Gut microbiota modulation with long-chain corn bran arabinoxylan in overweight individuals is linked to individualized temporal increase in fecal propionate

by

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ABSTRACT

Variability in the health effects of dietary fibers (DFs) might arise from inter-individual differences in the microbiome's ability to ferment these substrates into beneficial metabolites. However, our understanding of what drives this individuality is vastly incomplete. Here, we report results from a parallel-arm, randomized controlled trial aimed to characterize the effects of a long-chain, complex arabinoxylan (AX) isolated from corn bran on gut microbiota composition and short-chain fatty acid (SCFA) production as compared to microcrystalline cellulose (non-fermentable control). AX resulted in a global shift in the fecal bacterial community and the promotion of specific taxa, including OTUs related to *Bifidobacterium longum*, *Blautia obeum*, and *Prevotella copri*. AX further increased fecal propionate concentration (overall effect p=0.015, Friedman's test), an effect that showed two distinct temporal responses that allowed grouping of participants. The two groups showed significant differences in compositional shifts of the microbiome ($p \leq 0.025$, PERMANOVA), and multiple linear regression analyses revealed that the propionate response was predictable through shifts, and to a lesser degree, baseline composition of the microbiota. Pre-treatment dietary history was unable to predict the response. This study showed that individualized metabolic effects of DF on the gut microbiota are linked to both compositional shifts and its baseline composition, providing preliminary evidence that such responses might be predictable.

PREFACE

This thesis is an original work by Nguyen Khoi Nguyen. The randomized controlled trial (RCT) of this thesis, was prospectively registered on December 19, 2014 with ClinicalTrials.gov (NCT02322112) as part of a large parallel four-arm RCT: The Alberta FYBER (Feed Your Gut Bacteria morE fibeR) Study. The study protocol was approved by the Health Research Ethics Board of the University of Alberta (Pro00050274), with written informed consent obtained prior to participant enrollment. Due to comments of reviewers of a CHIR grant application (who criticized that the AX fiber used was not yet on the market in Canada and therefore cannot readily be implemented), the AX arm was separated from the original RCT and analyzed independently. Study visits were conducted in accordance with the principles of the Declaration of Helsinki at the University of Alberta Human Nutrition Research Unit in Edmonton, Alberta, Canada between September 2015 and October 2016.

For this thesis, my responsibilities included: DNA extraction and sequencing, microbiome analysis, bioinformatic and statistical analyses, data visualization, and writing. Much of this work was done in collaboration with Edward Deehan - a PhD candidate - who is co-first author of the proposed manuscript. Edward was responsible for managing all aspect of the project, was involved in developing and initiating the FYBER study, organizing the human study, data analysis, interpretation, and drafting the manuscript. As the clinical research coordinator for the FYBER study, Janis Cole contributed to the development and initiation of the study, and the acquisition of study funding. Dr. Zhengxiao Zhang was closely involved in network analysis, as well as drafting the manuscript. Dr. Mingliang Jin supported fecal sample processing, DNA extraction and sequencing, and fecal Short-Chain Fatty Acid quantification; with Nami Baskota - a MSc student – also contributing to the quantification of fecal SCFAs. Dr. Maria Elisa Perez-Muñoz and Dr.

Inés Martínez both supported the author's bioinformatic of microbiome analysis training, with Dr. Martínez was also involved in developing the study and optioning funding. Drs. Yunus Tuncil and Bruce Hamaker characterized the chemical structure of corn bran arabinoxylan, while Dr. Dan Knights supported the author's training on machine learning. Benjamin Seethaler - a PhD student with Dr. Stephan Bischoff - quantified fecal water content and contribute to the ERA-HDHL grant. Dr. Jeffrey Bakal supported the author's statistical training and supervised the statistical analyses for the project. Dr. Carla Prado is the co-PI of the FYBER study, providing supervision of the RCT and contributed to funding acquisition. Dr. Jens Walter is the study PI, and thus supervised all aspects of the project, including the author's training and the writing of the thesis and manuscript.

DEDICATION

“Two things are infinite: the universe and human stupidity; and I'm not sure about the universe.”

Albert Einstein

“Your work is going to fill a large part of your life, and the only way to be truly satisfied is to do what you believe is great work. And the only way to do great work is to love what you do. If you haven't found it yet, keep looking. Don't settle. As with all matters of the heart, you'll know when you find it.”

Steve Jobs

“*The passengers in our microbiome contain at least four million genes, and they work constantly on our behalf: they manufacture vitamins and patrol our guts to prevent infections; they help to form and bolster our immune systems, and digest food****.”***

Michael Specter

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Last but not least, nobody has been more important to me in the pursuit of this degree than my family, I would like to thank my parents, my partner (Ngoc Phuong Nguyen), whose love and guidance are with me in whatever I decide. They always are my unending inspiration.

Finally, I would like to thank all funding agencies (JPI-a healthy diet for a healthy life, Canadian Institutes of Health Research, Alberta Diabetes Institute, Muttart Diabetes Research and Training Center, Canadian Statistical Sciences Institute) and industry partners of the project (AgriFiber and Blanver) for their support in finance and research materials.

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List of Abbreviations

AX: Arabinoxylan

AICc: Akaike information criterion corrections

BL: Baseline

CARG: Co-Abundance Response Group

DF: Dietary fiber

MCC: microcrystalline cellulose

MLR: Multiple linear regression

NMDS: non-metric multidimensional scaling

OTU: Operational taxonomic unit

PCA: Principle component analysis

PC: Principle component

PERMANOVA: permutational multivariate analysis of variance

RS: Resistant Starch

SCFAs: Short chain fatty acids

W1: Week 1

W6: Week 6

CHAPTER 1: INTRODUCTION

1.1 Background

Obesity and its associated pathologies, such as cardiovascular disease and type 2 diabetes, have reached epidemic proportions within Canada [1] and worldwide [2]. In recent years, the development of obesity and its related pathologies has been linked to gastrointestinal (GI) microbiome - that is, the microbial community that inhabits the gut [3-6]. For example, faecal microbiota transplantations from healthy donors to metabolic syndrome recipients [5, 7, 8] resulted in improved insulin sensitivity, fasting blood glucose, and LDL cholesterol, indicating that the gut microbiota may be a potential target for strategies combating obesity. The mechanisms by which the gut microbiota influences obesity-related pathologies are not sufficiently understood. Mechanisms discussed include an impact on intestinal barrier integrity, regulation of gut hormones, and an influence on energy metabolism. Many of these mechanisms are mediated by microbial-derived metabolites, especially short-chain fatty acids (SCFA) (e.g. acetate, propionate, and butyrate)[3]. These SCFAs are produced by microbial fermentation of dietary fibre (DF), which has been shown to reduce inflammation, modulate the immune system, promote satiety, and modulate cancer homeostasis [9].

Epidemiologic studies consistently associate DF consumption with a reduced incidence of obesity and its associated pathologies [10, 11]. In large-scale observational studies, whole grains and cereal derived DFs (*e.g.* arabinoxylan [AX] and β-glucan) show stronger associations with reduced risk of developing cardiovascular diseases, type II diabetes, gastrointestinal cancers, and of all-cause mortality when compared to other DF sources [12-14]. A substantial body of animal research further consolidated the mechanisms by which DF reduces metabolic pathologies [15].

Despite these convincing associations, findings obtained from human dietary intervention trials that aimed to ameliorate risk markers of metabolic disease by supplementing isolated DFs remain inconsistent [16] potentially because of individualized clinical responses to DF [17, 18].

Owing to their chemical structure, DFs resist digestion in the small intestine, thus reaching the colon where they become substrates to the gut microbiota. Microbes ferment DF to SCFAs that have been implicate in the prevention of obesity-associated pathologies [19]. In this respect, especially propionate and butyrate are relevant, as they have been linked to beneficial immunometabolic effects [9]. Intervention studies with AX, for instance, have demonstrated increased fecal concentrations of both butyrate and propionate with long-chain AX isolated from wheat endosperm [20]. DFs can further modulate gut microbiota composition, leading to structure-dependent change in the proportion of bacterial taxa that benefit from substrate and tolerate the environmental changes caused through DF fermentation [15]. For example, dietary interventions with short-chain fractions of AX have shown an enriched abundance of bacterial species that can either utilize AX directly (*i.e. Bifidobacterium longum*, *Prevotella copri*, and *Blautia obeum*) or that benefits from metabolic products (*i.e. Faecalibacterium prausnitzii* and *Eubacterium hallii*) [21, 22]. Although DF-induced alterations to the gut microbiota are significant, the effects are also highly individualized [18], and this variability might have clinical ramifications that could explain the inconsistent health effects of DF [23].

To understand the individualized response of the gut microbiota to DF an ecological perspective is required, as microbiomes are vastly individualized and DF fermentation is determined by complex inter-species interactions between members of the gut microbiota [24]. Inter-individual compositional differences in the gut microbiome may result in the absence of important primary degraders (or 'keystone species') needed to initiate the degradation of recalcitrant DFs [25].

Individual microbiotas may also comprise of separate, potentially unrelated species able to compete for the same substrate [26], or strains of the same species with varying enzymatic capacity to metabolize the substrate [27]. These compositional variations may alter the network of interacting species (referred to as an ecological 'guild'), involved in DF fermentation by altering competitive or co-operative relationships between community members [28, 29], which influences metabolite outputs relevant to health (*i.e.* propionate or butyrate) [30].

1.2 Knowledge ands and research question

Although DF-induced alterations to the gut microbiota are well established in the recent literature and can lead to substantial alterations in the gut microbiota, the effects are also highly individualized [18]. This variability might have ramifications for the use of DF as it could explain the inconsistent health effects that have been shown in intervention studies [23]. To understand the individualized response of the gut microbiota to DF, an ecological perspective is required, as gut microbiomes are vastly individualized in their composition and DF fermentation is determined by complex inter-species interactions between members of the gut microbiota [31]. Inter-individual compositional differences in the gut microbiome may result in the absence of important primary degraders (or 'keystone species') needed to initiate the degradation of DFs [25]. In addition, individual gut microbiomes may comprise separate, potentially unrelated species that compete for the same substrate [26], or related strains of the same species with varying enzymatic capacities to metabolize the substrate [27]. These compositional variations are likely to alter the network of interacting species by altering competitive or co-operative relationships between community members [29]. These complex ecological interactions influence metabolite outputs that are relevant to health (i.e. SCFAs such as propionate or butyrate) [30]. Although inter-individual variation in the response of the gut microbiota to DF might constitute an important

factor influencing its health effects, this topic, and the underlying ecological principles, are poorly understood. The question remains of whether microbial interactions in the gut microbiota ecosystem can explain the individualized responses to DF.

1.3 Objective

The objective of this study was, therefore, to characterize the compositional and functional responses of the fecal microbiota to long-chain AX and apply an ecological framework to explain the individualized response of microbiota.

1.4 Specific aims & hypotheses

The first specific aim (Aim 1) of this thesis is to provide a descriptive overview of the gut microbiota compositional response to long-chain AX. I hypothesize that consuming high doses of long-chain AX will alter the composition of the gut microbiota at the community and species levels compared to both baseline and microcrystalline cellulose (MCC), a non-fermentable control DF.

The second specific aim (Aim 2) is to determine how long-chain AX supplementation affects fecal SCFA levels. I hypothesize that consuming long-chain AX will increase fecal SCFA concentrations compared to MCC.

The final aim (Aim 3) is to explore links between microbiome composition, SCFA levels, and dietary intake to explain individualized responses in the context of microbial ecology. I hypothesize that individualized responses in fecal SCFA concentrations can be explained by fecal microbiota composition and diet.

1.5 Outline of thesis

Chapter 2 – Literature review: This chapter will present the inter-relationship between DF, gut microbiome, SCFA levels, and obesity. Then, it will provide an overview of the inconsistent results

between observational and intervention studies regarding the health benefits of DF, and what factors may explain these inconsistent health effects.

Chapter 3 – Materials and methods: This chapter introduces the study design and statistical analyses of a DF intervention study that examined how the gut microbiota responded to a high dose of long-chain AX isolated from (females: 25 g/d; males: 35 g/d), compared to MCC.

Chapter 4 – Results: I present the effect of long-chain AX on the composition and function (SCFA production) of gut bacteria, and factors that explain the individualized response in propionate production.

Chapter 5 – Discussion: I begin the discussion by applying an ecological framework to explain the specific responses in the gut bacterial composition and function. Then, I discuss the factors that may predict the individualized response of the microbiota and end this section by highlighting the possible health benefits of long-chain AX.

Chapter 6 – General Discussion: This chapter provides a broader discussion of the findings in relation to other studies in the literature, as well as parallel projects conducted by the Walter lab. Furthermore, I discuss how my work is important for the use of DF in human nutrition and a potential personalization of diet and suggest future directions of research that will be necessary to progress towards this goal.

CHAPTER 2: LITERATURE REVIEW

2.1 Inter-relationship between microbiome composition and function in obesity

2.1.1 Microbiome and Obesity

Obesity and its related metabolic diseases have developed into an epidemic that damages individual health, family and society in general. According to a WHO report in 2017, these global diseases cause at least 2.8 million deaths each year and are present not only with high-income but also middle and low-income countries [32]. In 2016, the prevalence of obesity increased rapidly and tripled since 1975. The worldwide increase of obesity seriously affects health and reduces quality of life [33] because of its associated pathologies such as type-2- diabetes, cancer, work disability, osteoarthritis, sleep apnea and cardiovascular diseases [34]. Thus, efforts to prevent and manage the obesity epidemic are urgently needed.

Microbiota has been documented to be related closely to obesity and its associated pathologies. The term "Microbiota" refers to microbial communities that comprised of all taxa such as bacteria, archeae, fungi and protists while microbiome was used to refer to microbial gene and is also commonly used to mention the microorganism themselves [35]. Human has been recognised as an supraorganism – that is, a system of multiple organism that can be considered a single organism – which is composed of both human and microbial cells [36]. Thus, we are carrying 2 different sets of genes: those that belong to the human and microbiome. The former contains approximately 20,000 genes [37] [38] while the latter has a capacity of ~ 3 million genes [39], which performs multi-functions that are associated to host. Thus, the gut microbiota has been considered a vital partner of the human host and has been studied in, approximately 12900 studies within 5 years since 2013 [40].

In recent years, it has been well-documented in animal models that the gastrointestinal (GI) microbiome plays a crucial role in the development of obesity and its related pathologies [3-6]. Transferring microbiota from diet-induced obesity mice to lean germ-free recipients increased a greater fat accumulation than transplants from lean donors[41]. Faecal microbiota transplantations from healthy donors to metabolic syndrome recipients [5, 7, 8] resulted in improved insulin sensitivity, fasting blood glucose, and LDL cholesterol. Furthermore, the mechanisms by which the germ-free mice are protected from diet-induced obesity have been unveiled. They are 1) inducing peroxisomal proliferator-activated receptor coactivator (Pgc-1α) and enzymes involved in fatty acid oxidation. 2) promoting the activity of phosphorylated AMP-activated protein kinase (AMPK)[42]. In short, these findings suggest that the gut microbiota may be a potential target to combat obesity.

2.1.2 Microbiome and SCFAs

Dietary fiber (DF) is defined as carbohydrates (CHO) polymers with equal or more than ten monomeric unit, which are not digestible in the human small intestine[43]. DF does occur naturally in food (e.g. fruit, vegetable) or used as a supplement or food additive after being extracted or synthesized. To be considered DF, the two latter ones require proven physiological effects relevant towards health [44]. DF escape from the digestion of the small intestine and are metabolized by the microbiota in the cecum and colon [45] (figure A1). The major products from the microbial fermentation in the gut are SCFA – mainly, acetate, propionate and butyrate and their biosynthesis pathway are shown in Figure A2. Briefly, Pyruvate is the precursor of the three SCFAs. While acetate and butyrate can be produced from Acetyl-CoA, propionate can be formed by lactate and succinate via acrylate and succinate pathway, respectively. In addition, propionate can be produced from propanediol pathway using deoxyhexose sugars.

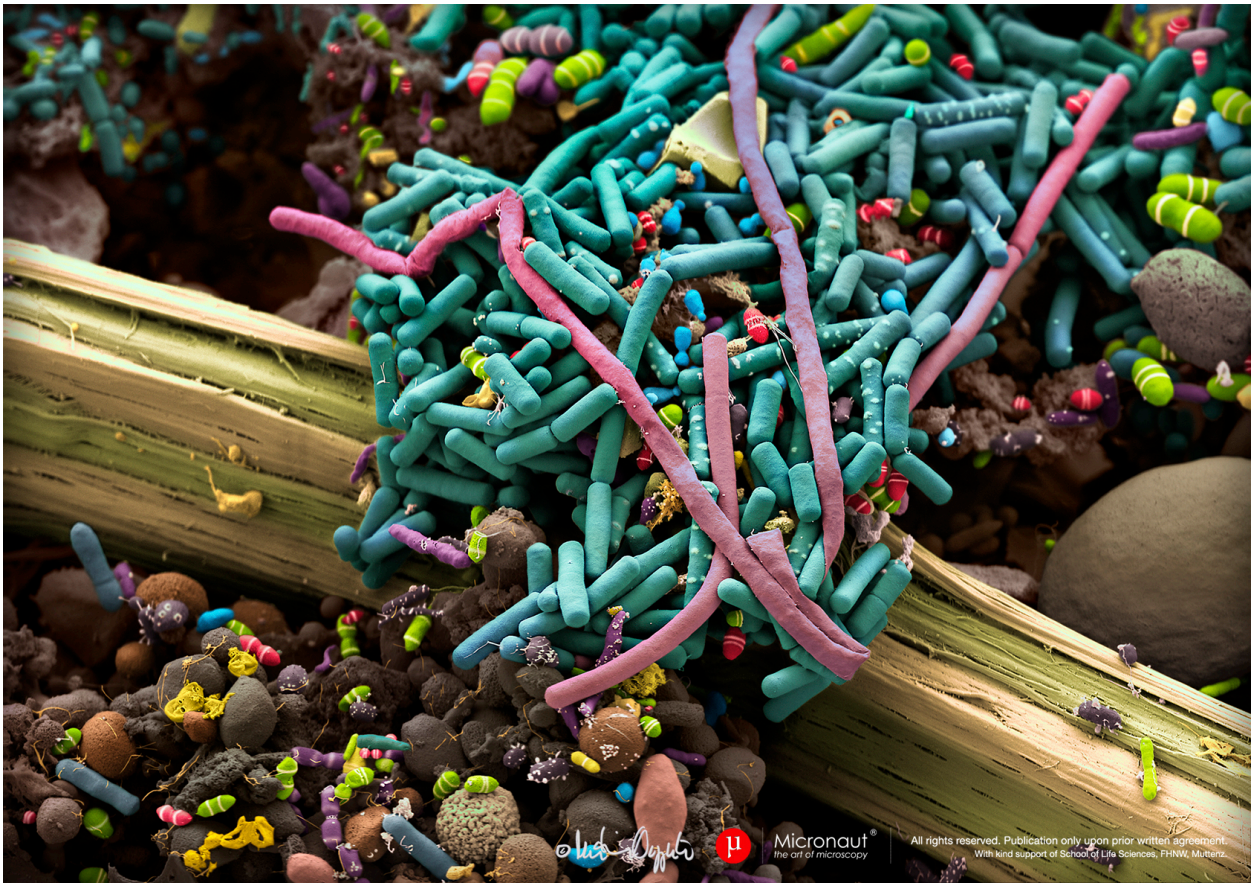


Figure A1: Human gut microbiome. Credit: © M. Oeggerli, supported by Pathology, University Hospital Basel and School of Life Science, FHNW.

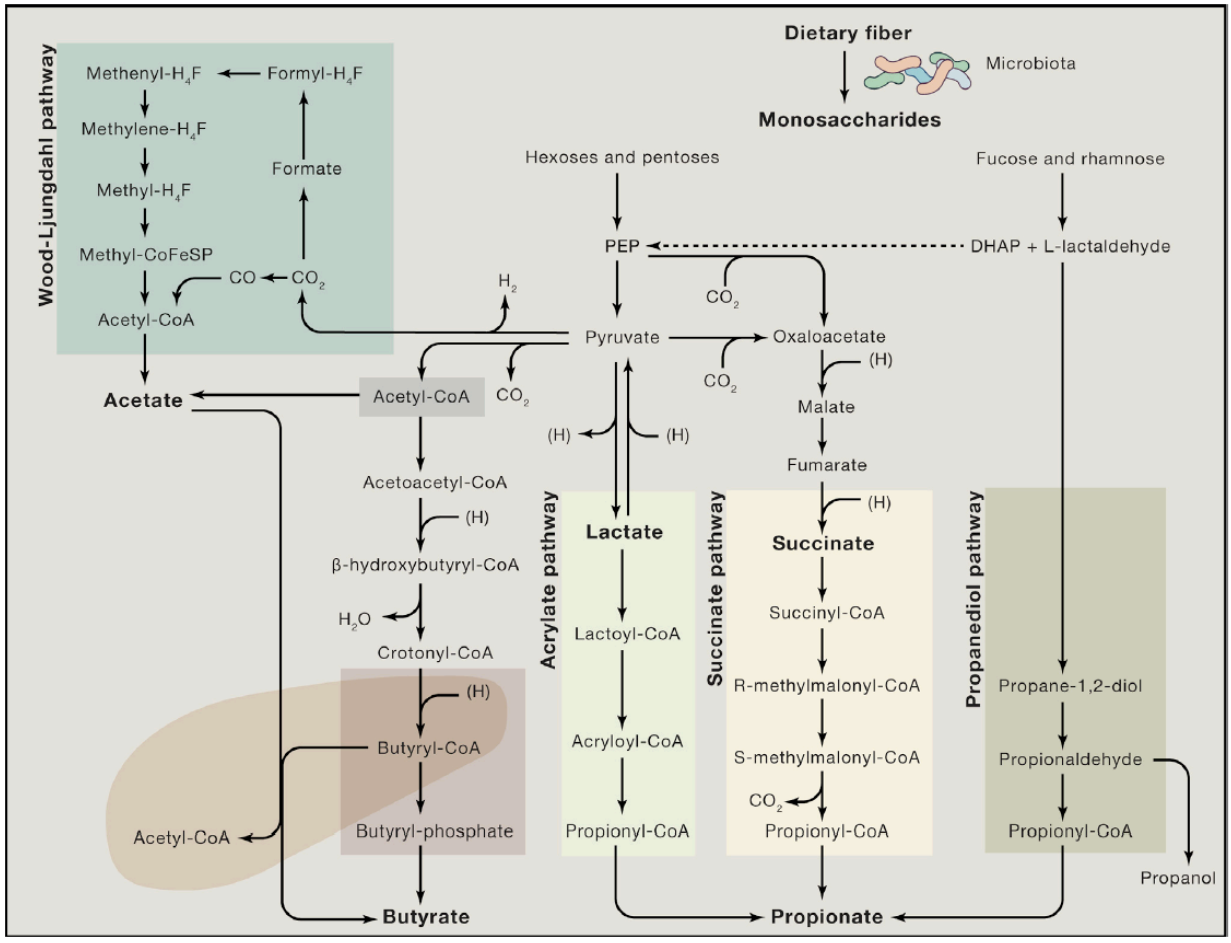


Figure A2: Known pathway for SCFA biosynthesis from Carbohydrate fermentation- Adopted from [9]. PEP: phosphoenolpyruvate; DHAP, dihydroxyacetonephosphate.

2.1.3 Association between SCFAs and Obesity and its associated pathologies

It has been shown that SCFAs impact on body weight by regulating appetite and therefore changing the host's energy intake as reviewed by Hernández and colleagues [46]. Mice studies showed that SCFAs activation of GPR41 stimulates the secretion of the gut hormone peptide YY (PYY), which induces satiety and reduces food intake [47]. In addition to increasing satiety, these hormones stimulate insulin secretion and decrease the secretion of glucagon from the pancreas [48].These findings were further confirmed in human studies showing that the administration of

propionate to patients with obesity led to enhanced PYY and Glucagon-like peptide 1 (GLP-1) secretion with significantly reduced adiposity and overall weight gain [49]. In mice, it was shown that supplementation of butyrate into the diet lowered fasting glucose and insulin levels and showed lower insulin resistance displayed in the HOMA-IR [50, 51]. Similar results on mitigated insulin resistance have been reported for butyrate supplementation to the diet in rats [52].

The effect of SCFAs on the host can be explained by two main mechanisms. They are 1) inhibiting histone deacetylase activity and its expression, and 2) binding to G-protein-coupled receptors (GPRs) including GPR41, GPR43, GPR109A [53]. The former has been indicated as the central mechanism by which SCFAs can stimulate the immune system which results in the inhibition of colon cancer [54]. The latter has different mechanism for three SCFAs. While butyrate is the only SCFA that can bind GPR109A to promote the anti-inflammation effect, both acetate and propionate can bind to GPR43 and release GLP-1 and PYY, increasing satiety. Acetate can bind to GPR41 to promote the production of dendritic cell and Propionate can bind to GPR41 activate the intestinal gluconeogenesis (IGN) which indirectly increase satiety (Figure A3).

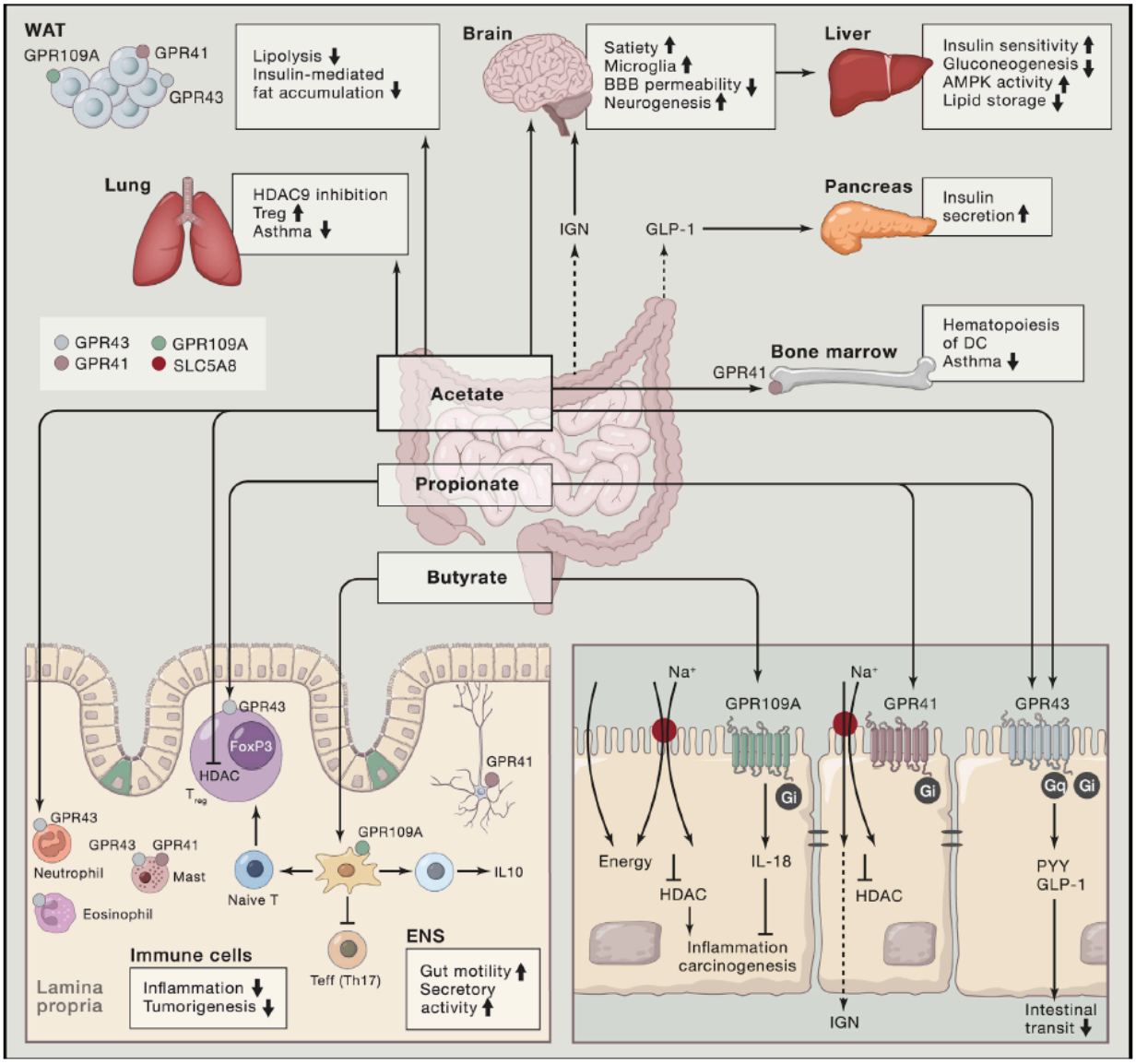


Figure A3: Mechanism of Action of Microbially Produced SCFAs **[9]**

The size of the boxes contains acetate, propionate and butyrate symbolizes the ratio between these SCFAs. In the distal gut, SCFA can pass through the cells by diffusion or using SCF5A8-mediated transport and act as 1) an energy source or 2) an HDAC inhibitor. Luminal acetate or propionate bind to GPR41 and GPR43 then release PYY and GLP-1, affecting satiety and intestinal transit. Butyrate promotes anti-inflammatory effects by increase IL-10 and reduce IL-18 via GPR109A and HDAC inhibition pathway. Furthermore, propionate bind to GRP41 and be converted into

glucose by IGN, indirect leading to satiety and decreased hepatic glucose production. Small amount of SCFAs (mostly acetate and possibly propionate) can reach the circulation and can also directly affect the adipose tissue, brain and liver, including overall beneficial effects. Solid arrows indicate the direct action of each SCFA, and dashed arrows from the gut are indirect effects.

2.2 What is known about Dietary fiber in the context of health benefits?

2.2.1 Health impact of Dietary Fiber in Observational studies

Increasing the dietary fiber intake or replacing refined grain by whole grain in daily diet have been encouraged as they are expected to benefit human health. According to an observational data of more than 4500 adults from 58 clinical trials, Reynold and colleagues observed a reduction from 15-30% in all-cause mortality including type 2 diabetes, cardiovascular diseases and color rectal cancer when comparing the highest and the lowest fiber consumers. Particularly, consuming 25g to 29g dietary fiber per day associated with the lowest risk of adverse outcomes [10]. Similar findings were obtained in a larger sample size study with more than 350,000 individuals, which revealed 17-19% risk reduction in all-cause mortality of highest whole grain, cereal fiber consumers when compared to those who eat least. This rate is even higher when assessing disease-specific mortality. Particularly, risk of having mortality caused by diabetes, an obesity-associated pathology, is 43% lower in individuals who consume highest amount of whole grain compared with never/rare consumers [55]. In addition, high intake of dietary fiber also reduces the probability of getting metabolic syndrome [56] - a common metabolic disorder that is strongly correlated with the prevalence of obesity [57]. Generally, observational studies consistently suggest that consuming DF benefits health and reduce prevalence of obesity and its associated pathologies.

2.2.2 Can DF modulate compositional and functional microbiome to combat obesity?

Understanding the inter-relationship between DF, microbiome, SCFAs, and obesity raises the question if we can fight against the disease by microbiome-targeted dietary intervention. Substantial number of animal studies have been performed to further strengthen the evidence to support the mechanisms by which DFs reduces metabolic disease by modulating the microbiome composition and function (SCFAs) [15]. For example, dietary interventions with short-chain fractions of AX have shown an enriched abundance of bacterial species such as *Bifidobacterium longum* [21, 22] which has been considered as a putative health promoting bacteria [58]. In another intervention study with long chain AX isolated from wheat endosperm [20], fecal concentration of both butyrate and propionate was increased. Despite of the promising findings the lack of consistency in evidence of human trial still remain[16], leading to difficulty to translate the knowledge to practical application.

2.2.3 Inconsistent findings on the health benefit of DF in interventional studies

Findings on the health benefits of DF that aimed to reduce the risk markers of metabolic diseases between interventional and observational studies are inconsistent. Particularly, while 13 of 16 observational studies reported the anti-inflammatory effect of DF, only 1 of 11 interventional studies resulted in a significant result [59]. This inconsistency could stem from individualized clinical responses to DF [17, 18]. DF intervention usually identify the responders and non-responders who showed very different effect in the primary outcome, host and microbiome response. Among 11 DF intervention studies from 2014 to 2017 reviewed by Healey and colleagues, there are 8 studies in which responders and non-responders showed different results in both host clinical out come and gut microbiome response and all studies revealed the different abundance of some taxa at baseline between responders and non-responders[18]. Although DF can

significantly shift some taxa of the gut microbiota its effects are also highly individualized [18]. This variability partly contributed by baseline microbiome might have clinical ramifications that could explain the inconsistent health effects of DF [23].

2.3 Attempts to understand the individualised response to explain inconsistent health effect of DF

Although a high degree of variability in host responses challenge dietary interventions, it opens a door for personalised nutrition approach to improve human health using individual characteristics (e.g. microbiome, genetic, phenotypic, medical information) to provide a more specific healthy eating guideline, nutritional product and service [60]. Despite some promising results in personalised nutrition field aimed to improved dietary behaviour [61] or ameliorated postprandial blood glucose level after a meal [62], more work is needed before nutritional intervention tailored to individual characteristic can be implemented [60], particularly in an overweight and obese population. Understanding of the factor the drives the individualised response could be a great help to ascertain the true efficiency of a given dietary intervention. Several attempts to understand the individualised response shall be discussed.

2.3.1 Comparing responder and non-responder

One of the easiest approaches to explore individualised response is by grouping the population based on their response of the primary outcome into responders and non-responder and comparing 2 groups in term of different contexts (e.g. Microbiome and diet feature) [18]. As mentioned earlier, all 11 DF interventional studies identified differences at baseline of bacteria communities between responders and non-responder, indicating that the inter-individual difference of baseline microbiota may also play a vital role in the gut microbiota response [63]. Although many studies found the different baseline microbiome in the response of microbiome or the host, only a few

studies further explore this relationship between baseline abundance of gut bacteria and the individualized responses by more a robust approach such as linear regression [64] or at least a correlation[65].

Regarding microbiome response, only 7 out of 20 papers reviewed by Healey et al. investigated the impact of diet history on the microbiome response. Interestingly, up to 6 papers found significant results[18]. These finding highlights the impact of baseline diet on the microbiome response and suggest that it should get more attention in the future studies.

Regarding host response, Healey et al. reviewed 17 papers and only 6 of them investigated the effect baseline diet [18]. Only 3 out of 6 studies showed the different dietary history between responders and non-responder and 1 used diet data to predict glycemic response [62]. It is clear that impact of dietary history on the response of microbiome and the host has not yet gained enough attention and, therefore, more studies should be conducted in this area.

2.3.2 Microbial ecology approach

Understanding microbial ecology is definitely an advantage to explain individualised response of bacteria to ferment DFs as this process is highly individualised and determined by a complex inter-species interaction between member of the gut microbiota [24]. Difference between responders and non-responders in term of microbiome response could be explained further by the presence of absence of essential primary degraders (or 'keystone species') needed to initiate the degradation of recalcitrant DFs [25]. Similar response in microbiome function could be resulted from very different microbiome compositions. Given the same DFs, unrelated spices in 1 community who possess the same trait to utilised DF could compete each other while in another microbiota, different species can co-operate to metabolise the substrate [26] [27]. Variability in the composition can alter the interaction between species or groups of species (ecological "guild")

[28, 29], involved in the fermentation of DF which influences in the SCFA production [30]. Given the importance of microbiome interaction and the ecological guild in the response of dietary intervention. Several studies have proposed the approach using co-occurrence network on the baseline abundance of bacteria to identify dietary enriched- microbial guilds that associate with the host response [66, 67]. Although microbial guild concept might play an important role that effect the host, this topic, and the underlying ecological principles, have received little attention.

CHAPTER 3: MATERIALS AND METHODS

3.1 Subject

Overweight and class-I obese (body mass index [BMI] 25.0 to 34.9 kg/m^2), otherwise healthy male and pre-menopausal, non-pregnant or lactating female, weight stable (± 3% for ≥ 1 month) subjects aged 19 to 50 years were recruited from the Edmonton area using campus-wide flyers, mailings to specific Listservs, local events, and word of mouth. Exclusion criteria included (1) history of GI disorders or surgeries; (2) history of diabetes mellitus; (3) chronic use of anti-hypertensive, lipid-lowering, anti-diabetic, analgesic, or laxative medications; (4) use of antibiotics in the three months prior to the start of the study; (5) use of probiotic, prebiotic, omega-3 fatty acid, or herbal supplements; (6) intolerance to corn; (7) vegetarian; (8) smoking; (9) alcohol intake ≥ 7 drinks/week; (10) > 3 hours of moderate-vigorous exercise per week.

3.2 Study design

This six-week, parallel two-arm, randomized controlled trial (RCT) was prospectively registered on December 19, 2014 with ClinicalTrials.gov (NCT02322112) as part of a large parallel four-arm RCT: The Alberta FYBER (Feed Your Gut Bacteria morE fibeR) Study [68]. Due to funding constraints, the AX arm was separated from the original RCT and analyzed independently. Study visits were conducted in accordance with the principles of the Declaration of Helsinki at the University of Alberta Human Nutrition Research Unit in Edmonton, Alberta, Canada between September 2015 and October 2016. Written informed consent was obtained from all study subjects prior to enrollment into the study.

Study subjects were required to attend five clinic visits (Figure 1). During a two-week screening/baseline period, potential subjects were pre-screened by telephone for initial eligibility and then attended a screening visit (visit 1) to confirm eligibility and receive study material

(including fecal collection supplies) to be complete prior to the baseline visit (visit 2). During the baseline visit, eligible subjects were enrolled, stratified based on sex, and then randomly assigned to either the AX or MCC arm. Random treatment allocation was accomplished using a computerized random number generator, in which two separate random allocation sequences (female and male sequence) were generated and concealed by a researcher not involved in subject allocation. Upon enrollment, subjects were then assigned to the next available randomization number by a study investigator blinded to these predetermined allocation sequences.

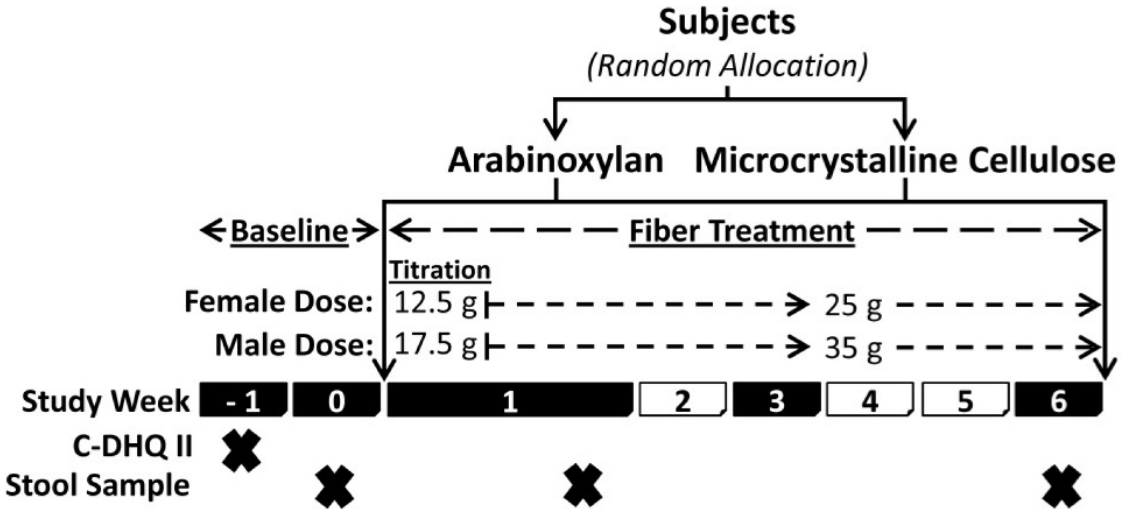


Figure 1. Study design of the human dietary intervention trial. The shaded study week block indicates a scheduled clinic visit. The 'X' indicates the specific task was completed during the study week. C-DHQ II; Canadian diet history questionnaire II.

Thirty-eight subjects were enrolled in the study and instructed to consume their corresponding supplement daily for six weeks at a DF dose of 25 g for females and 35 g for males, provided strictly as either AX or MCC. Half daily doses were provided for the first two days of treatment (12.5 g and 17.5 g, respectively), as this was shown by pilot data to be beneficial for diet incorporation. After one week of treatment, subjects returned to provide a second fecal sample and

to assess protocol compliance (visit 3), which was also assessed during their third week of treatment (visit 4). After six weeks of treatment, subjects returned to provide a third fecal sample and to assess overall protocol compliance (visit 5).

3.3 Treatments

AX was provided by Agrifiber Holdings LLC (Biofiber Gum, Illinois, USA) as a single batch. The long-chain AX product is a highly branched, alkali-extract soluble AX isolated from corn bran, and contained 88.8% soluble arabinoxylan (57.8% xylose; 32.5% arabinose; 9.7% galactose), 2.1% protein, and 6.3% lipids. The physiochemical properties of the AX concentrate (i.e. solubility, viscosity, and molecular weight) have been previously described by Kale and colleagues [69], with in vitro fecal fermentations suggesting a two-stage fermentation profile consisting of slow initial 4 h fermentation followed by faster final fermentation of the remaining AX [70]. MCC was provided by Blanver Farmoquimica Ltda. (Microcel MC-12, São Paulo, Brazil). The MCC is a large particle size (160-micron average), wood derived cellulose fiber processed with a dilute-acid to remove amorphous regions leaving only the recalcitrant crystalline regions, with preliminary in vitro fecal fermentations confirming resistance to microbial fermentation and therefore acts as an ideal non-fermentable control.

Both DFs were administered as powdered supplements incorporated daily into subject preferred foods and drinks. The treatments were not identical in their appearance or physiochemical properties, and therefore double-blinding was not possible. To achieve single-blinding, however, subjects were not informed of their DF treatment, and their weekly doses were provided in sealed opaque bags that contained individually packaged, ready-to-use sachets that provided the daily dose of 25 g/d or 35 g/d for female and male participants, respectively. Subjects were instructed

to return all provided sachets at their scheduled visits, where remaining DF was weighed to assess compliance.

3.4 Baseline Dietary Intake and Anthropometric Assessment

Subjects were asked to maintain their habitual diet and physical activity level during the intervention study. Baseline dietary intake was assessed by the online past-month Canadian Diet History Questionnaire II (C-DHQ II), a food frequency questionnaire adapted for the Canadian population from the validated US-DHQ-II [71]. Subjects' responses were analyzed using Diet*Calc software (Version 1.5.0) and the C-DHQ II specific nutrient database, previously updated to include eight new food group variables representing Canada's Food Guide serving size equivalents [72]. Prior to statistical analyses, C-DHQ II extracted data were assessed for extreme outliers using methods described by Kipnis and colleagues [73, 74], and then calorie-adjusted using methods described by Willett and Stampfer [75].

Anthropometric measurements were also obtained at baseline. Height and weight were measured, in light clothing, with empty pockets, and shoes removed, and used to calculate BMI. Waist circumference was measured using a Gulick II plus tape measure according to National Institutes of Health guidelines [76]. Body fat percentage was measured by bioelectrical impedance analysis (Tanita TBF-300A Body Composition Analyzer, Illinois, USA).

3.5 Fecal sample collection and processing

Fecal samples were collected at baseline, week 1 (W1), and week 6 (W6) using stool collection kits consisting of a stool specimen container, an air-tight bag (Fisher, Canada), and a GasPak™ EZ Anaerobe Sachet (BD, Canada) to generate an anaerobic environment within the container. Samples were delivered to researchers within 4 h of defecation. Upon receipt, fecal samples were processed immediately in an anaerobic chamber (Bactron™, Shel Lab, Oregon, USA) with an

anaerobic environment consisting of 5% H2, 5% CO2, and 90% N2. Raw fecal material was aliquoted for pH and moisture content measurements, and then diluted 1:10 in molecular grade phosphate-buffered saline (PBS) for DNA extractions and 1:5 5% phosphoric acid for SCFA quantification. Aliquots were stored at -80°C and kept frozen until further processing.

3.6 Fecal pH, SCFAs and dry mass quantifications

Raw fecal material was diluted 1:4 in distilled water to determine fecal pH using an Accumet AB150 pH meter (Fisher, Canada) as previously described [77, 78]. Quantification of fecal SCFAs was completed at the Agricultural, Food and Nutritional Science chromatography core facility of the University of Alberta as previously described [79]. Briefly, 1:5 dilution of fecal samples homogenized in 5% phosphoric acid were thawed and centrifuged, then 1000 µl of supernatant was mixed with 200 µl of internal standard (4-methyl-valeric acid). Subsequently, 0.2 µl of the mixture was injected into a Bruker SCION 456 gas chromatograph (Bruker Corporation, Massachusetts, USA). SCFAs were separated on a Stabilwax-DA column (30 m X 0.53 mm inner diameter X 0.5 µm film thickness, Restek Corporation, Pennsylvania, USA) with a flame ionization detector, and quantified by calculating response factors for each SCFA relative to 4-methyl-valeric acid using injections of pure standards. Total SCFA concentrations were determined as the sum of acetate, propionate, and butyrate; while the molar proportion of each SCFA was determined by dividing these individual SCFAs by total SCFAs. Total branched short-chain fatty acids (BCFA) concentrations were determined as the sum of isobutyrate and isovalerate. Fecal moisture content was determined by drying raw fecal material overnight in an oven at 103°C.

3.7 DNA Extraction, 16S Ribosomal RNA (rRNA) Gene Sequencing, and Data Processing for Microbiota Analysis

Bacterial DNA was extracted from fecal homogenates in phosphate-buffered saline (1:10) using the QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany) as previously described[80]. The V5-V6 regions of 16S rRNA gene were targeted for PCR amplification using primer pair 784F [5'-RGGATTAGATACCC-3'] and 1064R [5'-CGACRRCCATGCANCACCT-3']. 16S rRNA gene amplicons were sequenced by 300 bp paired-end sequencing on the MiSeq platform at the University of Minnesota Genomics Center (Minnesota, USA), with all samples of this study being included in the same run.

Sequences were trimmed to 210 bases long using FASTX-Toolkit, and paired-end reads were merged with the merge-illumina-pairs pipeline as previously described [80]. Samples exceeding 16,000 reads were subsampled to 16,000 using USEARCH v8.1 [81]. Removal of chimeric reads and clustering of OTUs (at a 98% pairwise identity threshold) were conducted using USEARCH, resulting in an average of 10,763 ± 670 high quality sequences per sample after quality control. Taxonomies from phylum to genus level were assigned using the entire sequence set by the Ribosomal Database Project Classifier [82]. OTUs were assigned taxonomy by using the Silva database (release 132 [83]) , and sequence identity at species level was confirmed using 16S rRNA databases on both EZ biocloud [84] and NCBI [85] platforms.

Prior to ordination and statistical analysis, OTU count data were converted into both relative abundance and CLR transformed to correct for compositionality [86]. Considering all fecal samples, OTUs with an average relative abundance below 0.15% were removed. This approach resulted in exactly 100 OTUs (referred to as 'all OTUs'), which were used in the downstream analysis, accounting for 88.1% of the approximately 1 million-curated reads.

3.8 Statistical analysis

All univariate analyses were performed by GraphPad Prism v.8.0.1 (GraphPad Software, California, USA), while multivariate analyses, regression models, and advanced statistics were performed and visualized using R v 3.5.3 (R Core Team, Vienna, Austria [87]) unless otherwise stated.

3.8.1 Bacterial community analysis

To explore the effect of DF on the bacterial community, we assessed overall β-diversity, dissimilarity between and within individuals, and α-diversity. To assess overall β-diversity, Euclidean distance between bacterial communities was first calculated from CLR-transformed data of all OTUs and then visualized using non-metric multidimensional scaling (vegan [88] and ggplot2 [89] packages). Differences in the communities of treatment and control groups at specific time points were compared by PERMANOVA using the Adonis function in vegan [88]. Euclidean distances were used to calculate inter-subject (between subjects at the same time point) and intra-subject (within subjects but at different time points) dissimilarity. Differences in inter-subject diversity were determined within each treatment group relative to baseline using GEE models (geepack [90] packages) followed by Bonferroni correction. Differences of intra-subject dissimilarity between AX and MCC treatments were compared using Mann-Whitney tests. α-diversity (Shannon index) and bacterial richness (OTU numbers) were determined using rarefied OTU data with the vegan package [88].

3.8.2 Bacterial composition and function analysis

Community membership of individual taxa was presented as relative abundance (mean ± SD), while CLR-transformed data was used for the statistical analyses. Comparisons of phyla, families, genera, and OTUs between baseline and W6 were performed by Wilcoxon signed-rank

tests, while comparisons of shifts (ΔW6-BL) between AX and MCC were performed by Mann-Whitney tests. P values were adjusted by Benjamini-Hochberg's false discovery rate (FDR) and considered significant when q values were <0.15. SCFA data were analyzed using the same approach without correction of p values. Differences at W1 and W6 in the effects of DF were determined using the Friedman's test followed by a Dunn's correction for multiple comparisons.

3.8.3 Co-abundance response group (CARG) and network analysis

Potential syntrophic interactions between bacterial taxa in their response to AX were assessed using co-occurrence network analysis [66]. To determine groups of interacting OTUs in their response to AX (thus potential ecological guilds) [29], CARGs were determined from the top OTUs impacted by the AX intervention (W6-BL unadjusted p<0.1; Wilcoxon test). Spearman's correlation analysis was performed between the shifts (ΔW6-BL) in these OTUs to construct a correlation matrix; then this matrix was converted into a distance matrix by (1 – correlation coefficients) [66]. Hierarchical clustering was then performed on the distance matrix to build a tree using the complete-linkage clustering algorithm [91]. Differences between distinct branches of the Hierarchical tree, and thus individual CARGs, were determined by PERMANOVA using a cut-off of p<0.05 [66]. Relative abundance of each CARG was calculated as the sum of the OTUs within each CARG prior to statistical analyses.

To visualize the interaction of OTUs within and between CARGs, Spearman's correlation network was calculated with a permutation test (1000x) using CoNet [92] as previously described [93]. Only OTUs with a significant interaction (i.e. FDR corrected q<0.05) were visualized in the network using Cytoscape v3.61.

3.8.4 Different in baseline and shift of gut bacteria and diet between W1- and W6-responders of propionate

To identify factors that contribute to the variation between two groups of responders in propionate, PERMANOVA was performed on Euclidian distances based on the baseline and shifts of total OTUs, significant OTUs, and CARGs, and baseline diet. The multivariate data of microbiome and diet were visualized on biplots of PCA using factoextra [94] and FactoMineR [95] packages.

3.8.5 Relationship between microbiome and SCFAs response with microbiome feature and diet history

To explain the individualized response of the microbiome to DF, MLR analyses were employed using R. In order to perform the analysis, dimensionality of the microbiome and diet data were reduced by PCA into PC1, PC2, and PC3. Microbiome compositional and SCFA response variables were used as dependent variables. Baseline and shifts of PC variables, CARGs, OTUs, and diet data were used as predictors. Subset selection in regression was applied to choose the best combination of predictors using the sequential replacement algorithm (leaps package [96]). Therefore, each MLR model presented only contained the top one or two predictor variables that explained the response variable the best. Dietary and microbiome-related predictors were treated separately in different models, and whole grains, total grains and total DF intake were used as single dietary predictors. All models were adjusted by sex/fiber dose and p values were corrected by FDR with statistical significance considered at $q<0.05$. To estimate the quality of each model in predicting the same dependent variable, AICc values were calculated using the AICcmodavg package [97]. AICc values were then converted to relative percentages by assigning the highest AICc value as 100%, and then remaining AICc values were calculated by (AICc value/Highest

AICc value) * 100. Thus, a lower AICc value would indicate a higher quality model. Residuals for all linear regression models were plotted to check for homogeneity of variance and normality.

CHAPTER 4: RESULTS

4.1 Subject Characteristics and Protocol Compliance

To compare the effects of AX and MCC, we conducted a six-week, parallel-arm, randomized controlled trial in healthy, overweight and class-I obese inviduals (Figure 1). Of the 38 subjects enrolled and randomized to a treatment arm, seven withdrew from the dietary intervention and were not included in the statistical analyses (AX arm: three due to challenges adding supplement and one due to constipation; MCC arm: two for personal reasons and one due to constipation) (Figure S1). Baseline characteristics of the 31 subjects are summarized in Table S1, which included 21 females and 10 males aged 32.9 ± 8.5 years with a BMI of 28.7 ± 2.3 kg/m^2. No significant differences were detected between the two intervention groups at baseline in the assessed demographics. Overall protocol compliance, assessed by the amount (weight) of returned supplement, was 94.7 ± 6.5% and 95.0 ± 5.6% in the AX and MCC arms, respectively.

4.2 Effect on the Composition of the Fecal Microbiome

4.2.1 Fecal Microbiota Diversity

NMDS analysis of Euclidean distances between subjects based on CLR transformed OTU data showed that the two treatment groups harbored bacterial communities that could not be differentiated at baseline (p=0.17, PERMANOVA; Figure 2A). One-week supplementation with AX altered the global fecal bacterial community, which became significantly different from the fecal microbiota of subjects receiving MCC (p=0.025). This effect was maintained until the end of the AX treatment (p=0.019). These changes were caused by AX inducing a temporal shift in β-diversity of the fecal microbiota in comparisons between treatments to baseline, which was significantly larger than the temporal shift observed in the MCC group (p<0.05, Mann–Whitney

test; Figure 2B). In addition, while MCC increased the inter-individual differences (β-diversity between subjects; $p<0.001$, GEE model), AX reduced it ($p\leq0.003$, Figure 2C).

Analysis of α-diversity showed that AX treatment significantly reduced fecal bacterial diversity (Shannon's index) but not the richness (total OTUs) after six weeks ($p=0.036$, GEE model; Figure 2D). Overall, while the non-fermentable MCC had no detectable effect on measures of fecal microbiota diversity, AX significantly altered the global bacterial community within one week, inducing significant temporal shifts in β-diversity, and a reduction of both inter-individual variation and α-diversity.

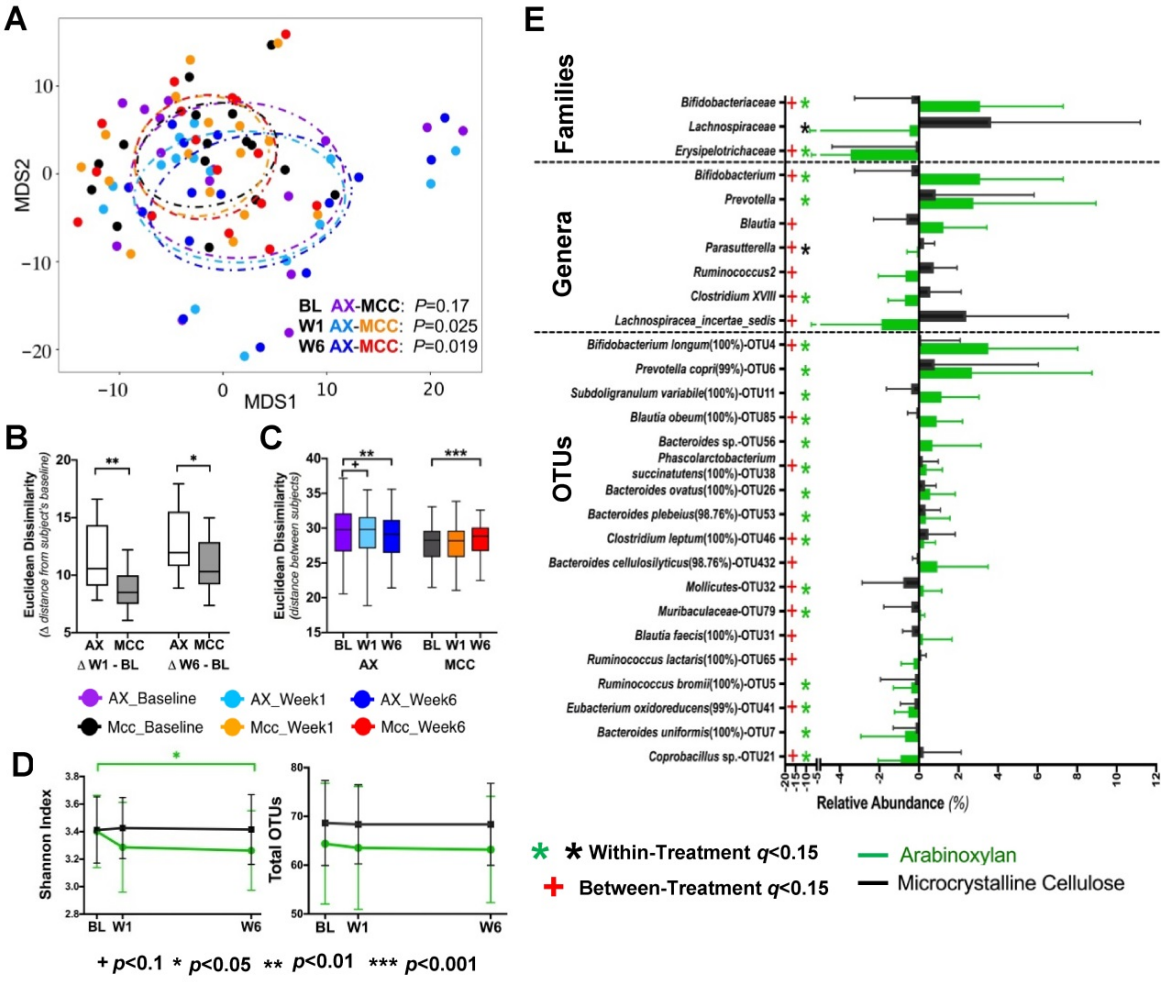


Figure 2. AX alters the global composition of fecal microbial communities and induces distinct shifts in bacterial taxa. (A) Non-metric multidimensional scaling (MNDS) plot based on Euclidean distance metrics of AX and MCC groups at each time point (inter-subject β-diversity) showing changes in the distance between subjects over time over time. Euclidean distances (B) between the fecal microbiomes of subjects at each time point (inter-subject) and (C) between each subject's fecal microbiome at baseline and during W1 or W6 treatment (intra-subject). (D) α-diversity (displayed as Shannon index and total OTUs) of the fecal microbiomes of subjects at each time point. (E) Absolute change (ΔW6–BL) in relative abundance of bacterial taxa significantly affected by the dietary intervention. Data analyzed for (A) using PERMANOVA, for (B,D) using GEE models (with Bonferroni correction), and for (C) using Mann-Whitney tests. For (E), data analyzed using either Wilcoxon tests to assess within-group changes relative to BL, or Mann-Whitney tests to assess between-group changes (i.e. AX vs. MCC; with FDR correction). β-diversity and compositional data were reported as mean ± SD and centered log-ratio transformed prior to the statistical analyses. BL; baseline; OTU, operational taxonomic unit; W1, week 1; W6, week 6.

4.2.2 Effect of AX and MCC on the Abundance of Bacterial Taxa and CARGs

Neither AX nor MCC significantly altered microbiota composition at the phylum level. At lower taxonomic levels, significant changes in the abundance of two bacterial families were detected at six weeks of AX when compared to baseline and MCC, namely an increase in *Bifidobacteriaceae* and a decrease in *Erysipelotrichaceae* ($q \leq 0.007$, Wilcoxon test; Figure 2E, Table S2). At the genus level, AX increased the genera Bifidobacterium and Prevotella in comparisons with both baseline and MCC; and it enriched *Blautia* when compared to MCC. Further, OTU level analysis revealed that 15 OTUs significantly changed during AX treatment relative to baseline (henceforth referred

to as 'significant OTUs'). In particular, AX significantly enriched OTUs related to *Bifidobacterium longum* (OTU4), *Prevotella copri* (OTU6), *Bacteroides plebeius* (OTU53), *Bacteroides* sp. (OTU56), *Bacteroides ovatus* (OTU26), Phascolarctobacterium succinatutens (OTU38), *Blautia obeum* (OTU85), Subdoligranulum variabile (OTU11), *Clostridium leptum* (OTU46), *Mollicutes* sp. (OTU32), and *Muribaculaceae* sp. (OTU79) ($q<0.15$). OTUs that were significantly reduced in abundance by AX were classified as *Ruminococcus bromii* (OTU5), *Eubacterium oxidoreducens* (OTU41), *Bacteroides uniformis* (OTU7), and *Coprobacillus* sp. (OTU21). Supplementation with MCC only increased the family *Lachnospiraceae* and the genus *Parasutterella* ($q=0.117$). Overall, the effect of AX was to a large degree specific to *B. longum* (OTU4) and *P. copri* (OTU6), as these taxa increased in relative abundance by 3.5% and 2.7%, while other OTUs only increased by ≤1.1%.

In an attempt to identify groups of cooperating species that could function as ecological guilds in the degradation of AX, we adapted a clustering approach conceptually similar to that described by Zhao et al.[66] but used AX-induced shifts and not absolute proportions of taxa to identify species whose responses were correlated. This analysis revealed seven CARGs (Figure 3A); of which five CARGs show significance responses to AX and none to MCC (Table S2). The CARG that showed the largest increase was CARG1 ($p=0.0003$, Wilcoxon test), which consisted of six out of the 11 OTUs increased by AX (Figure 3B). Among those six OTUs, *B. longum* (OTU4) exhibited the largest shift and showed significant connections to all but one member of CARG1, suggesting AX is degraded through cooperative interactions between these taxa. The second largest response was in CARG2, which only had three members with much weaker correlations than in CARG1. In CARG6, *P. copri* (OTU6) exhibited the largest response, but only showed a significant connection with one other member of the CARG, *Bacteroides massiliensis* (OTU98), which suggests *P. copri*

might act independently to degrade AX (Figure 3B). Remaining CARGs showed weaker increases. The majority of taxa that decreased after AX treatment, particularly *B. uniformis* (OTU7), clustered within CARG7 and showed negative correlations with taxa of CARG1, CARG2, and CARG6, suggesting competitive or antagonistic interactions.

4.2.3 Temporal Response of OTUs and CARGs

To determine if short- and long-term treatment with AX and MCC differed in its effects on the fecal microbiota, we compared shifts from baseline to W6 with those from baseline to W1. This analysis did not detect differences between the two time points ($q>0.25$, Wilcoxon test, data not shown). In addition, comparison of baseline, W1, and W6 values by Friedman's test indicated that the effects of AX occur rapidly within one week, with no further adaptations after six weeks (Figure 4A). Since there is no significant difference in microbiome compositional response between W1 and W6, below analysis on compositional changes were performed with W6 data unless otherwise stated.

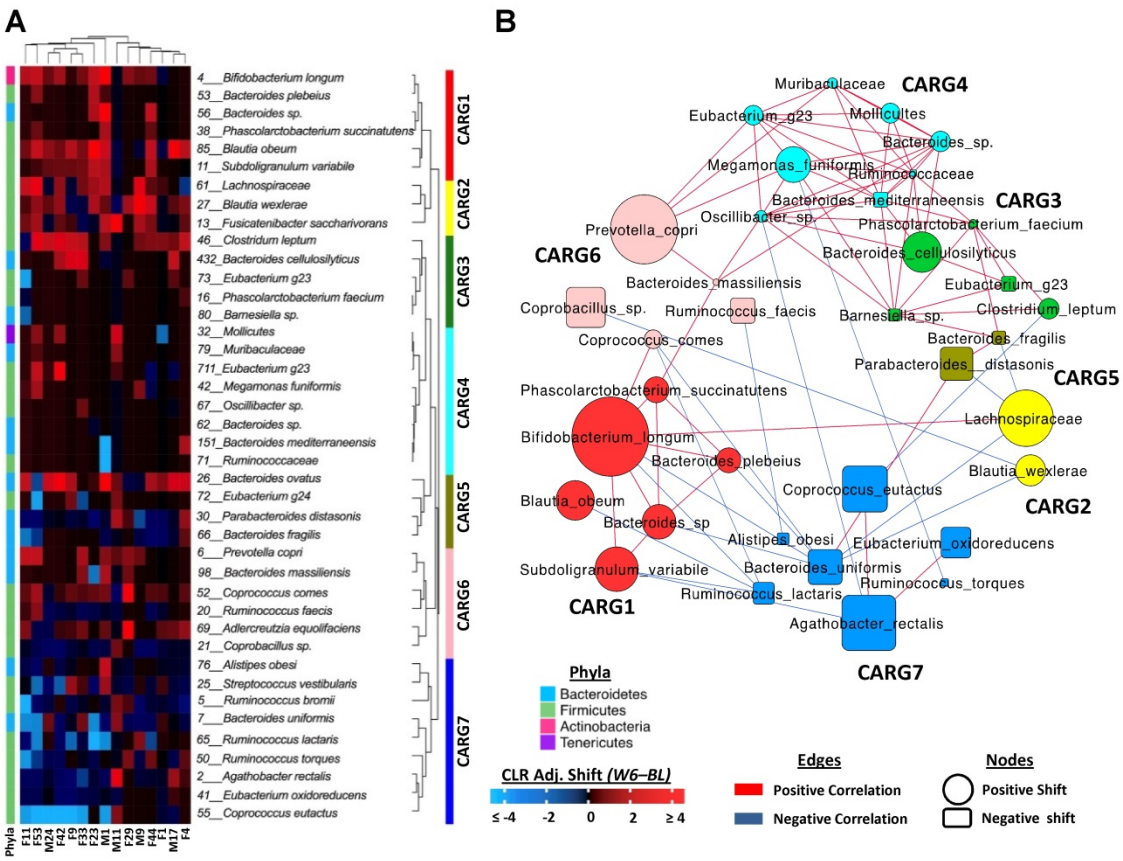


Figure 3. Identification of co-abundance response groups (CARGs) during AX supplementation. (A) Heatmap shows the change (ΔW6–BL) in centered log-ratio (CLR) adjusted abundance of 41 OTUs affected by AX treatment ($p<0.1$, Wilcoxon test). The Hierarchical dendrogram shows clustering of OTUs (rows) based on Spearman's correlation distances by the complete-linkage clustering algorithm, and then grouped on the dendrogram into seven CARGs by PERMANOVA ($p<0.05$). Subjects (columns) clustered based on Euclidean distances. Colors from blue to red indicate the direction and magnitude of change. (B) Co-response network analysis. Each node represents an OTU, where the size is proportional to the change (ΔW6–BL) in relative abundance, the shape indicates direction of change (positive: circle; negative: square), and the color references the respective CARG it was clustered to. Each edge represents a significant positive (red line) or

negative (blue line) Spearman's correlation between nodes (q<0.05). BL; baseline; OTU, operational taxonomic unit; W6, week 6.

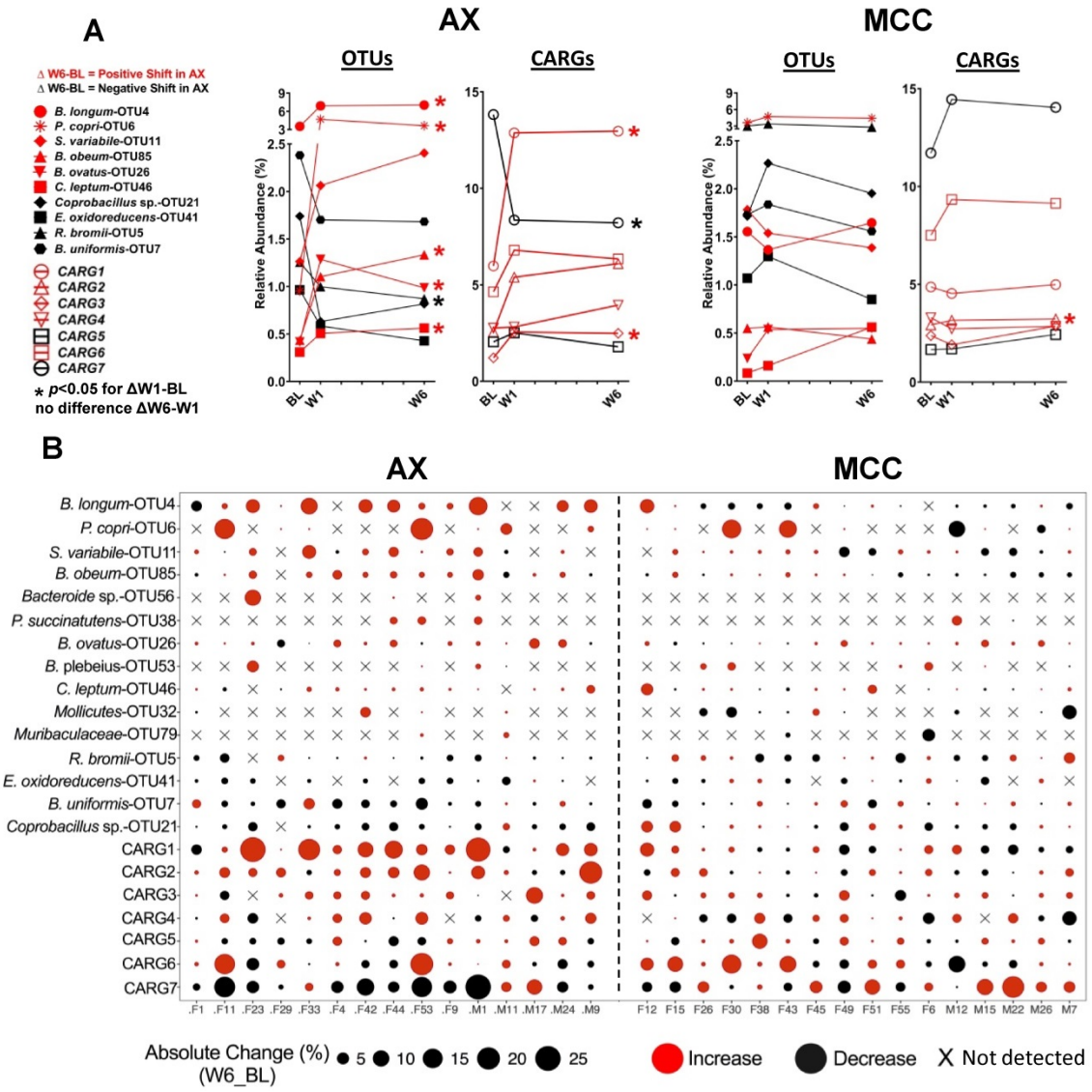


Figure 4. Temporal (A) and individualized responses (B) of OTUs significantly affected, and the respective CARGs, by AX and MCC treatment. (A) Line plots show the temporal response of the ten most abundant OTUs (detected in >25% of subjects) and the seven CARGs. Centered log-ratio (CLR) transformed data were analyzed by Friedman's test (with Dunn's correction) to assess within-group changes between time points (i.e. ΔW1–BL and ΔW6–W1). (B) Bubble plot shows

individualized differences (ΔW6–BL) in the proportions of OTUs (percentage of total microbiota composition) and CARGs (sum of OTUs) detected after six weeks AX and MCC supplementation. The size of the bubble is proportional to the change relative to baseline, while the color of the bubble represents the direction of the change (red: increase; black: decrease). BL; baseline; CARG, co-abundance response group; OTU, operational taxonomic unit; W1, week 1; W6, week 6.

4.2.4 Inter-individual variation in the response of AX treatment

In accordance with previous studies on the effect of DF on the human gut microbiome [18, 77, 98, 99], bacterial shifts in response to AX and their magnitude were highly individualized (Figure 4B). For instance, while absolute increases in relative abundance from approximately 5% to 10% (8 to 400 fold change) were detected for the OTU classified as *B. longum* (OTU4) in seven subjects, other subjects showed either a much smaller increase, a decrease, or the species was not detected at all. OTUs related to *B. obeum* (OTU85), *S. variabile* (OTU11*), B. ovatus* (OTU26), and *C. leptum* (OTU46) were enriched by AX in around two-thirds of the subjects. Less frequently enriched were OTUs classified as *P. copri* (OTU6), *B. plebeius* (OTU53), and *Bacteroides sp.* (OTU56). *P. copri* (OTU6) expanded beyond 10% of the total bacterial community in only three subjects, but enrichments were substantial, ranging from 1.5 to 17 times.

To determine drivers of this individualized response, we used MLR analysis to test if responses in the taxa that showed the numerically largest shifts (*P. copri*, *B. longum, B. obeum, S. variabile*) and CARGs with significant responses (CARG1, 2, 3, 6, 7) can be predicted by baseline diet or microbiome composition. Baseline microbiome variables (all OTUs and significant OTUs) and baseline dietary history were first reduced in their dimensionality by PCA and then treated as predictors. This analysis revealed that the individualized responses of bacterial taxa and CARGs

to AX and MCC could not be predicted through baseline dietary intake or microbiome composition ($q>0.05$; Figure S2).

4.3 Effect on Fecal SCFAs

4.3.1 Temporal Response of Fecal SCFAs

Fecal pH, fecal concentrations of SCFAs, and fecal moisture content did not significantly change after six weeks of either DF treatment ($p>0.1$, Wilcoxon test; Table S3). Considering that absolute concentrations of fecal SCFAs are affected by their absorption in the gut, we additionally assessed the molar proportions of acetate, propionate, and butyrate relative to total SCFA concentrations, which has been previously shown to vary little across colonic regions [100]. This analysis revealed an increase of propionate at W6 relative to baseline ($p=0.04$, Wilcoxon test) and MCC ($p=0.01$, Mann–Whitney test), as well as decrease of butyrate when compared to baseline ($p=0.018$), although this decrease does not reach significance in comparison with the MCC ($p=0.08$). Characterization of the temporal response in the three SCFAs revealed a significant increase in the fecal concentration of propionate by AX at W1 ($p=0.01$, Friedman's test) (Figure 5A). Although propionate concentrations remained elevated at W6, this increase was not statistically significant when compare to baseline ($p=0.15$). This loss of statistical significance was caused by an increase in the inter-individual variation at W6 (Figure 5B).

Visual evaluation of the individualized temporal response of propionate to AX revealed clear separation of subjects into two distinct patterns (Figure 5B), with one group showing little response at W1 but then an increase, while the other group showed an increase at W1 that then declined. Based on the direction of change from W1 to W6 (i.e. positive or negative), subjects were grouped into “W6-responders” (Δ W6-W1>0) and “W1-responders” (Δ W6-W1<0). W6-responders but not in W1-responders showed a significant higher output of propionate at W6 ($p<0.05$, Friedman's

test) but not at W1, and the two groups differed significantly by propionate concentrations at W6 (p=0.001, Mann–Whitney test).

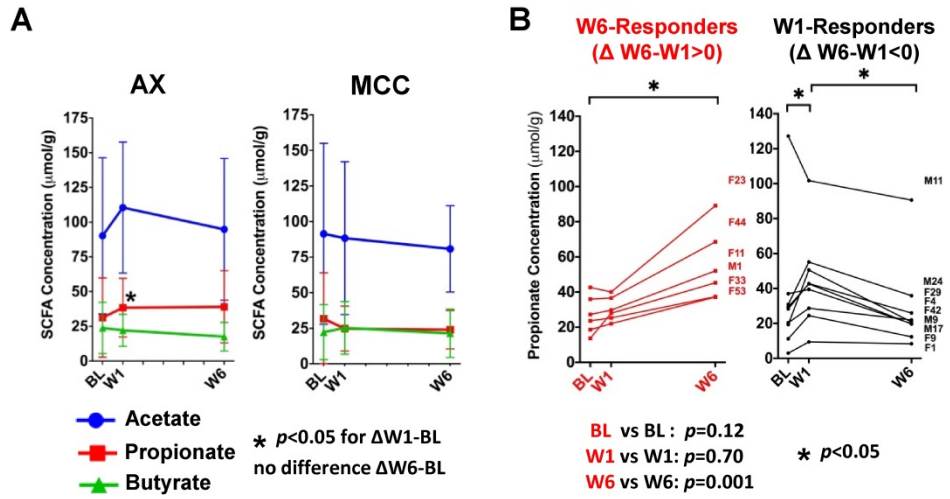


Figure 5. Temporal (A) and individualized (B) responses of fecal SCFA concentrations (µmol/g) to AX and MCC supplementation. (A) Line plots show the temporal response of acetate, propionate, and butyrate; reported as mean ± SD. (B) Temporal propionate response of W6-responders (red) and W1-responder (black) (grouped based on ΔW6–W1). Data analyzed for (A,B) using Friedman test (with Dunn's correction) to assess within-group changes between time points, and for (B) using Mann-Whitney tests to assess differences between-group at each time point. BL; baseline; CARG, co-abundance response group; OTU, operational taxonomic unit; W1, week 1; W6, week 6.

4.3.2 W1- and W6-responders differ in their microbiome response to AX

Microbiome compositional (baseline and shifts) and diet data were ordinated using PCA, and then differences between W1- and W6-responders were tested using PERMANOVA. This analysis

revealed that the bacterial communities of W6-responders were indistinguishable from W1-responders at baseline, but their response to AX was significantly different (ΔW6-BL; Figure 6). This was detected for all OTUs ($p=0.004$), the 15 significant OTUs ($p=0.025$) or the seven CARGs ($p=0.025$). In contrast, neither baseline microbiota composition (Figure 6) nor dietary factors (Figure S3A) separated according to W1 and W6 response ($p>0.1$). In addition, comparing W1- and W6-responders in term of their baseline whole grain, total grain, and total DF consumption did not reveal any significance either ($p>0.1$, Mann–Whitney test) (Figure S3B). Together these findings indicate that the temporal response in fecal concentrations of propionate is primarily associated with the shifts in the microbiome and not baseline microbiome composition or diet.

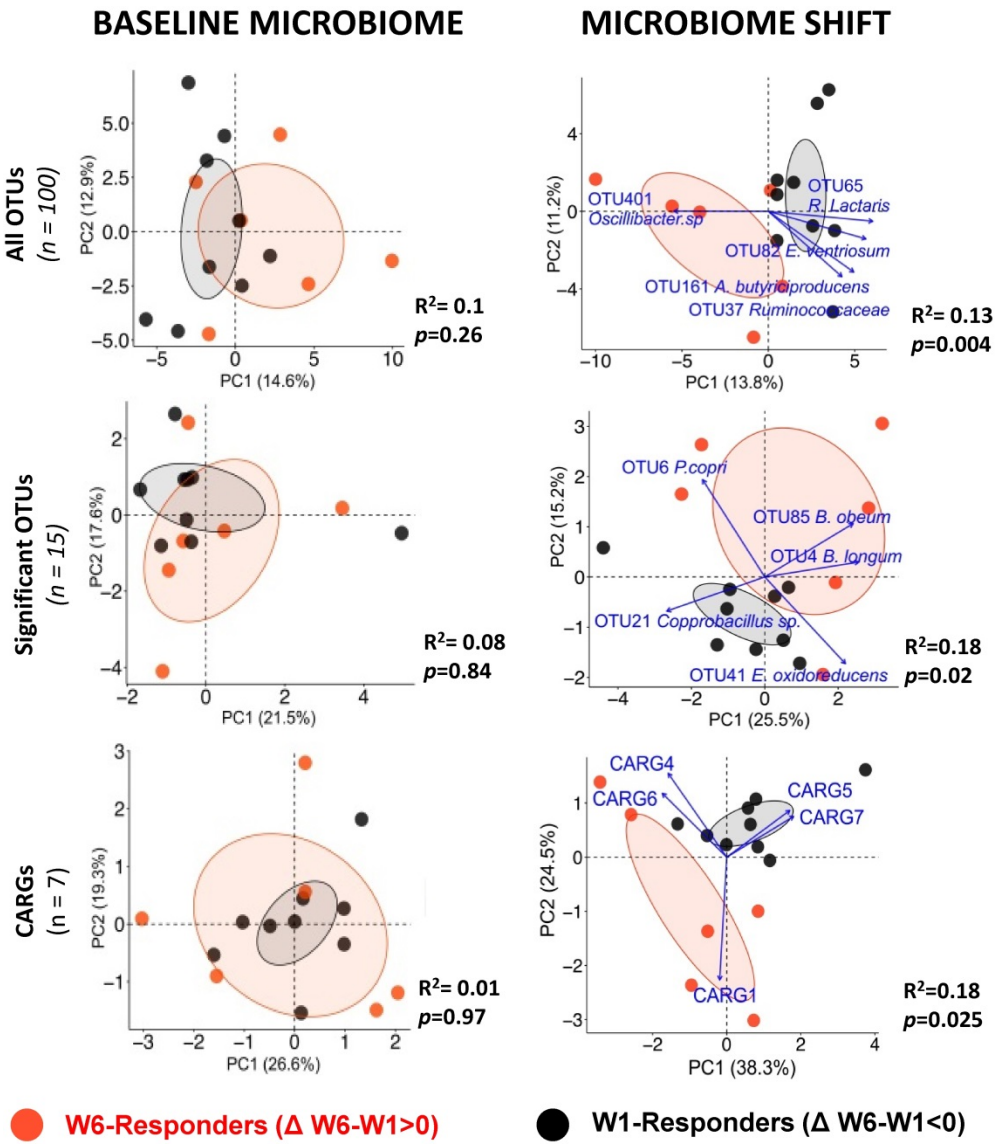


Figure 6. The individualized temporal propionate response to AX associates with compositional responses in the fecal microbiome. Principal component analysis plots based on Euclidean distance comparing the relative abundance of fecal microbiota, both at baseline and AX-induced shifts (ΔW6–BL), between W6-(red) and W1-(black) responders. When statistical significances were determined by PERMANOVA ($p<0.05$), microbiome variables (i.e. OTU or CARG) that contributed the most to inter-subject variation were shown as vectors on the plot. BL; baseline;

CARG, co-abundance response group; OTU, operational taxonomic unit; W1, week 1; W6, week 6.

4.3.2 Individualised responses of SCFAs can be explained by features of the gut microbiome

As with compositional responses, gut microbiota functional responses to DF interventions have been shown to be individualize [18, 99, 101], but what drives this variation is poorly understood. We applied MLR to determine whether SCFA responses from baseline to W6 could be explained by microbiome and diet related factors, and then compared the quality of the models using AICc values (where lower values mean higher quality). These analyses revealed that the fecal SCFA response to AX (i.e. ΔW6-BL) could be predicted by the fecal microbiome (Figures 7, S4A, and S4B) but not baseline diet (Figure S5). The best models were achieved for propionate, especially when PCs of shifts of either all or significant OTUs were used as predictors (Figure 7A). Models were of lower quality when CARGs or single OTUs were used, showing that overall community measures predict the response better then single or groups of taxa. Linear relationships between propionate responses and significant predictor using baseline (PC1 of All OTUs) and shift (CARG1) were further visualized using scatter plots (Figure 7B), confirming the analysis as a majority of subjects gather on the regression line or within the 95% confident interval area. Further analysis of the predictors revealed that *B. longum* (OTU4) contributed up to 50% of the total shift in CARG1 while other members account only for 5% to 16% (Figure 7B). This information highlights the importance of *B. longum* (OTU4) in CARG1 and in predicting the response of propionate.

Although the models that used baseline OTUs as predictors were of lower quality than those based on shifts, they are still valid, showing significant q values after FDR correction ($q<0.05$, MLR).

MLR did not detect any significant models for the MCC group, which is an indication that the statistical approach does not detect associations that are not related to DF fermentation. Lower quality models could also be designed for acetate and butyrate responses to AX, but not MCC. Interestingly, in contrast to propionate, the best models to predict butyrate responses were achieved using shifts of a single OTU (*E. oxidoreducens* [OUT41]) and CARG3 (Figure S4B). *E. oxidoreducens* (OUT41), an important butyrate producer [102], was significantly reduced through AX, thus providing a potential explanation for the reduction of butyrate proportions. In summary, while individualized responses in SCFAs showed no association with dietary history, they could be predicted by microbiome shifts and baseline composition.

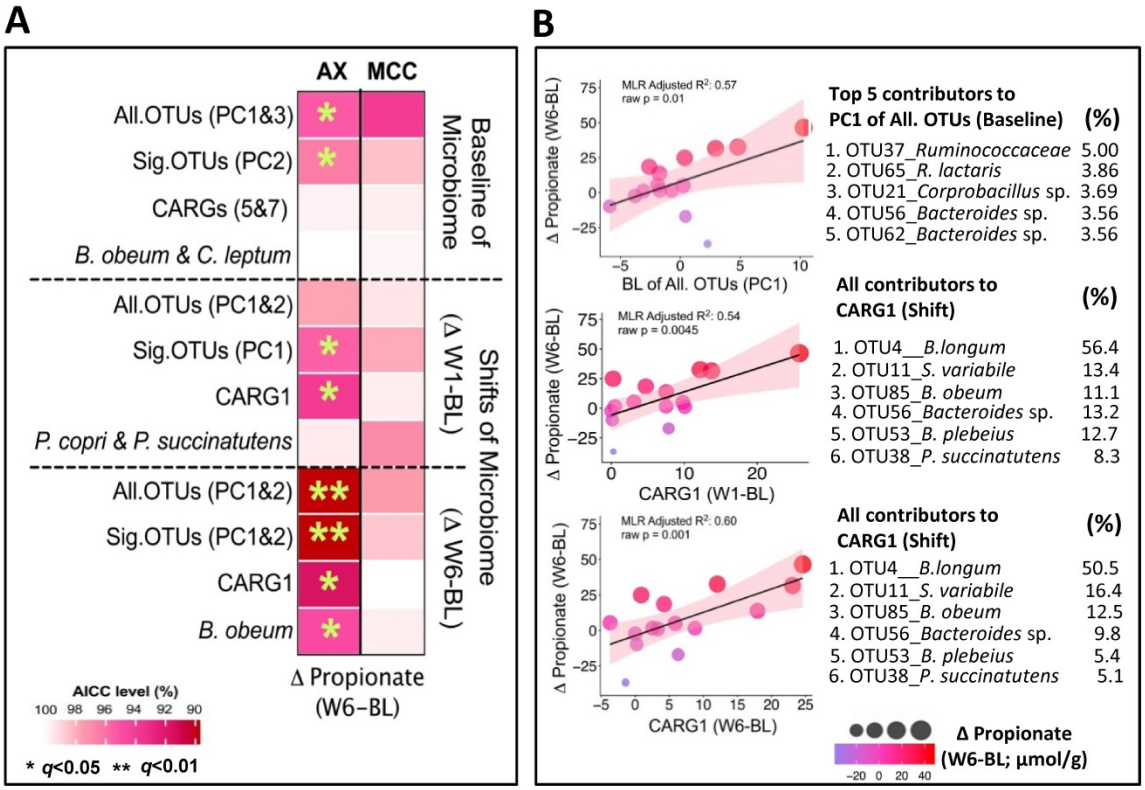


Figure 7. The individualized propionate response to AX could be explained by baseline and shifts of the gut microbiome. (A) Heatmap shows the associations between the individualized propionate response (ΔW6–BL; dependent variable; columns) and microbiome profiles (BL, ΔW1–BL,

ΔW6–BL; predictor variables; rows). Cells represent individual multiple linear regression models (with FDR correction) that assess whether the predictors explain the individualized propionate response. Multivariate microbiome data were simplified into principle component variables (i.e. PC1, PC2, PC3) prior to analysis. Each model contained the very best one or two predictors of PC variables, CARGs, or significant OTUs selected by stepwise regression. All models were adjusted by sex/fiber dose. Colors from white to red indicate relative AICc (Akaike information criterion corrections) values calculated by (AICc value/Highest AICc value) * 100. Lower AICc values (red) indicate higher quality models. (B) Scatter plots show the linear relationship between propionate response (ΔW6–BL) and either the baseline of all OTUs or the shift of CARG1. Color and size of each point indicate propionate response magnitude and the shaded area specifies the 95% confident interval. The top five OTUs that contributed the most to PC1 of either all OTUs or CARG1 are further provided. BL; baseline; CARG, co-abundance response group; OTU, operational taxonomic unit; W1, week 1; W6, week 6. Details of model components were presented in Table S4.

4.3.2 Using MLR models to determine the role of taxa in propionate production

MLR models were used to determine the connections between AX responders and propionate production. This analysis revealed that shifts in *P. copri* (OTU6) were a poor predictor of propionate response, while *B. longum* and correlated taxa in CARG1 resulted in much better models (Figure 8A). The highest quality models were obtained with *B. obeum* (OTU85), *B. plebeius* (OTU53), and *P. succinatutens* (OTU38), all of which encode metabolic pathways for propionate production [103], though *B. obeum* has only been shown to form propionate with deoxy sugars and otherwise produces acetate [104, 105]. *P. succinatutens* (OTU38) specifically, a propionate producer by means of succinate utilization [106], was estimated to increase fecal

propionate concentrations by 12 µmol/g per 1% increase in relative abundance (Figure 8A, p=0.04, MLR). Such analysis provides a potential explanation for the metabolic interactions between putative primary degraders, polysaccharide utilizers, oligosaccharide/sugars utilizers, and propionate producers that result in the promotion of propionate in response to AX (Figure 8B).

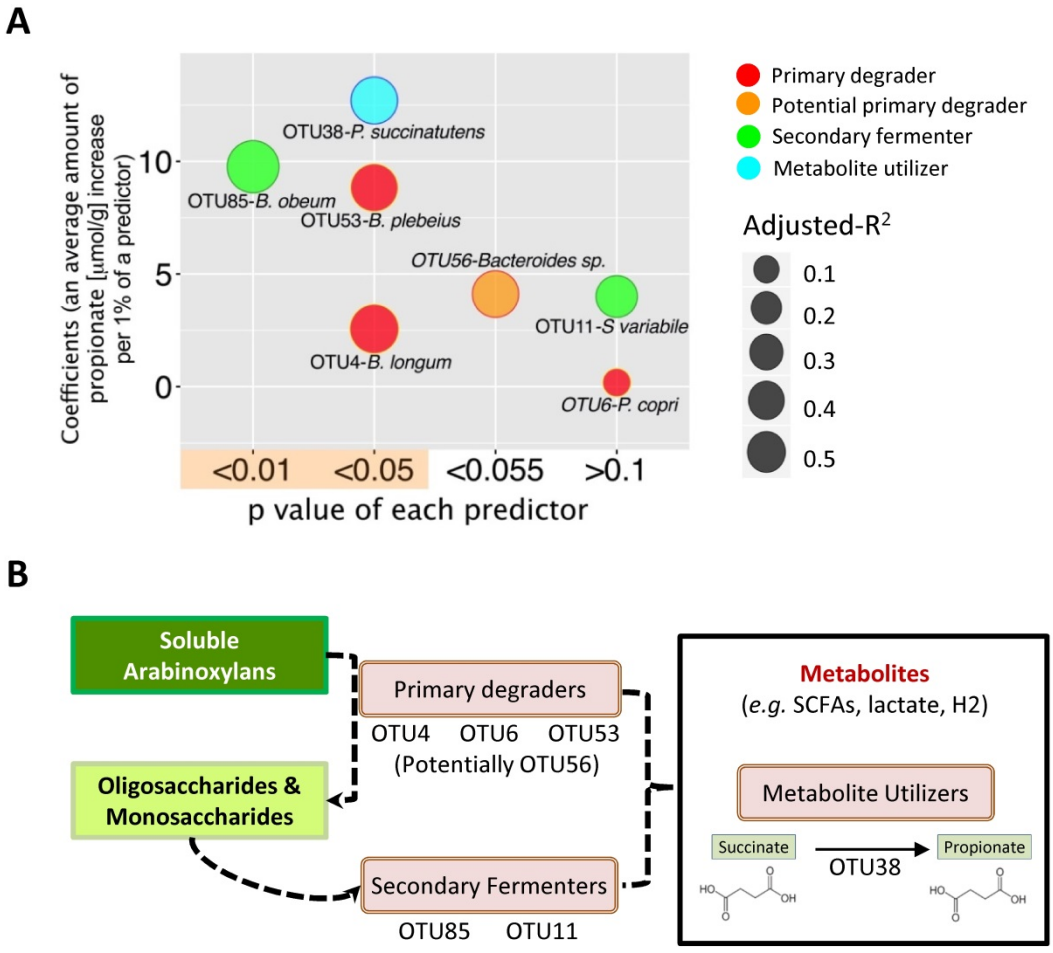


Figure 8. Relationship between propionate response during AX supplementation and putative primary degraders, secondary fermenters, and metabolite utilizers. (A) Individual multiple linear regression models determine OTU responses (ΔW6–BL;) that predict the fecal propionate response (ΔW6–BL). Y-axis shows the coefficient for each predictor, as in the average propionate response (µmol/g) when OTU relative abundance increases 1%. X-axis shows the p value (grouped into <0.01, <0.05, <0.055, and >0.1) for each predictor. All models were adjusted by sex/fiber

dose, where bubble size represents the adjusted-R^2. (B) Proposed model of bacterial cross-feeding in the gut during degradation of complex, soluble arabinoxylans.

4.4 Supplementary Tables & figures

Supplementary Table 1. Subject Characteristics at Baseline [1].

	Arabinoxylan	Microcrystalline Cellulose	Between *p* value [2]
Number	15	16	
Age (y)	33.7 ± 9.7	32.1 ± 7.4	0.91
Sex (F/M)	10/5	11/5	
Height (cm)	171.5 ± 8.4	168.8 ± 7.6	0.25
Weight (kg)	84.8 ± 12.3	81.9 ± 10.5	0.40
BMI (kg/m^2)	28.7 ± 2.7	28.7 ± 2.0	0.99
WC (cm)	95.7 ± 8.8	92.9 ± 6.0	0.30
Body fat (%)	33.0 ± 9.3	32.0 ± 7.3	0.63
Ethnicity (%)			0.56
White	60.0	43.8	
Asian	20.0	31.3	
Black	13.3	6.3	
Other	6.7	18.8	
Employment (%)			0.40
Student	26.7	50.0	
Employed	66.7	43.8	
Unemployed	6.7	6.3	

[1] Data presented as mean ± SD or as a percentage.

[2] Continuous variables were analyzed by Mann-Whitney test, and count variables were analyzed by Chi-Squared test. BMI, body mass index; WC, waist circumference.

Supplementary Table 2. Abundance of Bacterial Taxa Affected by the Dietary Intervention as Assessed by Illumina 16S rRNA Gene Sequencing [1].

	Proportion of bacterial taxa expressed in relative abundance (Mean ± SD)								
	Arabinoxylan (*n*=15)				Microcrystalline Cellulose (*n*=16)				Between
Taxonomic Group	Baseline	Week 6	Within *p* value (adj.)	Δ Taxa	Baseline	Week 6	Within *p* value (adj.)	Δ Taxa	*p* value (adj.)
Phyla									
Firmicutes	65.76 ± 7.49	59.80 ± 5.76	0.761(0.903)	-5.95 ± 8.59	64.2 ± 10.0	65.8 ± 9.05	0.143(0.457)	1.68 ± 6.82	0.281(0.856)
Bacteroidetes	17.19 ± 6.87	21.90 ± 7.44	0.021(0.215)	-4.70 ± 5.56	19.9 ± 6.57	21.8 ± 7.05	0.231(0.505)	1.91 ± 8.84	0.519(0.931)
Actinobacteria	11.41 ± 8.74	13.17 ± 8.60	0.072(0.300)	1.76 ± 5.01	7.67 ± 4.94	6.6 ± 4.45	0.403(0.743)	-1.12 ± 4.26	0.065(0.416)
Family									
Erysipelotrichaceae	7.27 ± 6.25	3.82 ± 3.52	**0.0001(0.004)**	-3.45 ± 3.98	5.56 ± 5.09	5.40 ± 4.05	0.820(0.957)	-0.17 ± 4.22	**0.015(0.149)**
Lachnospiraceae	30.66 ± 6.60	30.20 ± 4.33	0.359(0.567)	-0.46 ± 7.86	26.7 ± 10.1	30.4 ± 9.46	**0.002(0.117)**	3.65 ± 7.54	0.188(0.731)
Bifidobacteriaceae	5.41 ± 5.37	8.50 ± 7.15	**0.0026(0.036)**	3.08 ± 4.20	3.68 ± 4.16	3.3 ± 4.26	0.668(0.882)	-0.38 ± 2.86	**0.017(0.149)**
Genera									
Blautia	3.03 ± 2.18	4.28 ± 2.21	0.025(0.223)	1.24 ± 2.19	3.11 ± 3.1	2.40 ± 2.02	0.463(0.806)	-0.64 ± 1.64	**0.010(0.149)**
Clostridium XVIII	1.10 ± 1.14	0.40 ± 0.44	**0.0003(0.0007)**	-0.76 ± 0.85	0.78 ±1.21	1.37 ± 2.67	0.705(0.898)	0.58 ± 1.55	**0.011(0.149)**
Lachnospiracea incertae sedis	7.13 ± 4.42	5.24 ± 3.72	0.120(0.324)	-1.89 ± 5.59	5.57 ± 4.04	7.95 ± 6.90	0.044(0.310)	2.38 ± 5.15	**0.017(0.149)**
Ruminococcus2	2.10 ± 1.63	1.42 ± 1.32	0.041(0.258)	-0.67 ± 1.36	1.41 ± 0.73	2.16 ± 1.53	0.028(0.289)	0.74 ± 1.17	**0.005(0.149)**
Prevotella	0.99 ± 1.92	3.75 ± 7.66	**0.0001(0.006)**	2.75 ± 6.19	4.25 ± 8.49	5.10 ± 9.13	0.010(0.229)	0.85 ± 4.97	0.740(0.959)
Bifidobacterium	5.41 ± 5.37	8.50 ± 7.15	**0.002(0.003)**	3.08 ± 4.20	3.68 ± 4.17	3.29 ± 4.26	0.632(0.867)	-0.38 ± 2.86	**0.017(0.149)**
Megamonas	1.44 ± 4.21	2.21 ± 5.37	0.151(0.353)	0.76 ± 1.60	0.14 ± 0.51	0.31 ± 0.87	0.013(0.229)	0.17 ± 0.48	0.598(0.931)
Parasutterella	0.58 ± 0.88	0.54 ± 1.14	0.389(0.567)	-0.04 ± 0.55	0.40 ±0.80	0.66 ± 1.00	**0.003(0.117)**	0.25 ± 0.53	**0.011(0.149)**
Operational Taxonomic Units *(OTU Number, closest hit in database, % identity)*									
OTU6 (*Prevotella copri*, 99%)	0.95 ± 1.84	3.62 ± 7.41	**0.0001(0.006)**	2.67 ± 6.08	3.61± 7.53	4.41 ± 8.02	0.175(0.833)	0.79 ± 5.24	0.1194(0.398)
OTU79 (*Muribaculaceae*)	0.07 ± 0.21	0.14 ± 0.38	**0.0001(0.006)**	0.07 ± 0.21	0.50 ± 1.84	0.11 ± 0.45	0.596(0.932)	-0.38 ± 1.39	**0.0003(0.036)**
OTU11 (*Subdoligranulum variabile*, 100%)	1.26 ± 1.67	2.41 ± 3.27	**0.0020(0.042)**	1.14 ± 1.89	1.78 ± 1.89	1.38 ± 1.39	0.781(0.977)	-0.39 ± 1.24	0.1880(0.480)
OTU21 (*Coprobacillus* sp.)	1.74 ± 1.25	0.81 ± 0.69	**0.0026(0.042)**	-0.92 ± 1.13	1.71 ± 1.44	1.95 ± 2.52	0.433(0.833)	0.23 ± 1.90	**0.0105(0.105)**
OTU38 (*Phascolarctobacterium succinatutens*, 100%)	0.39 ± 0.95	0.77 ± 1.68	**0.0043(0.042)**	0.38 ± 0.80	0.25 ±1.00	0.44 ± 1.78	0.322(0.671)	0.19 ± 0.78	**0.0063(0.105)**
OTU4 (*Bifidobacterium longum*, 100%)	3.53 ± 4.35	7.04 ± 6.76	**0.0020(0.042)**	3.51 ± 4.51	1.55 ± 2.24	1.64 ± 3.96	0.375(0.833)	0.09 ± 1.99	**0.0105(0.105)**
OTU46 (*Clostridium leptum*, 100%)	0.31 ± 0.77	0.56 ± 1.31	**0.0043(0.042)**	0.25 ± 0.58	0.08 ± 0.08	0.56 ± 1.35	0.403(0.833)	0.47 ± 1.35	**0.0105(0.105)**
OTU53 (*Bacteroides plebeius*, 98.76%)	0.20 ± 0.55	0.56 ± 1.58	**0.0043(0.042)**	0.35 ± 1.20	0.72 ± 2.66	1.06 ± 2.97	0.297(0.833)	0.34 ± 0.74	0.4700(0.723)
OTU56 (*Bacteroides* sp.)	0.15 ± 0.66	0.84 ± 3.04	**0.0043(0.042)**	0.68 ± 2.44	BDL	BDL	BDL	BDL	BDL
OTU85 (*Blautia obeum*, 100%)	0.43 ± 0.53	1.33 ± 1.30	**0.0034(0.042)**	0.89 ± 1.31	0.55 ± 0.55	0.43 ± 0.37	0.375(0.833)	-0.11 ± 0.46	**0.0041(0.105)**

OTU32 (*Mollicutes*)	0.11 ± 0.43	0.35 ± 1.34	**0.0084(0.076)**	0.23 ± 0.91	1.59 ± 3.91	0.80 ± 1.95	0.073(0.671)	-0.79 ± 2.08	**0.0090(0.105)**

Supplementary Table 2. *Continued*

	Arabinoxylan (*n*=15)				Microcrystalline Cellulose (*n*=16)				
Taxonomic Group	Baseline	Week 6	Within *p* value (adj.)	Δ Taxa	Baseline	Week 6	Within *p* value (adj.)	Δ Taxa	Between *p* value (adj.)
OTU32 (*Mollicutes*)	0.11 ± 0.43	0.35 ± 1.34	**0.0084(0.076)**	0.23 ± 0.91	1.59 ± 3.91	0.80 ± 1.95	0.073(0.671)	-0.79 ± 2.08	**0.0090(0.105)**
OTU41 (*Eubacterium oxidoreducens*, 99%)	0.96 ± 1.08	0.42 ± 0.48	**0.0151(0.125)**	-0.53 ± 0.70	1.07 ±0.98	0.85 ± 0.80	0.705(0.977)	-0.22 ± 0.71	**0.0009(0.105)**
OTU26 (*Bacteroides ovatus*, 100%)	0.41 ± 0.91	0.98 ± 1.16	**0.0181(0.129)**	0.57 ± 1.26	0.26 ± 0.28	0.54 ± 0.69	0.024(0.356)	0.31 ± 0.55	0.3182(0.578)
OTU5 (*Ruminococcus bromii*, 100%)	1.25 ± 1.56	0.87 ± 1.31	**0.0181(0.129)**	-0.38 ± 0.90	2.98 ± 2.94	2.78 ± 3.30	0.743(0.977)	-0.19 ± 1.74	0.0710(0.326)
OTU7 (*Bacteroides uniformis*, 100%)	2.38 ± 1.99	1.68 ± 2.65	**0.0021(0.143)**	-0.70 ± 2.23	1.73 ± 2.22	1.55 ± 1.60	0.820(0.977)	-0.17 ± 1.12	0.0780(0.326)
OTU65 (*Ruminococcus lactaris*, 100%)	0.37 ± 0.65	0.09 ± 0.29	0.0353(0.185)	-0.27 ± 0.62	0.24 ± 0.32	0.34 ± 0.41	0.073(0.671)	0.11 ± 0.25	**0.0072(0.105)**
OTU432(*Bacteroides cellulosilyticus*, 98.76%)	0.16 ± 0.44	1.09 ± 2.99	0.0255(0.150)	0.93 ± 2.56	0.14 ± 0.27	0.06 ± 0.13	0.252(0.833)	-0.08 ± 0.24	**0.0105(0.105)**
OTU31 (*Blautia faecis*, 100%)	1.45 ± 1.63	1.64 ± 1.06	0.1876(0.360)	0.19 ± 1.47	0.94 ± 0.65	0.58 ± 0.45	0.015(0.356)	-0.36 ± 0.46	**0.0105(0.105)**
OTU10 (*Holdemanella biformis*, 99.58%)	4.52 ± 5.95	2.31 ± 3.36	0.1069(0.248)	-2.20 ± 3.25	1.98 ± 2.81	1.65 ± 2.48	0.018(0.356)	-0.33 ± 0.72	0.7700(0.906)
OTU116(*Eubacterium ramulus*, 100%)	0.22 ± 0.23	0.21 ± 0.29	0.6787(0.780)	-0.01± 0.23	0.11 ± 0.17	0.19 ± 0.18	0.018(0.356)	0.08 ± 0.15	0.0405(0.155)
OTU47 (*Bacteroides xylanisolvens*, 100%)	0.26 ± 0.36	0.58 ± 1.01	0.4887(0.634)	0.31 ± 0.95	0.33 ± 0.53	0.88 ± 1.53	0.007(0.356)	0.55 ± 1.09	0.0855(0.329)
OTU54 (*Parasutterella excrementihominis*, 100%)	0.58 ± 0.89	0.54 ± 1.15	0.3591(0.561)	-0.04 ± 0.55	0.38 ± 0.78	0.64 ± 0.97	0.013(0.356)	0.26 ± 0.54	0.0170(0.155)
Co-Abundance Response Groups (CARGs) (Sum of relative abundance of OTUs within each CARG)									
CARG1	5.97 ± 4.97	12.9 ± 11.02	**0.0034**	6.98 ± 8.76	4.86 ± 3.70	4.98 ± 4.72	0.97	0.12 ± 2.64	**0.0072**
CARG2	2.56 ± 1.21	6.09 ± 6.09	**0.0084**	3.53 ± 5.30	2.92 ± 2.51	3.22 ± 2.43	0.46	0.29 ± 1.19	**0.0170**
CARG3	1.21 ± 1.58	2.48 ± 3.58	**0.0200**	1.27 ± 2.83	2.37 ± 2.51	2.86 ± 2.94	0.07	0.48 ± 1.65	0.4600
CARG4	2.90 ± 4.09	3.94 ± 4.61	0.1600	1.04 ± 2.57	3.29 ± 4.20	2.85 ± 2.69	0.85	-0.43 ± 2.95	0.3100
CARG5	1.94 ± 1.90	1.78 ± 1.42	0.7100	-0.15 ± 1.82	1.66 ± 1.64	2.43 ± 2.86	0.14	0.77 ± 2.27	0.2400
CARG6	4.63 ± 3.59	6.34 ± 8.30	**0.0200**	1.70 ± 6.83	7.50 ± 7.18	9.14 ± 7.84	0.43	1.63 ± 5.76	0.4900
CARG7	13.82 ± 8.19	8.21 ± 6.49	**0.0200**	-5.60 ± 8.69	11.7 ± 5.93	14.4 ± 7.73	0.25	2.33 ± 6.20	**0.0063**

[1] Statistical significance of within-group shifts were determined by Wilcoxon signed-rank tests, while between-group shifts were determined by Mann-Whitney U tests. Data are presented as relative abundance, and were centered log-ratio (CLR) transformed prior to the statistical analyses. *p* values were adjusted by FDR, whereas FDR significance was set at $q<0.15$. BDL, below detection limit; Δ Taxa, absolute change in relative abundance from baseline to week 6.

Supplementary Table 3. Fecal pH, Moisture Content, and Concentration and Molar Proportion of Fecal Short-Chain Fatty Acids[1].

	Fecal pH, Moisture Content, and Short-Chain Fatty Acids (Mean ± SD)								
	Arabinoxylan				Microcrystalline Cellulose				Between *p* value
	Baseline	Week 6	Within *p* value	Δ SCFA	Baseline	Week 6	Within *p* value	Δ SCFA	
Fecal pH	6.8 ± 0.5	6.7 ± 0.5	0.57	-0.1 ± 0.6	6.8 ± 0.6	7.0 ± 0.6	0.18	0.3 ± 0.8	0.095
Moisture content (%)	71.68 ± 7.27	72.42 ±6.99	0.8	0.74 ± 6.68	72.23 ± 8.69	69.92 ± 7.2	0.403	-2.31 ± 11.18	0.52
Fecal Concentration *(µmol/g)*									
Total SCFA	145.3 ± 99.0	151.4 ± 80.7	0.59	6.2 ± 65.2	145.3 ± 111.9	126.2 ± 55.2	0.59	-19.0 ± 96.9	0.56
Acetate	90.2 ± 56.2	94.8 ± 50.9	0.99	4.6 ± 37.0	91.4 ± 63.6	80.8 ± 30.3	0.32	-10.6 ± 56.6	0.46
Propionate	31.2 ± 28.6	39.0 ± 26.0	0.15	7.8 ± 21.1	31.7 ± 32.4	24.0 ± 13.5	0.40	-7.7 ± 24.8	0.11
Butyrate	23.8 ± 18.4	17.6 ± 10.3	0.48	-6.3 ± 16.7	22.3 ± 19.3	21.4 ± 16.9	0.93	-0.8 ± 21.9	0.59
Valerate	3.1 ± 1.6	3.1 ± 1.7	0.93	-0.004 ± 1.1	3.5 ± 2.7	3.1 ± 1.6	0.59	-0.3 ± 2.4	0.76
Total BCFA	7.8 ± 3.6	8.4 ± 4.6	0.71	0.6 ± 3.8	7.4 ± 3.6	6.4 ± 2.5	0.43	-1.1 ± 4.8	0.39
Isobutyrate	3.1 ± 1.1	3.4 ± 1.6	0.52	0.3 ± 1.5	2.9 ± 1.3	2.6 ± 0.9	0.49	-0.2 ± 1.8	0.26
Isovalerate	4.7 ± 2.5	5.0 ± 3.0	0.97	0.3 ± 2.4	4.6 ± 2.5	3.7 ± 1.7	0.29	-0.8 ± 3.2	0.46
Molar Proportion of Total SCFA *(%)*									
Acetate	62.7 ± 6.4	62.7 ± 6.7	0.97	-0.04 ± 6.9	66.1 ± 6.1	66.6 ± 9.2	0.78	1.0 ± 8.3	0.66
Propionate	20.7 ± 6.3	25.0 ± 6.8	**0.04**	4.3 ± 7.8	18.9 ± 4.2	17.8 ± 5.6	0.23	-1.4 ± 4.5	**0.01**
Butyrate	16.6 ± 5.0	12.3 ± 5.2	**0.018**	-4.3 ± 6.4	15.0 ± 4.1	15.9 ± 7.1	0.89	0.4 ± 7.0	0.08

[1] Statistical significance of within-group shifts were determined by Wilcoxon tests, while between-group shifts (Δ Week 6 - Baseline) were determined by Mann–Whitney tests. Δ SCFA, absolute change from baseline to week 6.

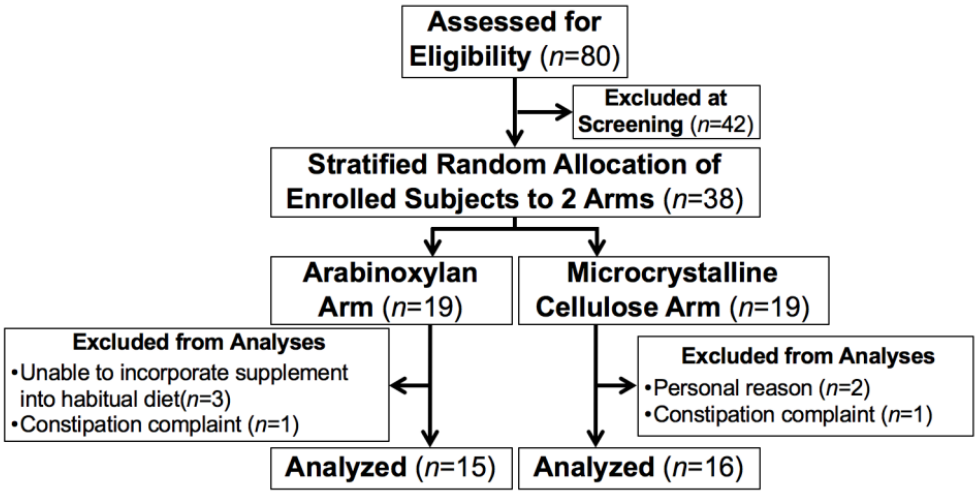
Supplementary Table 4. The association between individualised response of SCFA (W6-BL) and gut microbiome features (BL, W1-BL, W6-BL)*

Arabinoxylan			SCFA response (W6-BL)								
			Propionate			Acetate			Butyrate		
		Preditors	Coeff	p	AICc	Coeff	p	AICc	Coeff	p	AICc
Baseline Microbiome	All OTUs	PC1	2.86	0.01	133.2			146.2			113.3
		PC2				-4.15	0.03		-1.21	0.05	
		PC3	2.77	0.034							
	Sig. OTUs	PC1			134.5	-8.46	0.03	150	-2.85	0.02	115.6
		PC2	7.23	0.02		2.3	0.58		-0.8	0.52	
	CARGs	CARG5	5.54	0.07	139	10.16	0.013	144.2			110.3
		CARG6							-1.58	0.014	
		CARG7	1.43	0.03							
	Single OTUs	OTU85 *B. obeum*	-21.86	0.03	139.5			147			110.7
		OTU46 *C. leptum*	-9.28	0.17							
		OTU41 *E. oxidoreducens*				-13.48	0.046		-5.02	0.016	
Shifts of Microbiome (W1-BL)	All OTUs	PC1	-2.66	0.03	136.3			149.4			116.3
		PC2	-2.48	0.07							
		PC3				3.35	0.145		0.88	0.2	
	Sig. OTUS	PC1	-6.99	0.011	133.2			144.4			114.2
		PC2							2.67	0.08	
		PC3				12	0.01				
	CARGs	CARG1	1.89	0.0045	131.1			148.6			116.2
		CARG7				2.02	0.09		0.482	0.23	
	Single OTUs	OTU6 *P. copri*	-1.5	0.04	138.67			140.2			103.1
		OTU38 *P. succinatutens*	8.38	0.05							
		OTU7 *B. uniformis*				10.32	0.002				
		OTU41 *E. oxidoreducens*							7.22	0.0006	

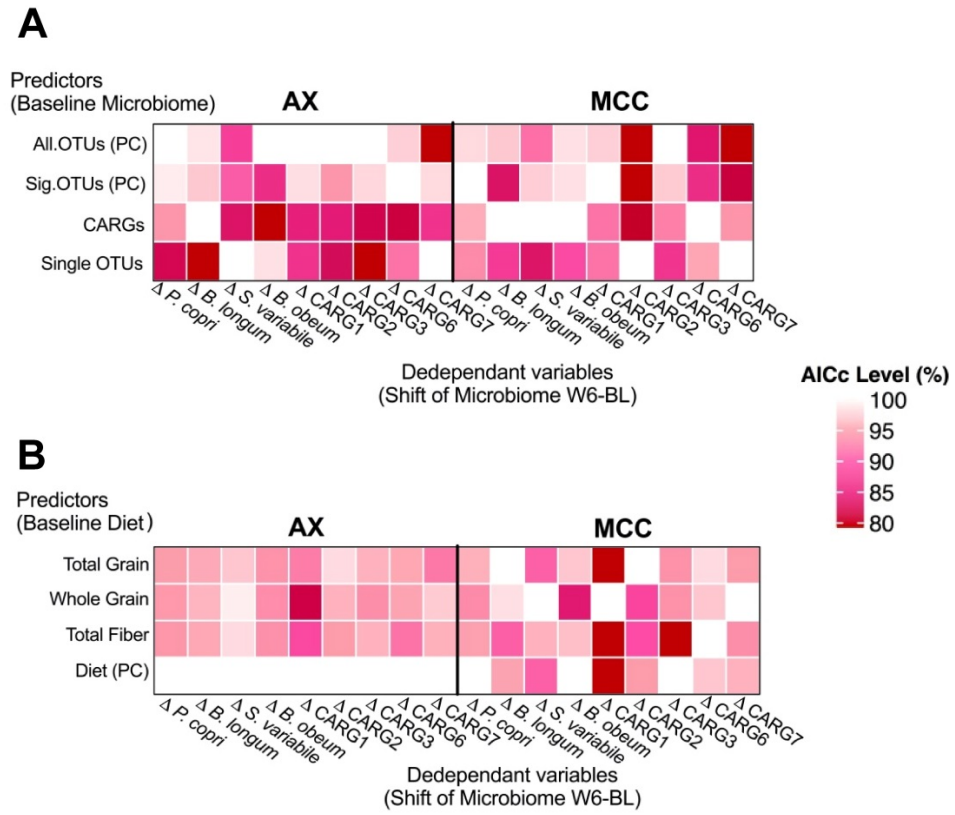
Supplementary Table 4: Continued

Shifts of Microbiome (W6-BL)	All OTUs	PC1	-4.23	0.00009	123.7			148.7		118
		PC2	-1.86	0.046		3.72	0.1			
		PC3							0.16	0.8
	Sig. OTUs	PC1	6.54	0.00062	125	8.05	0.02	148.7		112.6
		PC2	6.4	0.046		-3.92	0.35		-3.14	0.039
		PC3								
	CARGs	CARG1	1.63	0.0016	128.7	1.64	0.05	151.6		109.4
		CARG2				0.109	0.93			
		CARG3							2.13	0.009
	Single OTUs	OTU85 *B. obeum*	9.76	0.007	132.3			145.8		108.4
		OTU11 *S. variablile*				8.56	0.027			
		OTU41 *E. oxidoreducens*							8.48	0.006

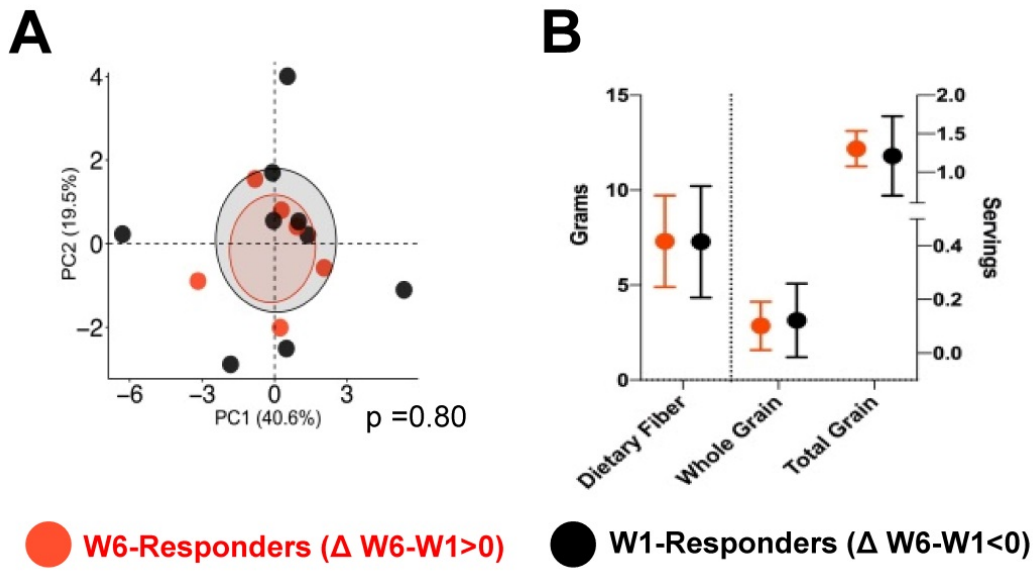
*Coefficient and p-value were provided for each predictor (row). Each model containes the very best one or two predictors of PC variables, CARGs or significant OTUs selected by stepwise regression. All models were adjusted by sex/fiber dose. Quality of each model was evaluated by AICc value. Coeff: Coefficient; p : p value; Sig.OTUs: significant Excluded from a model



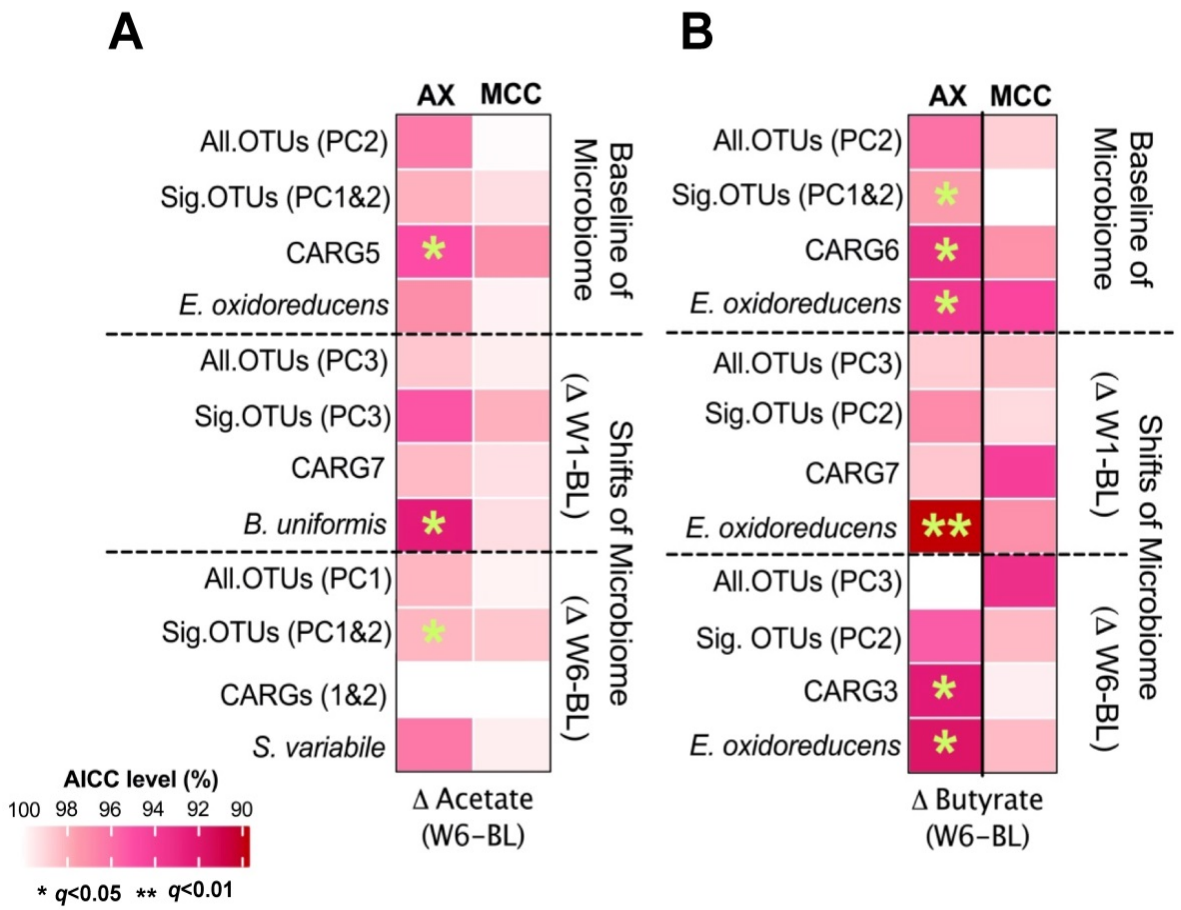
Supplementary Figure 1: Flow chart summarizing subject flow through study.



Supplementary Figure 2: Baseline fecal microbiome composition and diet showed no association with the individualized microbiome response to arabinoxylan. (A) Heatmap shows the associations between microbiome compositional shifts (ΔW6–BL; dependent variables; columns) and baseline microbiome profiles (predictor variables; rows). (B) Heatmap shows the association between microbiome compositional shifts (ΔW6–BL; dependent variables; columns) and baseline diet variables (predictor variables; rows). Cells represent individual multiple linear regression models (with FDR correction) that assess whether the predictors explain the individualized compositional shifts. Multivariate microbiome and diet data were simplified into principle component variables (*i.e.* PC1, PC2, PC3) prior to analysis. Each model contained the very best one to two predictors of PC variables (microbiome and diet), CARGs, or significant OTUs (predictors selected by stepwise regression), or either total grains, whole grains, or total fiber alone. All models were adjusted by sex/fiber dose. Colors from white to red indicate relative AICc (Akaike information criterion corrections) values calculated by (AICc value/Highest AICc value) * 100. Lower AICc values (red) indicate higher quality models. BL; baseline; OTU, operational taxonomic unit; W1, week 1; W6, week 6.

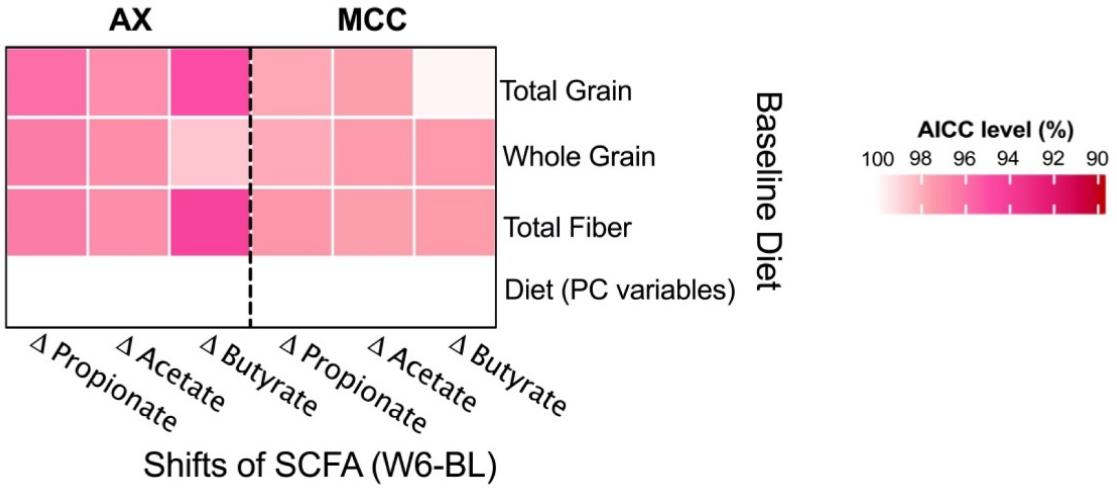


Supplementary Figure 3: Temporal propionate response to arabinoxylan supplementation showed no association with baseline diet. (A) Principal component analysis plot based on Euclidean distance comparing the baseline, calorie-adjusted intake of Canada's Food Guide food group and macronutrient variables between W6-(red) and W1-(black) responders. Data were analyzed using PERMANOVA. **(B)** Comparison between W6-(red) and W1-(black) responders in single dietary factors (total grains, whole grains, and dietary fiber) performed using Mann-Whitney tests. W1, week 1; W6, week 6.



Supplementary Figure 4: Individualized short-chain fatty acid response to arabinoxylan could be explained by baseline and shifts of the gut microbiome. Heatmap shows the associations between the individualized response of (A) Acetate and (B) Butyrate (ΔW6–BL; dependent variable; columns) and microbiome profiles (BL, ΔW1–BL, ΔW6–BL; predictor variables; rows). Cells represent individual multiple linear regression models (with FDR correction) that assess whether the predictors explain the individualized SCFA responses. Multivariate microbiome data were simplified into principle component variables (*i.e.* PC1, PC2, PC3) prior to analysis. Each model contained the very best one to two predictors of PC variables, CARGs, or significant OTUs selected by stepwise regression. All models were adjusted by sex/fiber dose. Colors from white to red indicate relative AICc (Akaike information criterion corrections) values calculated by (AICc

value/Highest AICc value) * 100. Lower AICc values (red) indicate higher quality models. BL; baseline; CARG, co-abundance response group; OTU, operational taxonomic unit; W1, week 1; W6, week 6. Details of model components were presented in Table S4.



Supplementary Figure 5: Individualized short-chain fatty acid response to arabinoxylan could not be explained by baseline diet. Heatmap shows the associations between the individualized SCFA response (acetate, propionate, butyrate; dependent variable; columns) and baseline diet variables (predictor variables; rows). Cells represent individual multiple linear regression models (with FDR correction) that assess whether the predictors explain the individualized SCFA responses. Multivariate diet data were simplified into principle component variables (*i.e.* PC1, PC2, PC3) prior to analysis. Each model contained total grains, whole grains, or total fiber, or the best one to two PC variables as the predictors (PCs selected by stepwise regression). All models were adjusted by sex/fiber dose. Colors from white to red indicate relative AICc (Akaike

information criterion corrections) values calculated by (AICc value/Highest AICc value) * 100. Lower AICc values (red) indicate higher quality models. BL; baseline; W6, week 6.

CHAPTER 5: DISCUSSION

5.1 Discussion

In the present study, we characterized the impact of a six-week, high-dose corn bran AX supplementation on the composition and function of the fecal bacterial community in healthy adults with overweight and class-I obesity. AX treatment changed community structure and induced specific shifts in the composition of the gut microbiota that manifested themselves after one week of treatment without further changes at W6. AX induced increases in propionate output. Both compositional and functional responses were highly individualized, and the propionate response showed two distinct temporal patterns. Although compositional responses to AX could not be predicted, and functional responses were independent of baseline diet, baseline microbiome composition and especially the compositional shifts explained the propionate response. The non-fermentable MCC showed virtually no effect on gut microbiota composition or function.

An understanding of compositional and functional responses of the gut microbiome towards diet requires an ecological framework [31]. The provision of AX provides resources that can be used by microbes that possess the traits to either access the chemical structures or competitively utilize public goods released during AX degradation [31]. In our study, the dominant effect of AX was directed towards two bacterial species, *B. longum* and *P. copri*, while nine additional OTUs showed smaller increases, including three *Bacteroides* spp. This high degree of specificity towards *B. longum* over other *Bifidobacterium* species is in agreement with other studies testing long-chain AXs [107, 108], as genes encoding AX-degrading glycosidase (*e.g.* β-xylosidase and α-arabinofuranosidase) are shown to be conserved and abundant only among *B. longum* strains [109, 110]. *P. copri* has also been described to efficiently utilize AX [111, 112], which can be explained by their polysaccharide utilization loci. This Bacteroidetes xylan utilization system is shown to be

conserved across xylan-degrading *Prevotella* [113, 114], and as *P. copri* is considered the only human gut symbiont in the *Prevotella* genus [115], it would explain its selective increase in our study. The xylan utilization system is also conserved across xylan-degrading *Bacteroides*, which are more abundant in the human gut microbiome, thus explaining the reduced specificity towards *Bacteroides* enrichment [113, 114]. Interestingly, the corn bran AX used in our study showed a much higher degree of specificity than wheat bran extracted AXOS, which promoted different species of *Bifidobacterium* and *Prevotella* along with other genera [21, 22]. This prominent difference in specificity is likely attributed to variations their structural features. Specifically, the corn bran AX exhibited a relatively high arabinose-to-xylose ratio of 0.56 and contained high amounts of galactose (9.7%), which suggests a heavily branched structure with complex side chains [70, 116, 117]. To access and utilize such complex structures, bacterial genomes must encode a more extensive repertoire of binding and degradation proteins relative to the machinery need for AXOS utilization, which are generally simpler in structure [118]. For instance, *Bifidobacterium adolescentis* has been shown to utilize simple AXOS both in monoculture [119] and during co-culture with *B. ovatus* on simple AXs, but not during co-culture on corn bran AX [120].

Exploring the response of the bacterial community in the context of ecological guilds provides a more complete view on the interactions among the bacterial species in the degradation of AX. This analysis showed the strongest response in CARG1 and CARG2, which further show extensive intra- and inter-guild associations. The response within CARG1 is dominated by *B. longum*, which showed connections to four out of five members within CARG1 (*B. plebeius*, *Bacteroides* sp., *P. succinatutens*, and S. *variabile*) and one member in CARG2 (an unclassified *Lachnospiraceae*), suggesting that its growth is closely connected to the growths of other

members. *B. longum* has been shown to be a primary degrader of AXs [109, 110], cleaving the complex AX structure by potentially secreting soluble AX-degrading glycosidase [121, 122], thus in theory making AXOS (or even xylose, arabinose, and galactose) accessible to secondary fermenters like *B. obeum* and *S. variabile* [105, 107, 123] (Figure 8B). This would simultaneously make corn bran AX more accessible to xylan-utilizing *Bacteroides*, for instance *B. plebeius*, which would likely be capable of degrading AX on their own [114, 124]. This cross-feeding would explain the strong positive associations between *B. longum* and the other OTUs within CARG1. In contrast, *P. copri* also increased and is also likely a primary AX degrader, but showed very few positive interactions within CARG6, suggesting the bacterium behaves 'selfishly' [111]. Our findings suggest that no singular 'keystone species' initiates the degradation of AX, as it has been described for type III resistant starches [25]. Most likely, a few primary degraders, including *B. longum*, *P. copri*, and certain *Bacteroides* species, assume this task.

The ecological connections described above provide a basis to understand the effects of AX on microbiome metabolism, which is characterized by an increase in propionate. The specificity of long-chain AXs for propionate has been previously described [125], and is affiliated with a higher presence of arabinose side-chains [14, 126]. Although *P. copri* is a primary degrader, the bacterium does not produce propionate [103], and therefore does not explain the fecal propionate response in our study. However, it indirectly contribute to propionate production by producing succinate [103], which can be converted to propionate by metabolite-utilizers like *P. succinatutens*.

Metabolic interactions are likely more relevant within CARG1. Although the numerically dominant responder within this CARG, *B. longum* does not produce propionate by itself, but its enrichment is closely connected to the growth of other members that encode in their genomes metabolic pathways for propionate production (*i.e. B. obeum*, *P. succinatutens*, *B. plebeius*, and

Bacteroides sp.) [104, 106]. In accordance, MLR models based on *B. obeum*, *B. plebeius*, and *P. succinatutens* are better predictors of propionate response than *B. longum*. Although MLR results in significant models with single taxa, models using CARG1 are better at predicting shifts of propionate, indicating that groups of bacteria collaborate in producing propionate. Overall, our analyses on ecological guilds suggest cooperative and syntrophic interactions among *B. longum*, *B. obeum*, *P. succinatutens*, and some *Bacteroides* species in the degradation of AX to produce propionate, while *P. copri* displays a more competitive phenotype during AX degradation.

A reduction in the proportion of butyrate could be the result of the decrease of several butyrate-producing bacteria in CARG7, such as *E. oxidoreducen* [102], which was the best predictor of the butyrate response. In our network, CARG7 represented the only 'guild' that was strongly reduced by AX and showed a high number of negative correlations to bacteria from CARGs that were promoted by AX. This finding, and the fact that bacteria in CARG7 have been shown to have either slower or no growth on AX (*e.g. B. uniformis*, *Agathobacter rectalis* [reclassified from *Eubacterium rectale*] and *Ruminococcus bromii* [127-129]) suggests that they were outcompeted by taxa able to competitively utilize AX . Interestingly, AXOS supported a wide range of butyrate-producing bacteria, such as *Eubacterium hallii*, *F. prausnitzii*, *A. rectalis*, and *Roseburia faecis* [21, 22]. In short, the complex structure of corn bran AX likely restricted the access of butyrate-producing bacteria known to grow on simple linear AXs, such as *A. rectalis* and *Roseburia* sp. [128, 130], which in turn affected butyrate levels while selecting for propionate producers.

Although significant findings on both specific taxa and propionate production were discovered, these variables displayed a high degree of individuality. In terms of taxa, this might be driven by the inter-individual differences in baseline microbiome composition and diet [18]. Although the responses of *P. copri* were strictly linked to the presence of the species at baseline,

as only individuals that already possessed *P. copri* showed a response, our MLR models showed no significant association between the baseline and individualized responses. However, some models showed *p* values below 0.001 before FDR correction, suggesting that associations between the compositional response to AX and the baseline microbiome exist but could not be detected with the sample size of our study. Therefore, future studies on the individualized response of DF should be conducted with larger sample sizes.

Individuality was especially pronounced when looking at metabolite production. Stratifying the population based on their SCFA shifts clearly identified W6- and W1-responders that differed in how their microbiome responded to AX, but not in baseline microbiome composition or diet. Our MLR analyses revealed that shifts in propionate output (ΔW6-baseline) could be explained by W6 shifts in the microbiome, and to a lesser degree W1 shifts and baseline composition, but not diet. PCs generated from the entire microbiome performed better than individual CARGs or taxa, confirming our conclusion from above that propionate production is the result of AX utilization by ecological guilds. This supports the application of an ecological framework to interpret the outcome of DF fermentation by the human microbiome.

Dietary history in our study did not predict propionate responses or contributed to the quality of the models. This could be reflective of the fact that diet is only one of many contributors to the variation of microbiomes [131, 132], although we cannot exclude that our small sample size and limitations in the self-reported dietary intake data contributed to the lack of signal [133].

Put together, our analyses showed that the effects of AX were specific for the promotion of *B. longum* and *P. copri*, and for an increased propionate level. These findings have implications in the targeted use of AX to modulate the gut microbiome for improved health. Probiotic treatment

with *B. longum* strains has been shown to be health-promoting in a variety of contexts [58], including gastrointestinal [134, 135], immunological (*e.g.* anti-allergy and anti-inflammatory [136, 137]), and psychological (*e.g.* depression and anxiety [138, 139]) disorders. The specific enrichment of this species supports the use of AX in synbiotic applications with *B. longum*. Another finding that warrants attention in the context of health is the increase in *P. copri*. The species was associated with the improved glucose metabolism after whole grain barley kernel treatment [140], and correlated with weight loss in volunteers that consumed diets high in whole grains [64, 65]. *Prevotella* is a genus that has been consistently-negatively associated with an industrialized lifestyle [141, 142]. The reason for this reduction is unknown, but it has been speculated that reduced consumption of plant-based, high-DF foods is responsible [143]. The increase of *P. copri* after supplementing through AX supports this hypothesis, as AX is a dominant DF in whole grains, which are reduced in the westernized diet. The increased production of propionate would have implications for the treatment of obesity and related metabolic and immune alterations, as it has been shown in mice to enhance satiety via induction of anorectic gut hormones [49, 144], improve glucose metabolism via upregulation of intestinal gluconeogenesis [145], and support regulatory T cell development [146] and suppress proinflammatory interleukin-8 levels [147]. Overall, our findings suggest that AX has prebiotic properties in that it specifically promotes putatively health-promoting organisms and the increase of propionate, which makes it a candidate for targeted applications such as the prevention of obesity.

5.2 Conclusion

Supplementing AX promoted the increase of *B. longum*, *P. copri*, and propionate, which are considered beneficial microbes and a health relevant SCFA, respectively. By stratifying the population based on their response, our study revealed two distinct temporal response patterns in

fecal propionate. These findings are relevant as they provide a potential explanation for the inconsistent effect of DF in human intervention studies [16]. If metabolic functions relevant for the physiological effects of DF (*e.g.* propionate) are individualized, then effects might not be detectable without stratifying the human population. An understanding of the factors that explain the propionate responses to AX might allow the development of a framework to personalize the use of DF. Our findings that propionate responses can potentially be predicted through the baseline microbiome is therefore relevant as it provides a basis for the personalized use of AX based on an individual's fecal microbiome. However, larger studies are needed to develop robust machine learning algorithms to predict the health outcomes of DF based on microbiome characteristics.

CHAPTER 6: GENERAL DISCUSSION

6.1 General Discussion

Apart from providing insight into the effects of AX on the gut microbiota from an ecological perspective, this study provides information on the health effects of AX. AX supplementation has previously been shown to improve glucose metabolism[148, 149] and insulin sensitivity[148, 149], induce satiety [4, 150], and improve cholesterol [151] and lipid metabolism [152]. These effects could be linked to the gut microbiota, a topic that has been addressed by other research groups for different structures and sources of AX [65, 111, 140, 150, 153]. In parallel with the study conducted in my thesis, we also collected blood samples before and after the intervention to evaluate the effect of long-chain AX on host physiology. This study is hereon referred to as the AX-health study. This study is in the final stages of data analysis (Deehan et al., manuscript in preparation) and, therefore, is not presented this thesis. However, the two studies are very connected and can be discussed together to enhance the impact of the findings discussed in this thesis and open new directions for future studies.

Generally, AX has been shown to induce satiety [4, 150] and improve insulin sensitivity [148, 149], in both published literature and our AX-health study, and AX produced from wheat endosperms has an EFSA claim on the improvement of insulin sensitivity [9]. Improvements in both satiety and insulin sensitivity have been linked to the gut microbiome and to microbiome features impacted by long-chain AX (Deehan et al., manuscript in preparation). The findings presented in this thesis are, therefore, directly relevant. In my study, AX promoted the growth of *P. copri*, and this species has been linked improved glucose metabolism after consumption of barley kernel-based bread [140]. In addition, results obtained from our AX-health study show significant improvements in insulin resistance induced through AX (measured by HOMA-IR:

homeostasis model assessment of insulin resistance), suggesting that AX beneficially affects glucose metabolism. These improvements might be linked to the effects of AX on *P. copri*. To test this hypothesis, Deehan and colleagues are examining associations between markers of glucose metabolism and gut microbiome features. Regarding SCFA production, propionate has been reported to induce satiety [9], possibly by regulating PYY and GLP-1[49]. In our AX-health study, we also observed increased satiety (30-60 minutes after meal) as a result of AX consumption. These connections warrant investigations into the relationships between propionate levels and satiety in our study. Exploring the response of *P. copri* and propionate and how this is linked with health markers provides a more complete view of the impact of long-chain AX on host physiology and the gut microbiota, which may help inform dietary strategies that target obesity and its associated pathologies.

Recent evidence suggests that SCFAs play an important role in the regulation of immunometabolic markers of health and, therefore, modulating SCFA levels may be one strategy to address obesity-associated pathologies [9, 154]. Propionate reduced the proinflammatory cytokine interleukin-8 [147] and improved glucose metabolism via upregulation of intestinal gluconeogenesis [145], and support regulatory T cell development [146] and suppress proinflammatory interleukin-8 levels [147]. If SCFAs are critical intermediates for the health effects of DFs, but their production is vastly different between individuals, then this individuality could be a potential reason for the inconsistent effects of DF in human intervention trials and may justify the personalization of DF in nutrition strategies. Our findings provide evidence that such a personalization could be achieved by using the baseline microbiome, which is more practical than using AX-induced microbiome shifts, although the latter resulted in higher quality models in our study. Fecal microbiomes could be characterized at baseline and then used to stratify human

populations and assign them to different DF sources. However, additional studies are needed to establish predictive models to achieve such personalization.

Alternatively, different routes of SCFA supplementation could be explored. There are two methods which could be employed in interventional studies to accomplish this. (1) Consuming DFs that are fermented into SCFAs by the gut microbiota, (2) consuming SCFA-binding dietary fiber such as inulin propionate ester (IPE) [147] and (3) consuming SCFA-containing tablets such as sodium propionate [155] and sodium butyrate [156]. Apart from providing DFs as a major quantitative source of SCFAs, consuming SCFAs directly might be more convenient for users compared to taking high doses of DF. In addition, consuming sodium butyrate tablets can reduce the inflammation of acute ulcerative colitis [156] and supplementing propionate has been reported to improve glucose metabolism [157-159] and increase resting energy expenditure through the modulation of whole-body lipid oxidation [155]. However, while butyrate is well-known as the main energy source for colonocytes [160], butyrate taken from tablets would be absorbed in the small intestine, which would result in the loss of benefits for the colonocytes. Therefore, to provide energy for colonocytes, it is likely better for butyrate to be produced by microbiota from fermenting DF in the colon.

Although it might not be favorable for consumer when taking a high dose of DF daily, I still believe that consuming a high DF diet can provide the benefits from SCFAs as well as benefits that are independent to SCFA production. First, a DF-deficient diet has been linked to a thinner mucus layer [161], and a high-DF diet in germ-free mice has been shown to protect this layer from the consumption of introduced *Bacteroides thetaiotaomicron*, which is a dominant member of a normal human gut microbiota [162], compared to the polysaccharides-free diet[163] and simple sugar-diet [164]. In fact, gut bacteria are known to be able to degrade and consume intestinal mucus

layer for over five decades [165, 166].Therefore, a lack of DF forces the microbiota to switch to using the human mucus layer for nutritional support [167]. The mucus layer of the human colon maintains gut homeostasis, and the loss of this barrier integrity allows microbes and their products (lipopolysaccharides) to reach and interact with the epithelial layer, which can result in a pro-inflammatory response [168].

Second, consuming DF promotes laxation, increases fecal bulk, and reduces transit time; hence, it helps to ameliorate constipation [169] and improve bowel function [170, 171]. Third, the mechanisms by which DF, especially soluble and viscous DFs, can lower glucose and cholesterol levels are related to their physico-chemical properties. Some DFs can form a viscous layer that reduces starch digestion and, therefore, glucose absorption [148]. Viscous DFs also reduce the reabsorption of bile acids and enhances their excretion, which in turn promotes heptaic synthesis of bile acids from cholesterol, which lowers systemic cholesterol levels [172, 173]. Fourth, the next evidence came from an animal model when Bindels et al studied the effect of resistant starch (RS- a type of DF) in both conventionalized and germ-free mice. Their results showed that feeding germ-free mice RS type 4 improved insulin sensitivity [174], which further supports that some health benefits of DF are independent of the gut microbiota and SCFA production. In short, many health benefits of consuming DF are independent of the gut microbiota and SCFA production, indicating that there are more advantages in consuming a high DF diet compared to consuming only SCFA-containing tablets.

6.2 Implications

By promoting specific taxa and SCFA production, as well as improving host glucose metabolism and satiety, long-chain AX can be considered as a candidate for targeted applications in the treatment of obesity-associated insulin resistance. Since both the compositional changes

(e.g. promotion of *B. longum* and *P. copri*) and metabolic functional changes (propionate production) induced by AX are considered beneficial, the findings in this study support the consideration of this long-chain isolated AX as a prebiotic.

By stratifying the population based on their responses, our study revealed two distinct temporal response patterns in fecal propionate production. These findings may be implicated the inconsistent effects of DF supplementation in human intervention studies [16]. If metabolic functions relevant for the physiological effects of DF (e.g. propionate production) are individualized, then effects might not be detectable without stratifying the human population.

Prediction of SCFA responses based on baseline fecal microbiome composition would allow us to estimate the shifts of SCFA concentration prior to consuming a DF supplement, which could save time, money, and effort for both physicians and patients (if these responses are linked to physiological effects). Although the shifts of bacterial taxa predict SCFA levels better than baseline composition, to obtain such data requires that the dietary intervention is conducted for at least one week, which is expensive and, therefore, means it is unnecessary to obtain prediction models when actual SCFA data after treatment is available. Generally, the integrated data from this research established a basis to understand factors that explain propionate responses, providing a framework to personalize the use of AX.

6.3 Limitations

In the context of data analysis, stratifying the 15 subjects based on their patterns of response might reduce the power of the test to detect the differences in microbiota or diet between two groups. Moreover, 15 observations are not enough to develop and validate robust machine learning algorithms to predict the health outcomes of DF based on microbiome characteristics.

6.4 Future directions

When assessing how the gut microbiota composition influences responses to dietary interventions, it is highly recommended that future studies increase the sample size to 30 to expect around 15 samples for each responder and non-responders group or use a crossover study design if a larger sample size is not feasible.

Long-chain AX improved glucose metabolism and increased satiety that should be further explored as a potential therapy for obesity-associated insulin resistance. On the other hand, long-chain AX increased satiety and promoted the growth of *P. copri*, which has been linked to weight loss [65]. Therefore, a future study could examine the role of *P. copri* and AX, provided as a synbiotic, in weight management. Alternatively, with a large enough sample size, future studies could develop models based on *P. copri* abundance at baseline to predict the success of weight loss programs. My study suggested that baseline microbiota can predict the level of propionate produced from consuming long-chain AX. Therefore, future studies should assess whether the baseline microbiota can predict health outcomes, such as an individual's response to certain types of DF using more robust predictive modelling like machine learning. In the long term, this knowledge obtained from these prediction models could be directly applied to personalized nutrition strategies for individuals.

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