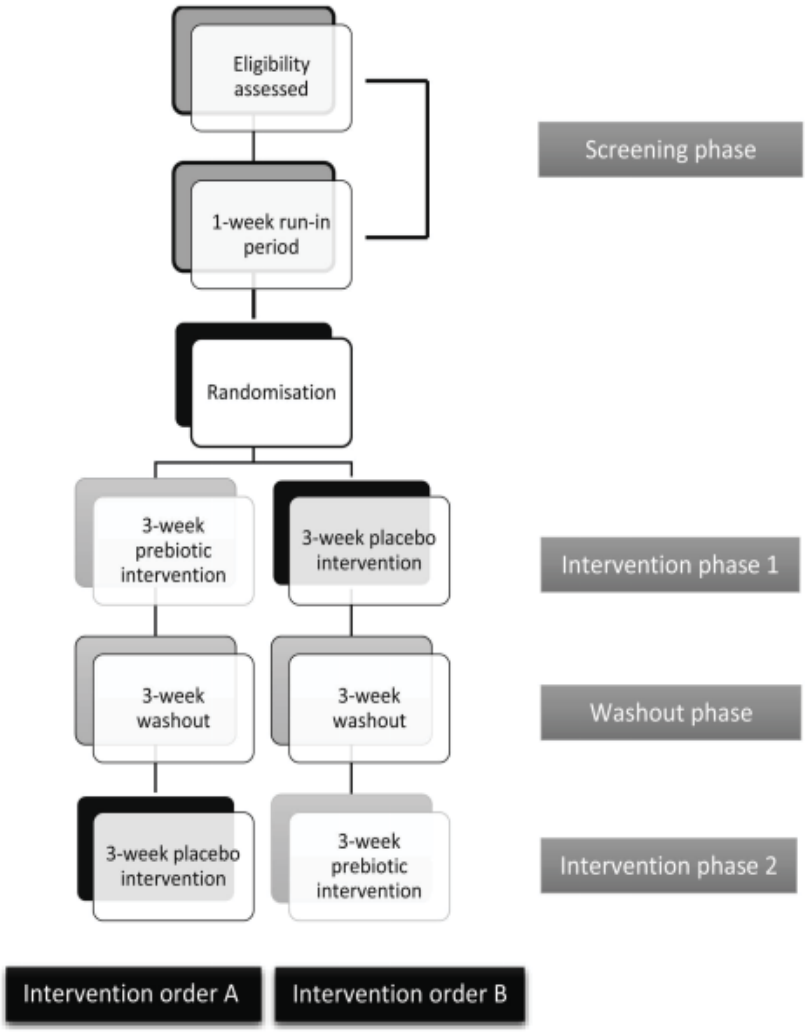

Figure 1 Flow diagram summarising the four separate study phases including the two possible intervention orders. The intervention orders may not be as described within the figure as they are blinded to the lead researcher, analysts and participants.

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.[37] The low dietary fibre intake cut-offs were

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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 complete a 3-day diet record and appetite questionnaire, and a fructan intake FFQ (FI-FFQ)[39] by a registered dietitian, as well as materials and 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 initiation of IP1 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 prebiotic[15] 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

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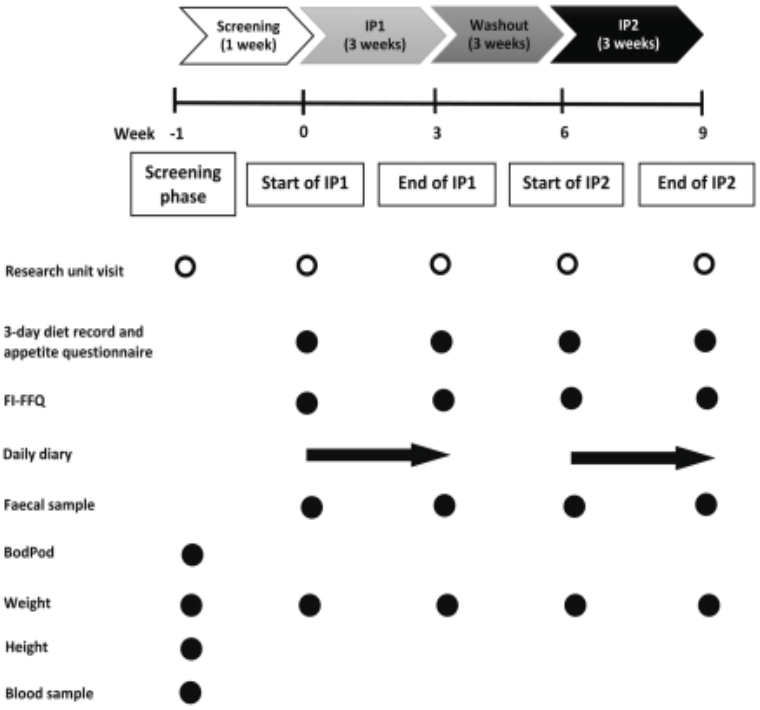

Figure 2 Participant flow through the study including measurements, questionnaires and samples taken at each Human Nutrition Research Unit visit. BodPod, air displacement plethysmography; FI-FFQ, fructan intake food frequency questionnaire; IP, intervention phase.

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, borborygmi and bloating.[23 41] 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.[42]

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.

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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 IP1 (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[43] 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.

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BMJ Open

Influence of habitual dietary fibre intake on the responsiveness of the gut microbiota to a prebiotic: protocol for a randomised, double-blind, placebo-controlled, cross-over, single-centre study

Genelle Healey, Louise Brough, Chrissie Butts, Rinki Murphy, Kevin Whelan and Jane Coad

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Appendix 6-2. Statement of contribution to doctoral thesis containing publications

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Genelle Healey

Name/Title of Principal Supervisor: Professor Jane Coad

Name of Published Research Output and full reference:

Healey G, Brough L, Butts C, et al. Influence of habitual dietary fibre intake on the responsiveness of the gut microbiota to a prebiotic: protocol for a randomised, double-blind, placebo-controlled, cross-over, single-centre study. BMJ Open 2016; 6: e012504. doi:10.1136/bmjopen-2016-012504

In which Chapter is the Published Work: Chapter 6

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Involved in the conception, study design and writing the manuscript.

Candidate's Signature — Date: 27/4/17

Jane Coad — Principal Supervisor's signature — Date: 27.4.17

GRS Version 3– 16 September 2011

Appendix 7-1. Human intervention study advertisement

Plant & Food RESEARCH
RANGAHAU AHUMĀRA KAI

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Participants Needed

What you eat influences your gut bacteria and health

We are looking for…

- **healthy** volunteers
- aged between **19-65 years**
- who eat a LOT or VERY LITTLE **fruit, vegetables, wholegrains, nuts, seeds and legumes**

You will receive a **voucher** for your contribution in this study.

Please contact: Genelle Healey via email on **adaptstudy2016@gmail.com**

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 15/34. If you have any concerns about the conduct of this research, please contact Mr Jeremy Hubbard, Acting Chair, Massey University Human Ethics Committee: Southern A, telephone 04 801 5799 ext 63487, email humanethicsouthb@massey.ac.nz

Appendix 7-2. Human intervention study consent form

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Plant & Food RESEARCH
RANGAHAU AHUMĀRA KAI

ADAPT (hAbitual Diet And PrebioTic) study

This consent form will be held for a period of five (5) years

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

All collected samples (i.e. blood and faeces) are kept in a containment laboratory and therefore cannot be returned.

I consent for data to be stored for future research (please tick): Yes ☐ No ☐

I consent for faecal and blood samples to be stored for up to 2 years for future research (please tick): Yes ☐ No ☐

I agree to participate in this study under the conditions set out in the Information Sheet.

Signature: _____ **Date:** _____

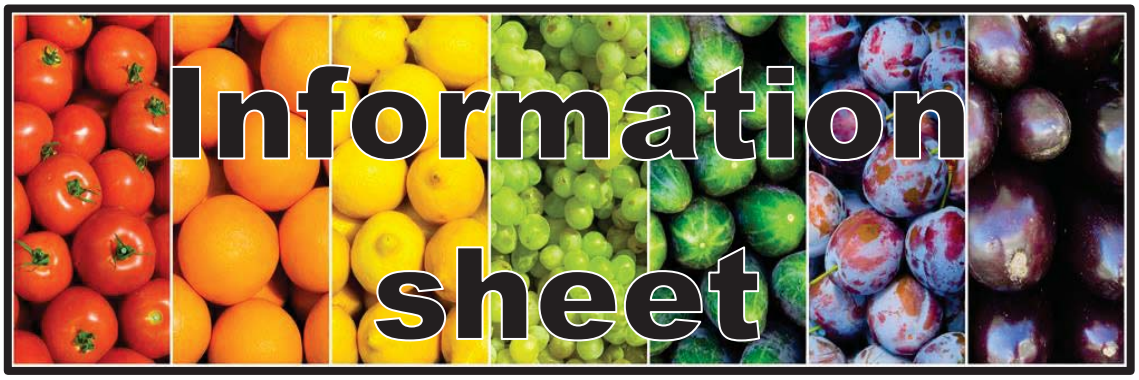
Printed full name: _____

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 15/34. If you have any concerns about the conduct of this research, please contact Mr Jeremy Hubbard, Acting Chair, Massey University Human Ethics Committee: Southern A, telephone 04 801 5799 ext 63487, email humanethicsoutha@massey.ac.nz

Appendix 7-3. Human intervention study information sheet

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Plant & Food RESEARCH
RANGAHAU AHUMĀRA KAI



Study title: ADAPT (hAbitual Diet And PrebioTic) study

Lead researchers details

Name: Genelle Healey **Contact phone number:** 06 355 6108

Email address: adaptstudy2016@gmail.com

Supervisor's details

Name: Professor Jane Coad **Contact phone number:** 06 951 6321

We would like to invite you to participate in our study which aims to investigate whether long term dietary fibre intake influences how the bacteria within your gastrointestinal system (gut) respond to a prebiotic supplement.

Read through the information sheet carefully before making a decision on whether or not to participate. The information sheet will inform you on what the purpose of the study is, whether you meet the study inclusion criteria, what is involved in the study, the risks and benefits of participating in the study and participant rights.

If you agree to participate in the study you will need to complete a Consent Form.

WHAT IS THE PURPOSE OF THE STUDY?

Research has shown that what we eat can influence the type of bacteria in our gut. It has also been shown that the bacteria in our gut can have an influence on our health.

The presence of "unfavourable" bacteria within the gut has been linked to obesity, diabetes and some gastrointestinal disorders such as inflammatory bowel disease and irritable bowel syndrome. Researchers are therefore trying to determine what can be done to ensure the bacteria living in our gut are "favourable" to help optimise our health. A number of dietary fibre supplements, known as prebiotics, have been shown to improve our health through their actions on "favourable" gut bacteria. Prebiotics are not digested by humans and therefore reach the large bowel where they are used by the gut bacteria as a source of energy.
On a day to day basis we consume dietary fibre from the foods we eat. Some people consume a large amount of dietary fibre while others consume small amounts. We believe that if someone is already consuming foods high in dietary fibre then the gut bacteria will likely respond in a different way to a prebiotic than the gut bacteria of an individual with a low intake of dietary fibre foods. The aim of the study is to determine what influence a person's long term dietary fibre intake has on how their gut bacteria respond to a prebiotic supplement.

PARTICIPATION IN THE STUDY

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- Decline to answer any particular question
- Withdraw from the study at any time
- Ask any questions about the study at any time during participation
- Provide information on the understanding that your name will not be used unless you give permission to the researcher
- Be given access to a summary of the findings of the study when it is concluded

You are welcome to bring along a family/whanau member or support person to each of your visits to the Massey University Human Nutrition Research Unit.

At the end of the study you will receive a **$25 grocery or petrol voucher for each research unit visit** (of which there are 5 in total) you attended to contribute towards your travel costs and the time you have committed to participating in the study.

All samples collected (i.e. blood and faecal) are stored in a containment laboratory and therefore cannot be returned at the end of the study.

STUDY INCLUSION CRITERIA

To be able to participate in this study you need to meet the following criteria:

- Aged between 19 and 65 years
- Not taken antibiotics within the last 6 months
- Not taken laxatives, gastric motility medications, prebiotic or probiotic containing foods or supplements (i.e. Symbio probalance, Bio yoghurt, Yoplait Elivae, Activate, Bio farm organic, Yakult, Kefir, Psyllium, Sauerkraut, Kimchi, Kombucha) within the last month*
- Healthy

- BMI between 18.5 and 30kg/m^2
- No significant weight loss or weight gain within the past year
- No significant dietary change within the past year
- Not pregnant, breastfeeding or planning a pregnancy in the next 3 months
- No food intolerances which cause gastrointestinal symptoms (i.e. lactose intolerance, gluten sensitivity)
- Non-smoker
- Do not have a high alcohol intake
- Have either a low or high dietary fibre intake (consume either a high or low amount of fruit, vegetables, legumes, wholegrains, nuts and seeds)

***Please note:** If you are taking prebiotic or probiotic containing foods or supplements and are willing to stop taking these foods or supplements for a month prior to starting the study and during the study then you are also eligible to take part in the study.

WHAT IS INVOLVED IN THE STUDY?

If you decide to participate in the study you will initially be asked to complete a screening questionnaire to ensure you are able to be involved in the study.

If you appear to be eligible to participate in the study the researcher will make an appointment with you to visit the Palmerston North Massey University Human Nutrition Research Unit.

At your first visit you will:

- Be weighed and your height will be measured
- Fill out a participant questionnaire
- Be provided with instructions on how to complete the appetite questionnaire and 3 day diet record, and fructan intake food frequency questionnaire (FI-FFQ)
- Be provided with instructions and equipment to collect a faecal sample. The faecal sample will be used to analyse the bacteria in your gut.
- Have your body composition measured using the BodPod which will involve wearing a swimsuit or tight clothing (i.e. gym tights)
- Have a fasted blood sample (15ml will be taken which is around 3 tsp) taken by a qualified phlebotomist to check your health. The following tests will be undertaken- liver function test, calcium, creatinine, C-reactive protein, glucose, complete blood count, potassium and sodium. You will need to fast overnight for the blood test. Breakfast will be provided after the blood sample has been taken

The first visit will take approximately 1 hour.

Within a week of the first visit you will be invited back to the Research Unit for a second visit.
During the second visit you will:

- Bring along a faecal sample which was collected at home. The faecal sample may need to be stored in your freezer. The sample will be triple contained which means it will be stored hygienically in your freezer

- Return a completed 3 day diet record and appetite questionnaire
- Return a completed FI-FFQ
- Be weighed

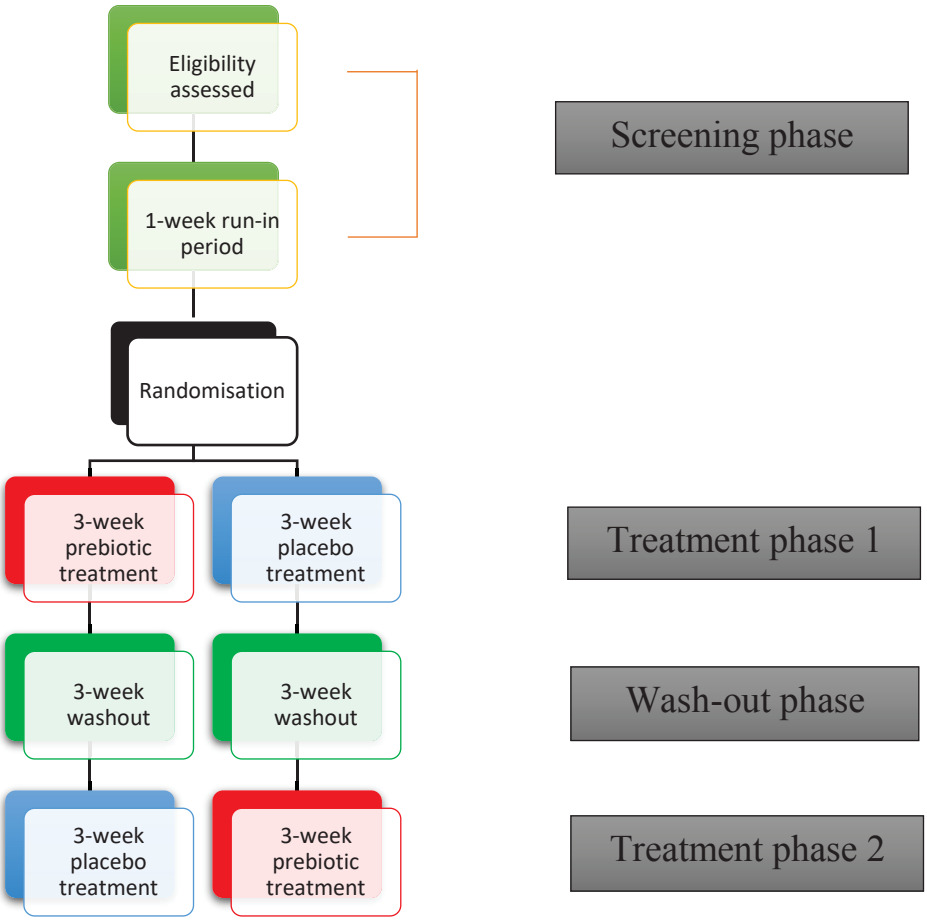
This visit will take around 30 minutes.

A picture of someone sitting inside the BodPod

During this visit your blood test results will be reviewed. If any of the blood tests are not in the normal range you will not be eligible for inclusion in the study and you will be provided with a letter that you can take with you when you next visit your general practitioner or on request the letter can be sent to your medical practice directly.

If you are eligible for inclusion in the study you will be randomly allocated a treatment order so you receive the prebiotic and placebo in a random order. You will take both treatments over the course of the study. You and the researcher will not know what treatment you are on. This will help ensure that you and the researcher have no influence on the effect of the treatment. The total length of the study is 10 weeks. There will be a 1 week run in period, two 3 week treatment periods with a 3 week no treatment period (washout) between the treatments.
During the 3 week prebiotic treatment period you will be required to take 8g of prebiotic powder twice a day (16g in total; equivalent to around 4 teaspoons) mixed into hot and cold liquids (i.e. yoghurt, tea, coffee etc.). During the 3 week placebo treatment period you will be required to take 8g of placebo powder twice a day (16g in total; equivalent to around 4 teaspoons) mixed into hot and cold liquids. You will not receive a treatment during the 3 week no treatment period.

The diagram below helps demonstrate how the study will progress.



Throughout the study three additional visits to the Research Unit will be organised with you (5 visits in total). The third visit will occur at the end of the first 3 week treatment period. The fourth visit will occur at the start of the second treatment period which will also be the end of the 3 week no treatment period. The final visit will occur at the end of the second treatment period.

At each of these visits you will:

- Bring along a faecal sample which was collected at home. The faecal sample may need to be stored in your freezer. The sample will be triple contained which means it will be stored hygienically in your freezer
- Return a completed 3 day diet record and appetite questionnaire
- Return a completed FI-FFQ
- Be weighed

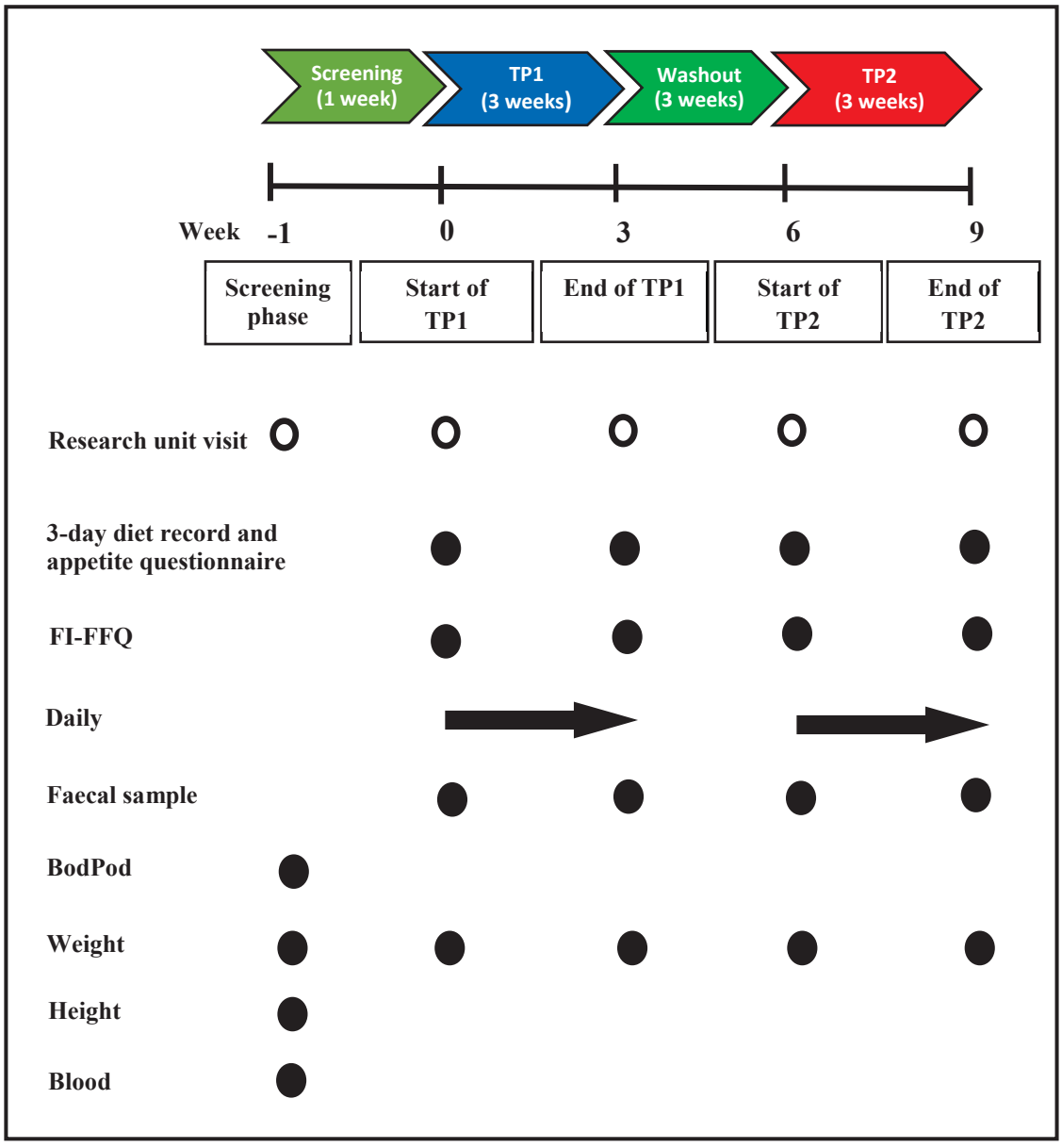
These visits will take around 30 minutes each.

The 3 day diet record and appetite questionnaires are estimated to take around 45 minutes each to complete (3 hours over the duration of the study).

The FI-FFQ is estimated to take around 10 minutes to complete (40 minutes over the duration of the study).

Also over the course of the study you will be asked to complete a daily diary to record when you took the treatments and whether you experience any gastrointestinal symptoms. This will only take around 5 minutes per day to complete.

The diagram below helps demonstrate the flow of the study.



Abbreviations: TP- treatment phase, FI-FFQ- fructan intake food frequency questionnaire

WHAT ARE THE BENEFITS AND RISKS OF TAKING PART IN THIS STUDY?

Benefits

A potential benefit of being involved in this study is that you will contribute to gaining a better understanding of what influence long term dietary fibre intakes have on the responsiveness of the gut bacteria to a prebiotic.

If you are interested you can also request to be sent information relating to your body composition measurements and dietary intake analysis.

You will also receive a summary of the main findings of the study which will either be emailed or posted to you.

Risks

Some people may experience mild to moderate gastrointestinal symptoms, such as diarrhoea, bloating and flatulence/wind, as a result of taking the treatment. Often these symptoms only last a few days while your gastrointestinal system adjusts. If any adverse events occur during the study you will be immediately withdrawn.

As with all blood tests there may be some discomfort when the needle is inserted. You may also receive a bruise after the blood sample is taken, however this is unlikely.

ETHICS COMMITTEE APPROVAL

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 15/34. If you have any concerns about the conduct of this research, please contact Mr Jeremy Hubbard, Acting Chair, Massey University Human Ethics Committee: Southern A, telephone 04 801 5799 ext 63487, email humanethicsoutha@massey.ac.nz

PROJECT PROCEDURES

The data will be used only for the purposes of this study and no individual will be identified. Only the researchers and administrators of the study will have access to personal information and this will be kept secure and strictly confidential. Participants will be identified only by a participant identification number. Results of this study may be published or presented at conferences or seminars. No individual will be able to be identified.

At the end of this study the list of participants and their study identification number will be disposed of. Any raw data on which the results of the study depend will be retained in secure storage for 10 years, after which the data will be destroyed.

COMPENSATION FOR INJURY

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Injury Prevention, Rehabilitation and Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

If your ACC claim is not accepted you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

Thank you very much for considering participating in this study

Appendix 7-4. Human intervention study screening questionnaire

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Plant & Food RESEARCH
RANGAHAU AHUMĀRA KAI

ADAPT (hAbitual Diet And PrebioTic) study

Thank you for expressing an interest in participating in our research project. To ensure you are eligible to participate in the research project we would appreciate it if you can answer the following questions.

If you have any comments or questions relating to the research project or the questionnaire please feel free to contact **Genelle Healey** during working hours on **06 355 6108** or email **adaptstudy2016@gmail.com**

OR

Professor Jane Coad during working hours on **06 951 6321**

Name: _____

Email address: _____

Postal address: _____

Day time phone number: _____

General practitioner: _____

Date of birth: _____

Current weight: _____kg **Height**: _____cm

Gender (please tick): Male ☐ Female ☐

Ethnicity (please tick all that apply):

NZ European ☐ Maori ☐ Samoan ☐ Cook Island Maori ☐

Tongan ☐ Indian ☐ Chinese ☐ Other ☐ please specify:_____

Do you smoke cigarettes (please tick)? Yes ☐ No ☐

Do you drink alcohol (please tick)? Yes ☐ No ☐

If yes, how many standard drinks do you consume per week? _____

(1 standard drink is 1 can/bottle of standard beer (330ml), 100ml wine or 30ml of spirits)

If yes, on how many occasions would you drink alcohol per week? _____

Have you been diagnosed with or experienced any of the following (tick for yes)?

Heart disease	
Stroke	
High cholesterol	
High blood pressure	
Kidney disease	
Cancer	
Diabetes (type 1 or 2, or prediabetes)	
Inflammatory bowel disease	
Irritable bowel syndrome	
Bowel or gastrointestinal surgery	
Food intolerance or allergies causing diarrhoea, bloating, cramping or constipation	
Long term diarrhoea or constipation	
Autoimmune disease (e.g. Coeliac disease, Rheumatoid arthritis, Multiple sclerosis)	
Liver disease	
Other (please specify):	

Are you taking any medications (traditional or homeopathic) **or nutritional supplements?**

Type of medication/supplement	**Taking?** (please tick)	**If you have answered <u>YES</u> for any of the medication or supplement options please provide the below information**	
		Medication/supplement name	***Dose and frequency***
Antibiotics	Yes☐ No☐		
Blood pressure lowering	Yes☐ No☐		
Cholesterol lowering	Yes☐ No☐		
Vitamins or minerals	Yes☐ No☐		
Laxatives	Yes☐ No☐		
Metamucil or Benefibre	Yes☐ No☐		
Phloe or Kiwicrush	Yes☐ No☐		
Probiotics	Yes☐ No☐		
Prebiotics	Yes☐ No☐		
Antacids or anti-reflux	Yes☐ No☐		
Anti-inflammatory	Yes☐ No☐		
Other	Yes☐ No☐		
Other	Yes☐ No☐		

Have you consumed prebiotic or probiotic yoghurt, or fermented drinks or foods (i.e. Symbio probalance, Bio yoghurt, Yoplait Elivae, Activate, Bio farm organic, Yakult, Kefir, Sauerkraut, Kimchi, Kombucha) **within the <u>past month</u>** (please tick)?

Yes ☐ No ☐

If yes, please specify the product name and frequency of consumption: _____

_____

Have you taken antibiotics within the <u>last 6 months</u> (please tick)?

Yes ☐ No ☐

If yes, please specify the name of the antibiotic and when you last took it:

_____

Are you pregnant or breastfeeding, or planning to become pregnant within the <u>next 3 months</u> (please tick)?

Yes ☐ No ☐

Has your weight been stable over the <u>past year</u> (please tick)? Yes ☐ No ☐

If no, please record the amount of weight you have lost or gained? _____kg

Have you made any significant changes to your food intake (i.e. become vegetarian/vegan, stopped consuming gluten, dairy or sugar, increased your fruit and vegetable intake or increased/decrease the amount of food you are eating) **over the <u>past year</u>** (please tick)?

Yes ☐ No ☐

If yes, what changes to your food intake have you made? _____

_____

Do you regularly experience any of the following (please tick all that apply)?

Abdominal pain ☐ Abdominal bloating ☐

Flatulence/wind ☐

If you experience abdominal pain, bloating or flatulence/wind is it mild (nagging/annoying)**, moderate** (strong negative influence on your daily living) **or severe** (disabling) (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Abdominal pain				
Abdominal bloating				
Flatulence/wind				

On average, over the <u>past year,</u> how many serves of the following food groups have you consumed?

Food group	**1 serve equals...** (these are examples only)	**Frequency of consumption (serves)-** tick the box which applies for each food group											
		Never	< 1/ month	1-3/ month	1/ week	2-4/ week	5-6/ week	1/ day	2/ day	3/ day	4/ day	5/ day	6+/ day
Fruit	1 medium piece of fruit- i.e. apple, banana, orange, tomato or pear <u>OR</u> 2 small pieces of fruit- i.e. apricot, kiwifruit or plum <u>OR</u> ½ cup fresh, frozen, tinned or stewed fruit- i.e. berries, tinned peaches <u>OR</u> 1 small handful dried fruit- i.e. sultanas												
Vegetables	1 medium potato, kumara, yam, taro or carrot <u>OR</u> ½ cup cooked broccoli, peas, corn, pumpkin, spinach <u>OR</u> 1 cup salad												
Breads and cereals	<u>Wholemeal/wholegrain-</u> 1 slice of bread, 1 small roll or 1 wrap <u>OR</u> <u>White-</u> 2 slices of bread, 2 small rolls or 2 wraps <u>OR</u> <u>Rice/Pasta-</u> ½ cup cooked brown rice or wholemeal pasta or 1 cup white rice or white pasta <u>OR</u> <u>Cereals-</u> ½ cup porridge or muesli, ⅓ cup All/Sultana/San Bran, 2 Weet-Bix												
Nuts and seeds	2 Tb peanut butter <u>OR</u> ⅓ cup nuts or seeds (e.g. cashews, almonds, pistachio, brazil nuts, macadamia nuts, hazel nuts, chia seeds, sunflower seeds, pumpkin seeds, sesame seeds)												
Legumes- beans, peas and lentils	¾ cup tofu <u>OR</u> ¾ cup cooked legumes (e.g. kidney beans, chickpeas, green/brown/red lentils, hummus, baked beans, split peas, canned bean mix, broad beans, white or black beans)												

Thank you very much for taking the time to complete the screening questionnaire. We will be in contact with you shortly.

Appendix 7-5. Human intervention study participant questionnaire

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Plant & Food RESEARCH
RANGAHAU AHUMĀRA KAI

ADAPT (hAbitual Diet And PrebioTic) study

Participant identification number: _____

If you have any comments or questions relating to the research project or the questionnaire please feel free to contact **Genelle Healey** during working hours on **06 355 6108** or email **adaptstudy2016@gmail.com**

OR

Professor Jane Coad during working hours on **06 951 6321**

Have you consumed prebiotic or probiotic yoghurt, or fermented drinks or foods (i.e. Symbio probalance, Bio yoghurt, Yoplait Elivae, Activate, Bio farm organic, Yakult, Kefir, Psyllium, Sauerkraut, Kimchi, Kombucha) **at <u>any time in your life</u>** (please tick)?

Yes ☐ No ☐

If yes, please specify the product name/s and when you last consumed the product/s:

_____

_____

Do you regularly remove the skin from any of the following fruit and/or vegetables before you eat them (please tick the ones that apply)?

Apple	☐	Cucumber	☐	Carrot	☐
Kiwifruit	☐	Kumara	☐	Parsnip	☐
Peach	☐	Pear	☐	Potato	☐
Pumpkin	☐				

Are you a vegetarian (do not consume meat, fish or chicken) **or vegan** (do not consume meat, fish, chicken, eggs or dairy) (please tick one)?

No ☐ Yes, vegetarian ☐ Yes, vegan ☐

Do you regularly skip meals (please tick)?

Yes ☐ No ☐

If yes, please indicate which meals you regularly skip (please tick all that apply):

Breakfast ☐ Lunch ☐ Dinner ☐

How many snacks would you consume on average <u>per day</u> (please tick one)?

None ☐ One ☐ Two or Three ☐ Four or more ☐

What statement would best describe your day to day activity level (please tick one)?

Chair-bound or bed-bound	
Seated work with **no option of moving** around and **little or no** strenuous leisure activity	
Seated work with **no option of moving** around but **strenuous leisure activity**	
Seated work with some **moving around** but with **little or no** strenuous leisure activity	
Seated work with some **moving around** and **strenuous leisure activity**	
Standing work with **little or no** strenuous leisure activity	
Standing work with **strenuous leisure activity**	
Strenuous work **or** very active leisure	
Strenuous work **and** very active leisure	

Please note: **strenuous leisure activity** is defined as 30-60 minutes of activity (i.e. walking) or sport, 4-5 times a week. **Very active leisure** would exceed strenuous activity requirements.

Have you ever had to take antibiotics as a child or adult (please tick)?

Yes ☐ No ☐

If yes, please estimate on how many separate occasions you have taken antibiotics over your lifetime (please tick one):

1-4 times ☐ 5-10 times ☐ 11-14 times ☐ >15 times ☐

On average how many bowel motions do you pass a week? _____

When you pass a bowel motion do you have a sensation of complete bowel emptying (please tick one)?

Yes, with all bowel motions ☐

Yes, with around half of all bowel motions ☐

Yes, but infrequently ☐

No ☐

Do you usually have to strain to pass a bowel motion (please tick one)?

Yes, with all bowel motions	☐
Yes, with around half of all bowel motions	☐
Yes, but infrequently	☐
No	☐

Do you ever need to take laxatives to help you pass a bowel motion (please tick)?

Yes ☐ No ☐

If yes, please estimate how many days per year you take laxatives (please tick one):

1-4 times ☐ 5-10 times ☐ 11-14 times ☐ >15 times ☐

Can you please choose which bowel motion consistency best describes your bowel motions (*refer to the Bristol Stool Chart on the following page*) (please tick one):

Type 1- Separate hard lumps, like nuts (hard to pass)	☐
Type 2- Sausage-shaped but lumpy	☐
Type 3- Like a sausage but with cracks on its surface	☐
Type 4- Like a sausage or snake, smooth and soft	☐
Type 5- Soft blobs with clear-cut edges (passed easily)	☐
Type 6- Fluffy pieces with ragged edges, a mushy stool	☐
Type 7- Watery, no solid pieces. Entirely Liquid	☐

Bristol Stool Chart

Type 1		Separate hard lumps, like nuts (hard to pass)
Type 2		Sausage-shaped but lumpy
Type 3		Like a sausage but with cracks on its surface
Type 4		Like a sausage or snake, smooth and soft
Type 5		Soft blobs with clear-cut edges (passed easily)
Type 6		Fluffy pieces with ragged edges, a mushy stool
Type 7		Watery, no solid pieces. **Entirely Liquid**

Thank you very much for taking the time to complete the questionnaire

Appendix 7-6. Human intervention 3-day diet record and appetite questionnaire

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Plant & Food RESEARCH
RANGAHAU AHUMĀRA KAI

ADAPT (hAbitual Diet And PrebioTic)

Instructions for completing the 3 day diet record section

- Record **everything you eat and drink** over **three days**. It is really important that you **do not adjust what you eat or drink** because you are keeping a record. We are only interested in your normal eating habits not the perfect diet.
- Please record food **at the time of eating** and **not by memory** at the end of the day. This will help improve accuracy.
- Start each day at **midnight** and finish 24 hours later.
- The **more detail** you can provide the **better**.
- Please record any **dietary supplements** taken, i.e. vitamin C, iron etc.

On the diet record sheets include:

- The time of day the food was eaten
- The food or drink consumed, including a precise description of the food or drink (variety, brand, cut of meat etc.)
- The way the food or beverage was prepared (fried, steamed, boiled)
- The amount eaten (weight, measures etc.)

Please note:

- **Eat normally.** You will not be judged on the food you eat.
- Remember to record all drinks, **including water**.
- **Measure foods and fluids where possible**, e.g. how many cups of breakfast cereal, rather than writing "a bowl"; how many cups or scoops of ice cream, rather than writing "a dessert plate". Use **household measures**, e.g. teaspoon, tablespoon, cup.

- Many purchased foods and drinks state the **weight or volume on the labels**, these can be used directly. Empty packages can be stapled to the form.
- **Recipes** can be printed out or attached to the diet record. Make sure you indicate on the diet record the proportion of the recipe you consumed, i.e. the recipes serves 4 and 1 serving eaten and recorded on the diet record.

Diet record example:

Time	**Food or drink-** including description (variety, brand, cut of meat)	**Food or beverage preparation**	**Amount consumed** (weight, measures etc)
7:20 AM	Porridge (Harraways wholegrain oats) with brown sugar (Pams) and trim milk (Meadow Fresh)	Cooked on the stove	1 cup cooked porridge 1 Tsp brown sugar ½ cup milk
" "	Orange juice (Citrus tree)		1 glass (275 ml)
10 AM	Gala apple	Raw- core removed	1 medium (150g)
12 noon	Sandwich – bread (Pams), ham (shaved, deli), lettuce, tomato and tasty cheese (Mainland)		2 slices of wholemeal bread Ham – 1 slice Lettuce – 1 leaf Tomato – 1 small Cheese – 1 slice (20g)
" "	Chocolate mint biscuit (Arnotts)		2 biscuits
4 PM	Lite Greek yoghurt (Yoplait)		1 pottle (125g)
	Banana	Raw	1 small banana (120g)
7.30 PM	Spaghetti Bolognese- Meat sauce (see attached recipe) Spaghetti pasta (Diamond) Tasty cheese (Mainland) Salad- lettuce, tomato, red onion and French dressing (Eta) Carrot	 Slow cooker Boiled Grated Raw	 1 serve 1 cup (cooked) Around 1 slice (20g) Lettuce- 2 leaves Tomato- 1 small Red onion- 1/20th of an onion Dressing- 1 Tsp 1 medium
Over the day	Water	Tap	1500ml

Recipe example:

Spaghetti Bolognese meat sauce recipe		**Serves 4**
500g lean beef mince	2 cloves garlic, minced	1 onion, chopped
2 tins of chopped tomatoes	4 Tsp tomato puree	1 tsp dried oregano

Add all ingredients to a slow cooker and mix to combine. Set the slower cooker to low heat and simmer for 6-8 hours.

Instructions for completing the Appetite questionnaire section

- Please complete the appetite questionnaire **on the same three days** you **record your dietary intake** using the **3 day diet record**
- The appetite questionnaire is to be completed **6 times per day- 30 minutes before and 30 minutes after breakfast, lunch and dinner**

An example of how to use the appetite scale

Use an X on the scale to indicate how hungry you are feeling.

1. **How hungry do you feel?**

Day One- Appetite questionnaire

Date: _____

Please answer the following questions **30 minutes BEFORE** consuming **BREAKFAST**

1. **How hungry do you feel?**

I am not hungry at all	———	**I have never been more hungry**

2. **How satisfied do you feel?**

I am completely empty	———	**I cannot eat another bite**

3. **How full do you feel?**

Not at all full	———	**Totally full**

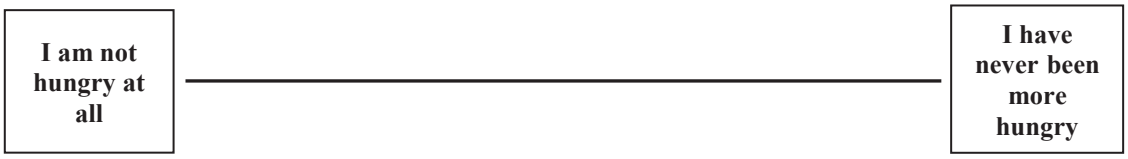
4. **How much do you think you can eat?**

Not at all	———	**A lot**

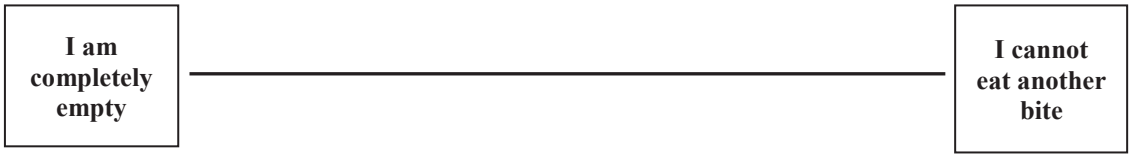
Please take the treatment after you have completed this section

Please answer the following questions **30 minutes AFTER** consuming **BREAKFAST**

1. **How hungry do you feel?**

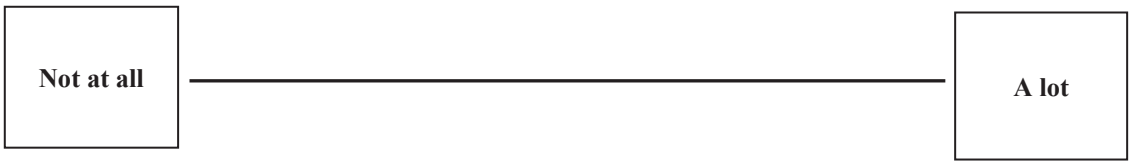


2. **How satisfied do you feel?**



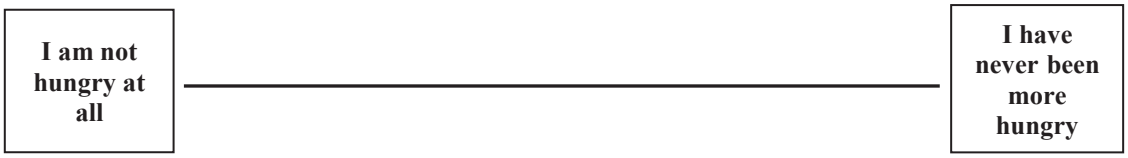
3. **How full do you feel?**

4. **How much do you think you can eat?**

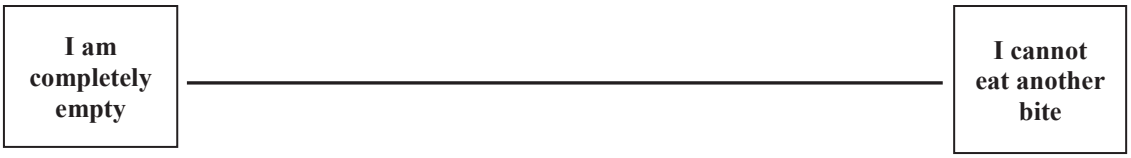


Please answer the following questions **30 minutes BEFORE** consuming **LUNCH**

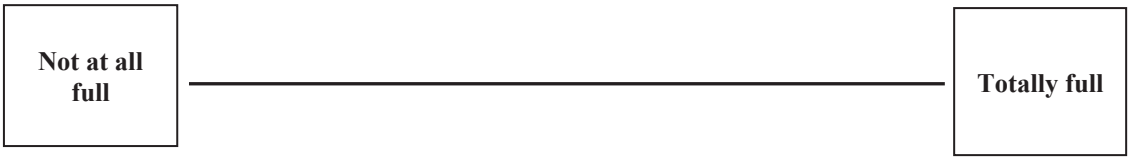
1. **How hungry do you feel?**



2. **How satisfied do you feel?**



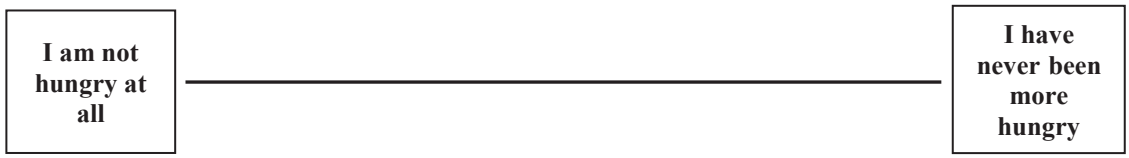
3. **How full do you feel?**



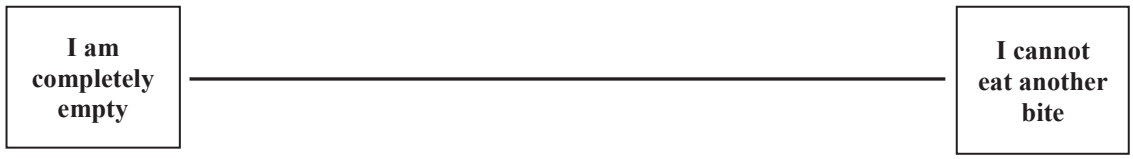
4. **How much do you think you can eat?**

Please answer the following questions **30 minutes AFTER** consuming **LUNCH**

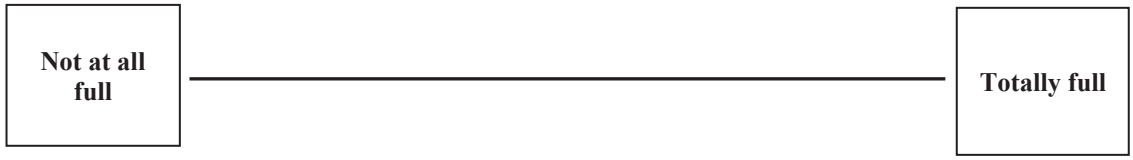
1. **How hungry do you feel?**



2. **How satisfied do you feel?**



3. **How full do you feel?**



4. **How much do you think you can eat?**

Please answer the following questions **30 minutes BEFORE** consuming **DINNER**

1. **How hungry do you feel?**

I am not hungry at all	———	**I have never been more hungry**

2. **How satisfied do you feel?**

I am completely empty	———	**I cannot eat another bite**

3. **How full do you feel?**

Not at all full	———	**Totally full**

4. **How much do you think you can eat?**

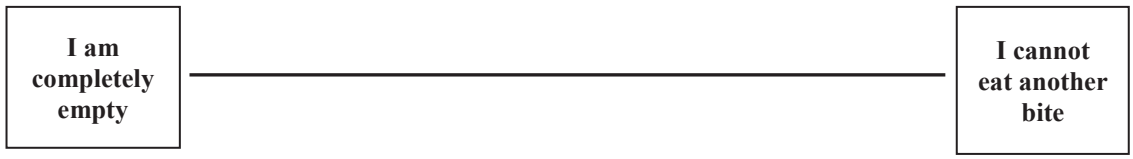
Not at all	———	**A lot**

Please take the treatment after you have completed this section

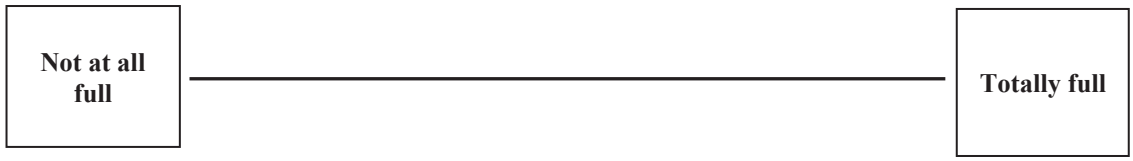
Please answer the following questions **30 minutes AFTER** consuming **DINNER**

1. **How hungry do you feel?**

2. **How satisfied do you feel?**



3. **How full do you feel?**



4. **How much do you think you can eat?**

Day One- 3 day diet record

Date: _____ Weekday / Weekend (please circle one)

Time	**Food or drink- including description** (variety, brand, cut of meat etc.)	**Food or beverage preparation**	**Amount consumed** (weight, measures etc.)

Day One continued...

Date: _____ Weekday / Weekend (please circle one)

Time	**Food or drink- including description** (variety, brand, cut of meat etc.)	**Food or beverage preparation**	**Amount consumed** (weight, measures etc.)

Day Two- Appetite questionnaire Date: _____

Please answer the following questions **30 minutes BEFORE** consuming **BREAKFAST**

1. **How hungry do you feel?**

I am not hungry at all	———	**I have never been more hungry**

2. **How satisfied do you feel?**

I am completely empty	———	**I cannot eat another bite**

3. **How full do you feel?**

Not at all full	———	**Totally full**

4. **How much do you think you can eat?**

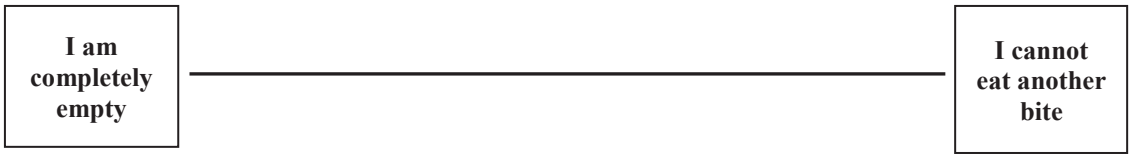
Not at all	———	**A lot**

Please take the treatment after you have completed this section

Please answer the following questions **30 minutes AFTER** consuming **BREAKFAST**

1. **How hungry do you feel?**

2. **How satisfied do you feel?**



3. **How full do you feel?**

4. **How much do you think you can eat?**

Please answer the following questions **30 minutes BEFORE** consuming **LUNCH**

1. **How hungry do you feel?**

I am not hungry at all	———	**I have never been more hungry**

2. **How satisfied do you feel?**

I am completely empty	———	**I cannot eat another bite**

3. **How full do you feel?**

Not at all full	———	**Totally full**

4. **How much do you think you can eat?**

Not at all	———	**A lot**

Please answer the following questions **30 minutes AFTER** consuming **LUNCH**

1. **How hungry do you feel?**

I am not hungry at all	———	**I have never been more hungry**

2. **How satisfied do you feel?**

I am completely empty	———	**I cannot eat another bite**

3. **How full do you feel?**

Not at all full	———	**Totally full**

4. **How much do you think you can eat?**

Not at all	———	**A lot**

Please answer the following questions **30 minutes BEFORE** consuming **DINNER**

1. **How hungry do you feel?**

I am not hungry at all	———	**I have never been more hungry**

2. **How satisfied do you feel?**

I am completely empty	———	**I cannot eat another bite**

3. **How full do you feel?**

Not at all full	———	**Totally full**

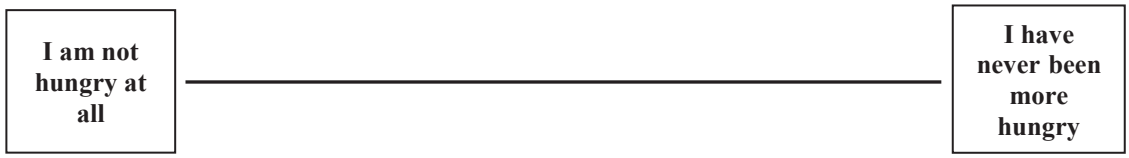
4. **How much do you think you can eat?**

Not at all	———	**A lot**

Please take the treatment after you have completed this section

Please answer the following questions **30 minutes AFTER** consuming **DINNER**

1. **How hungry do you feel?**



2. **How satisfied do you feel?**

3. **How full do you feel?**

4. **How much do you think you can eat?**

Day Two- 3 day diet record

Date: _____ Weekday / Weekend (please circle one)

Time	**Food or drink- including description** (variety, brand, cut of meat etc.)	**Food or beverage preparation**	**Amount consumed** (weight, measures etc.)

Day Two continued...

Date: _____ Weekday / Weekend (please circle one)

Time	**Food or drink- including description** (variety, brand, cut of meat etc.)	**Food or beverage preparation**	**Amount consumed** (weight, measures etc.)

Day Three- Appetite questionnaire

Date: _____

Please answer the following questions **30 minutes BEFORE** consuming **BREAKFAST**

1. **How hungry do you feel?**

I am not hungry at all	———	**I have never been more hungry**

2. **How satisfied do you feel?**

I am completely empty	———	**I cannot eat another bite**

3. **How full do you feel?**

Not at all full	———	**Totally full**

4. **How much do you think you can eat?**

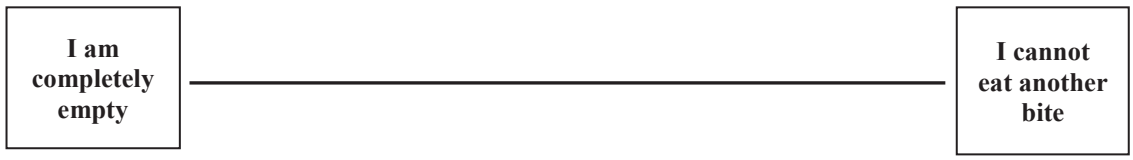
Not at all	———	**A lot**

Please take the treatment after you have completed this section

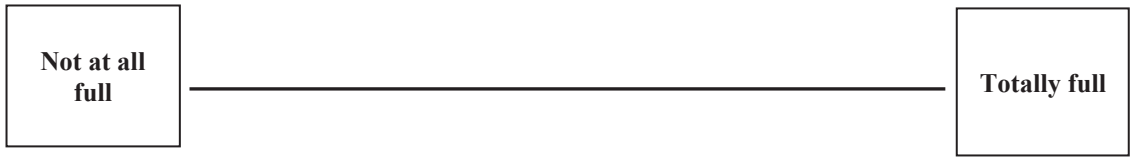
Please answer the following questions **30 minutes AFTER** consuming **BREAKFAST**

1. **How hungry do you feel?**

2. **How satisfied do you feel?**



3. **How full do you feel?**

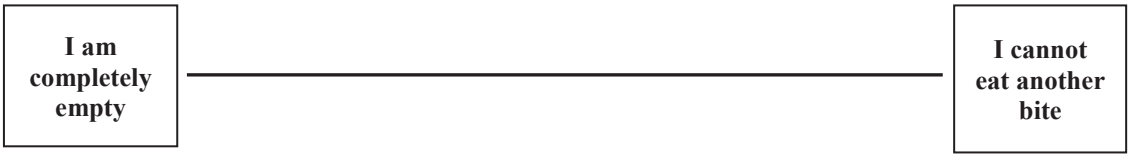


4. **How much do you think you can eat?**

Please answer the following questions **30 minutes BEFORE** consuming **LUNCH**

1. **How hungry do you feel?**

2. **How satisfied do you feel?**

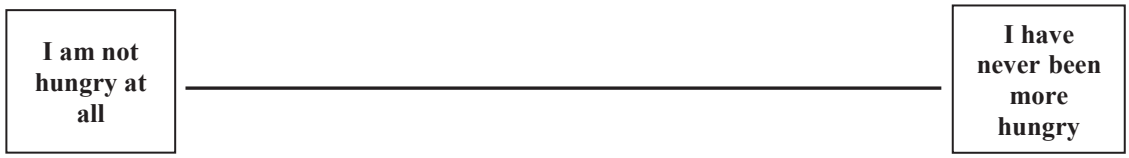


3. **How full do you feel?**

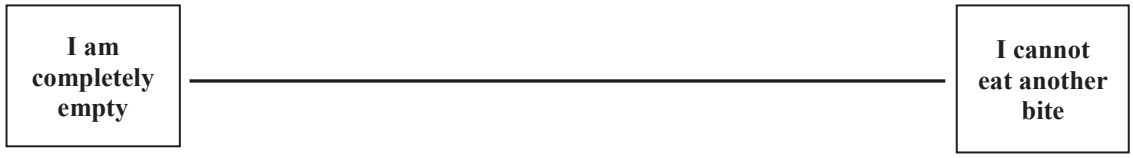
4. **How much do you think you can eat?**

Please answer the following questions **30 minutes AFTER** consuming **LUNCH**

1. **How hungry do you feel?**

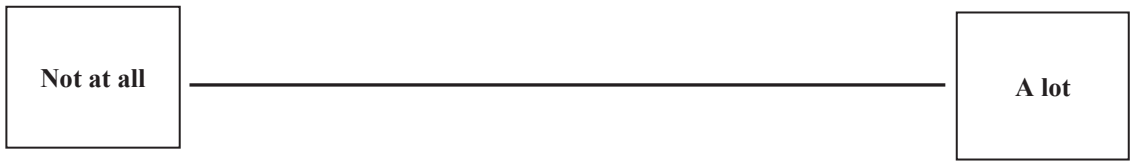


2. **How satisfied do you feel?**



3. **How full do you feel?**

4. **How much do you think you can eat?**



Please answer the following questions **30 minutes BEFORE** consuming **DINNER**

1. **How hungry do you feel?**

I am not hungry at all	———	**I have never been more hungry**

2. **How satisfied do you feel?**

I am completely empty	———	**I cannot eat another bite**

3. **How full do you feel?**

Not at all full	———	**Totally full**

4. **How much do you think you can eat?**

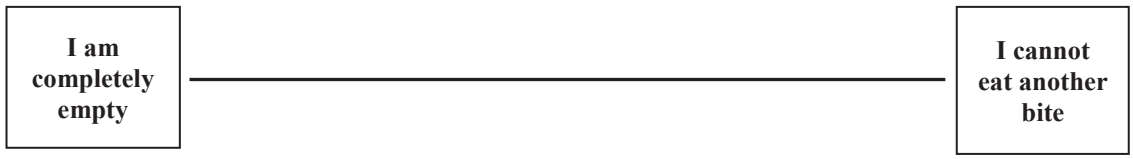
Not at all	———	**A lot**

Please take the treatment after you have completed this section

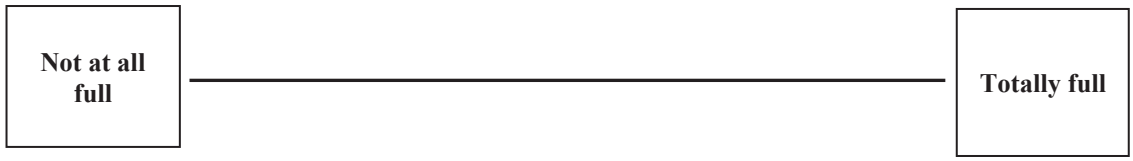
Please answer the following questions **30 minutes AFTER** consuming **DINNER**

1. **How hungry do you feel?**

2. **How satisfied do you feel?**



3. **How full do you feel?**



4. **How much do you think you can eat?**

Day Three- 3 day diet record

Date: _____ Weekday / Weekend (please circle one)

Time	**Food or drink- including description** (variety, brand, cut of meat etc.)	**Food or beverage preparation**	**Amount consumed** (weight, measures etc.)

Day Three continued...

Date: _____ Weekday / Weekend (please circle one)

Time	**Food or drink- including description** (variety, brand, cut of meat etc.)	**Food or beverage preparation**	**Amount consumed** (weight, measures etc.)

Appendix 7-7. Human intervention study fructan intake food frequency questionnaire

ADAPT (hAbitual Diet And PrebioTic) study

Instructions for completing the fructan food frequency questionnaire

- Please think about your food intake **over the last 7 days only** when completing the fructan food frequency questionnaire.
- For **each food** please **circle** what your **usual portion size** is (i.e. Breads- 1 thick slice [large]) **AND how many portions** of the food you have eaten **in the last 7 days** (i.e. Six [6])**.**

Example

Food	**What is your usual portion size? (please circle)**			**How many such portions have you eaten in the last 7 days?**								
	Small	Medium	Large	None	One	Two	Three	Four	Five	Six	Seven	More than 7 (please state)
Breads *e.g. white, brown, wholemeal*	1 thin slice	1 medium slice	1 thick slice	0	1	2	3	4	5	6	7	……..

FRUCTAN FOOD FREQUENCY QUESTIONNAIRE Date: _____ Participant ID: _____

Food	What is your usual portion size? (please circle)			How many such portions have you eaten in the last 7 days?								
	Small	Medium	Large	None	One	Two	Three	Four	Five	Six	Seven	More than 7 (please state)
Breads *e.g. white, brown, wholemeal*	1thin slice	1 medium slice	1 thick slice	0	1	2	3	4	5	6	7	……..
Other Breads *e.g. Chapatti, Naan, Pitta bread, Baguette*	½ chapatti ¼ naan 1 mini pitta 2" slice baguette	1 chapatti ½ naan 1 small pitta ½ baguette	2 chapattis 1 naan 1 large pitta 1 baguette	0	1	2	3	4	5	6	7	……..
Rye breads and crispbreads *e.g. Ryvita, other crispbread*	1 bread	2 breads	3 breads	0	1	2	3	4	5	6	7	……..
Wheat cereals *e.g. Weetabix, Shredded Wheat, Special K, Fruit 'n Fibre, Cheerios, Cereal bars*	1 biscuit 2 tablespoon	2 biscuits 3 tablespoon	3 biscuits 4 tablespoon	0	1	2	3	4	5	6	7	……..
Pizza	1 slice of pizza	½ medium pizza	1 medium pizza	0	1	2	3	4	5	6	7	……..
Pasta - boiled *e.g. spaghetti, pasta shells*	2 heaped tablespoons	3 heaped tablespoons	4 heaped tablespoons	0	1	2	3	4	5	6	7	……..
Noodles – boiled *e.g. chow mein, pot noodle, super noodles*	½ takeaway box ½ sachet noodles	1 takeaway box 1 sachet noodles		0	1	2	3	4	5	6	7	……..
Bulgar wheat, cracked wheat or couscous - cooked	1 heaped tablespoon	2 heaped tablespoons	3 heaped tablespoons	0	1	2	3	4	5	6	7	……..
Cakes, muffins, buns, doughnuts, pancakes	1 small slice ½ bun	1 medium slice 1 small bun	1 large slice 1 large bun	0	1	2	3	4	5	6	7	……..
Pastry products – savoury or sweet *e.g. meat pie, cheese pasty, apple pie, Danish pastry*	1 small slice ½ individual pie or pasty	¼ medium pie 1 individual pie or pasty	½ medium pie 2 individual pies or pasties	0	1	2	3	4	5	6	7	……..

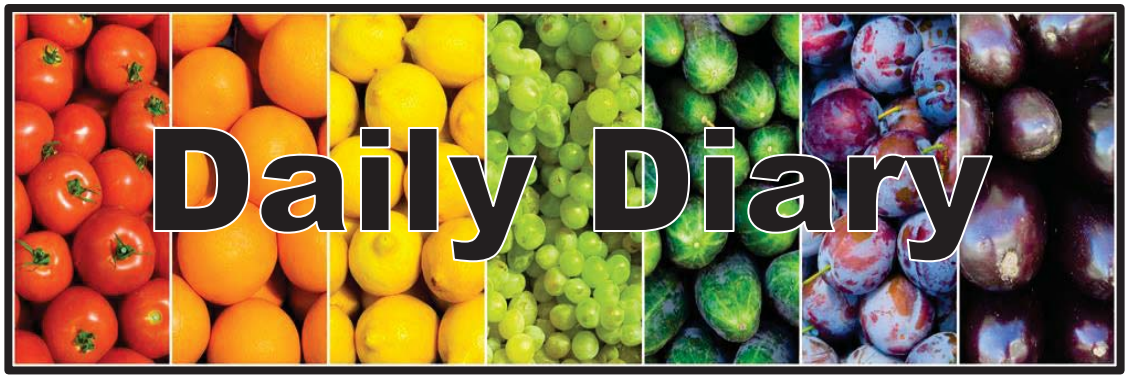
Food	What is your usual portion size? (please circle)			How many such portions have you eaten in the last 7 days?								
	Small	Medium	Large	None	One	Two	Three	Four	Five	Six	Seven	More than 7 (please state)
Flour-based puddings *e.g. sponge, semolina, bread and butter*	1 tablespoon	2 tablespoons	3 tablespoons	0	1	2	3	4	5	6	7	……..
Biscuits *e.g. digestives, rich tea, shortcake*	1 biscuit	2 biscuits	3 biscuits	0	1	2	3	4	5	6	7	……..
Biscuit-based chocolates *e.g. Maltesers, Twix, Fingers*	½ bag 1 Twix finger	1 bag 2 Twix fingers		0	1	2	3	4	5	6	7	……..
Beer	½ pint beer	1 pint beer		0	1	2	3	4	5	6	7	……..
Onion	1 small	1 medium	1 large	0	1	2	3	4	5	6	7	……..
Banana	1 small	1 medium	1 large	0	1	2	3	4	5	6	7	……..
Asparagus	4 spears	6 spears	8 spears	0	1	2	3	4	5	6	7	……..
Garlic	½ clove	1 clove		0	1	2	3	4	5	6	7	……..
Jerusalem artichoke	½ bulb	1 bulb		0	1	2	3	4	5	6	7	……..
Globe artichoke, artichoke hearts	½ globe heart	1 globe heart		0	1	2	3	4	5	6	7	……..
Leeks	½ leek	1 leek		0	1	2	3	4	5	6	7	……..
Chicory root	½ root	1 root		0	1	2	3	4	5	6	7	……..
Specialist chicory coffee *e.g. Camp chicory & coffee liquid*	1 espresso cup	1 coffee cup	1 mug	0	1	2	3	4	5	6	7	……..

Appendix 7-8. Human intervention daily diary

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Plant & Food RESEARCH
RANGAHAU AHUMĀRA KAI

ADAPT (hAbitual Diet And PrebioTic) study



Treatment phase 1

Participant ID number: _____

Date treatment started: _____

Instructions for taking the treatment:

- Take 1 sachet **30 minutes before breakfast** and 1 sachet **30 minutes before dinner** (take 2 sachets per day)
- Mix the powder into hot or cold beverages such as fruit juice, coffee, tea, milo or milk
- Make sure the powder is mixed in well so there are no lumps. To avoid lumps add a small amount of liquid to the powder, mix it to a paste and then add the rest of the liquid

If you have any comments or questions relating to the research project or the daily diary please feel free to contact **Genelle Healey** during working hours on **06 355 6108** or email **adaptstudy2016@gmail.com**

Day 1

Please complete the daily diary at the end of each day you are taking a treatment.

	Did you take the treatment? (please tick)	**Amount of sachet taken?** (please tick one)	**What time was the treatment taken?**	**What did you mix the treatment into** (i.e. cup of tea, yoghurt)?
AM sachet (Breakfast)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		
PM sachet (Dinner)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		

How many bowel motions have you passed today? _____

Has the consistency of your bowel motion/s changed today compared to your usual bowel motion consistency (please tick one)?

No ☐ Yes, more loose ☐ Yes, more firm ☐

Have you needed to take laxatives, Metamucil or Benefibre to pass a bowel motion today (please tick)?

Yes ☐ please specify: _____ No ☐

Have you experienced any of the following today (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling stomach				
Abdominal cramps				
Abdominal pain				
Bloating				

Definitions: **Mild-** Nagging or annoying, **Moderate-** Strong negative influence on your daily living, **Severe-** Disabling

Please record any comments or concerns you may have: _____

_____

_____

Day 2

Please complete the daily diary at the end of each day you are taking a treatment.

	Did you take the treatment? (please tick)	**Amount of sachet taken?** (please tick one)	**What time was the treatment taken?**	**What did you mix the treatment into** (i.e. cup of tea, yoghurt)?
AM sachet (Breakfast)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		
PM sachet (Dinner)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		

How many bowel motions have you passed today? _____

Has the consistency of your bowel motion/s changed today compared to your usual bowel motion consistency (please tick one)?

No ☐ Yes, more loose ☐ Yes, more firm ☐

Have you needed to take laxatives, Metamucil or Benefibre to pass a bowel motion today (please tick)?

Yes ☐ please specify: _____ No ☐

Have you experienced any of the following today (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling stomach				
Abdominal cramps				
Abdominal pain				
Bloating				

Definitions: **Mild-** Nagging or annoying, **Moderate-** Strong negative influence on your daily living, **Severe-** Disabling

Please record any comments or concerns you may have: _____

_____

_____

Day 3

Please complete the daily diary at the end of each day you are taking a treatment.

	Did you take the treatment? (please tick)	**Amount of sachet taken?** (please tick one)	**What time was the treatment taken?**	**What did you mix the treatment into** (i.e. cup of tea, yoghurt)?
AM sachet (Breakfast)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		
PM sachet (Dinner)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		

How many bowel motions have you passed today? _____

Has the consistency of your bowel motion/s changed today compared to your usual bowel motion consistency (please tick one)?

No ☐ Yes, more loose ☐ Yes, more firm ☐

Have you needed to take laxatives, Metamucil or Benefibre to pass a bowel motion today (please tick)?

Yes ☐ please specify: _____ No ☐

Have you experienced any of the following today (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling stomach				
Abdominal cramps				
Abdominal pain				
Bloating				

Definitions: **Mild-** Nagging or annoying, **Moderate-** Strong negative influence on your daily living, **Severe-** Disabling

Please record any comments or concerns you may have: _____

_____

_____

Day 4

Please complete the daily diary at the end of each day you are taking a treatment.

	Did you take the treatment? (please tick)	**Amount of sachet taken?** (please tick one)	**What time was the treatment taken?**	**What did you mix the treatment into** (i.e. cup of tea, yoghurt)?
AM sachet (Breakfast)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		
PM sachet (Dinner)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		

How many bowel motions have you passed today? _____

Has the consistency of your bowel motion/s changed today compared to your usual bowel motion consistency (please tick one)?

No ☐ Yes, more loose ☐ Yes, more firm ☐

Have you needed to take laxatives, Metamucil or Benefibre to pass a bowel motion today (please tick)?

Yes ☐ please specify: _____ No ☐

Have you experienced any of the following today (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling stomach				
Abdominal cramps				
Abdominal pain				
Bloating				

Definitions: **Mild-** Nagging or annoying, **Moderate-** Strong negative influence on your daily living, **Severe-** Disabling

Please record any comments or concerns you may have: _____

_____

_____

Day 5

Please complete the daily diary at the end of each day you are taking a treatment.

	Did you take the treatment? (please tick)	**Amount of sachet taken?** (please tick one)	**What time was the treatment taken?**	**What did you mix the treatment into** (i.e. cup of tea, yoghurt)?
AM sachet (Breakfast)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		
PM sachet (Dinner)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		

How many bowel motions have you passed today? _____

Has the consistency of your bowel motion/s changed today compared to your usual bowel motion consistency (please tick one)?

No ☐ Yes, more loose ☐ Yes, more firm ☐

Have you needed to take laxatives, Metamucil or Benefibre to pass a bowel motion today (please tick)?

Yes ☐ please specify: _____ No ☐

Have you experienced any of the following today (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling stomach				
Abdominal cramps				
Abdominal pain				
Bloating				

Definitions: **Mild-** Nagging or annoying, **Moderate-** Strong negative influence on your daily living, **Severe-** Disabling

Please record any comments or concerns you may have: _____

_____

_____

Day 6

Please complete the daily diary at the end of each day you are taking a treatment.

	Did you take the treatment? (please tick)	**Amount of sachet taken?** (please tick one)	**What time was the treatment taken?**	**What did you mix the treatment into** (i.e. cup of tea, yoghurt)?
AM sachet (Breakfast)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		
PM sachet (Dinner)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		

How many bowel motions have you passed today? _____

Has the consistency of your bowel motion/s changed today compared to your usual bowel motion consistency (please tick one)?

No ☐ Yes, more loose ☐ Yes, more firm ☐

Have you needed to take laxatives, Metamucil or Benefibre to pass a bowel motion today (please tick)?

Yes ☐ please specify: _____ No ☐

Have you experienced any of the following today (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling stomach				
Abdominal cramps				
Abdominal pain				
Bloating				

Definitions: **Mild-** Nagging or annoying, **Moderate-** Strong negative influence on your daily living, **Severe-** Disabling

Please record any comments or concerns you may have: _____

_____

_____

Day 7

Please complete the daily diary at the end of each day you are taking a treatment.

	Did you take the treatment? (please tick)	**Amount of sachet taken?** (please tick one)	**What time was the treatment taken?**	**What did you mix the treatment into** (i.e. cup of tea, yoghurt)?
AM sachet (Breakfast)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		
PM sachet (Dinner)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		

How many bowel motions have you passed today? _____

Has the consistency of your bowel motion/s changed today compared to your usual bowel motion consistency (please tick one)?

No ☐ Yes, more loose ☐ Yes, more firm ☐

Have you needed to take laxatives, Metamucil or Benefibre to pass a bowel motion today (please tick)?

Yes ☐ please specify: _____ No ☐

Have you experienced any of the following today (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling stomach				
Abdominal cramps				
Abdominal pain				
Bloating				

Definitions: **Mild-** Nagging or annoying, **Moderate-** Strong negative influence on your daily living, **Severe-** Disabling

Please record any comments or concerns you may have: _____

_____

_____

Day 8

Please complete the daily diary at the end of each day you are taking a treatment.

	Did you take the treatment? (please tick)	**Amount of sachet taken?** (please tick one)	**What time was the treatment taken?**	**What did you mix the treatment into** (i.e. cup of tea, yoghurt)?
AM sachet (Breakfast)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		
PM sachet (Dinner)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		

How many bowel motions have you passed today? _____

Has the consistency of your bowel motion/s changed today compared to your usual bowel motion consistency (please tick one)?

No ☐ Yes, more loose ☐ Yes, more firm ☐

Have you needed to take laxatives, Metamucil or Benefibre to pass a bowel motion today (please tick)?

Yes ☐ please specify: _____ No ☐

Have you experienced any of the following today (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling stomach				
Abdominal cramps				
Abdominal pain				
Bloating				

Definitions: **Mild-** Nagging or annoying, **Moderate-** Strong negative influence on your daily living, **Severe-** Disabling

Please record any comments or concerns you may have: _____

_____

_____

Day 9

Please complete the daily diary at the end of each day you are taking a treatment.

	Did you take the treatment? (please tick)	**Amount of sachet taken?** (please tick one)	**What time was the treatment taken?**	**What did you mix the treatment into** (i.e. cup of tea, yoghurt)?
AM sachet (Breakfast)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		
PM sachet (Dinner)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		

How many bowel motions have you passed today? _____

Has the consistency of your bowel motion/s changed today compared to your usual bowel motion consistency (please tick one)?

No ☐ Yes, more loose ☐ Yes, more firm ☐

Have you needed to take laxatives, Metamucil or Benefibre to pass a bowel motion today (please tick)?

Yes ☐ please specify: _____ No ☐

Have you experienced any of the following today (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling stomach				
Abdominal cramps				
Abdominal pain				
Bloating				

Definitions: **Mild-** Nagging or annoying, **Moderate-** Strong negative influence on your daily living, **Severe-** Disabling

Please record any comments or concerns you may have: _____

_____

_____

Day 10

Please complete the daily diary at the end of each day you are taking a treatment.

	Did you take the treatment? (please tick)	**Amount of sachet taken?** (please tick one)	**What time was the treatment taken?**	**What did you mix the treatment into** (i.e. cup of tea, yoghurt)?
AM sachet (Breakfast)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		
PM sachet (Dinner)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		

How many bowel motions have you passed today? _____

Has the consistency of your bowel motion/s changed today compared to your usual bowel motion consistency (please tick one)?

No ☐ Yes, more loose ☐ Yes, more firm ☐

Have you needed to take laxatives, Metamucil or Benefibre to pass a bowel motion today (please tick)?

Yes ☐ please specify: _____ No ☐

Have you experienced any of the following today (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling stomach				
Abdominal cramps				
Abdominal pain				
Bloating				

Definitions: **Mild-** Nagging or annoying, **Moderate-** Strong negative influence on your daily living, **Severe-** Disabling

Please record any comments or concerns you may have: _____

_____

_____

Day 11

Please complete the daily diary at the end of each day you are taking a treatment.

	Did you take the treatment? (please tick)	**Amount of sachet taken?** (please tick one)	**What time was the treatment taken?**	**What did you mix the treatment into** (i.e. cup of tea, yoghurt)?
AM sachet (Breakfast)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		
PM sachet (Dinner)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		

How many bowel motions have you passed today? _____

Has the consistency of your bowel motion/s changed today compared to your usual bowel motion consistency (please tick one)?

No ☐ Yes, more loose ☐ Yes, more firm ☐

Have you needed to take laxatives, Metamucil or Benefibre to pass a bowel motion today (please tick)?

Yes ☐ please specify: _____ No ☐

Have you experienced any of the following today (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling stomach				
Abdominal cramps				
Abdominal pain				
Bloating				

Definitions: **Mild-** Nagging or annoying, **Moderate-** Strong negative influence on your daily living, **Severe-** Disabling

Please record any comments or concerns you may have: _____

_____

_____

Day 12

Please complete the daily diary at the end of each day you are taking a treatment.

	Did you take the treatment? (please tick)	**Amount of sachet taken?** (please tick one)	**What time was the treatment taken?**	**What did you mix the treatment into** (i.e. cup of tea, yoghurt)?
AM sachet (Breakfast)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		
PM sachet (Dinner)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		

How many bowel motions have you passed today? _____

Has the consistency of your bowel motion/s changed today compared to your usual bowel motion consistency (please tick one)?

No ☐ Yes, more loose ☐ Yes, more firm ☐

Have you needed to take laxatives, Metamucil or Benefibre to pass a bowel motion today (please tick)?

Yes ☐ please specify: _____ No ☐

Have you experienced any of the following today (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling stomach				
Abdominal cramps				
Abdominal pain				
Bloating				

Definitions: **Mild-** Nagging or annoying, **Moderate-** Strong negative influence on your daily living, **Severe-** Disabling

Please record any comments or concerns you may have: _____

_____

_____

Day 13

Please complete the daily diary at the end of each day you are taking a treatment.

	Did you take the treatment? (please tick)	**Amount of sachet taken?** (please tick one)	**What time was the treatment taken?**	**What did you mix the treatment into** (i.e. cup of tea, yoghurt)?
AM sachet (Breakfast)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		
PM sachet (Dinner)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		

How many bowel motions have you passed today? _____

Has the consistency of your bowel motion/s changed today compared to your usual bowel motion consistency (please tick one)?

No ☐ Yes, more loose ☐ Yes, more firm ☐

Have you needed to take laxatives, Metamucil or Benefibre to pass a bowel motion today (please tick)?

Yes ☐ please specify: _____ No ☐

Have you experienced any of the following today (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling stomach				
Abdominal cramps				
Abdominal pain				
Bloating				

Definitions: **Mild-** Nagging or annoying, **Moderate-** Strong negative influence on your daily living, **Severe-** Disabling

Please record any comments or concerns you may have: _____

_____

_____

Day 14

Please complete the daily diary at the end of each day you are taking a treatment.

	Did you take the treatment? (please tick)	**Amount of sachet taken?** (please tick one)	**What time was the treatment taken?**	**What did you mix the treatment into** (i.e. cup of tea, yoghurt)?
AM sachet (Breakfast)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		
PM sachet (Dinner)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		

How many bowel motions have you passed today? _____

Has the consistency of your bowel motion/s changed today compared to your usual bowel motion consistency (please tick one)?

No ☐ Yes, more loose ☐ Yes, more firm ☐

Have you needed to take laxatives, Metamucil or Benefibre to pass a bowel motion today (please tick)?

Yes ☐ please specify: _____ No ☐

Have you experienced any of the following today (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling stomach				
Abdominal cramps				
Abdominal pain				
Bloating				

Definitions: **Mild-** Nagging or annoying, **Moderate-** Strong negative influence on your daily living, **Severe-** Disabling

Please record any comments or concerns you may have: _____

_____

_____

Day 15

Please complete the daily diary at the <u>end of each day</u> you are taking a treatment.

	Did you take the treatment? (please tick)	**Amount of sachet taken?** (please tick one)	**What time was the treatment taken?**	**What did you mix the treatment into** (i.e. cup of tea, yoghurt)?
AM sachet (Breakfast)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		
PM sachet (Dinner)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		

How many bowel motions have you passed <u>today</u>? _____

Has the consistency of your bowel motion/s changed <u>today</u> compared to your usual bowel motion consistency (please tick one)?

No ☐ Yes, more loose ☐ Yes, more firm ☐

Have you needed to take laxatives, Metamucil or Benefibre to pass a bowel motion <u>today</u> (please tick)?

Yes ☐ please specify: _____ No ☐

Have you experienced any of the following <u>today</u> (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling stomach				
Abdominal cramps				
Abdominal pain				
Bloating				

<u>Definitions:</u> **Mild-** Nagging or annoying, **Moderate-** Strong negative influence on your daily living, **Severe-** Disabling

Please record any comments or concerns you may have: _____

_____

_____

Day 16

Please complete the daily diary at the <u>end of each day</u> you are taking a treatment.

	Did you take the treatment? (please tick)	**Amount of sachet taken?** (please tick one)	**What time was the treatment taken?**	**What did you mix the treatment into** (i.e. cup of tea, yoghurt)?
AM sachet (Breakfast)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		
PM sachet (Dinner)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		

How many bowel motions have you passed <u>today</u>? _____

Has the consistency of your bowel motion/s changed <u>today</u> compared to your usual bowel motion consistency (please tick one)?

No ☐ Yes, more loose ☐ Yes, more firm ☐

Have you needed to take laxatives, Metamucil or Benefibre to pass a bowel motion <u>today</u> (please tick)?

Yes ☐ please specify: _____ No ☐

Have you experienced any of the following <u>today</u> (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling stomach				
Abdominal cramps				
Abdominal pain				
Bloating				

<u>Definitions:</u> **Mild-** Nagging or annoying, **Moderate-** Strong negative influence on your daily living, **Severe-** Disabling

Please record any comments or concerns you may have: _____

_____

_____

Day 17

Please complete the daily diary at the end of each day you are taking a treatment.

	Did you take the treatment? (please tick)	**Amount of sachet taken?** (please tick one)	**What time was the treatment taken?**	**What did you mix the treatment into** (i.e. cup of tea, yoghurt)?
AM sachet (Breakfast)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		
PM sachet (Dinner)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		

How many bowel motions have you passed today? _____

Has the consistency of your bowel motion/s changed today compared to your usual bowel motion consistency (please tick one)?

No ☐ Yes, more loose ☐ Yes, more firm ☐

Have you needed to take laxatives, Metamucil or Benefibre to pass a bowel motion today (please tick)?

Yes ☐ please specify: _____ No ☐

Have you experienced any of the following today (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling stomach				
Abdominal cramps				
Abdominal pain				
Bloating				

Definitions: **Mild-** Nagging or annoying, **Moderate-** Strong negative influence on your daily living, **Severe-** Disabling

Please record any comments or concerns you may have: _____

_____

_____

Day 18

Please complete the daily diary at the end of each day you are taking a treatment.

	Did you take the treatment? (please tick)	**Amount of sachet taken?** (please tick one)	**What time was the treatment taken?**	**What did you mix the treatment into** (i.e. cup of tea, yoghurt)?
AM sachet (Breakfast)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		
PM sachet (Dinner)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		

How many bowel motions have you passed today? _____

Has the consistency of your bowel motion/s changed today compared to your usual bowel motion consistency (please tick one)?

No ☐ Yes, more loose ☐ Yes, more firm ☐

Have you needed to take laxatives, Metamucil or Benefibre to pass a bowel motion today (please tick)?

Yes ☐ please specify: _____ No ☐

Have you experienced any of the following today (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling stomach				
Abdominal cramps				
Abdominal pain				
Bloating				

Definitions: **Mild-** Nagging or annoying, **Moderate-** Strong negative influence on your daily living, **Severe-** Disabling

Please record any comments or concerns you may have: _____

_____

_____

Day 19

Please complete the daily diary at the end of each day you are taking a treatment.

	Did you take the treatment? (please tick)	**Amount of sachet taken?** (please tick one)	**What time was the treatment taken?**	**What did you mix the treatment into** (i.e. cup of tea, yoghurt)?
AM sachet (Breakfast)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		
PM sachet (Dinner)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		

How many bowel motions have you passed today? _____

Has the consistency of your bowel motion/s changed today compared to your usual bowel motion consistency (please tick one)?

No ☐ Yes, more loose ☐ Yes, more firm ☐

Have you needed to take laxatives, Metamucil or Benefibre to pass a bowel motion today (please tick)?

Yes ☐ please specify: _____ No ☐

Have you experienced any of the following today (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling stomach				
Abdominal cramps				
Abdominal pain				
Bloating				

Definitions: **Mild-** Nagging or annoying, **Moderate-** Strong negative influence on your daily living, **Severe-** Disabling

Please record any comments or concerns you may have: _____

_____

_____

Day 20

Please complete the daily diary at the end of each day you are taking a treatment.

	Did you take the treatment? (please tick)	Amount of sachet taken? (please tick one)	What time was the treatment taken?	What did you mix the treatment into (i.e. cup of tea, yoghurt)?
AM sachet (Breakfast)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		
PM sachet (Dinner)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		

How many bowel motions have you passed today? _____

Has the consistency of your bowel motion/s changed today compared to your usual bowel motion consistency (please tick one)?

No ☐ Yes, more loose ☐ Yes, more firm ☐

Have you needed to take laxatives, Metamucil or Benefibre to pass a bowel motion today (please tick)?

Yes ☐ please specify: _____ No ☐

Have you experienced any of the following today (please tick the boxes that apply)?

	Absent	Mild	Moderate	Severe
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling stomach				
Abdominal cramps				
Abdominal pain				
Bloating				

Definitions: **Mild-** Nagging or annoying, **Moderate-** Strong negative influence on your daily living, **Severe-** Disabling

Please record any comments or concerns you may have: _____

_____

_____

Day 21

Please complete the daily diary at the end of each day you are taking a treatment.

	Did you take the treatment? (please tick)	**Amount of sachet taken?** (please tick one)	**What time was the treatment taken?**	**What did you mix the treatment into** (i.e. cup of tea, yoghurt)?
AM sachet (Breakfast)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		
PM sachet (Dinner)	Yes ☐ No ☐	All ☐ Half ☐ Less than half ☐ Not taken ☐		

How many bowel motions have you passed today? _____

Has the consistency of your bowel motion/s changed today compared to your usual bowel motion consistency (please tick one)?

No ☐ Yes, more loose ☐ Yes, more firm ☐

Have you needed to take laxatives, Metamucil or Benefibre to pass a bowel motion today (please tick)?

Yes ☐ please specify: _____ No ☐

Have you experienced any of the following today (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling stomach				
Abdominal cramps				
Abdominal pain				
Bloating				

Definitions: **Mild-** Nagging or annoying, **Moderate-** Strong negative influence on your daily living, **Severe-** Disabling

Please record any comments or concerns you may have: _____

_____

_____

Appendix 7-9. Human intervention faecal sample collection instructions

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Plant & Food RESEARCH
RANGAHAU AHUMĀRA KAI

ADAPT (hAbitual Diet And PrebioTic) study

Faecal samples need to be **collected prior to your visits** to the Massey University Human Nutrition Research Unit.

Over the course of the study you will need to collect **four separate faecal samples**. Refer to your **participant schedule** for information relating to when the samples need to be collected.

If you regularly have a bowel motion in the morning please collect the faecal sample on the morning of your visit, otherwise collect the faecal sample the day before your visit.

Once the faecal sample has been collected it will need to be **frozen** and kept frozen.

You have been provided with the following:

- A pair of disposable gloves
- A plastic rectangular container
- Plastic scoop
- A sterile plastic jar
- Anaerobic bag
- Anaerobic sachet
- A cooler bag
- A freezer pack
- A brown paper bag

Instructions:

1. Use the **disposable gloves** while collecting the faecal sample.
2. To obtain the faecal sample (try to fill the **sterile plastic jar** to at least a 1/3 full):
    a. Catch the faecal sample using the **plastic rectangular container** before it reaches the toilet water. Use the **plastic scoop** to transfer some of the faecal sample to the **sterile plastic jar** (be careful not to contaminate the sample with any urine).

    OR

    b. Void the faecal sample straight into the **sterile plastic jar** (be careful not to contaminate the sample with any urine).
3. Ensure the lid on the **sterile plastic jar** is secure and then seal the **sterile plastic jar** in the **anaerobic bag** with the **anaerobic sachet** inside (remove the tinfoil outer packaging to reveal the white anaerobic sachet before putting the anaerobic sachet in the anaerobic bag). **Disposable gloves** can be removed at this point.
4. The **anaerobic bag** can then be put into the **cooler bag** prior to putting it into the freezer. The faecal sample is now triple contained and can be handled safely.
5. Put the contained faecal sample into the freezer, as well as the **freezer pack** so it is frozen when you transport the sample.
6. Excess faeces can be disposed of down the toilet and any used items can be put into the **brown paper bag** provided.
7. The **brown paper bag** can then be disposed of in the rubbish.
8. When transporting the faecal sample to the Massey University Human Nutrition Research Unit, for your study visit, put the frozen **freezer pack** into the **cooler bag** with the contained faecal sample to ensure it stays frozen.

Appendix 7-10. Dietary intake comparison table

Comparison of dietary intakes throughout the course of the study in the whole cohort[1]

	Prebiotic (n = 34)				Placebo (n = 33)				
	Before intervention		After intervention		Before intervention		After intervention		
Dietary intake	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**P value**
Energy (kJ/d)	8839.2	2916.4	9120.5	3067	9042.2	2800	8825.3	2284.3	0.963
Protein (g/d)	100.5	41.6	100.4	47.5	100.3	38.8	96.1	32.9	0.963
Total fat (g/d)	84.3	31.2	89.9	39.2	93.6	39.5	88.5	35.7	0.775
Saturated fat (g/d)	30.5	14.3	34.2	16.3	32.9	14.4	33.5	14.1	0.757
Polyunsaturated fat (g/d)	14	5.9	13.6	6.6	15.7	7.2	14	7.1	0.599
Monounsaturated fat (g/d)	32.4	12	34.3	17.2	37.2	18.7	33.2	14.9	0.642
Carbohydrate (g/d)	215.2	88.2	222.7	89.1	209.5	75.8	215.1	69.1	0.93
Sugars (g/d)	94.5	44.8	94.8	36.7	94.4	43.8	98	40.4	0.983
Starch (g/d)	118.5	54.3	125.7	65.2	113.4	49.2	115.8	44.5	0.808
Dietary fibre (g/d)	30.1	14.4	28.2	12.2	30.3	14	28	11.9	0.845
Water (g/d)	2479.5	1236.1	2467.7	1219.7	2523.8	1251.6	2525.5	1368	0.997
Alcohol (g/d)	3.8	10.3	3.2	7.3	2.9	7.5	1.9	3.8	0.779
Energy from protein (%)	19.7	6.1	18.5	5.4	19.4	6.8	18.7	5	0.814
Energy from fat (%)	35.3	7.3	35.8	8.9	37.4	7.8	36.6	9	0.733
Energy from saturated fat (%)	12.7	4.2	13.7	4.3	13.3	4	13.8	3.8	0.628
Energy from carbohydrate (%)	39.8	8.7	40.8	9.3	38.6	8.3	40.5	10.5	0.76
Energy from alcohol (%)	1.1	2.6	1	2.4	0.9	1.9	0.6	1.4	0.808
Energy from fibre (%)	2.7	0.9	2.6	0.9	2.7	0.9	2.5	0.8	0.702

[1] One-way repeated measures ANOVA. P value <0.05 is considered significant. SD: standard deviation

Appendix 7-11. Alpha diversity table

Alpha diversity comparisons at baseline (before intervention), and before and after the prebiotic intervention in the low and high dietary fibre groups[1]

	Low dietary fibre (n=14)					High dietary fibre (n=20)					
	Before intervention		After intervention			Before intervention		After intervention			
Alpha diversity index	**Mean**	**SD**	**Mean**	**SD**	**P value#**	**Mean**	**SD**	**Mean**	**SD**	**P value#**	**P value^{+}**
Observed species (OTUs)	801.1	156.2	841.7	147.7	0.531	841.5	133.5	836.2	160.3	0.911	0.442
Shannon index	6.08	0.48	5.87	0.45	0.263	6.28	0.50	5.99^	0.47	0.060	0.261
PD_whole tree	83.61	15.79	87.50	13.12	0.512	87.30	14.22	86.14	13.74	0.794	0.487
Chao index	5655	817	6977^	2300	0.060	6624	1640	5783	1516	0.100	0.796

[1] Non-parametric two-sample t-test (using QIIME). Mean values are significant different between the low and high dietary fibre groups at baseline ($^{+}$; before *versus* before) and from the before intervention sample within a dietary fibre group (#; before *versus* after). *p < 0.05, ^trend towards significance (p < 0.1). SD: standard deviation

Appendix 7-12. Gastrointestinal symptom table

Gastrointestinal symptom differences between the placebo and prebiotic intervention phase for the whole cohort and the low and high dietary fibre groups[1]

	Whole cohort			**Low dietary fibre**			**High dietary fibre**		
	Placebo (n = 33)	**Prebiotic (n = 34)**		**Placebo (n = 14)**	**Prebiotic (n = 14)**		**Placebo (n = 19)**	**Prebiotic (n = 20)**	
	%	**%**	**P value**	**%**	**%**	**P value**	**%**	**%**	**P value**
Mild (total)	79	79	1.000	79	64	0.687	79	89	0.625
Nausea	9	9	1.000	0	0	1.000	16	16	1.000
Diarrhoea	21	18	1.000	21	0	0.250	21	32	0.687
Flatulence	61	70	0.375	57	50	1.000	63	84	0.125
Gurgling	42	52	0.549	43	43	1.000	42	58	0.375
Cramps	30	27	1.000	43	14	0.219	21	37	0.375
Pain	27	33	0.754	14	21	1.000	37	42	1.000
Bloating	39	45	0.687	29	21	1.000	47	63	0.375
Moderate (total)	12	42	**0.013**	14	21	1.000	11	58	**0.004**
Nausea	0	3	1.000	0	7	1.000	0	0	1.000
Diarrhoea	6	0	0.500	7	0	1.000	5	0	1.000
Flatulence	3	30	**0.012**	7	21	0.625	0	37	**0.016**
Gurgling	3	6	1.000	0	0	1.000	5	11	1.000
Cramps	0	3	1.000	0	0	1.000	0	5	1.000
Pain	0	15	0.062	0	0	1.000	0	26	0.062
Bloating	3	12	0.375	0	7	1.000	5	16	0.625
Severe (total)	0	6	0.500	0	7	1.000	0	5	1.000
Nausea	0	0	1.000	0	0	1.000	0	0	1.000
Diarrhoea	0	3	1.000	0	0	1.000	0	5	1.000
Flatulence	0	3	1.000	0	7	1.000	0	0	1.000
Gurgling	0	3	1.000	0	0	1.000	0	5	1.000
Cramps	0	0	1.000	0	0	1.000	0	0	1.000
Pain	0	0	1.000	0	0	1.000	0	0	1.000
Bloating	0	0	1.000	0	0	1.000	0	0	1.000

[1] McNemar test. Significant results ($p < 0.05$) are in **bold**. %: the percentage of participants who experienced mild (nagging or annoying), moderate (strong negative influence on daily living) and severe (disabling) gastrointestinal symptoms at least once during the placebo and prebiotic intervention phases. SD: standard deviation

Appendix 7-13. Appetite rating table- Whole cohort

Appetite rating (fullness, hunger, much [how much can you eat], satisfaction ratings using an 100 mm anchored visual analogue scale) changes 30 mins before and 30 mins after breakfast, lunch and dinner during the placebo and prebiotic intervention phases in the whole cohort[1]

	Placebo (n = 33)				Prebiotic (n = 34)			
	Before intervention		After intervention		Before intervention		After intervention	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Before Breakfast								
Fullness	31.7	14.0	31.2	14.1	31.0	18.4	31.1	17.4
Hunger	52.1	17.3	53.3	20.0	53.2	19.9	50.2	22.1
Much	59.2	16.4	58.7	17.6	58.1	19.3	57.1	18.2
Satisfaction	34.4	12.2	34.4	14.5	37.0	17.9	35.5	18.8
After Breakfast								
Fullness	70.7	13.0	71.4	15.9	68.3	14.7	70.6	19.2
Hunger	21.1	13.5	20.2	13.1	23.3	15.6	20.3	14.2
Much	30.3	15.0	28.1	15.8	28.4	16.4	27.0	15.2
Satisfaction	72.5	12.6	73.8	15.3	70.3	15.3	71.1	18.1
Before Lunch								
Fullness	32.7	13.2	33.1	16.4	30.4	14.3	30.1	15.9
Hunger	57.9	15.9	60.1	19.6	59.0	18.3	61.8	18.2
Much	61.1	15.8	62.0	19.6	63.0	16.0	65.6	15.8
Satisfaction	35.9	12.0	36.1	15.7	34.8	17.2	31.6	16.0
After Lunch								
Fullness	71.3	12.7	68.6	14.3	71.5	13.5	73.8	14.0
Hunger	19.8	13.1	19.9	12.7	21.8	14.2	19.3	11.7
Much	27.9	13.9	28.3	16.1	28.1	18.5	24.3	16.2
Satisfaction	74.2	12.4	71.3	14.3	71.3	14.0	74.8	15.2
Before Dinner								
Fullness	32.3	11.1	30.7	13.5	30.6	14.9	28.5	14.3
Hunger	61.5	13.2	62.1	16.7	59.9	16.8	62.8	18.6
Much	66.1	14.1	66.4	15.7	64.4	16.2	68.8	15.7
Satisfaction	33.6	11.8	33.1	12.1	33.5	14.4	29.9	15.3
After Dinner								
Fullness	78.5	17.0	76.6	14.4	76.8	13.2	78.4	15.2
Hunger	13.1	9.1	17.2*	11.1	17.3	13.6	16.0	9.3
Much	19.8	11.3	22.1	12.8	20.3	13.1	19.2	11.4
Satisfaction	79.2	16.8	78.2	14.1	77.5	15.7	79.7	12.7

[1] Two-way repeated-measures ANOVA (blocked by participant) and least significant difference test. Mean value is significantly different from before intervention within a particular intervention phase; * $p < 0.05$. SD: standard deviation

Appendix 7-14. Appetite rating table- Low dietary fibre

Appetite rating (full, hungry, much [how much can you eat], satisfied ratings using an 100 mm anchored visual analogue scale) changes 30 mins before and 30 mins after breakfast, lunch and dinner during the placebo and prebiotic intervention phases in the low dietary fibre group[1]

	Placebo (n = 14)				Prebiotic (n = 14)			
	Before intervention		After intervention		Before intervention		After intervention	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Before Breakfast								
Fullness	32.1	13.0	34.1	13.2	35.5	19.6	33.9	13.9
Hunger	56.0	15.5	54.1	15.9	47.2	19.0	46.5	18.6
Much	59.9	16.9	56.6	14.6	51.8	17.9	54.4	14.5
Satisfaction	32.2	11.8	33.4	11.6	39.6	18.5	36.3	16.2
After Breakfast								
Fullness	70.5	15.2	72.8	17.0	69.4	18.2	65.4	24.5
Hunger	21.5	13.9	23.2	14.5	25.2	21.1	24.8	14.2
Much	29.8	18.9	30.3	18.7	27.0	20.0	29.7	18.4
Satisfaction	71.1	14.9	72.1	17.6	71.4	18.2	67.2	23.4
Before Lunch								
Fullness	38.5	10.6	36.1	19.6	32.2	15.8	35.4	18.8
Hunger	50.5	17.0	53.6	23.3	58.0	19.7	57.3	17.6
Much	54.6	16.6	54.4	22.7	61.4	16.3	62.2	14.5
Satisfaction	42.8	10.3	38.4	18.3	32.6	16.5	35.6	17.5
After Lunch								
Fullness	71.0	13.2	75.5	15.3	73.3	17.1	73.8	15.4
Hunger	21.1	16.1	18.1	11.3	20.4	17.4	18.9	13.6
Much	25.1	15.6	22.1	14.3	25.2	22.1	23.2	19.6
Satisfaction	74.1	13.0	75.1	15.5	74.2	15.8	75.0	16.3
Before Dinner								
Fullness	32.3	11.5	34.6	15.5	33.0	17.1	30.1	17.5
Hunger	62.1	14.6	59.3	20.4	57.0	19.2	61.7	20.5
Much	62.3	15.6	60.7	17.8	62.1	17.1	63.9	19.3
Satisfaction	35.0	13.4	36.0	13.9	33.4	15.3	30.9	18.1
After Dinner								
Fullness	82.2	19.1	80.5	18.6	83.2	10.2	78.8	20.0
Hunger	13.7	10.8	14.5	10.4	13.1	11.0	16.2	12.2
Much	18.0	13.9	17.2	13.9	15.4	10.3	17.2	13.7
Satisfaction	80.3	20.2	80.3	19.5	83.4	10.0	79.8	17.0

[1] Two-way repeated-measures ANOVA (blocked by participant) and least significant difference test. Mean values were not significantly different ($p > 0.05$) from each other. SD: standard deviation

Appendix 7-15. Appetite rating table- High dietary fibre

Appetite rating (full, hungry, much [how much can you eat], satisfied ratings using an 100 mm anchored visual analogue scale) changes 30 mins before and 30 mins after breakfast, lunch and dinner during the placebo and prebiotic intervention phases in the high dietary fibre group[1]

	Placebo (n = 19)				Prebiotic (n = 20)			
	Before intervention		After intervention		Before intervention		After intervention	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Before Breakfast								
Fullness	31.4	15.1	29.0	14.7	27.7	17.1	29.1	19.6
Hunger	49.3	18.4	51.6	23.1	57.5	19.9	52.8	24.5
Much	58.7	16.5	61.2	19.9	62.7	19.5	59.1	20.7
Satisfaction	36.0	12.5	35.3	16.8	35.1	17.8	34.8	21.0
After Breakfast								
Fullness	70.9	11.4	71.1	15.4	67.5	12.0	74.4	13.7
Hunger	20.7	13.7	17.0	11.7	21.9	10.3	17.0	13.6
Much	30.6	12.0	25.9	13.4	29.4	13.7	25.0	12.4
Satisfaction	73.6	10.9	75.8	13.6	69.5	13.4	74.1	12.8
Before Lunch								
Fullness	28.6	13.6	30.4	13.7	29.2	13.5	26.4	12.8
Hunger	63.1	13.2	64.7	15.2	59.7	17.8	64.9	18.4
Much	65.7	13.9	68.5	15.3	64.2	16.0	67.9	16.6
Satisfaction	31.1	10.9	33.8	13.9	36.3	17.9	28.8*	14.7
After Lunch								
Fullness	71.5	12.7	64.3**	11.4	70.4	10.6	73.9**	13.4
Hunger	18.9	11.0	20.5	13.8	22.8	11.9	19.6	10.6
Much	29.9	12.7	32.0	16.2	30.1	15.7	25.1	13.8
Satisfaction	74.2	12.2	69.0*	13.1	69.3	12.5	74.7*	14.8
Before Dinner								
Fullness	32.3	11.2	28.1	11.4	28.9	13.3	27.5	12.0
Hunger	61.1	12.5	63.4	13.6	62.0	15.0	63.6	17.6
Much	68.8	12.6	70.6	12.8	66.0	15.8	72.2	12.0
Satisfaction	32.6	10.8	30.9	10.4	33.6	14.2	29.2	13.5
After Dinner								
Fullness	75.9	15.2	74.5	10.0	72.4	13.5	78.2	11.4
Hunger	12.4	7.9	19.2**	11.5	20.3	14.8	15.8**	7.1
Much	21.0	9.1	25.5	10.9	23.7	14.0	20.6	9.5
Satisfaction	78.4	14.3	76.6	8.7	73.3	17.8	79.6	9.1

[1] Two-way repeated-measures ANOVA (blocked by participant) and least significant difference test. Mean values are significantly different from before intervention within a particular intervention phase; * $p < 0.05$, ** $p < 0.01$.
SD: standard deviation

Appendix 7-16. Washout phase table

Short-chain fatty acid concentrations and bacterial taxa at baseline and post washout in the whole cohort[1]

	Baseline		Post washout		
	Mean	**SD**	**Mean**	**SD**	**P value**
Short-chain fatty acids (µmol/g)					
Acetate	30.3	17.9	33.0	18.8	0.497
Butyrate	8.8	5.7	9.4	6.0	0.679
Propionate	9.5	6.1	10.7	7.9	0.401
Sum of short-chain fatty acids	52.8	28.4	57.1	31.4	0.573
Phylum (% relative abundance)					
Actinobacteria	10.9	6.6	10.9	7.7	0.827
Bacteroidetes	15.7	13.8	13.1	8.1	0.695
Firmicutes	71.4	12.3	73.9	9.3	0.466
Proteobacteria	0.5	0.6	0.5	0.5	0.866
Verrucomicrobia	0.3	0.6	0.3	0.5	0.399
Genus (% relative abundance)					
Bifidobacterium	7.0	5.7	6.3	6.0	0.521
Collinsella	2.9	2.4	3.5	2.9	0.623
Bacteroides	7.2	4.1	6.0	3.8	0.209
Prevotella	5.8	13.5	4.7	6.8	0.876
Lactobacillus	0.3	1.1	0.2	0.5	0.418
Lachnospiraceae, other, unknown genus	1.9	1.3	2.0	1.0	0.163
Lachnospiraceae, unknown genus	12.6	5.7	13.2	6.3	0.770
Blautia	10.5	5.4	10.2	5.4	0.837
Coprococcus	3.8	1.9	4.5	2.5	0.366
Dorea	1.6	0.8	1.8	1.0	0.400
Ruminococcus (Lachnospiraceae)	2.0	1.8	1.9	1.4	0.789
Ruminococcaceae, unknown genus	15.9	4.8	15.7	4.1	0.571
Faecalibacterium	0.4	0.2	0.5	0.3	0.132
Oscillospira	1.1	0.7	1.1	0.5	0.886
Ruminococcus (Ruminococcaceae)	5.3	3.8	5.8	3.7	0.554
Dialister	1.0	1.7	0.9	1.2	0.995

[1] Mann-Whitney test. A p value of > 0.05 is considered significant. SD: standard deviation

Appendix 7-17. Placebo intervention phase table

Bacterial taxa and short-chain fatty acid concentrations before and after the placebo intervention phase in the whole cohort[1]

	Before intervention		After intervention		
	Mean	**SD**	**Mean**	**SD**	**P value**
Short-chain fatty acids (µmol/g)					
Acetate	31.78	17.80	33.80	18.97	0.610
Butyrate	9.75	6.12	9.44	5.62	0.871
Propionate	10.09	6.19	11.63	7.78	0.414
Sum of short-chain fatty acids	55.52	28.69	59.48	32.28	0.619
Phylum (% relative abundance)					
Actinobacteria	10.88	6.43	10.84	7.24	0.839
Bacteroidetes	14.30	12.09	13.09	8.39	0.646
Firmicutes	72.90	11.31	73.85	11.02	0.770
Proteobacteria	0.43	0.40	0.51	0.61	0.858
Verrucomicrobia	0.33	0.66	0.38	0.85	0.684
Genus (% relative abundance)					
Bifidobacterium	6.56	5.21	6.50	5.88	0.819
Collinsella	3.36	2.52	3.15	2.80	0.592
Bacteroides	6.49	3.81	6.45	4.31	0.980
Prevotella	5.36	12.16	3.66	5.98	1.000
Lactobacillus	0.24	0.92	0.44	1.96	0.194
Lachnospiraceae, other, unknown genus	2.07	1.11	1.91	1.24	0.358
Lachnospiraceae, unknown genus	13.27	5.79	13.43	5.62	0.980
Blautia	10.78	5.81	9.45	4.43	0.507
Coprococcus	3.80	1.83	4.16	2.20	0.637
Dorea	1.65	0.86	1.61	0.86	0.889
Ruminococcus (Lachnospiraceae)	1.85	1.66	1.95	1.64	0.949
Ruminococcaceae, unknown genus	16.33	4.82	16.86	4.29	0.858
Faecalibacterium	0.47	0.32	0.53	0.30	0.256
Oscillospira	1.10	0.67	1.11	0.70	0.858
Ruminococcus (Ruminococcaceae)	5.60	3.73	5.52	4.00	0.839
Dialister	0.77	1.15	1.00	1.56	0.346

[1] Mann-Whitney test. A p value of > 0.05 is considered significant. SD: standard deviation

Appendix 7-18. PICRUSt analysis table

Bacterial substrate metabolism specific KEGG orthology groups predicted by PICRUSt analysis (% relative abundance) between the low and high dietary intake groups during the prebiotic intervention phase[1]

	High dietary intake				Low dietary intake				
	Before intervention		After intervention		Before intervention		After intervention		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P value
Amino Acid metabolism									
Amino acid related enzymes	1.490	0.172	1.536	0.034	1.475	0.314	1.611	0.279	0.295
Arginine and proline metabolism	1.281	0.147	1.302	0.031	1.289	0.285	1.384	0.268	0.338
Cysteine and methionine metabolism	0.939	0.111	0.975	0.024	0.936	0.197	1.032	0.184	0.284
Glycine, serine and threonine metabolism	0.799	0.094	0.821	0.015	0.794	0.169	0.866	0.148	0.278
Histidine metabolism	0.638	0.073	0.649	0.019	0.640	0.139	0.691	0.137	0.299
Lysine biosynthesis	0.917	0.104	0.942	0.025	0.928	0.205	1.000	0.187	0.385
Lysine degradation	0.104	0.018	0.102	0.017	0.098	0.023	0.104	0.024	0.239
Phenylalanine metabolism	0.162	0.025	0.162	0.019	0.158	0.034	0.168	0.037	0.292
Phenylalanine, tyrosine and tryptophan biosynthesis	0.899	0.101	0.920	0.024	0.895	0.200	0.973	0.196	0.285
Tryptophan metabolism	0.110	0.021	0.110	0.019	0.106	0.023	0.113	0.014	0.265
Tyrosine metabolism	0.322	0.038	0.342	0.016	0.330	0.067	0.368	0.073	0.389
Valine, leucine and isoleucine biosynthesis	0.798	0.090	0.830	0.039	0.816	0.181	0.883	0.165	0.459
Valine, leucine and isoleucine degradation	0.164	0.036	0.172	0.035	0.152	0.036	0.171	0.030	0.212
Carbohydrate metabolism									
Amino sugar and nucleotide sugar metabolism	1.393	0.168	1.453	0.055	1.382	0.291	1.531	0.251	0.275
Ascorbate and aldarate metabolism	0.088	0.015	0.090	0.013	0.088	0.016	0.106	0.015	0.099
Butanoate metabolism	0.564	0.069	0.576	0.025	0.567	0.119	0.616	0.110	0.266
C5-Branched dibasic acid metabolism	0.340	0.037	0.357	0.018	0.351	0.080	0.379	0.066	0.581
Citrate cycle (TCA cycle)	0.474	0.089	0.475	0.065	0.452	0.098	0.491	0.108	0.221
Fructose and mannose metabolism	0.985	0.121	1.005	0.062	1.002	0.208	1.092	0.191	0.245
Galactose metabolism	0.801	0.095	0.854	0.047	0.812	0.178	0.916	0.161	0.290
Glycolysis / Gluconeogenesis	1.117	0.133	1.163	0.040	1.128	0.235	1.254	0.257	0.242
Glyoxylate and dicarboxylate metabolism	0.497	0.058	0.502	0.018	0.496	0.111	0.534	0.107	0.284
Inositol phosphate metabolism	0.081	0.015	0.089	0.014	0.082	0.017	0.095	0.014	0.289
Pentose and glucuronate interconversions	0.510	0.067	0.526	0.034	0.529	0.117	0.581	0.128	0.332
Pentose phosphate pathway	0.910	0.108	0.938	0.026	0.920	0.198	1.017	0.206	0.224
Propanoate metabolism	0.479	0.057	0.488	0.023	0.483	0.096	0.524	0.080	0.248
Pyruvate metabolism	1.041	0.121	1.047	0.035	1.052	0.221	1.132	0.231	0.251
Starch and sucrose metabolism	1.112	0.129	1.176	0.072	1.132	0.255	1.248	0.250	0.447
Energy metabolism									
Carbon fixation in photosynthetic organisms	0.649	0.079	0.669	0.026	0.642	0.137	0.711	0.140	0.199
Carbon fixation pathways in prokaryotes	0.904	0.116	0.907	0.050	0.882	0.187	0.942	0.163	0.279
Methane metabolism	1.348	0.159	1.347	0.066	1.360	0.293	1.449	0.250	0.272
Nitrogen metabolism	0.645	0.077	0.657	0.024	0.643	0.139	0.685	0.104	0.420
Oxidative phosphorylation	1.055	0.137	1.048	0.086	1.040	0.232	1.072	0.157	0.523
Sulfur metabolism	0.257	0.030	0.263	0.011	0.252	0.059	0.270	0.052	0.382
Glycan biosynthesis and metabolism									
Glycosaminoglycan degradation	0.032	0.020	0.029	0.016	0.025	0.016	0.024	0.012	0.705
Glycosphingolipid biosynthesis ganglio series	0.022	0.015	0.018	0.010	0.016	0.012	0.015	0.008	0.766
Glycosphingolipid biosynthesis globo series	0.090	0.020	0.092	0.012	0.086	0.021	0.091	0.020	0.498
Glycosyltransferases	0.284	0.042	0.281	0.020	0.277	0.064	0.289	0.062	0.373
Lipopolysaccharide biosynthesis	0.087	0.071	0.080	0.063	0.060	0.039	0.065	0.029	0.349
Lipopolysaccharide biosynthesis proteins	0.161	0.074	0.157	0.059	0.136	0.048	0.146	0.039	0.369
N-Glycan biosynthesis	0.019	0.007	0.020	0.007	0.017	0.006	0.018	0.004	0.970
Other glycan degradation	0.243	0.039	0.245	0.016	0.235	0.063	0.255	0.058	0.294
Peptidoglycan biosynthesis	0.850	0.099	0.869	0.023	0.845	0.181	0.920	0.174	0.269
Various types of N-glycan biosynthesis	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.154
Lipid metabolism									
Alpha-Linolenic acid metabolism	0.001	0.002	0.001	0.003	0.001	0.001	0.001	0.001	0.601
Arachidonic acid metabolism	0.013	0.011	0.012	0.010	0.009	0.007	0.008	0.004	0.970
Biosynthesis of unsaturated fatty acids	0.109	0.019	0.114	0.020	0.109	0.023	0.119	0.023	0.487
Ether lipid metabolism	0.002	0.001	0.001	0.000	0.002	0.001	0.002	0.000	0.215
Fatty acid biosynthesis	0.481	0.056	0.477	0.029	0.478	0.103	0.502	0.112	0.363
Fatty acid elongation in mitochondria	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.948
Fatty acid metabolism	0.212	0.030	0.234	0.025	0.219	0.045	0.251	0.041	0.434
Glycerolipid metabolism	0.419	0.053	0.425	0.020	0.429	0.095	0.465	0.102	0.258
Glycerophospholipid metabolism	0.578	0.068	0.584	0.023	0.576	0.137	0.611	0.137	0.406
Linoleic acid metabolism	0.070	0.010	0.069	0.006	0.072	0.016	0.077	0.017	0.242
Lipid biosynthesis proteins	0.565	0.072	0.573	0.040	0.557	0.123	0.596	0.142	0.386
Primary bile acid biosynthesis	0.037	0.007	0.039	0.007	0.038	0.009	0.044	0.009	0.086
Secondary bile acid biosynthesis	0.037	0.007	0.039	0.007	0.038	0.009	0.044	0.009	0.083
Sphingolipid metabolism	0.208	0.035	0.221	0.027	0.205	0.049	0.224	0.046	0.680
Steroid biosynthesis	0.001	0.003	0.001	0.003	0.000	0.001	0.000	0.001	0.629
Steroid hormone biosynthesis	0.009	0.011	0.009	0.014	0.005	0.004	0.005	0.003	0.901
Synthesis and degradation of ketone bodies	0.028	0.006	0.027	0.006	0.027	0.009	0.027	0.008	0.792

[1] Two-way repeated-measures ANOVA (blocked by participant). P values < 0.05 are considered significant. SD: standard deviation

Appendix 7-19. Change in *Bifidobacterium* table

Changes in *Bifidobacterium* relative abundance (%) during the placebo intervention phase by participant

Participant ID	Change in *Bifidobacterium*
ADAPT02	2.17
ADAPT03	-1.00
ADAPT04	8.23
ADAPT05	-0.17
ADAPT06	0.09
ADAPT08	-0.37
ADAPT09	2.45
ADAPT10	1.82
ADAPT11	2.17
ADAPT12	-4.94
ADAPT13	-1.38
ADAPT14	0.22
ADAPT15	0.96
ADAPT16	3.25
ADAPT17	1.27
ADAPT19	2.67
ADAPT20	-0.04
ADAPT21	6.63
ADAPT23	-8.30
ADAPT24	-1.94
ADAPT25	-0.03
ADAPT26	-2.46
ADAPT28	-1.09
ADAPT29	8.63
ADAPT30	2.20
ADAPT31	-5.76
ADAPT32	-2.41
ADAPT33	-0.85
ADAPT34	-7.07
ADAPT37	-1.65
ADAPT38	4.15
ADAPT40	-0.15
ADAPT42	-9.35

Appendix 7-20. Poster presented at conference

Habitual dietary fibre intake influences gut microbiota response to a fructan-based prebiotic

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Introduction

An aberrant host-microbe relationship has been implicated in a number of disease states such as obesity[1], type 2 diabetes[2], colon cancers[3] and inflammatory bowel disease[4]. It is well-recognised that diet is one of the main factors leading to the inter-individual variability seen in gut microbiota composition and function. Therefore, dietary interventions, including prebiotic supplementation, are being used to target the gut microbiota to improve human health. Previous research has demonstrated that there are inconsistencies in how the gut microbiota respond to dietary change, making it very difficult to predict how the gut microbiota will react to a dietary intervention[5,6]. Inter-individual differences in baseline gut microbiota composition, function and diversity[7–9] as well as habitual diet[10,11] have been implicated in gut microbiota responses, however, no studies have been conducted in humans with the primary aim of exploring whether differing habitual dietary fibre intakes influence gut microbiota responsiveness to a dietary intervention. The aim of this study was to assess whether low *versus* high habitual dietary fibre intakes influence how the gut microbiota respond to a fructan-based prebiotic.

Methods

In this randomised, double-blind, placebo-controlled, cross-over study, 34 healthy participants were grouped as low (LDF) or high dietary fibre (HDF) consumers. Participants were considered to be LDF consumers (n=14) if their dietary fibre intake was <18 g/day for females or <22 g/day for males and HDF consumers (n=20) if their dietary fibre intake was ≥25 g/day for females or ≥30 g/day for males. Gut microbiota composition (Illumina MiSeq 16S rRNA bacterial gene sequencing) and organic acid concentrations (gas chromatography) were assessed following three weeks of either daily prebiotic supplementation (Beneo Orafti® Synergy 1; 16 g/day) or placebo (Roquette Glucidex® 29 Premium; 16 g/day) as well as after three weeks of the alternative intervention following a three-week washout period. Mann-Whitney tests were used to determine whether the prebiotic intervention led to significant bacterial changes in the whole cohort and whether there were significant differences in baseline bacterial composition, organic acid concentrations and dietary intakes between the LDF and HDF groups. Repeated-measures ANOVA was used to assess changes after the prebiotic intervention between LDF and HDF groups.

Results

Prebiotic supplementation led to significant ($p<0.05$) changes in the relative abundance of several bacterial taxa including *Bifidobacterium, Blautia, Dorea* and *Faecalibacterium* but not organic acid concentrations in the whole cohort (Table 1). Significant baseline (before intervention) differences in an unknown genus of *Lachnospiraceae* (other) (Table 2) and dietary intakes (e.g. fruits, vegetables and dietary fibre) were demonstrated between dietary fibre groups (Table 3). The gut microbiota responded differently between dietary fibre groups for *Lactobacillus,* an unknown genus of *Ruminococcaceae* and *Faecalibacterium* (Figure 1). Minimal change (0.1%) in the relative abundance of *Lactobacillus* occurred in HDF consumers; however, in LDF consumers *Lactobacillus* increased from 0.6 to 3.0% ($p=0.025$). An unknown genus of *Ruminococcaceae* increased slightly (+0.8%) in the HDF group but reduced in the LDF group (-2.9%) ($p=0.018$). *Faecalibacterium* increased more markedly in the HDF than in the LDF group (0.4 to 0.7% and 0.4 to 0.5%, respectively) ($p=0.009$) (Table 2).

Discussion and Conclusions

The present study demonstrates that habitual dietary fibre intakes influence how the gut microbiota respond to a fructan-based prebiotic. Gaining a better understanding of the factors implicated in gut microbiota responsiveness may help improve dietary intervention success and subsequently enhance human health outcomes. Therefore, future studies aiming to modulate the gut microbiota should take habitual dietary fibre intake into account.

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Tables and figure

Table 1- Organic acid concentrations (μmol/mL) and bacterial taxa (% relative abundance) before and after the prebiotic intervention for the whole cohort (n=34). Significant results ($p<0.05$) are in **bold**. Abbreviation: SD- standard deviation.

	Before intervention		After intervention		
	Mean	**SD**	**Mean**	**SD**	**P value**
Organic acids					
Acetate	31.5	19.0	39.5	21.0	0.115
Butyrate	8.5	5.5	10.2	5.6	0.202
Propionate	10.0	7.8	11.9	7.5	0.176
Sum of organic acids	54.3	31.2	65.5	32.5	0.130
Phylum					
Actinobacteria	11.0	7.9	20.0	10.2	**< 0.001**
Bacteroidetes	14.6	10.7	12.5	8.3	0.514
Firmicutes	72.4	10.8	65.7	11.0	**0.011**
Proteobacteria	0.5	0.7	0.4	0.4	0.224
Verrucomicrobia	0.3	0.5	0.2	0.3	0.307
Genus					
Bifidobacterium	6.7	6.4	15.1	8.5	**< 0.001**
Collinsella	3.1	2.8	3.8	2.8	0.253
Bacteroides	6.8	4.2	5.9	3.4	0.498
Prevotella	5.2	9.1	4.9	8.0	0.206
Lactobacillus	0.3	0.9	1.3	3.8	1.000
Lachnospiraceae, other, unknown genus	1.9	1.2	1.6	0.6	0.396
Blautia	9.9	4.8	7.7	3.9	**0.034**
Coprococcus	4.5	2.5	3.6	1.7	0.189
Dorea	1.8	1.0	1.2	0.7	**0.004**
Ruminococcus (Lachnospiraceae)	2.1	1.6	1.2	1.0	**0.003**
Ruminococcaceae, unknown genus	15.2	4.0	14.5	4.1	0.554
Faecalibacterium	0.4	0.2	0.6	0.3	**0.009**
Oscillospira	1.1	0.5	0.8	0.5	**0.019**
Ruminococcus (Ruminococcaceae)	5.5	3.8	4.4	3.3	0.224
Dialister	1.1	1.7	0.9	1.6	0.072

Table 2- Organic acid concentrations (μmol/mL) and bacterial taxa (% relative abundance) before and after the prebiotic intervention in low (n=14) and high (n=20) dietary fibre groups. Bacterial taxa which were significantly different between the low and high dietary fibre groups are in **bold** ($p<0.05$). P values- *Compares before intervention (baseline) differences between low and high dietary fibre consumers, #Compares change between low and high dietary fibre consumers. Abbreviation: SD- standard deviation.

	High dietary fibre					Low dietary fibre						
	Before intervention		After intervention		Change	Before intervention		After intervention		Change		
	Mean	SD	Mean	SD		Mean	SD	Mean	SD		* P value	# P value
Organic acids												
Acetate	33.3	19.7	43.2	19.7	9.9	29.0	18.2	34.3	22.3	5.3	0.592	0.534
Butyrate	9.1	5.8	11.5	5.6	2.4	7.8	5.1	8.3	5.3	0.5	0.545	0.375
Propionate	10.1	8.9	12.7	7.8	2.6	10.0	6.3	10.9	7.1	0.9	0.666	0.424
Sum of organic acids	56.6	33.1	71.1	31.1	14.5	51.0	29.0	57.6	34.0	6.6	0.877	0.475
Phylum												
Actinobacteria	8.9	6.0	17.7	10.2	8.8	14.0	9.4	23.2	9.6	9.2	0.104	0.907
Bacteroidetes	16.8	11.4	14.5	9.5	-2.3	11.3	8.9	9.6	5.4	-1.7	0.169	0.829
Firmicutes	72.1	12.2	65.5	12.1	-6.6	72.8	8.8	66.0	9.7	-6.9	0.931	0.933
Proteobacteria	0.5	0.5	0.4	0.5	-0.1	0.7	0.9	0.3	0.4	-0.4	0.823	0.188
Verrucomicrobia	0.3	0.3	0.2	0.2	-0.1	0.3	0.6	0.2	0.5	-0.1	0.304	0.947
Genus												
Bifidobacterium	4.5	4.1	13.0	8.6	8.5	9.8	7.8	18.0	7.9	8.2	0.066	0.900
Collinsella	3.2	2.7	3.7	2.5	0.6	3.0	3.1	3.9	3.2	1.0	0.616	0.681
Bacteroides	6.8	3.7	6.0	3.1	-0.9	6.8	5.0	5.7	3.9	-1.0	0.931	0.909
Prevotella	6.8	11.0	6.5	9.7	-0.3	2.9	4.9	2.5	3.6	-0.5	0.666	0.898
Lactobacillus	0.0	0.1	0.1	0.2	0.1	0.6	1.3	3.0	5.6	2.4	0.609	**0.025**
Lachnospiraceae, other, unknown genus	1.5	0.8	1.4	0.5	-0.2	2.4	1.5	1.8	0.6	-0.5	**0.043**	0.261
Blautia	9.5	4.4	7.4	3.8	-2.2	10.4	5.6	8.1	4.1	-2.3	0.569	0.917
Coprococcus	5.0	2.7	3.9	1.4	-1.1	3.8	2.1	3.1	1.9	-0.8	0.204	0.65
Dorea	1.6	0.7	1.2	0.8	-0.4	2.0	1.2	1.2	0.5	-0.8	0.341	0.253
Ruminococcus (Lachnospiraceae)	1.8	1.2	1.1	0.8	-0.7	2.6	2.0	1.2	1.3	-1.3	0.306	0.249
Ruminococcaceae, unknown genus	14.7	3.1	15.5	3.3	0.8	16.0	5.1	13.1	4.8	-2.9	0.569	**0.018**
Faecalibacterium	0.4	0.2	0.7	0.3	0.3	0.4	0.3	0.5	0.3	0.1	0.500	**0.009**
Oscillospira	1.0	0.4	0.7	0.5	-0.3	1.2	0.7	0.9	0.5	-0.3	0.478	0.986
Ruminococcus (Ruminococcaceae)	5.3	3.5	3.9	2.8	-1.3	5.8	4.3	5.0	4.0	-0.8	0.849	0.617
Dialister	1.1	1.8	0.8	1.1	-0.3	1.0	1.7	1.1	2.1	0.1	0.568	0.356

Table 3- Baseline differences in fructan, food group serves and nutrient intakes after the prebiotic intervention between the low (n=14) and high (n=20) dietary fibre groups. Significant results ($p<0.05$) are in **bold**. Abbreviation: SD- standard deviation.

	High dietary fibre		Low dietary fibre		
	Mean	**SD**	**Mean**	**SD**	**P value**
Fructan intakes from diet					
Total Inulin (g)	2.9	1.1	3.1	1.3	0.796
Total oligofructose (g)	2.8	1.0	3.0	1.2	0.769
Food group serves					
Grains	6.6	3.5	5.3	2.4	0.359
Refined grains	3.6	2.6	3.8	2.6	0.641
Wholegrains	3.0	2.6	1.5	1.2	0.064
Fruit	2.2	1.7	1.0	0.9	**0.009**
Vegetables	6.1	3.3	2.4	1.6	**<0.001**
Dark green vegetables	0.5	0.4	0.2	0.3	**0.007**
Red orange vegetables	1.7	1.9	0.7	0.7	**0.039**
Tomatoes	0.4	0.4	0.2	0.2	0.06
Starchy vegetables	1.0	1.4	0.6	0.7	0.737
Potatoes	0.8	1.2	0.5	0.6	0.671
Legumes (vegetable)	0.6	0.8	0.2	0.3	0.144
Protein foods	3.7	1.7	2.4	1.7	**0.036**
Red meats	0.7	0.6	0.9	0.9	0.548
Poultry	0.6	0.7	0.4	0.6	0.217
Eggs	0.4	0.4	0.3	0.4	0.169
Processed meats	0.3	0.4	0.3	0.3	0.495
Nuts and seeds	1.0	1.0	0.2	0.2	**0.001**
Legumes (protein)	0.4	0.5	0.1	0.2	0.155
Dairy	1.9	1.2	1.4	1.1	0.18
Nutrient intakes					
Energy (kJ)	10013.9	2769.8	7161.1	2285.2	**0.002**
Protein (g)	112.7	45.5	83.1	28.5	0.066
Total fat (g)	95.9	29.7	67.8	26.0	**0.012**
Saturated fat (g)	33.3	14.6	26.5	13.3	0.259
Polyunsaturated fat (g)	16.3	5.9	10.8	4.2	**0.005**
Monounsaturated fat (g)	38.0	10.8	24.6	8.9	**0.001**
Carbohydrate available (g)	241.3	84.0	178.0	83.0	**0.015**
Sugars (g)	106.2	40.4	77.8	47.0	0.051
Starch (g)	132.0	59.9	99.2	39.5	0.104
Water (g)	2781.4	1428.1	2048.2	746.3	0.104
Alcohol (g)	4.2	12.3	3.1	6.8	0.565
Dietary fibre (g)	38.6	13.0	18.0	3.4	**<0.001**
Energy from protein (%)	19.1	4.5	20.6	8.0	0.877
Energy from fat (%)	35.9	8.5	34.4	5.2	0.986
Energy from saturated fat (%)	12.3	4.5	13.1	3.9	0.457
Energy from carbohydrate (%)	39.3	7.4	40.6	10.4	0.823
Energy from alcohol (%)	1.0	2.7	1.2	2.6	0.601
Energy from fibre (%)	3.1	0.8	2.2	0.7	**<0.001**
Dietary fibre (g) per 1000kJ	3.9	1.0	2.7	0.8	**<0.001**

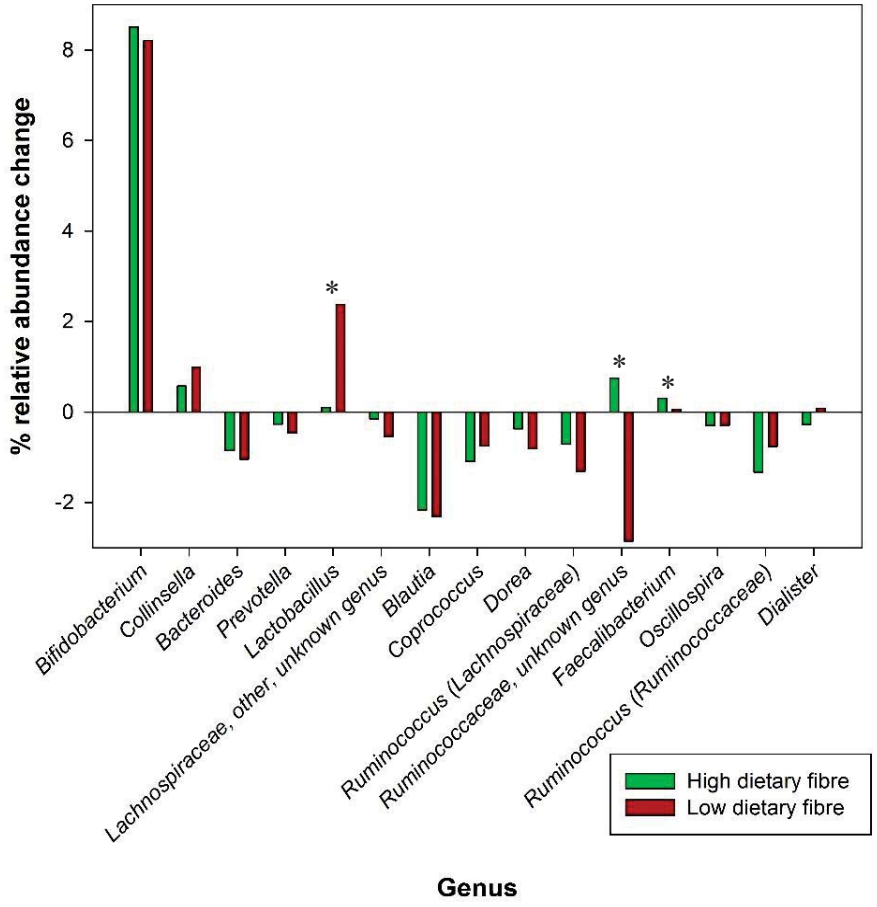

Figure 1- Genus level changes after prebiotic intervention for the low (n=14) and high (n=20) dietary fibre groups. Changes that are significantly different ($p<0.05$) between dietary fibre groups are indicated with an asterisks (*)